PROCEEDINGS

American Academy of Forensic Sciences

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PROCEEDINGS
of the American Academy of Forensic Sciences 70th Annual Scientific Meeting

The *Proceedings* of the American Academy of Forensic Sciences is an official publication of the American Academy of Forensic Sciences (AAFS). It is devoted to the publication of the abstracts of technical oral papers and posters presented at the AAFS Annual Scientific Meeting. These include various branches of the forensic sciences such as anthropology, criminalistics, digital evidence, engineering, immunology, jurisprudence, odontology, pathology, psychiatry, questioned documents, and toxicology. Similar submissions dealing with forensic-oriented aspects of the social sciences are also included.

Please note that some of the abstracts included in the *Proceedings* deal with topics, results, and/or conclusions that are controversial. The publication of abstracts does not imply that the AAFS, its sections, or the individual section program chairs/committee members have verified or agree with the studies, results, and/or conclusions of each abstract. During the process of planning a scientific program, it is impossible to "peer-review" each abstract and presentation to the degree that is accomplished during manuscript review. Abstracts and presentations are accepted, in part, so they can be critiqued and reviewed by other scientists. Thus, a forum is created to discuss controversial issues.

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Articles from the AAFS *Proceedings* should be officially cited in the following format:

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S1 Raising the Bar in Forensic Science

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The goals of the 2018 Interdisciplinary Symposium (IDS) are to provide attendees with an overview on how forensic science is viewed inside and outside the forensic arena and to present ideas on what the forensic science community can learn from the broader scientific community regarding transparency, research integrity, and a strong commitment to education.

This presentation will impact the forensic science community by providing attendees with the perspectives of scientists from major scientific organizations inside and outside of forensic science and from attorneys from leading legal associations. Attendees will receive information on how to address strengths and weaknesses in the forensic sciences and should then be able to apply this knowledge in their own research and casework.

The 2018 IDS will comprise several distinguished scientists from within and outside of forensic science. The speakers will address perceived research gaps in the forensic sciences and discuss how to close these gaps.

To complement the 2018 American Academy of Forensic Sciences theme, *Science Matters*. the theme of the 2018 IDS is *Raising the Bar.* This symposium will highlight collaboration with the broader scientific community as a means to strengthen forensic science. Popular television shows have had a generally positive impact (for forensic science) on how the public views forensic science and forensic scientists. Despite the public perception of the near-infallibility of forensic science to detect and solve crimes, there has been criticism of forensic science from the scientific and legal communities. The criticism often notes that there is too much emphasis on the forensic aspect of various disciplines and not enough emphasis on foundational sciences. The National Commission on Forensic Science (NCFS), in which many AAFS members were active, provided a forum for members of the broader scientific community to contribute to recommendations designed to foster the foundational sciences of forensic science and to strengthen the forensic science community. Although the NCFS was not renewed upon the expiration of its charter in the spring of 2017, collaboration with the broader scientific community remains an important objective of AAFS.

At the 2018 IDS, a distinguished panel of researchers and speakers from professional organizations in the broader scientific community will address their perceptions of the strengths and challenges of the forensic science community. The 2018 speakers include the current leaders of the American Chemical Society (ACS), the American Physical Society (APS), the American Academy of Psychiatry and the Law (AAPL), the National Association of Criminal Defense Lawyers (NACDL), the Criminal Justice Section of the American Bar Association (ABA), and an AAFS past president. These speakers will discuss the efforts of their respective organizations to strengthen forensic science and the importance of "outside" voices in the advancement of forensic science. For example, with the APS, that could involve a discussion of what forensic science can learn from the broader scientific community about transparency, research integrity, and a strong commitment to education.

The keynote speaker is Vernon M. Neppe, MD, Director of the Pacific Neuropsychiatric Institute in Seattle, WA, Adjunct Professor of Psychiatry and Human Behavior, St. Louis University School of Medicine, St. Louis, MO, and former Director, Division of Neuropsychiatry, University of Washington, Seattle, WA. Dr. Neppe has contributed internationally in the specialties of neuropsychiatry and behavioral neurology, psychopharmacology, forensic psychiatry, anomalistic psychology, and epileptology. A distinguished psychiatrist, author, playwright, and philosopher, Dr. Neppe's presentation will highlight the importance of groundbreaking paradigm shifts to the advancement of scientific theory and practice.

Forensic Science, Reliability, Admissibility

S2 Research in Science: How Young Scientists Can Shape a Better Future

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After attending this presentation, attendees will better understand the novel research being conducted by both young and leading scientists within a variety of forensic science disciplines and how important that research is to the field.

This presentation will impact the forensic science community by educating young scientists on the importance of scientific research and the effects it has on the community at large. This special session also provides students and scientists with the opportunity to network with professionals in a unique setting that focuses on bridging the gap between established professionals and researchers and those aspiring to enter and thrive in the field.

Calling all students, young researchers, and those new to forensic science! The Young Forensic Scientists Forum (YFSF) focuses on providing young professionals and students continued education in different aspects of forensic science at each AAFS Annual Scientific Meeting. Registration for the YFSF Special Session includes a day-long session on Tuesday and a Breakfast Session on Thursday. The full-day special session will highlight the novel and exciting research being conducted by Academy members and students. The YFSF also hosts Bring Your Own Slides (BYOS) and Bring Your Own Posters (BYOP) sessions that provide young professionals and students with the opportunity to present their research.

The 2018 YFSF Special Session, *Research in Science: How Young Scientists Can Shape a Better Future*, is a day-long session that will leave attendees invigorated and excited about the research being conducted by Academy members and students. Participants will have a more complete understanding of the variety of disciplines that make up the Academy. The selection of speakers represents nearly every AAFS section, which makes for a highly diverse and unique suite of presentations.

The morning session will include an assortment of topics ranging from how to become an AAFS member to how 3D imaging can be used to analyze fingerprints. Following a delicious lunch, included with registration, attendees will hear about the Innocence Network and case-driven research. This day-long session will conclude with a Q&A session in which attendees can engage speakers and panelists in an open discussion.

YFSF, Education, Research

BS1 Analytical Thinking Skills: Essential Training for 21st-Century Forensic Scientists

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The goals of this presentation are to: (1) provide attendees with an overview on creating a stand-alone analytical thinking course in graduate curriculums; (2) instruct attendees on structuring a course to address real-world forensic science problems; and, (3) inform attendees on teaching students a specific methodology to break down complex problems.

This presentation will impact the forensic science community by providing students the skills for working in the field in the 21st century.

Forensic science can be considered a three-pronged science designed to train students in theory, research, and application; however, students can face particularly significant challenges without analytical thinking skills and understanding how to apply them to real-world forensic problems.

While analytical thinking skills are important for all scientists, they are particularly critical for forensic science students who must be able to comprehend complex scientific theories, principles, and methodologies, synthesize all of their knowledge, and apply it in real-world situations with unpredictable challenges. To complicate this challenge, many forensic scientists are regularly expected to testify, under oath in court, as to the analytical thinking process they applied to the problem. A weakness or flaw in their analytical thinking or in the application of the science to real-world problems, and the subsequent flawed or weak explanations to the trier of fact, could result in a serious appellate issue for the case, which could summarily damage or even end a young scientist's career.[1]

Forensic scientists are also often asked to serve on multidisciplinary commissions, panels, etc., to collaborate on specific forensic science problems, identify causation, and recommend viable solutions.

Dr. Richard Bloom, a well-known psychologist and educator, created the *Taxonomy of Educational Objectives Book*, which identified levels of cognition ranging from basic comprehension of scientific theories and principles to much more complicated levels of cognition.[1] Later revised, the book identified analytical thinking, creativity, and evaluation as the highest levels of cognition requiring specific teaching methodologies in order to develop and maintain these skills.[2]

In their 1992 paper presented at the annual meeting of the American Educational Research Association, Franklin and Theall noted that college instructors in soft disciplines utilized a wider range of teaching behaviors than those utilized by instructors in science disciplines.[3] Twenty-two years later, Benton and Cashen opined that Science, Technology, Engineering, and Math (STEM) instructors relied predominately on lectures in their courses, rather than more advanced levels of instructional behaviors to help students reach higher levels of cognition, including analytical and creative thinking.[4] This study conducted a review of ten forensic science graduate programs. Five programs were Forensic Science Education Programs Accreditation Commission (FEPAC) -accredited, and five were not. Results indicated none of these programs included a stand-alone analytical thinking course in their curriculums.

Part 1: Demonstrate how to break down a real-life forensic science problem into its most basic component parts in order to identify causation. Students will see that most real-life forensic science problems are complicated and multilayered, and they need a method to break the problem into manageable parts.

Part 2: Students will learn how to develop hypotheses to test causation using research methodologies, both qualitative and quantitative.

Part 3: Using a collaborative and multidisciplinary framework, students will learn how to use analytical and creative skills to identify relevant conclusions and, from those conclusions, develop creative but scientifically sound recommendations for solutions.

Currently, many graduate forensic science programs do not offer stand-alone analytical thinking courses. These skills are either not directly taught or are minimally covered in other courses. Without a solid understanding of analytical thinking skills, forensic science students are not being adequately equipped to face career challenges in the 21st century.

Reference(s):

1. Bloom, B. 1956. Taxonomy of Educational Objectives Book, Handbook 1: Cognitive Domain (New York: Longman).
2. Anderson, Lorin W., and David R. Krathwohl. 2001. *A Taxonomy for Learning, Teaching, and Assessing: A Revision of Bloom's Taxonomy of Educational Objectives.* (New York: Longman).
3. Franklin, Jennifer, and Michael Theall. *Disciplinary Differences: Instructional Goals and Activities, Measures of Student Performance, and Student Ratings of Instruction.* (1992).
4. Stephen Benton and William Cashin, *Student Ratings of Instruction in College and University Courses in Higher Education: Handbook of Theory and Research, ed. Michael Paulsen* (New York: Springer, 2014), 29.

Analytical, Challenges, Methodology

BS2 My Experiences as a Forensic Science Consultant for Crime Drama Television Series

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The goal of this presentation is to educate attendees on the processes that occur in the creation of a crime drama episode and why certain aspects of the "reality" of forensic science do not necessarily get translated to the screen. This presentation will discuss the real-life experiences of a forensic science consultant for such television shows as: *CSI: Crime Scene Investigation; CSI: Miami; Law and Order; BONES; Killer Instinct; Vanished; The Mob Doctor; Rizzoli and Isles; Drop Dead Diva; The Blacklist;* and *Rosewood*. Clips from various episodes of these shows will be presented with a discussion on what is real and what is "Hollywood Real." Attendees will be taken on a virtual tour of what happens in the writers' room and on the set.

This presentation will impact the forensic science community by providing attendees with a better understanding of the reason forensic science is portrayed in a certain manner on television crime dramas.

Forensic science flew under the radar for a number of decades. A brief spike in interest of the discipline occurred for a span of seven years from 1976 to 1983. *Quincy ME* was a very popular television show. It was Quincy played by Jack Klugman that fostered the career interest of this presenter. It was not until October of 2000 that the breakout television series *CSI: Crime Scene Investigation* hit the television airwaves. This megahit show took the world by storm. At one point, *CSI: Crime Scene Investigation* was the number-one rated show in the world and/or the most-watched television crime drama. Soon after, the television series led to a number of spinoffs: *CSI: Miami, CSI: New York*, and the much less-known *CSI: Cyber*, gathering number-one ratings for the CBS television network. Not to be outdone, the Fox Television Network launched its own hit forensic science series, *BONES*. On the cable networks, docudrama series such as *Forensic Files* aka *Medical Detectives, Cold Case Files,* and *Extreme Forensics* became extremely popular, with the HLN network broadcasting multiple episode reruns of *Forensic Files* nightly for six years after the original series ended. This presenter has had the distinct honor of having been featured in three episodes. Another hit television show, *NCIS*, and its spinoffs, *NCIS: Los Angeles*, and *NCIS: New Orleans*, feature forensic science components.

For the disciplines of forensic science, these television shows created a wave — perhaps a tsunami — of interest. Young people suddenly began to seriously look at career paths in forensic science. Colleges and universities began seriously looking at either creating or expanding programs in forensic science. During the 2000s, forensic science was in vogue. It was cool to work in a crime lab or be a crime scene investigator. Crime labs were seeing a tremendous uptick in applications for criminalist positions. Colleges and universities began to develop curricula to meet Forensic Science Education Program Accreditation Commission (FEPAC) guidelines. With success comes criticism, and, not surprisingly, the legal community and its allies in the legal educational community began their quest to diminish forensic science. The specter of the "CSI Effect" was raised because it was felt that forensic scientists, when testifying as expert witnesses, were seen as too believable by juries. Juries had unreasonable expectations when forensic science was not introduced in certain trials. Then, many disciplines were being questioned as to whether they were scientific or employing valid scientific techniques. Forensic science was now under the microscope.

For the past 17 years, I have served as a forensic consultant to such television shows as *CSI: Crime Scene Investigations, CSI: Miami, BONES, Rizzoli and Isles, Law and Order, Rosewood*, and *The Blacklist*, in addition to appearing on several forensic science-themed docudramas. As a television consultant, I would either respond to email questions from writers and producers, review scripts, work on-set advising the director or the set dresser, or explain how to perform a technique to the actors. While most of these shows have now been canceled, they do appear on cable television networks and appear to have a strong following. Binge watching these shows can be accomplished by live streaming or DVD rental. While no longer in the forefront of television series lineups, forensic science appears as an ancillary subject in television crime dramas, such as *The Blacklist* and *Lucifer*. So, to an extent, I still keep somewhat busy. Through the use of personal stories and video clips from some of these shows, this presentation will reveal both the real and farcical aspects of television crime dramas.

Forensic Science, Consultant, Television

BS3 The Lawyers Always Win

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The goal of this presentation is to reinforce the nature of science as transparent and objective. The legal system will ultimately turn to recognized criteria based in the scientific method and academic science to judge the utility and validation of forensic science. Forensic science can profit from associations that will quantify and qualify the limits of forensic examinations. This argues that there is no valid basis to resist examination of forensic science practice by traditional science and legal scholars.

This presentation will impact the forensic science community by demonstrating that it is ultimately the law and law-trained persons who determine the criteria for admissibility of forensic science evidence in court.

In 1923, the *Frye* case judged a challenge to a nascent technology purporting to detect deception in a subject.[1] *Frye* held that evidence produced from novel techniques based in scientific supposition must be judged by the general acceptance of a relevant scientific community related to the technique in question to be admitted. Polygraphy was sidelined; "general acceptance by a relevant community" became a criterion for the admissibility of novel scientific evidence. Physiologists and psychologists were considered the relevant touchstone for "expert testimony deduced from the discovery, development, and experiments thus far made."[1] Polygraphy is still judged by these objective disciplines, but not so much the polygraphers themselves.[2] The law has a suspicion of self-referenced validation.

In 2009 and 2016, failures of forensic claims to validity and reliability in their theories, applications, and results caused two blue-ribbon commissions to review the state of forensic science, and various pattern-matching disciplines specifically.[3,4] In the latter, the President's Council of Advisors on Science and Technology (PCAST) identified two gaps for these disciplines: (1) the need for clarity regarding the scientific standards for the validity and reliability of forensic methods; and, (2) the need to evaluate specific forensic methods to determine whether they have been scientifically established to be valid and reliable. It cast these concepts as "foundational validity" and "validity as applied."[5]

Forensic practitioners criticized these reports as reflecting the views of persons from outside the practice of forensic science. Citing "unprecedented (and unrelenting) challenges from legal professionals, research academics, and the popular press" promotes an idea that only those who practice the particular discipline can establish or judge its validity, not statisticians or scientists from academia, and most particularly, not lawyers.[6] These critics do not recall that forensic science itself is a collection of applied disciplines whose goal is to explain case phenomena in ways relevant and helpful to a court. For years, forensic science escaped much critical evaluation because its genesis, practitioners, and proponents in court were, for the most part, on the same side. This changed in the 1990s when an academically validated and objective scientific technique — DNA analysis — was used not to convict, but to exonerate persons who had been wrongfully convicted. In half of those cases, false and overstated forensic opinions contributed to the injustice. At that point, the legal profession began looking to objective scientific evaluation of claims to legitimacy to which some more subjective (i.e., pattern-matching) disciplines of forensic science laid claim. Prosecutors paid attention to avoid reversals and defense attorneys to call "foul" on unsupported testimony.

Wrongful convictions are a stain on the judicial system, which is self-policing. In cases in which trust in forensic science has been shaken, the law looks to established scientific practice to evaluate and change it. The Los Alamos National Laboratories teaches judges that foremost, science is an open process in which theories and methods must be open to testing by any interested party. The manner in which statistics can validate investigative conclusions compels the use of likelihood ratios, and expressions of limitations on conclusions become requisite to expert testimony. Academic scientists, therefore, inform us as to what validity, repeatability, and reliable process is, and what should be used in the important work of administering justice.

The practice and fate of the forensic sciences is in the hands of lawyers, who are its end consumers, and who are awakening to the need to validate forensic specialties that have been shown susceptible to bias, subjectivity, and lack of enforceable standards for practice. Admissibility of forensic results is in the hands of judges, who look to statisticians, behavioral scientists, and academic disciplines to quantify and qualify validity and reliability of forensic techniques and results. For forensic scientists to keep their research cards close to the vest ignores the open nature of science and sacrifices collaboration with academic scientists to develop acceptable standards for the practice. Forensics' validity in court can be admitted as valid and reliable within its limits, so long as the limits are properly expressed.[7] A bunker mentality that closes out scrutiny and validation can only result in the march of the law going around the bunker.

Reference(s):

1. *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923).
2. National Research Council. 2003. *The Polygraph and Lie Detection*. Washington, DC: The National Academies Press. https://doi.org/10.17226/10420.
3. National Research Council. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press, 2009.
4. https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/PCAST/pcast_forensic_science_report_ final.pdf (last accessed 7/31/17).
5. Id., Note 4 at x.
6. Chumbley, S., Zhang, S., Morris, M., Spotts, R. and Macziewski, C. (2017), Development of a Mobile Toolmark Characterization/Comparison System. *J Forensic Sci*, 62: 83–91.
7. E.g., *United States v. Monteiro*, 407 F. Supp. 2d 351 (D. Mass. 2006) (holding although tool mark analysis is sufficiently valid and reliable to be admissible, the expression of results was not in accord with standards and was excluded).

Law, Forensic Science, Admissibility

BS4 YFSF's Wake Up to Professional Development... and Bacon!

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After attending this presentation, attendees will have gained knowledge pertaining to professional development and understand how to better prepare themselves for a career in forensic science. Additionally, attendees will be informed of practical applications by professionals established within several disciplines of forensic science.

This presentation will impact the forensic science community by educating young scientists on the importance of personal and professional developments and on the positive effects they can have on the forensic science community. This breakfast seminar provides students and scientists with the opportunity to network with professionals in a unique setting that focuses on bridging the gap between established professionals and young scientists, both in higher education and in the beginning of their careers.

The Young Forensic Scientists Forum (YFSF) Breakfast Session is a morning session that will leave attendees motivated and enthusiastic about their future successes and accomplishments. This session will focus on bridging the gap between academics and early career growth. The goal is to provide young forensic scientists, whether students, near-graduates, or recent employees, with practical skills and knowledge associated with this transition period. Topics of interest will include the application and interview processes, early success and development as a scientist in a respective field, and the overall feelings and emotions that can often overwhelm young scientists. The speaker presentations will conclude with an open Q&A session, where attendees will be able to interact with the speakers to gain additional information.

The YFSF Breakfast Session will conclude, as always, with the popular résumé review session, pioneered by Academy scientists and peers from across several disciplines and career paths. This session is a rare opportunity for young professionals and students to discuss and improve their résumés with established professionals and leaders in the fields of forensic science. Attendees will sit down one-on-one with résumé reviewers to gain imperative knowledge regarding important aspects and areas to highlight or improve upon within their achievements and qualifications.

YFSF, Network, Forensic Careers

BS5 The Making of an Opioid Crisis in America? Why Research, Policy, and Practice Matter

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After attending this presentation, attendees will be able to analyze and discuss the features and characteristics of the opioid crisis in the United States. Attendees will also be able to summarize successful implementation of policies and practices at the federal, state, and local levels.

This presentation will impact the forensic science community by providing an open forum for forensic practitioners of many disciplines to discuss the impacts of the opioid crisis to the criminal justice system and how improved reporting, surveillance, research, analytical testing, technology, and policy can help mitigate the challenges of use and misuse of these drugs.

Nearly three million Americans reported a substance use disorder to prescription pain relievers or heroin in 2015, fueling a steady increase in fatalities to an estimated 91 United States deaths daily. These rates are not slowing. In fact, alarming increases in 2015 also resulted in drug overdoses becoming the leading cause of accidental death in the United States, with more than half attributed to heroin and prescription pain relievers (33,091 of 52,404 total drug overdoses).[1] Effective strategies begin with understanding the factors that drive the interrelated problems our nation faces with the ever-increasing opioid crisis in public health and the criminal justice system.

Law enforcement, medical professionals, laboratories, and legal agencies are battling with unmanageable caseloads, economic shortfalls, and challenges for safety, analytical preparedness, and basic education and training. Confronted with the fast-paced emerging drug life cycles, reliable surveillance and intelligence are needed more than they have ever been. The legislative quagmire is just as burdensome, as policy change cannot happen without the data to support change.

This breakfast seminar is an ongoing effort of the National Institute of Justice's Forensic Technology Center for Excellence and the American Academy of Forensic Sciences Synthetic Opioids Ad Hoc Committee to heighten awareness in our communities and encourage working together to bring about necessary research and positive changes to policy and practice. This seminar will offer a multifaceted perspective to the manner in which diverse criminal justice disciplines are addressing these challenges, sharing their knowledge, and advancing science, technology, and law. Dealing with the impacts of the opioid crisis to the criminal justice system requires better reporting, surveillance, research, technology, and policy than are currently in use. This type of forum is the kind of effective public safety strategies identified by the National Governors Association to reduce the illicit supply of and demand for opioids by implementing best practices and ensuring inter-governmental cooperation in criminal and death investigations as well as establishing and enhancing stakeholder coalitions.[2] The need to understand the epidemic and its effects goes beyond knowing your own profession — it takes a global perspective to fully act and make a difference.

Reference(s):

1. Rudd R.A., Seth P., David F., Scholl L. Increases in Drug and Opioid-Involved Overdose Deaths — United States, 2010–2015. *Morb Mortal Wkly Rep (MMWR)* 2016;65:1445–1452. DOI:http://dx.doi.org/10.15585/ mmwr.mm655051e1.

2. National Governors Association. *Finding Solutions to the Prescription Opioid and Heroin Crisis: A Road Map of States.* 2016. https://www.nga.org/files/live/sites/NGA/files/pdf/2016/1607NGAOpioidRoadMap.pdf.

Opioids, Policy, Practice

L1 Post-Conviction DNA Testing in an Ever-Advancing DNA World

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After attending this presentation, attendees will better understand the complexities and challenges faced in processing post-conviction DNA cases and will be presented with real-life case examples.

This presentation will impact the forensic science community by showcasing the need for the continued processing of post-conviction cases so forensic science can, through the development and deployment of new technologies, continue to serve both the criminal justice system and society.

DNA testing has become one of the most important forensic tools available in solving, prosecuting, and preventing crime. It is relied upon heavily in the justice system to aid in convicting the guilty and freeing the innocent; however, the usefulness of DNA testing has only been routinely demonstrated within the past decade because of advancements in DNA technology. Such advancements have led to a renewed interest in wrongful convictions. This interest is especially strong in those cases in which convictions are based solely on eyewitness accounts. With the implementation of new DNA technology, physical evidence at crime scenes can be reassessed to see what is possible in terms of examining evidence for biological fluids and/or biological material.

Post-conviction investigations face many of the same basic challenges as new investigations in terms of DNA testing; however, there are additional complexities. Processing a post-conviction case involves greater cooperation among law enforcement, the crime laboratory, and the district attorney's office. This is due to the fact that simply locating the items of evidence to be processed can be both time-consuming and challenging. Just as time-consuming and challenging is locating previous reports and previous laboratory testing results. From past reports and considering previously tested and untested pieces of evidence, if they are found, the crime laboratory can make a determination as to what DNA testing or additional testing is now plausible. Challenges are then faced in the processing of evidence that is old and may only result in the recovery of degraded DNA. Difficulties also arise when dealing with items of evidence that may have been unknowingly contaminated at the scene in a pre-DNA world where there was less emphasis on proper protective equipment. A DNA profile may be able to be recovered from the item of evidence tested; however, obtaining these elimination samples from witnesses or law enforcement personnel, who may have come into contact with the item, may be impossible. Oftentimes, processing post-conviction cases can encounter obstacles in obtaining funding. Laboratories are frequently backlogged with current cases and often do not have the funding to look back, vet, and process old cases.

With all the hurdles faced by crime laboratories, several post-conviction cases remain dormant, their probative evidence locked in unprocessed DNA to this day. The wrongfully convicted continue to serve their sentences in jail, harboring the hope that DNA will one day help them in their quest for justice. Similarly, the families of victims of violent crimes, in too many instances, remain deprived of the closure of knowing the true identities of actual perpetrators. This Luncheon Seminar will present post-conviction case examples that will highlight the challenges faced in processing such cases. This seminar will also showcase the need for the continued processing of post-conviction cases so forensic science can, through the development and deployment of new technologies, continue to serve the criminal justice system and society.

Post-Conviction, DNA, Advancing

L2 Understanding the Impact of Human Factors on Forensic Science: Case Studies in Fingerprint and Handwriting Examination

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After attending this presentation, attendees will better understand: (1) the general themes of human factors and organizational theory; and, (2) the findings and recommendations of the Expert Working Groups on Human Factors in Latent Print and Handwriting Examinations.

This presentation will impact the forensic science community by helping forensic professionals understand the impact of human factors on every aspect of the evidence examination process.

Forensic science plays a vital role in the criminal justice system by providing scientifically based information through the analysis of physical evidence; however, several high-profile cases in the United States and abroad have highlighted the fact that human errors can occur. Human error is an inevitable part of everyday life; however, in certain endeavors, such as forensic analysis, in which errors may lead to the loss of life or liberty, error prevention is imperative. Human factors analysis can advance the understanding of the nature of errors in complex work settings. The study of human factors is the scientific discipline concerned with the understanding of interactions among humans and other elements of a system and is the profession that applies theory, principles, data, and other methods to design in order to optimize human well-being and overall system performance. The forensic science community can benefit from the application of the substantial body of human factors research to advance the understanding of the nature of errors, enhance productivity and quality in forensic examinations, and reduce the consequences and likelihood of human error in the interpretation of evidence.

The National Institute of Justice and the National Institute of Standards and Technology have partnered to sponsor a series of expert working groups to examine the effects of human factors in forensic analyses and recommend practices to reduce the likelihood of error. Each discipline-specific working group will be comprised of experts from relevant forensic disciplines, statisticians, psychologists, researchers, and other scientific experts, in addition to representatives from the legal community, professional organizations, and other identified stakeholder groups. To date, reports, including recommendations, have been published in the areas of fingerprint and handwriting examinations. The next working groups in this series will focus on DNA mixture interpretation and tool mark examinations.

This presentation will provide the general themes of human factors and organizational theory. The findings and recommendations of the Expert Working Groups on Human Factors in Latent Print and Handwriting Examinations will be presented. A range of issues affecting forensic science disciplines in the areas of work environment, training, emerging technology, and research needs will also be covered.

This presentation will further assist forensic examiners in understanding the purposes and value of reporting and documenting examinations and will provide recommendations for standardizing the content of these materials. Presenters will discuss methods to improve trial and pretrial communications between relevant parties — the experts, lawyers, judges, and juries.

Human Factors, Handwriting Examination, Latent Print Examination

ES1 Science in the Public Eye: Diversity, Research, and Communication

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The goal of this presentation is to provide information, based on the experience of national leaders in media, activism, and advocacy, regarding how the use of communication, research, and a commitment to diversity can move the objectives forward and enhance the view of forensic science in the public eye.

This presentation will impact the forensic science community by revealing how the national news media and political activists influence public opinion. The community can apply the experiences, perspectives, and skills demonstrated to implement new methods of accurately presenting the work of forensic scientists in a manner lay people can understand.

Program Description: "A reporter, an activist, and a producer walk into a bar … " Please join us for a slightly different twist on this year's meeting theme, *Science Matters*. The focus of the AAFS 2018 program is diversity, research, and communication in forensic science. By tapping resources from other professions, this presentation seeks to broaden the understanding of how media plays a pivotal role in the general public's *perception* of forensic science and the criminal justice system. This session's speakers come to the table (or bar) bringing their unique experiences regarding how to communicate the work of forensic scientists, their research, and their diversity to affect change in society.

Ryan Gabrielson is a journalist with the independent, non-profit investigative newsroom ProPublica that was awarded the 2017 Pulitzer Prize for public service. Mr. Gabrielson won the 2009 Pulitzer Prize for stories that exposed how immigration enforcement in Maricopa County, AZ, undermined both emergency response and law enforcement investigations. Mr. Gabrielson's reporting has called attention to the apparent failure of the criminal justice system to understand the difference between a presumptive roadside drug test kit and the certainty of confirmatory testing. This lack of communication between science, law enforcement, and the courts has resulted in countless people being unnecessarily detained as well as documented cases of wrongful conviction. Mr. Gabrielson's work has resulted in changes in policy and procedures in the criminal justice system throughout the country.

Aretha Marshall is an executive producer at Peacock Productions, NBC's non-fiction production company, and is responsible for all aspects of talent development, casting for content, and programming. She previously worked as the managing editor for *Dateline*, winning the 2010 Emmy for Outstanding Coverage of a Breaking News Story in a News Magazine. Ms. Marshall's recent work as Executive Producer of Booking on the History Channel's *Navy Seals: America's Secret Warriors* demonstrates the importance of having appropriate, knowledgeable people supplying facts to televised documentaries.

Kevin Oliver is a Washington state cannabis activist. Mr. Oliver is the executive director of the Washington state National Organization for the Reform of Marijuana Laws (NORML) and Political Action Committee (PAC). NORML's mission is to move public opinion sufficiently to legalize the responsible use of marijuana. During his career, Mr. Oliver has relied on grassroots organizing and providing information, based on data and research, to achieve legal reform and to ensure that consumers have access to safe, convenient, and affordable marijuana. In advocating individual legal rights, law reform, and best practices, he brings a different perspective to the forensic science point of view of cannabis identification and toxicology.

Michelle Richmond is a television news producer specializing in legal, crime, and justice news shows. She provided editorial guidance and post-production review for such non-fiction programs as MSNBC's *O.J. Simpson Chasing Freedom* and Lifetime's *JonBenet's Mother: Victim or Killer* to ensure the accuracy and quality of the productions. She also covered the Casey Anthony trail, analyzing witness testimony, motions, closing arguments, and jury instructions for news packages on *Good Morning America, World News Tonight,* and *Nightline*. Ms. Richmond routinely attends forensic conferences to develop storylines and content for news productions and brings in-depth knowledge regarding how forensic science is presented in the media.

Communication, Diversity, Research

W01 Proposed Revisions to the Federal Bureau of Investigation (FBI) Quality Assurance Standards – DNA

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After attending this presentation, attendees will be informed of the proposed changes to the FBI Quality Assurance Standards for Casework and Databasing laboratories. Laboratory personnel will be aware of any changes to policies and procedures that may be necessary in order to comply with the new standards.

This presentation will impact the forensic science community by providing an opportunity to learn about the proposed changes to these quality standards in advance. All National DNA Index System (NDIS) -participating laboratories are required to adhere to and be audited against the revised standards when these standards become effective.

The Scientific Working Group on DNA Analysis Methods (SWGDAM) is revising and updating the Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories and the Quality Assurance Standards for DNA Databasing Laboratories. Upon completion, SWGDAM will forward the revised QAS to the Director of the FBI and recommend them for issuance. Once approved and an effective date is established, all NDIS-participating laboratories must adhere to and be audited against the revised QAS by the effective date.

The last major revision of the QAS occurred in 2009, with additional revisions issued in 2011. Many changes in technology, interpretation approaches, and casework applications have occurred since then. These include such topics as the development of sophisticated software programs for interpretation and statistics, the expansion of the Combined DNA Index System (CODIS) core Short Tandem Repeat (STR) loci, the emergence of legacy data, and the implementation of Rapid DNA technology. In addition, next generation sequencing and non-STR markers could be adopted in forensic casework or databasing laboratories in the near future. As a result, efforts have been made over the past two years to bring the standards up to date with the demands of today's laboratories and to look forward to tomorrow's needs. This has involved some restructuring and re-organizing of the QAS document and the associated audit document.

Information from past audits, the feedback posted on the SWGDAM Frequently Asked Questions website, and suggestions gathered from laboratories were used to clarify standards that have led to confusion for laboratories and auditors. Where appropriate, elements of the discussion sections of the audit document were incorporated into the standards to better ensure compliance. The goal of these revisions is a document that is adaptable to new advances in the field of human DNA identification yet strict enough to retain confidence that the highest quality testing results are being reported.

Workshop discussion will include the revision process, the revisions under consideration, and the anticipated timeline for approval, issuance, and compliance. This workshop will provide insight into the thought processes behind the proposed changes and will allow workshop participants to collaborate on methods to meet and ensure continued compliance to the standards within their laboratories.

This workshop is being provided with the goal of giving laboratories a closer look at the new QAS in order for participants to initiate preparations that may be needed in their laboratories to achieve compliance to these new standards.

DNA, Quality, Audit

W02 Heavy Petting: A Forensic Expert's Guide to Bestiality and Zoophilia

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After attending this presentation, attendees will understand the history of human-animal sexual intercourse in various cultures across time as well as the development of laws around the world to prosecute this behavior. Attendees will learn current conceptualizations of bestiality and the related diagnosis of zoophilia from the psychiatric perspective. Lastly, attendees will be able to describe current approaches to the evaluation of bestiality cases from the point of view of a forensic pathologist and law enforcement officer.

This presentation will impact the forensic science community by improving the understanding of bestiality, a rarely discussed sexual behavior, and its relevance to the fields of forensic mental health, forensic pathology, and criminal investigation.

This multidisciplinary session featuring forensic mental health experts, a forensic pathologist, and a law enforcement officer will educate attendees about bestiality (i.e., human-animal sexual intercourse). The history of bestiality across cultures as well as the development of legislation to prosecute this behavior will be described. The body of literature on individuals who engage in bestiality will be reviewed, providing practical recommendations for psychiatric and medical evaluation and treatment. The Enumclaw horse sex case will be reviewed from a forensic pathologist's perspective and current practices and examples of the forensic investigation of such cases will be described.

Human-animal intercourse, or bestiality, has occurred since earliest recorded human history. Some ancient cultures were permissive of such behavior, most commonly in the setting of religious practice. On the other hand, many early civilizations punished individuals accused of having sex with animals. Over time, the majority of nations around the world have established legislation to punish individuals deemed to have engaged in bestiality. Various legal grounds for punishing acts of bestiality exist, including moral or religious proscriptions, treating animals as property, and considerations of animal rights.

Though many nations and states have severe sentences for violations of anti-bestiality law, the scientific community knows relatively little about the people who have sex with animals. Alfred Kinsey's original research indicated that human-animal sexual contact was a relatively common phenomenon, at least among farm-raised boys.[1] More recent research has identified various types of individuals who have sex with animals, including self-identified "zoophiles" who report being sexually attracted to or having relationships with their pets as well as incarcerated sexual offenders who report histories of bestiality in addition to other forms of animal cruelty. Case reports describe individuals with autism spectrum disorder who engage in sexual acts with animals, possibly as a component of restricted or atypical interests. Lastly, some individuals have sex with animals for secondary gain, such as financial compensation from pornography or sex shows.

Despite research providing an incomplete understanding of bestiality, there have been multiple efforts to categorize those who have sex with animals. In terms of psychiatric nosology, the *Diagnostic and Statistical Manual, Fifth Edition* classifies "zoophilic disorder" under the diagnosis of "other specified paraphilic disorder," which requires that an individual's sexual interest in animals causes distress or impairment or results in harm or risk of harm to self or others. Notably, the diagnosis may fail to capture all individuals who engage in sex with animals, depending on whether or not a clinician determines that bestiality incurs a risk of harm to others. In 2011 Aggrawal proposed a novel categorization of bestiality based on his necrophilia classification scheme, which he stated could be used for sentencing offenders, despite the lack of any evidence that his classification separated offenders based on their risk of violence or recidivism.[2] In 2017, Holoyda and Newman summarized the current understanding of bestiality in a classification scheme based on offenders' motivations.[3]

In 2005, the Washington state Enumclaw horse sex case brought bestiality into the public eye. A 2007 movie, *Zoo*, described the life and death of Kenneth Pinyan, an American man whose anal intercourse with a horse led to the perforation of the man's colon.[4] Subsequent to Mr. Pinyan's death, Washington state passed its own anti-bestiality bill. Though somewhat rare, law enforcement agencies do investigate reports of animal cruelty that involve bestiality. For example, in 2015, investigators identified two adult males who engaged in sexual intercourse with a mini-mare on a farm in Whatcom County, WA. Utilizing an online forum for individuals interested in bestiality, the detectives were able to obtain evidence incriminating the two defendants. Further analysis of the forum revealed ties to the original Enumclaw animal sex farm as well as another case in which a man was running a dog brothel in Sumas, WA.

Poorly understood and in need of further research, bestiality remains a taboo topic in much of the world. Despite the lack of scientific evidence, societies and law enforcement agencies have developed — and, in some cases, been forced to develop — legislation and investigative techniques to identify and punish individuals who have sex with animals. Further research will spur improved scientific and legal understanding and help guide legal bodies in their management of offenders.

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Bestiality, Zoophilia, Sexual Offender

W03 Alternate Light Source (ALS) Photography: Ultraviolet (UV), Infrared Radiation (IR), Lights, and Filters

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After attending this presentation, attendees will understand how to properly image trace evidence by ALS, utilizing a Digital Single Lens Reflex (DSLR) cameras, ALS kits, and filters.

Professionals charged with processing crime scenes and forensic evidence are often expected to understand, but are not properly trained in, the use of ALS, including UV and IR. This presentation will impact the forensic science community by providing a broad understanding of the capabilities of standard and modified DSLR cameras in their ability to image evidence outside the visible spectrum.

While most crime scene and evidence photography is conducted in the visible spectrum of light, much of the most valuable evidence (biological fluids, trace hair/fibers, firearms/explosive residue, inks, etc.) must be visualized and thus imaged outside of the visible spectrum. This ALS photography session will expose attendees to UV and IR photography through instruction and hands-on practical exercises on the use of DSLR cameras, ALS, and specialized filters.

Crime scene and forensic photography within the visible spectrum of light remains the most utilized and thorough means of documenting crime scenes, autopsy findings, physical injury, and forensic evidence. Despite being the most utilized method of documentation, the lack of proper understanding, training, experience, and equipment results in photography being underutilized or at least not utilized to its fullest potential.

Whatever the level of understanding of photography may be in the visible spectrum, the crime scene professionals' understanding of photography in the ultra-violet and infrared spectrums, or spectra outside the visible, remains an elusive concept. With this lack of understanding comes missed opportunities to locate, visualize, and properly capture photographically evidence that is likely crucial in verifying that a reported crime occurred or to refute a false allegation.

After attending this presentation, attendees will understand how to properly visualize, and image evidence requiring the use of ALS, UV, and IR energy. Common types of evidence requiring the use of ALS for visualization and imaging include semen, saliva, urine, gunshot and explosive residue, fluorescent fingerprint powders, hairs/fibers, inks utilized in fraudulent document cases, and other trace evidence.

This workshop will include a short review of photography principles, including the exposure triangle, an introduction (or "tour") of the Nikon® D7000, and topic lectures by faculty members, followed by hands-on practical exercises.

Sufficient DSLR cameras, lenses, tripods, alternate light source kits, specific nanometer "barrier" filters, and props will be provided for every two to three attendees.

Forensic science professionals in all disciplines that are charged with imaging crime scenes, or forensic evidence, will benefit from this workshop. Jurors want and even expect to see high-quality images of crime scenes, injuries, and other physical evidence. All too often, images exposed in an attempt to capture the various forensic evidence found at crime scenes and other forensic settings are lacking in quality or do not meet the basic legal standards required. In response to these recognized deficiencies, this workshop has been designed for attendees to learn the basic legal requirements for introducing properly formatted images into the courtroom, including when to utilize RAW uncompressed (i.e., lossless) settings. Informal surveys of forensic science and law enforcement professionals have shown repeatedly that most law enforcement and crime scene photographers do not understand the nuances in compression levels, such as when to utilize JPEG vs. RAW in general crime scene work versus when capturing critical comparison or evidence-quality images.

ALS, Photography, UV and IR

W04 Applications of Raman Spectroscopy for Trace Evidence Examinations

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This presentation focuses on applications of Raman spectroscopy for the analysis of various types of materials that may be encountered as trace evidence. This presentation is intended to provide trace evidence examiners with a better understanding of this underutilized analytical method, which has seen significant developments in instrument technology in the past couple of decades. After attending this presentation, attendees will gain: (1) a better understanding of the theory, principles, and instrumentation of Raman spectroscopy; and, (2) a greater appreciation of Raman spectroscopy's capabilities and limitations for the characterization, comparison, and identification of various types of trace evidence.

This presentation will impact the forensic science community by increasing participant knowledge and information as well as providing a framework upon which examiners can better utilize this method for casework and to correctly interpret the spectral data of their evidence.

The principles and applications of Raman spectroscopy for the analysis of various materials encountered as trace evidence are presented in this workshop. The emphasis is on spectral interpretation and explanations of the underlying reasons as to why Raman and infrared spectra differ. Because the two methods present data in formats that — superficially, at least — appear identical, the differences between the two methods are often a source of confusion.

All instructors of this workshop have experience as both trace evidence examiners and as researchers who have sought new applications of Raman spectroscopy. Collectively, the types of materials they have examined with this technique include textile fibers, paint, polymers, pigments and dyes, cosmetics, explosives, nanoparticles, and general unknowns. These instructors thus provide perspectives as both practitioners and researchers who have applied Raman spectroscopy for the analysis of a wide variety of materials.

Probably the most significant development that permitted the practical utilization of the very weak Raman effect, which consists of inelastic scattering from a sample, has been the advent of stable lasers, which provide intense monochromatic excitation sources. Use of such lasers for Raman spectroscopy began in the 1970s, and since that time, there have been a number of other significant developments in commercial Raman instrumentation. These include holographic gratings, Charge-Coupled Device (CCD) detector arrays, efficient notch and edge filters to remove Rayleigh scattering lines (without which Fourier Transform (FT) -Raman spectroscopy would not be possible), lasers covering a wide range of excitation wavelengths, and microscope attachments that allow spectral data to be collected from diffraction-limited spatial areas. These developments have transformed what was once a time-consuming analysis requiring hours to one in which data can now be obtained in minutes. More importantly, the lower laser power levels now permitted have expanded the range of materials from which useable Raman data can be obtained without destroying samples.

Because certain types of trace evidence may consist of very complex matrices, a battery of analytical techniques is typically employed to obtain more complete information about the sample. The types of information provided by a Raman analysis of various types of evidence are described, with particular emphasis on how this complements, supplements, or augments data obtained from other methods. Using Raman spectroscopy, trace examiners can probe very small areas of their evidence with minimal sample preparation in a non-destructive manner. Raman spectroscopy is thus ideally suited for the analysis of certain types of trace evidence, but it is currently an underutilized technique in the forensic science laboratory. This workshop is intended to help rectify this situation.

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Raman Spectroscopy, Trace Evidence, Identification

W05 Ohio's Assertive Approach to Scheduling Opioids and Fentanyl Analogs

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After attending this presentation, attendees will: (1) recognize the role of state government in the scheduling of drugs; (2) understand the importance of rapid scheduling of drugs for law enforcement purposes; (3) identify the parts of the molecular structure of drugs that are responsible for pharmacological interactions; and, (4) realize the importance of communication and cooperation between forensic laboratories and government entities.

This presentation will impact the forensic science community by providing an in-depth examination of how one state is combating the opioid epidemic.

Opioid overdoses in the United States have quadrupled since 1999. Among the 47,055 drug overdose deaths in the United States that occurred in 2014, 28,647 (60.9%) involved an opioid. Opioids were involved in 33,091 overdose deaths in the United States. in 2015.[1]

Ohio was one of the five states with the highest rate of death due to drug overdose in 2015 (West Virginia: 41.5 per 100,000; New Hampshire: 34.3 per 100,000; Kentucky: 29.9 per 100,000; Ohio: 29.9 per 100,000; and Rhode Island: 28.2 per 100,000).[1] In response to the alarming trend in opioid deaths, government agencies in Ohio worked with forensic scientists across the state to combat this epidemic through rapid and novel legislation.

In 2016, confirmed cases of U-47700 opioid fatalities in Ohio initiated drug scheduling research to be conducted by the Ohio Attorney General's Bureau of Criminal Investigation (BCI). After BCI's research was provided to the State of Ohio Board of Pharmacy, the Board cast a swift and unanimous vote which classified U-47700 as a Schedule I opium derivative under rule 4729-11-02 of the Ohio Administrative Code.[2] The next day, Ohio Governor John Kasich signed an executive order authorizing the Board to take emergency action and subjecting U-47700 to criminal drug penalties as of May 4, 2016. This action occurred six months before the United States Drug Enforcement Administration placed U-47700 into Schedule I of the Controlled Substances Act on November 14, 2016.

Another combined effort in Ohio to combat the influx of newer drug analogs was the development of the "pharmacophore rule."[3] The Office of the Ohio Attorney General working together with the Ohio State Board of Pharmacy developed this unique method of establishing guidelines for the scheduling of newer drug analogs based upon the scientific principles of drug design.

Drugs elicit their mechanism of action through biochemical and physiological interactions with drug targets. The pharmacophore of a drug molecule is the portion responsible for producing a pharmacological response and provides the core scaffold to which functional groups are added. Functional groups provide atoms for interacting with drug targets, such as receptors. The binding of a drug to a receptor produces most of the pharmacologic and toxicologic effects of the drug.

The ''pharmacophore rule'' addresses the fentanyl analog problem at the level of pharmacology before the compounds have even been identified in forensic laboratories. The rule is written such that a forensic scientist can identify the basic structural elements required for a fentanyl analog to bind to the drug receptor. If the binding elements are met as outlined in the Ohio Administrative Code, a forensic scientist can report the fentanyl analog as a Schedule I controlled substance.

The ability of government entities in Ohio to rapidly schedule newer opioids, in addition to the ability of forensic scientists in Ohio to utilize the "pharmacophore rule" to immediately classify new fentanyl analogs as Schedule I drugs, allows for rapid action on the part of law enforcement officials in the state.

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Opioid, Pharmacophore, Drug Scheduling

W06 Machine-Readable Technologies in Travel and Identity Documents

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After attending this presentation, attendees will understand how contemporary machine-readable technologies used in travel and identity documents function and the circumstances under which the encoded data can be accessed using software and/or hardware readers.

This presentation will impact the forensic science community by describing novel travel and identity documents examination methods that revolve around document reader technology instead of microscopic and other conventional methods for the examination of questioned documents.

Drivers for the adoption of machine-readable security features in identity and travel documents generally include a desire for better facilitation and/or improved security. In context, facilitation means improved speed in the processing of documents and their bearers through border control environments and similar contact points, coupled with a reduced risk of typographical or clerical errors that result from human involvement in data entry. Security means the adaptation of machine-readable features to help a document authenticate itself to a machine reader and/or human inspector or the inclusion of biometric technologies that can bind the document to its bearer to reduce the risk of successful impostor fraud. The nature of machine-readable technologies used in travel and identity documents has changed considerably over the past four decades, and which technologies may be present in a particular document is a function of the document type, its intended usage, and the governing technological standards of the era in which the document was issued.

Passports, visas, birth records, identity cards, and other identity and travel documents have been manufactured with a diverse array of machine-readable technologies that are capable of very different functions. These include optical character recognition fonts, machine-readable text zones, magnetic stripes, barcodes, optical strips, and both contact and contactless smart chips. The function and usage of each of these technologies in the context of identity and travel documents will be described on a technical level, including how the technology encodes data, the limits of how much and the nature of the data that can be encoded, and how the feature may or may not facilitate biometric comparisons. Additionally, the technologies will be considered from the point of view of document design standards promulgated by the International Civil Aviation Organization (ICAO), the American Association of Motor Vehicle Administrators (AAMVA), and other bodies, and as they relate to the REAL ID Act of 2005 and the Western Hemisphere Travel Initiative.

Clearly, machine-readable technologies are not designed for human examination and most cannot be deciphered using conventional questioned document methods, such as microscopy; however, this does not mean that machine-readable document features are inaccessible to questioned document examiners. Although highly sophisticated document reading systems are being deployed for use in border control environments, expensive and complicated readers are not always necessary to access individual machine-readable technologies. Many machine-readable features can be decoded using inexpensive equipment ranging from small magnetic stripe readers to commercial barcode software to a variety of smartphone applications. Similarly, it is often not necessary to have a detailed knowledge of computer science or cryptography to obtain useful information from machine-readable technologies, particularly if the primary benefit of the technology is related to facilitation (as opposed to security). The final goals of this workshop are to describe straightforward methods for accessing machine-readable document features using low-cost tools and to explain when such an approach is likely or unlikely to be successful in regard to different circumstances.

Document, Security, Facilitation

W07 Data Standards, Archiving, and Analytics in Forensic Anthropology

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After attending this presentation, attendees will understand the benefits of a unified data architecture and ontology of forensic anthropology data, which enables the development and implementation of software applications for data analytics. Attendees of this session will join a community of users and will gain access to open source software for recording and managing biometric data in forensic anthropology.

This presentation will impact the forensic science community by providing access to an ecosystem of software applications for forensic anthropology that facilitates casework analyses. Casework efficiencies are realized through a common ontology, enabling data sharing and opportunities for new methods. The ecosystem of applications is based on open source software that fosters collaboration and community engagement via appropriate interfaces and Application Programming Interfaces (APIs).

Disparate and fractured datasets preclude collaboration among various research groups. The purpose of this workshop is to promote a unified data architecture and ontology for forensic anthropology. In doing so, the consistency, quality, and usability of skeletal data improves, providing for more robust reporting and analytics capability, and enhances collaboration and efficiency among globally distributed forensic scientists, researchers, networks, and infrastructures.

This workshop begins with a series of presentations introducing the concepts of: (1) a unified data architecture and its application to forensic anthropology; (2) a well-defined data ontology; and, (3) an overview of data analytics and project pipelines. These concepts are then reinforced through tutorials and hands-on demonstrations.

The Resource Description Framework (RDF) is a standard for data integration, serving as a formal representation of knowledge and a conceptual data model in knowledge management systems. The RDF-based data standard (i.e., RDFBones) is presented with research data from the Forensic Anthropology Center Texas State (FACTS). The Commingled Remains and Analytics (CoRA) is an open source software application for recording and managing data from skeletal specimens. CoRA is a web-enabled relational database built on the open source PHP Laravel framework. It was created to manage multiple data types obtained from skeletal specimens in a large commingled assemblage. In doing so, data attributes (i.e., metric and non-metric data) are then searchable and filterable to propose linkages among specimens associated to single individuals. During these presentations, attendees will gain access to software, create user accounts, and realize the benefits by performing data input and aggregation into various information systems and run complex data queries across various data types.

The later presentations build upon the previously introduced concepts by demonstrating the benefits through analytical packages developed for specific tasks. A short hands-on introductory session to R programming will be provided to introduce the basics of computer programming to attendees and ensure users can draw on extensibility functions of R and hacking R program source code for their own research purposes. The analytical packages OsteoSort, TDStats, Skelet-o-matic, and recent updates to FORDISC® will be discussed.

OsteoSort is an osteological sorting package for R (www.osteosort.net) and provides tools to conduct pair-matching, articulation, and association analyses on large commingled assemblages. A related package, OsteoShiny, provides a graphical user interface to OsteoSort. Hands-on demonstrations will involve conducting analyses within R and through the graphical interface.

TDStats is an R application for standardizing and automating facial soft tissue thickness analysis for craniofacial identification and is freely available from the craniofacial identification resource hub CRANIOFACIALidentification.com. It is built under an exploratory data analysis framework and heavily utilizes plots rather than statistical significance tests to encourage comprehensive understanding of the data to draw scientific inferences and generate enhanced central tendency statistics for use in casework. TDStats uses base tcl/tk for graphical user interfaces.

Skelet-o-matic is a Microsoft® Excel® macro-enabled program that enables inventory of skeletal remains and the automatic generation of a colored skeletal homunculus. As an Excel® spreadsheet with set cells for data values, completed inventory forms can be easily data mined in R.

FORDISC® software is used to assess sex and ancestry and estimate stature, and upcoming versions will add modules for: (1) age estimation using transition analysis; (2) ancestry estimation using macromorphoscopic traits of the skull; and, (3) ancestry estimation using dental morphology traits. These modules will be available as free standalone programs.

Tying separate and related analytical packages together demonstrates the utility and efficiencies gained in analytical processes linked via a computing ecosystem. By creating a community of forensic anthropology users who contribute data to unified data architecture patterns, human skeletal variation among spatially and temporally disparate collections becomes attainable.

Information Management, Data Analytics, Forensic Anthropology

W08 Innovative Teaching With Active Learning Methods — Implementation in Forensic Science Education

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After attending this presentation, attendees will be able to: (1) describe general principles of learning, including examples related directly to scientific disciplines; (2) identify and describe a variety of active learning methods; (3) distinguish between active and non-active learning methods; (4) cite examples of useful teaching methods dependent on the environment; (5) evaluate innovative teaching styles that could be incorporated into their classroom; and, (6) generate lesson plans that could be integrated into their classroom.

This presentation will impact the forensic science community by exploring a variety of teaching strategies to gain insight into a broader scope of active learning methods useful in forensic science teaching. Attendees will learn how these teaching methods can help forensic science instructors adapt their lesson plans to rapidly changing learning environments. By discussing multiple learning techniques and the application of teaching methods in forensic science education, attendees will be able to incorporate new tools into their forensic science classroom.

Many instructors still teach the way they were taught — lecture and test — without realizing that more active learning methods exist. This session, organized jointly by the Council of Forensic Science Educators (COFSE) and the Forensic Science Education Programs Accreditation Commission (FEPAC), will explore innovative teaching strategies currently being used in science education. The active learning methods presented are applicable to undergraduate and graduate level forensic science programs, educational workshops for forensic science practitioners, and forensic laboratory trainees. Active learning methods improve a student's critical thinking skills, problem-solving abilities, and long-term retention of the material. Such methods include flipped classroom techniques, large lecture teaching methods, service-based learning, gamed-based learning, direct experimentation, and a writing-based approach to scientific learning. Each discussion will include real classroom examples of how the teaching method is implemented within the forensic curriculum and how to apply the teaching methods to any discipline.

This presentation explores what learning scientists have discovered regarding how individuals process new knowledge, reason with information, problem solve, and engage in professional practices. Theories about "what counts" as knowledge and learning will be explored, including individual cognition, sense-making, and apprenticeship.

A "flipped" classroom employs recorded lectures to deliver information outside of class time. Class time can then be used for other learning activities, such as case studies, discovery-based activities, writing-to-learn exercises, class discussions, and in-class office hours.

Teaching 15-30 students is much different from teaching 300-400 students. Instructors want to engage students in the material. This presentation will focus on methods used in large lectures to engage students through active learning techniques. Topics will include group discussion, useful technology to engage attendees in presentations, and response systems in the classroom.

This workshop will provide a framework for utilizing service-learning projects as an active learning methodology in the forensic science classroom. Service learning offers transformative learning experiences to students by developing "beyond-disciplinary skills," broadening students' perspectives in relation to themselves and their community, integrating meaningful community service, and placing students at the "center of their own active and reflective learning experience."

Forensic science education naturally lends itself to game-based learning. The games can be a re-imagination of a board game or be based on current technology. Additionally, hands-on laboratories and case studies allow instructors to incorporate vastly different game components. Assessment of student mastery of the material can be accomplished simply by the student successfully completing the game. The knowledge, skills, and abilities students gain from the game would complement any current curriculum.

Discussion will include the use of small-group activities interspersed within the standard lecture format. The different outcomes of providing groups with very detailed instructions regarding how to conduct an experiment compared to groups given simplified instructions will be discussed. Items to be covered will include creating groups, design strategies, and methods of evaluation.

Scientific writing is one of the necessities of the working life, yet teaching this subject to students proves to be a challenge. A journal-based approach yields higher grades and greater involvement for the students and leads to higher impact learning. Such an approach will be outlined, using a custom journal aimed at undergraduate level research, peer reviewed, and edited by undergraduates in an upper division science writing course.

Forensic Education, Active Learning, Teaching Strategies

W09 Putting the Expert on Trial

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After attending this presentation, attendees will: (1) recognize and understand the significance of various pediatric injuries; (2) understand the process of discerning inflicted from accidental injuries causing death; (3) appreciate the effective use of consultants in certain pediatric deaths; (4) realize the potential legal risks of providing opinions as practitioners and consultants; and, (5) be informed about appropriate practices to follow in the event of subsequent allegations of improperly practicing the forensic sciences and providing opinions.

This presentation will impact the forensic science community by clarifying the recognition and significance of various pediatric injuries, improving the determination of inflicted from accidental injuries, and preparing practitioners and experts for potential litigation against them for providing their opinions.

The juxtaposition of medical science and justice is fraught with challenges. Whereas scientific reasoning seeks to objectively explore theory with empirical observation, American justice is adversarial by design. This contrast is particularly problematic in homicidal abuse, with a helpless and preverbal victim on the one hand, and an accused caregiver on the other; one life lost and the other hanging in the balance. Adding to the incendiary environment is a recent Supreme Court dissent claiming that "doubt has increased within the medical community," which in turn has led to doubts about the existence of abusive head trauma in general. Assertions of flawed studies, circular reasoning, wrongful incarceration, and miscarriages of justice occur with regularity, on the part of doctors and lawyers alike. It is therefore not that surprising that the justice system on occasion might turn on the primary interpreters and put the forensic pathologist on trial; however, the educational value of such a case is immense.

This workshop is centered on the case of a 20-month-old toddler who was found dead in bed. Autopsy examination disclosed many hallmarks of abuse and the death was certified as homicide. The boyfriend of the deceased's mother was eventually charged and incarcerated; however, after receiving opposing opinions from experts for the defense, the prosecutor dismissed all charges with prejudice, after which four pathologists, the arresting officer, a municipality, a county, and a hospital were sued in federal court for due process violations, under auspices of the 4th, 6th, and 14th amendments of the United States Constitution.

This workshop will review the multiple aspects of medical science raised in this case, with strict adherence to the scientific method and quality of evidence — in effect a systemic review of all relevant literature. The topics will include: clinical and social context of child abuse, perpetrator data, cutaneous bruising in physical abuse, impact-related abusive head trauma vs. shaken baby syndrome, subdural hematoma, severe hemorrhagic retinopathy, mechanisms of parenchymal injury in pediatric head trauma, short distance falls, lucid intervals vs. pathological timing of injuries, brain injury biomechanics, second impact syndrome, coagulopathy (intrinsic versus drug-induced versus trauma-induced), and child abuse mimics. Included in this workshop will be an experienced attorney who devoted numerous hours to the civil case on the defense side researching case law, engaging in motion practice, and incorporating medical science. This attorney will explore in-depth the concept of qualified immunity, areas of inherent risk and exposure to due process and malpractice claims targeting forensic pathologists in child abuse and other cases, tactics and philosophies of personal injury lawyers, and strategies for pre-emptive protection of forensic pathologists in a hyper-litigious environment.

After considering the scientific underpinnings of the manner of death assessment, a number of additional questions will be raised for discussion, including, but not limited to, the following: (1) Is the American justice system capable of litigate medical science?; (2) What is the risk to forensic pathologists of certifying a child abuse case as homicide?; (3) What is the level of adherence to scientific principles by lawyers seeking damages and/or their retained experts?; (4) How often is medical science co-opted by retained experts relying on *ipse dixit* opinion and low evidence quality?; (5) What is the extent of the chilling effect on the willingness to properly certify manner of death and/or offer objective opinion as an expert if the scientific conclusion is homicidal child abuse?; (6) Are personal and pecuniary biases on the part of retained experts corrupting otherwise robust science in civil and criminal justice?; (7) Should expert panels be employed to litigate medical science instead of individual retained experts?; and, (8) What justice systems in other countries may serve as better models to deal with the juxtaposition of medical science and justice?

Child Abuse, Forensic Pathology, Jurisprudence

W10 A Multidisciplinary Approach to Dogfighting Cases

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After attending this presentation, attendees will: (1) possess a basic understanding of dogfighting within the United States, including the breeds of dogs utilized, housing, breeding, training and conditioning of dogs, the rules and procedures of a fight, and recognition of paraphernalia associated with this crime; (2) be familiar with investigative techniques specific to dogfighting; (3) recognize the unique aspects of dogfighting crime scenes; (4) understand evidence analyses typically associated with dogfighting case work; (5) recognize the pattern of injury and animal behavior consistent with dogs utilized in organized dogfighting; (6) understand the link between dogfighting and other violent crimes; and, (7) better understand major legal issues in dogfighting investigations and prosecutions.

This presentation will impact the forensic science community by enabling recognition of this clandestine crime and elucidating the most current research and techniques utilized when investigating and prosecuting dogfighting. Additionally, this presentation will illustrate the benefits of a multidisciplinary approach for the most favorable outcome to dogfighting criminal cases.

Organized dogfighting is intentional, severe abuse of animals. Although animal fighting is illegal in all 50 states and the federal Animal Welfare Act prohibits animal fighting ventures, this organized abuse of animals is still rampant throughout the United States and rarely occurs in isolation. Organized crimes, such as racketeering, illegal gambling, illicit drugs, and firearm offenses, are frequently associated with animal fighting.[1-3] Therefore, it is critical that law enforcement, veterinarians, prosecutors and others involved possess a basic understanding regarding the animals utilized, how they are maintained, trained and conditioned, fought, and the pattern of injuries and the behavior associated with these illegal fights.

In the United States, the American Pit Bull Terrier is the breed most commonly associated with organized dogfighting.[1-4] Dogfighting can be divided into three main categories: street fighting, hobbyist fighting, and higher level "professionals."[1,3,5] Dogs are prepared for a fight in a fairly routine pattern; however, this varies depending on the category in which they fall. A wide array of equipment, techniques, supplements, and drugs are used to condition a dog for a fight. It is of the upmost importance that those involved in the investigation and prosecution of this crime be familiar with these items and information that may be inferred from them. Dogfighting crime scenes are unique and ideally involve a multidisciplinary approach that includes a variety of experts.

In addition to the items of physical evidence that might be encountered on the crime scene, it is important to remember that the animals themselves are also evidence and may have important information to be considered. Veterinarians involved in the examination of live and deceased animals should be familiar with the wound patterns characteristic of this activity. Fighting dogs typically display scars and/or wounds caused by the teeth of their opponent; however, typically, not all dogs in a dogfighting yard will have wounds and/or scars present, due to age and/or use.[6] The extent and distribution of wounds and/or scars can be very informative. Initial research has demonstrated that there is a distinct pattern of injury associated with organized dogfighting, which has been differentiated from spontaneous dogfights.[6-7] Additionally, underlying fractures or other skeletal trauma is not uncommon and should be documented through advanced imaging or skeletal analysis of remains.

Normal canine aggression is highly ritualized, but a fighting dog is more likely to rush directly at its opponent without hesitation, target vulnerable areas of the opponent's body with deep-mouthed, hard bites, and continue to fight even if the opponent signals submission and defeat. Dogs seized in fighting cases should be systematically evaluated by experienced behavior specialists in order to document these unique behavioral characteristics, as this information can be used to support charges that the dogs were used for organized fighting.

Additionally, there are a variety of major legal issues involved in the investigation and prosecution of dogfighting, both at the state and federal level. Those involved in these areas should have a basic understanding of the issues at hand and how they may impact a case. This workshop will address the link between dogfighting and other crimes, provide attendees with a basic understanding of the animals utilized, how they are maintained, trained and conditioned, fought and the pattern of injuries and the behavior associated with these illegal fights as well as discuss major legal issues in dogfighting investigations and prosecutions.

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Dogfighting, Veterinary Forensic Science, Animal Cruelty

W11 Some Like It Hot: A Forensic Analysis of Burnt Remains

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After attending this presentation, attendees will: (1) be introduced to fire scene investigation involving burnt human remains and their recovery; (2) recognize the micro- and macroscopical changes undergone by the body, skeletal, and dental hard tissues when subjected to fire; (3) understand the different techniques, approaches, and challenges for the identification of burnt human remains, including the practical issues; (4) learn what additional information about the incineration event can be gained through burnt bone analysis; and, (5) gain insights through the discussion of forensic casework.

This presentation will impact the forensic science community through the presentation of multidisciplinary and innovative approaches to tackle complex cases of burnt human remains with a more holistic approach to not only facilitate the identification of remains, but to also glean insight into the reconstruction of the incineration conditions.

Mass disasters, aircraft accidents, or motor vehicle collisions can involve fire; sometimes, a fire may have been set to destroy forensic evidence, including to prevent the identification of the deceased. When the fire affects a large area, it often cannot be extinguished for many hours or even days. As a consequence, some bodies are subjected to prolonged high temperatures and, in many cases, be reduced to extremely fragile skeletal elements. Thus, the identification of human remains subjected to incineration depends on the degree of destruction of the remains, which is affected by the intensity and duration of the fire. In fact, the loss of soft tissues destroys visual and fingerprint clues, leaving odontology, anthropology, and DNA as the only possible identifiers.

Subsequent to the destruction of skin and soft tissues, the skeletal remains exposed to fire or high temperatures undergo changes both on a macro- as well as a microscopical level. Macroscopically, high-temperature exposure modifies the bone structure in size, color, and shape. At the microscopic level, there are different changes in chemical and crystalline structures. All of these changes complicate the estimation of sex, age, and stature, challenging the accurate identification of the remains. The same changes are found in teeth. Although genetic identification can be an option in some circumstances, DNA extraction, yield, and quality is also affected by the intensity and duration of the fire.

Therefore, due to the fragility of skeletal and dental remains, the maximization of collection and stabilization are required at the very early stages of scene recovery; attention to detail and care throughout the analyses to maximize the extractable information is critical.

Based on these issues and challenges, a multidisciplinary team effort of forensic pathologists, anthropologists, odontologists, and analytical scientists is essential to facilitate the correct identification of the victims and to reconstruct the incineration conditions.

This workshop will illustrate this multidisciplinary effort, explaining the recovery of burnt human remains from the fire scene and the analysis of these remains at different levels — macroscopic, microscopic/biochemical, and genetic — to facilitate the identification of the victims, applying anthropological, odontological, and genetic techniques to finally illustrate the discussed approach and challenges through practical case studies.

Fire Scene, Burnt Remains, Forensic Identification

W12 Eric Zimmerman's Open Source Forensic Tools Library

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After participating in this workshop, attendees will better understand the challenges facing digital forensic software developers to both design and maintain open source tools and will understand how to prepare and use an open source digital forensic toolkit to conduct examinations. Attendees will better understand the relative advantages of commercial forensic packages and open source tools and where they can be used in concert to impact the work of digital forensic examiners. Those attending will learn the specific capabilities and methods of use of one of the most well-known open source libraries of digital forensic software. Issues vital to those who would develop and maintain forensic software, such are architecting through plug-ins to evolve functionality as opposed to monolithic executables, will be discussed as well as how that architecture can allow forensic examiners to expand the tools' capabilities by developing plug-ins to meet their particular casework needs and challenges. Finally, this session will help forensic examiners better understand how growing complexity and evidence volumes are making the ability to perform triage and focus on those elements most likely to be relevant to the investigation. This session will help the forensic community understand the balance between thoroughness and timeliness that is the hallmark of real-world cases that our examiner community faces every day.

This presentation will impact the forensic science community by demonstrating that freely available, open source forensic tools and forensic libraries can help both public and private sector organizations.

Software initially developed to support investigations into online sexual exploitation of children (which in one year resulted in the rescue of at least 45 children, the execution of 300 search warrants, and 222 arrests of suspects) was recognized as having value to the forensic community as a whole and was evolved into an open source library. Challenges faced in child trafficking and child pornography investigations — such as the need to quickly and efficiently parse hundreds of thousands of files when time and resources are limited — have become challenges for all forensic examiners as typical storage drives have come to contain hundreds of thousands of system, application, and user files. Attendees will also learn how open source forensic software can be created and modified as new operating system versions and forensic challenges arise. These tools are now used by thousands of forensic examiners in more than 50 countries; this workshop will help make the knowledge of how to employ these important tools available to forensic investigators worldwide. Because of the use of both open source and plug-in architecture, forensic examiners can develop and publish their own plug-ins to the benefit of the forensic community as a whole.

Tools, Computer, Open Source

W13 Moving From the Combined Probability of Inclusion (CPI) to Probabilistic Genotyping for DNA Mixture Interpretation

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After attending this presentation, attendees will understand the current limitations of interpreting DNA mixtures using "binary" approaches in which alleles are either "included" or "excluded" from analysis. Attendees will develop an understanding and overview of probabilistic approaches that consider missing data (allele drop-out) or spurious alleles (allele drop-in) to interpret DNA profiles.

This presentation will impact the forensic science community by providing an overview of the limitations of current statistical approaches and promises for the future of DNA mixture interpretation for forensic DNA analysts, DNA technical leaders, laboratory directors, prosecutors, defense attorneys, and judges.

This workshop is targeted to attendees that have not committed to a probabilistic genotyping software but would like to have an introduction to improve their knowledge base.

Several high-profile closures of forensic DNA laboratories in the United States over the past few years have now focused the forensic DNA community on the challenges associated with the interpretation of mixtures. Many of these challenges involve the use of the CPI or the Combined Probability of Exclusion (CPE) on complex mixtures where allele drop-out is reasonable. In 2010, the Scientific Working Group on DNA Analysis Methods (SWGDAM) published a set of guidelines for autosomal Short Tandem Repeat (STR) interpretation, updated in January of 2017. The SWGDAM guidelines, along with the recommendations of the International Society for Forensic Genetics (ISFG) in 2006, have recommended the use of a stochastic threshold to consider the possible loss of alleles in a DNA profile (i.e., allele drop-out).

With the recent improvements in both STR multiplex chemistry and Capillary Electrophoresis (CE) instrumentation, the interpretation of highly complex mixtures such as "touch" items with (1) two or more contributors, and/or (2) low-level contributors with possible dropout has become a greater challenge for the analyst to interpret. Methods that treat alleles probabilistically instead of with a threshold-based approach have gained acceptance around the world over the past few years as a way forward for DNA mixture interpretation. The statistical output of a probabilistic genotyping software is the Likelihood Ratio (LR), which evaluates the evidence under two mutually exclusive hypotheses.

This workshop is targeted for laboratories considering a move to a probabilistic genotyping system for mixture interpretation or for individuals in the legal community wanting to learn more about this approach compared to the current methods of interpretation. This workshop will begin with a general review of probability, provide an introduction of the LR, and examine the limits of CPI, the modified Random Match Probability (RMP), and the binary LR. This workshop will work specific examples by hand, so attendees are encouraged to bring a calculator or have a calculator app on their smartphone. Finally, attendees will examine two approaches to probabilistic interpretation: the discrete (semi-continuous) and the fully continuous methods of interpretation.

DNA Mixture Interpretation, Likelihood Ratio, Probabilistic Genotyping

W14 Pharmacogenomics — Uses in Forensic and Clinical Toxicology

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After attending this presentation, attendees will be able to develop effective approaches for the development and validation of genetic assays for drug monitoring applications, identify the benefits associated with pharmacogenetic testing and understand their relevance to the practice of medicine, and utilize toxicology and pharmacogenetic test results in conjunction with other case histories for decisions regarding cause- and manner-of-death determinations.

This presentation will impact the forensic science community by illustrating the benefits and utility of pharmacogenetic testing. Case studies will be provided to assist attendees with the interpretation of toxicology and pharmacogenetic test results and explain how these findings can impact death investigation outcomes.

The purpose of this presentation is to familiarize participants with the study of pharmacogenomics and to cultivate an understanding about how an individual's genotype affects an individual's health status and influences their response to drugs. Without the benefit of an analytical test to help provide guidance, it is challenging at best, and perhaps not even possible, to predict who will benefit from a medication, who will not respond at all, and who will experience symptoms associated with an adverse drug reaction. To provide a working knowledge of pharmacogenomics, speakers will detail the development, validation, and utility of predictive genetic tests, address how genetic makeup influences drug metabolism, disease states and their progression, and describe how test results can have implications for cause- and manner-of-death determinations.

Pharmacogenetics is the specialized area of pharmacology concerned with the effect of genetic influences on reactions to medications and other drugs. In essence, genetic variability means that not all people within a population will react to the same drug in the exact same manner. Incorporation of genetic test outcomes into the prescribing process is one important aspect of personalized medicine and can improve efficacy while minimizing adverse drug reactions and therapeutic failures. Alternate uses involve the identification of genetic abnormalities associated with a life-threatening or a lethal outcome. Taking into account the possibilities for why this type of testing must be performed, laboratories need to identify those genetic factors (e.g., enzyme polymorphisms) that are most relevant to creating a personalized treatment approach to the practice of medicine, then develop and validate those testing procedures. This workshop will enable attendees to understand how metabolic and genetic influences affect the overall health of an individual, specifically in regard to drug metabolism, drug-drug interactions, and the disease process. In addition to using genetic testing results prior to prescribing a drug, test results can also be applied to interpretation of toxicology results in conjunction with provided case history. This becomes relevant to death investigations in which one main goal is to determine an individual's cause and manner of death. Was a drug purposely consumed in an overdose amount or was the individual as a direct consequence of their genotype unable to properly metabolize the drug? Can genetic testing be used in the event of a negative autopsy to identify a familial disease that contributed to death? Overall, this workshop will provide a thorough overview of pharmacogenomics from start to finish and provide an introduction to pharmacogenomics, cover development and validation of laboratory testing, and address where and how this genetic approach can be applied to both patient treatment and the death investigation process. Finally, this workshop will benefit attendees by broadening their approach when interpreting toxicology results.

Pharmacogenomics, Pharmacogenetics, Toxicology

W15 Postmortem Monocular Indirect Ophthalmoscopy (PMIO)

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After attending this presentation, attendees will be able to: (1) differentiate between direct and indirect ophthalmoscopy, noting advantages and limitations of each technique for the postmortem detection of retinal hemorrhages; (2) discuss the fundal location of retinal hemorrhages relative to their projected aerial image during monocular indirect ophthalmoscopy; and, (3) accurately draw retinal abnormalities observed during monocular indirect ophthalmoscopy on a fundal diagram and capture the projected aerial image with a smartphone.

This presentation will impact the forensic science community by providing an overview of PMIO, promoting skill acquisition, evaluating practical training, and facilitating imaging techniques with fundal diagrams and a smartphone.

Postmortem examination of the retina has relied on ocular enucleation. In most medical examiner/coroner jurisdictions ocular evisceration is not a standard autopsy procedure unless child abuse is suspected, thus creating observational bias when citing the prevalence of postmortem fundal findings such as retinal hemorrhages (subinternal limiting membrane, flame-shaped or splinter, and dot/blot), perimacular retinal folds, retinoschisis, and postmortem artefactual retinal folds.

PMIO permits examination of the decedent's posterior fundus and portions of the peripheral retina. The required equipment necessary for PMIO is relatively inexpensive and, when compared to direct ophthalmoscopy, the technique is less affected by corneal clouding, lens opacity, or vitreous hemorrhage. PMIO uses a focal light source and an aspheric, convex condensing lens. An excellent source of paraxial illumination is a smartphone or a surgical or procedural headlamp using a halogen or xenon light source. The headlamp light source creates a collimated beam of light and permits the examiner to stabilize the condensing lens with both hands. Current aspheric lenses range from +14 to +40 diopters and come in different diameters permitting a field of view of 35°–55°. Postmortem corneal opacity may cause the fundus to appear hazy; however, by gently removing the epithelial layer of the cornea, the emergent image is usually of adequate quality to readily detect lesions such as fundal hemorrhages and retinal folds.

Learning how to perform and become proficient at PMIO can be perplexing and intimidating. Most pathology residents and forensic pathology fellows have limited exposure to indirect ophthalmoscopy. Because the projected aerial image is inverted and laterally reversed, precise descriptions or recordings of fundal abnormalities can be challenging. Unlike binocular indirect ophthalmoscopy with a teaching mirror attachment, an instructor and the fellow or resident cannot view the projected aerial image simultaneously during PMIO. To address these learning obstacles, it is necessary to develop tools and models to facilitate skill acquisition. An hour or two with an inexpensive ocular model can shift the learning curve of the resident, fellow, or forensic pathologist substantially to the right in how to correctly position the light source and hold the indirect lens.

This workshop consists of an initial discussion and didactic presentation reviewing the technique of PMIO, highlighting the optics, equipment, and examples of abnormal fundal findings found at autopsy by PMIO, and the use of a smartphone to capture the projected aerial image. Next, attendees will have a realistic learning experience by practical hands-on training with a procedural headlamp, an aspheric indirect lens, and simple ocular models containing a variety of retinal abnormalities observed at autopsy. Attendees will receive assistance in positioning the procedural headlamp, holding the indirect lens, viewing the projected aerial image, and accurately recording the retinal abnormalities on fundal diagrams and their smartphones. Attendees with smartphones can practice still image acquisition and video recording of fundal images produced by PMIO and will learn how to hold and stabilize the smartphone while imaging the fundal findings in the ocular models.

Following practice visualizing and diagramming numerous fundal images, attendees will have the option of being evaluated with a series of unknowns. Self-assessment of technical skill training and review of the unknown retinal findings concludes the workshop. Attendees will be provided fundal diagrams and articles on PMIO.

Indirect Ophthalmoscopy, Retinal Hemorrhage, Smartphone

W16 Forensic Image Processing

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The goal of this presentation is to provide a working knowledge of forensic image processing to enable an analyst to apply the optimum image processing algorithms to surveillance video and digital photography.

This presentation will impact the forensic science community by providing basic forensic image processing skills for the analyst. Surveillance video is nearly ubiquitous and a successful analyst is one who is familiar with forensic image processing and how to use it.

Contrast: A histogram can provide information about the amount of contrast in an image. Since image contrast is the difference in brightness between pixel values in a scene, the shape of the histogram is directly related to image contrast. For example, an image with a high level of contrast will have a broad-shaped histogram, but an image with low contrast will have a narrow histogram.

Pixel values in an image can be redistributed to a different range in an output image (i.e., histogram modification). This process has the effect of "stretching" or "compressing" the intensity range in the output image. If the pixel values are stretched to the full available range, then all pixel intensity values are utilized. This process can optimize the contrast and brightness of the output image.

Spatial Filters: Spatial filtering, or convolution, is an aspect of image processing. The process of filtering involves a moving window of array coefficients (i.e., weights). The size of an array, or kernel, is usually an odd number of pixels such as 3 x 3, 5 x 5, or 7 x 7. As the kernel is incrementally positioned through an image, the value of the pixel at the center of the kernel is multiplied by the value of the corresponding pixel in the image. For a given kernel position, all the kernel values are multiplied similarly and summed to produce a new output image. The next kernel step associates the next image pixel with the center of the kernel, and this process continues for the entire image.

The value of an output pixel from spatial filter is a function of the adjacent pixels in the original image. Spatial filters can be used to isolate the high and low frequency components of an image. High frequency components can be removed by either a low-pass filter or a rank filter.

Low-Pass Filters: Low-pass spatial filters are typically used to minimize Gaussian, or random noise. Ideally, the frequency of the noise is different from the frequency of the information in the image. The output pixel from a low-pass filter is a weighted sum of the adjacent pixels in the input image.

Edge Operations: Edge operations are types of spatial filtering in which each pixel is replaced with a weighted sum of the adjacent pixels. The type of edge operation is controlled by the weights that are applied to each of the adjacent pixels. For example, vertical or horizontal edge detection can be performed by choosing specific weights. There are two general objectives for edge operations: (1) increase image contrast; and, (2) detect edges within an image.

Smoothing: Image noise suppression via smoothing is a fundamental procedure in image enhancement. The trade-off for noise suppression is image blurring, which can be problematic for edges in a digital image. Noise suppression is usually accomplished with an averaging filter or a Gaussian filter.

Reference(s):

1. Borengasser, M., 2018. *Forensic Image Processing*, in preparation.

Image Processing, Surveillance Video, Digital Photography

W17 An Introduction to Lean Fundamentals and Six Sigma Operational Improvement

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After attending this presentation, attendees will better understand the common tools and techniques used in a Lean Six Sigma (LSS) project to increase productivity and efficiency of the laboratory without increasing employees or purchasing new equipment or software.

This presentation will impact the forensic science community by introducing participants to a logical, step-wise procedure to greatly improve the understanding (through actual data collection) of the current laboratory system and to provide a structured method to eliminate waste and make improvements for a more efficient and effective laboratory process.

Attendance at this full-day workshop will introduce the participants to the LSS philosophy of continuous improvement and its methodology to achieve rapid and lasting process improvements while producing a positive culture change. Attendees will receive lectures and take part in practical exercises that will demonstrate the principles of LSS and clarify how the adoption of this philosophy can have a marked improvement in laboratory throughput. Finally, attendees will be presented with the LSS projects of modern crime laboratories. Participants will learn by using the Define, Measure, Analyze, Improve, Control (DMAIC) process, a system the labs were able to develop that increased productivity and morale without compromising quality.

LSS methodologies are used globally to improve testing laboratory processes, reduce turnaround time, increase productivity, and enhance morale. This approach also upholds the highest regulatory compliance while actually increasing quality.

The term Lean has its origins in "lean production" or "lean manufacturing" and was widely developed, implemented, and disseminated by Toyota®, although Toyota® learned its roots from Henry Ford. Lean is more than a set of tools to improve efficiency. It is a philosophy that understands that value must be interpreted from the customer's viewpoint. In order to add value, waste — those activities that don't add value — must be eliminated or minimized. This is accomplished by creating continuous flow of value-adding activities to increasing throughput.

The DMAIC process is the Six Sigma methodology used to discover the current state of the laboratory, the location of its primary bottlenecks, and the waste inherent in the production system. It is used to create a process in which samples flow through the laboratory in an efficient and proficient manner. Six Sigma is a rigorous performance improvement approach that uses a customer-focused and data-driven understanding of process variation and process capability.

Both Lean and Six Sigma supports quality management systems, including accreditation programs by the American National Standards Institute-American Society of Quality (ANSI-ASQ) National Accreditation Board (ANAB), the American Association for Laboratory Accreditation (A2LA), the American Board of Forensic Toxicology (ABFB), the National Association of Medical Examiners (NAME), and others.

Concurrently, the organizational culture will change to one of identifying problems and finding solutions to those problems. The quality of the work product will increase by designing quality checks throughout the process and by having all scientists perform their work in a similar standard way. Finally, employee morale will increase by creating a positive teamwork atmosphere and having each employee perform at their designed level.

Lean, Six Sigma, Process Improvement

W18 Domestic Violence and Child Abuse Deaths

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After attending this presentation, attendees will understand the biomechanics of accelerative/blunt force injury in infants and children; the dynamics of Munchausen Syndrome by Proxy; multiple descriptions of child interview techniques; psychological analysis, which can be conducted in child death investigations; and the Fatality Review Board (FRB) process within the United States Department of Defense. This session will also include homicide presentations by special agents and crime scene investigators who process domestic violence and child abuse death crime scenes.

This presentation will impact the forensic science community by providing insight into the many disciplines utilized in domestic violence deaths. The presentations will leave a lasting impact on attendees through the intricate details of each topic, discussing the criminal, physiological, and psychological aspects of domestic violence.

A group of psychologists, a forensic pathologist, and forensic science special agents, all bringing decades of forensic science and federal law enforcement experience, will present technical material pertaining to Munchausen Syndrome by Proxy, child interviews, accelerative/blunt force trauma, and psychological analysis used in solving domestic violence investigations. This group will present an FRB process that brings together subject matter experts and leaders to reduce the number of deaths in a community using a multi-modal approach. Special agents will provide three separate case presentations of domestic violence deaths they investigated, sharing their insight of the forensic science process in domestic violence homicides.

Features of Munchausen Syndrome by Proxy (now referred to as Factitious Disorder Imposed on Another in the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*) will be described. Typical case findings will be discussed, along with offender psychological characteristics and implications for investigation and interview strategies. Illustrative examples will also be used from past cases.

A case presentation from a 2013 child death involving a 14-month-old male murdered while in the care of his grandmother will be reviewed. The boy, born from an incestuous relationship between the subject's daughter and husband, became a victim of the subject's anger. The initial autopsy report listed the cause and manner of death as undetermined and the case grew cold; however, 18 months after the child's death, the case was renewed by investigators, resulting in the manner of death changing to homicide and prison time for subject.

A history of child interviews and research conducted in the past 30 years will also be discussed. This segment will explore the type and value of open-ended questions and the amount of information gained when compared to traditional direct questions, and elements that are necessary in all structured child interviews will be discussed.

The Army FRB will then be presented. The FRB conducts a review of all domestic violence and child abuse deaths in order to establish trends or patterns that may help in formulating training or preventative methods for the future. An overview of recent trends in regard to child abuse deaths will also be provided, as will recommendations, then the importance of utilizing the results of FRBs in order to establish processes and training that help in the reduction of these types of deaths will be examined.

The biomechanics of accelerative and blunt force injuries in infants and children will be discussed, with a focus on head and neck injury. Landmark articles and "controversies" over the past several decades will be discussed. Additionally, the classification of a death as Sudden Unexplained Infant Death and the necessary documentation will be reviewed.

Next, the response by special agents to a suspected double homicide and suicide will be presented. The subject was a spouse of a service member and the mother of the two victims, ages 3 and 18 months. In the weeks leading up to the deaths, the subject was observed exhibiting increasingly erratic behavior, incoherent conversations, and religious paranoia. Investigators utilized scene reconstruction to replace displaced items as well as to conduct a bloodstain pattern analysis and shooting incident reconstruction.

The final case review details a 28-day-old infant death. The topics discussed will include a review of the mother's pre-incident behaviors, all circumstantial evidence pertaining to the case, analysis of the 911 call, video reenactment, and post-incident behaviors.

Child, Violence, Death

W19 The Evolution of Fire Investigation From the Perspective of Science: Why Science Matters in the Search for Justice

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After attending this presentation, attendees will have an understanding of how: (1) past anecdotally based methods resulted in potentially erroneous convictions; (2) scientific research discredited those methods; (3) applicable standards have evolved as a result; and, (4) science-based research in many disciplines continues to improve our understanding of fire science and its proper application in fire investigation.

This presentation will impact the forensic science community by explaining the importance of empirical scientific research as well as how and why the results of such research must be applied when investigating fire scenes, analyzing fire debris and other evidence, and crafting valid conclusions in any investigative discipline.

Numerous organizations, academics, and fire investigators have contributed to an ever-growing body of empirical research in fire science, some of which contradicted many previously accepted anecdotally derived fire behavior beliefs; however, this scientific information, even when available for use in fire investigations, has not always been applied nor always accepted in contemporary court proceedings. This workshop will look at the methods, processes, and reasoning used in past fire investigations and compare them to countervailing science-based analyses elucidated, primarily, during post-conviction appeals. Faulty fire investigations are not limited to criminal matters; they are equally likely to affect insurance-related civil litigation; however, criminal court records and information are more accessible and, since the fire investigation techniques are the same, this workshop uses mostly (though, not exclusively) criminal case examples.

To illustrate the effect that empirical, scientific research has had on fire investigations, the fatal arson conviction of Adam Gray will be examined.[1] Gray was arrested in March 1993 following a fire in the back of a house that killed two people. Investigation indicated the fire had been intentionally set and there was a confession from Gray. Two fire investigators found alligator charring and deep burn patterns at the scene and concluded that was evidence of a hot fire set with an accelerant. A milk jug found in the alley behind the home was believed to contain the accelerant. A gas station clerk said Gray bought gas shortly before the fire. In his confession, Gray admitted purchasing gasoline and dousing the enclosed second floor back porch and stairs. Gray was convicted of arson and murder; he was 14 years old. On appeal, Gray contended that his conviction was based on a coerced confession, faulty laboratory analysis, and flawed fire behavior conclusions. Despite the proffer of research-based evidence, a trial-level judge denied Gray's motion for a new trial. The charges against Gray were only dropped in May 2017 after prosecution and defense jointly sought dismissal from an appellate court.[2]

These contrasts will be further illustrated by examining other fatal arson convictions subsequently affected by science-driven changes, including Angela Garcia, Katherine Bunch, Earnest Ray Willis, and Han Tak Lee.[3-6]

Presenters will also examine the following: how science-based research significantly altered fire science concepts and fire investigation; how the research is driving standards development by the National Fire Protection Association (NFPA), the American Society for Testing and Materials (ASTM), and others; the role of certifying organizations, such as the International Association of Arson Investigators (IAAI) and others, in promoting acceptance of science-based concepts; recent and on-going research using computer simulation, electron microscopy, Gas Chromatography/Mass Spectrometry (GC/MS), and real-world live-fire experimentation by Underwriters Laboratories, the Bureau of Alcohol, Tobacco, Firearms and Explosives, and other entities; human bias issues affecting cause determinations and their use in court proceedings; and contemporary issues of reliability, validity, and accuracy of fire investigations and their acceptance in court proceedings.

Since trial court judges are evidentiary gate keepers, determining, as a matter of law, who is qualified to be an expert witness and whether an expert's opinion is admissible, throughout the workshop speakers will explore questions pertaining to admissibility: Should fire investigators be subjected to *Daubert* challenges? What qualifies a fire investigator to be considered an expert witness? Are fire investigators "forensic science practitioners" or "forensic scientists"? Does it matter? What constitutes an admissible origin and cause determination?

Reference(s):

1. *People v. Adam Gray*, No: 94-CR-279301, Cir. Ct. Cook County, Ill., 2017.
2. Joint Motion to Vacate Convictions and Enter an Order of *Nolle Prosequi*, *People v. Gray,* No 16-3218, Ill. App. 1st, 2017.
3. *Ohio v. Garcia*, CR-00-387760-ZA, Ct. C.P. Cuyahoga County, 2013.
4. *Bunch v. State*, 964 N.E.2d 274, Ind. Ct. App., 2012.
5. *Willis v. Cockrell*, No. P-01-CA-20, (W.D.Tex. Aug. 09, 2004).
6. John J. Lentini, A Calculated Arson, *49 Fire & Arson Investigator 2*, April 1999, pp. 20-25.

Fire/Arson Investigation, Wrongful Convictions, Fire Science Research

W20 Fentalogs: The Chemistry, Pharmacology, and Toxicology of Illicit Fentanyl and Emerging Opioids

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After attending this presentation, attendees will be able to describe the origination of fentanyl and fentanyl analogs and describe their pharmacology as well as identify and implement methods for the safe handling of these compounds. In addition, attendees will be able to describe methods used to profile and disseminate information on emerging opioids, assess the findings of fentanyl and fentanyl analogs in casework, and implement appropriate analytical techniques used in their identification

This presentation will impact the forensic science community by providing current information on the opioid epidemic with a specific focus on fentanyl and fentanyl analogs and their chemistry, pharmacology, and toxicology seen in forensic casework.

The opioid epidemic, which has recently proliferated to include fentanyl and its analogs, is a serious public health concern as many users are unknowingly ingesting these drugs under the misconception they are using heroin. The rate with which these compounds are being illicitly and clandestinely synthesized creates a major challenge for seized drug, forensic, and clinical toxicology laboratories in identifying the compounds and developing methods for their analysis. Fentanyl and fentanyl analogs or "fentalogs," many of which are several times more potent than traditional opioids, have been implicated in several case reports and adverse events associated with overdoses. Limited information related to their pharmacology, often embedded in the pharmaceutical patents from which they are derived, further complicates interpretation related to these cases. In just the past year, case reports of fentanyl analogs, including furanyl fentanyl, carfentanil, butyryl fentanyl, para-isobutyryl fentanyl, cyclopropyl fentanyl, acryl fentanyl, and the reemergence of 3-methylfentanyl, have been implicated in postmortem and Driving Under the Influence (DUI) cases, demonstrating the high rate of turnover and overall prevalence.

This workshop will provide background information related to origin of fentalogs, history derived from the patents where they are originally described, the pharmacology related to receptor binding, and potency. The actual and perceived risk of accidental overdoses of first responders, police officers, and laboratory personnel encountering these substances will be presented, and current recommendations regarding the safe handling of these substances will be discussed. Additional topics included in this workshop will focus on early detection and dissemination of the identity of novel opioids, methodologies used in profiling seized drug materials, and analytical approaches for detecting and confirming the presence of fentanyl-related analogs. This workshop will conclude with case reports, including toxicological data for incidents involving fentanyl and its analogs.

Fentanyl Analogs, Novel Opioids, Opioid Epidemic

W21 Macromorphoscopic (MMS) Traits: Data Collection and Analysis

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After attending this presentation, attendees will: (1) understand the history and theoretical concepts of ancestry estimation via cranial morphology, particularly in reference to MMS traits; (2) have gained experience scoring macromorphoscopic traits in human crania using recently developed software; (3) have learned the basic strategies of various statistical procedures to estimate ancestry with MMS trait scores; and, (4) have acquired working knowledge of the application of MMS trait data in casework.

This presentation will impact the forensic science community by providing up-to-date methodologies and theoretical considerations in ancestry estimation using MMS trait data. Additionally, attendees will learn how to record, analyze, and report MMS trait data using the appropriate statistical framework. Attendees will be introduced to a worldwide reference dataset that permits ancestry estimations beyond the historical three-group classifications used in ancestry estimation from cranial morphology.

This presentation will focus on MMS trait data collection and analysis, particularly as these slight variations in cranial form relate to the estimation of ancestry from human skeletal remains. The once-subjective nature of MMS data has shifted to more objective methods through the introduction of standard data collection protocols; however, observer experience, expertise, and training still impact data collection. To that end, attendees will gain a deeper understanding of MMS trait analysis by understanding where and how biases can be introduced.

Following a general introduction, a series of lectures provided by scholars in MMS trait analysis will outline MMS trait manifestations, trait distributions, and illuminate aspects of ancestry estimations from different viewpoints and through multiple forms of analysis. Previously, approaches to ancestry estimation using MMS traits did not detail the various manifestations, or character states, of each trait. Instead, these approaches relied on extreme expressions and trait lists. In other words, analysts needed an expert-level surety of human variation. Attendees will be guided through the complete analytical process performed in forensic casework. Using a variety of examples and hands-on material, attendees will learn to assess MMS trait data using standardized data collection protocols. In concurrence with this training, attendees will become familiar with newly developed data collection software freely available to practitioners. Statistical procedures will be reviewed, both theoretically and practically, which have been deemed most appropriate for MMS trait data analysis. Attendees will then learn to effectively report ancestry classifications using a worldwide reference sample using observed trait scores and statistical analyses.

Lecturers will conclude the session by demonstrating the ways in which MMS data have been applied in current research. This will include geographic patterning of MMS traits, observer error, and potential secular change inherent in MMS data. Becoming familiar with and understanding these nuances allows practitioners to make more meaningful interpretations of ancestry classifications using MMS data.

Estimating ancestry from the skull need not be difficult. In much the same way students first learn metric analysis (e.g., “GOL is measured between these two landmarks and entered into a computer program to obtain an estimate”), macromorphoscopic trait analysis requires visual learning and a hands-on approach. The goal of this session is to increase the utilization of an empirical method to ancestry estimation via cranial morphological traits in the forensic sciences by training a subset of the community to correctly perform the method. In turn, this information can then be disseminated to colleagues and students.

Ancestry, Macromorphoscopic Traits, Human Variation

W22 Science Matters to Everyone: Victims, Offenders, and the Public

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The goal of this presentation is to educate attendees regarding systemic issues in forensic science and the strides being taken to address them.

This presentation will impact the forensic science community by exploring how the criminal justice system can or should respond in the face of backlogs, lab scandal, error, and publicity involving forensic science.

For a number of years, the forensic science community has grown and advanced and the demand for forensic evidence in courtrooms has dramatically increased. As a result, the forensic community has faced challenges: (1) involving discoveries that the strength of the forensic evidence testified to at trial may not have been as strong or as certain as was thought; (2) working through backlogs in testing, such as sexual assault kits; or, (3) in some cases, regarding simply untruthful or deceptive analysts, which has led to laboratory shutdowns and more.

This workshop will look first at the impact of increased volume and demand for forensic services that has impacted communities; specifically, the impact of backlogs in sexual assault kits. This has led to the re-traumatization of victims and to offenders being on the street to offend again. Speakers will then address the responses that have been developed, recommendations for best practices in the handling of sexual assault kits, how to work in a multidisciplinary fashion using a trauma-informed approach to reduce backlogs and streamline the processing of sexual assault kits and notification of victims.

In addition to backlogs, forensic science faces challenges stemming from the fact that what is known in forensic science is evolving. New technology and new revelations about a forensic discipline or even an unethical forensic scientist can lead to questions concerning the integrity of convictions relying on this evidence. This workshop will focus on the changes that have taken place in this area surrounding arson cases. When assumptions used to determine whether a fire was an intentional fire were discovered to be erroneous, the integrity of convictions came to light. The justice system had to determine what to do regarding the integrity of convictions that had been secured and how to go about addressing issues as they arise. The lessons learned within arson review provide guidance for addressing conviction integrity in all areas. Speakers will discuss what a conviction integrity unit is within a prosecutor's office, what should be considered when creating these units, including partnerships among stakeholders, and will address the state response in Texas, including the creation of a Science Advisory Work (SAW) group.

When carried over into the tapestry of the trial, where the various portions of the cloth of justice are woven into a final piece, science, medicine, dentistry, and jurisprudence result in a single verdict. When a conviction is sewn together from flawed fabric, the victim, the defendant, the courts, the justice system, and society itself suffer. One of the most often-questioned areas of forensic evidence involves bitemark evidence. Such cases may have relied on flawed analysis and/or questioned testimony to establish guilt. As the discipline consensus changes regarding the reliability, reproducibility, and significance of the data, the question remains as to how the witness can and should respond to prior convictions and practice pre-emptive analysis to prevent potential future miscarriages. Less highly publicized, but no less challenging, are cases in which the medical "gold standard" of the autopsy is challenged. Forensic pathologists, like everyone else, are subject to flawed analyses and conclusions resulting from human shortcomings and/or advances in understanding. These might involve inaccurate/incomplete interpretations of findings (both inclusion and exclusion), improved analytic methodologies, additional testing, etc. Handling situations in which the data doesn't match the testimony must be addressed — both as a forensic pathologist during the death investigation and as an expert witness on the stand.

Finally, the news media and public perception come into play. High-profile cases with extensive media attention can also lead to analytical errors, flawed understandings, hasty conclusions, and more. Media exposure may have immediate real and lasting effects; for example, by contaminating a potential jury pool necessitating a change in venue, sequestration, and the like. More subtle, but perhaps more lasting, could be the contamination of the public perception of the case, such that results of the trial are questioned and an acquittal is viewed with skepticism with the result being that a jury's verdict (guilty or innocent) remains in question, damaging public confidence in the jury system, sprouting conspiracy theories, and so forth.

Sexual Assault, Conviction Integrity Unit, Errors

W23 Cardiovascular Pathology for Medical Examiners and Coroners: Basic and Advanced Techniques for the Investigation of Sudden Cardiac Death

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After attending this presentation, attendees will: (1) understand basic cardiac anatomy relevant to the investigation of sudden cardiac death; (2) be able to apply basic and select advanced cardiac dissection techniques useful for the investigation of sudden cardiac death; (3) identify situations in which postmortem genetic testing may be useful; (4) appreciate the challenges of interpretation of genetic testing results with respect to determining mechanism of death, underlying cause of death, and contributory cause of death; and, (5) recognize situations in which consultation with a cardiovascular pathologist is warranted.

This presentation will impact the forensic science community by providing up-to-date knowledge and practical techniques in cardiovascular pathology and molecular genetics that will assist medical examiners, coroners, death scene investigators, and others involved in the investigation of sudden death.

The spectrum of cardiovascular disease which may present in the forensic setting ranges from common entities such as coronary artery atherosclerosis and hypertension to less common entities such as infective endocarditis or viral myocarditis, and more esoteric entities such as various congenital heart diseases, the dilated, hypertrophic, and arrhythmogenic forms of cardiomyopathy, arrhythmia syndromes such as Long QT syndrome or catecholaminergic polymorphic ventricular tachycardia (CPVT), and aortopathy syndromes such as Marfan Syndrome or Loeys-Dietz Syndrome. It is especially important for medical examiners and coroners to be familiar with inheritable cardiovascular diseases, which may be treatable in surviving family members, thereby directly fulfilling the ultimate goal of quality death investigation — to help the living.

The diagnosis of many cardiovascular diseases at autopsy has become increasingly complicated. Critical morphologic information suggestive of a particular disease may be observed at the time of autopsy, but confirmation of the disease usually requires a comprehensive evaluation incorporating medical history, scene investigation, and multiple diagnostic modalities, which may include histologic examination, microbiology testing, enzymatic assays, and genetic testing. The increasing use of postmortem genetic testing in the evaluation of sudden unexplained death in the young is a particularly challenging area for medical examiners and coroners and is potentially subject to error due to misinterpretation of genetic variants whose significance is unknown. The failure to consider the presence of inheritable disease and retain appropriate specimens for genetic testing or subspecialist consultation remains a persistent risk for medical examiners and coroners who are not familiar with the spectrum of cardiovascular disease which may present in a forensic setting.

This workshop is intended to be a practical introduction to the investigation of sudden cardiac death. The presenters include practicing cardiovascular and forensic pathologists with extensive experience in autopsy pathology, death investigation, surgical cardiovascular pathology, and molecular genetics. The basic foundations of cardiovascular pathology, including normal cardiac anatomy and histology, normal anatomic variants, and standard cardiac dissection methods, will be reviewed. Advanced dissection techniques will also be taught, including long-axis cuts (four-chamber and left ventricular outflow cuts), base of heart dissection for demonstrating valvular heart disease, gross dissection and histologic examination of the cardiac conduction system, and histologic examination of valves, myocardium, and aorta. Both common and rare entities in the differential diagnosis for sudden cardiac death will be discussed, including atherosclerotic coronary artery disease, hypertensive heart disease, hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic cardiomyopathy, inherited arrhythmia syndromes, and inherited aortopathy syndromes.

The role of genetic testing in the diagnosis of inherited cardiomyopathy, arrhythmia, and aortopathy syndromes will also be discussed, including the challenges associated with the interpretation of genetic test results that are equivocal for pathogenicity, or when pathogenic variants are discovered in the setting of alternative and equally compelling causes of death. A framework for communication of results to families will also be provided. Guidelines for specimen retention and cardiovascular pathology subspecialist consultation will also be discussed. This session will incorporate didactic lectures, informal question-and-answer sessions with questions solicited from the audience, and hands-on tutorials utilizing 3D scanned and printed models.

Forensic Pathology, Cardiovascular Pathology, Sudden Cardiac Death

W24 Think Tank on the Leading Edge of Forensic Science: Drones, Autonomous Vehicles, Big Data/Big Problems, National Security Globalization Into Protrusionism Privacy, Dirty Bombs, and Microbial Forensics

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After attending this presentation, attendees will understand how the rapid rate of change in society may impact several fields in forensic science.

This presentation will impact the forensic science community by demonstrating how the rate of change in society provides new challenges for forensic science. The development of designer drugs as well as the rapid development of methods to extract information from large amounts of data should be considered and perhaps prompt needed changes in laws. The issues with investigation of Chemical, Biological, Radiological, and Nuclear (CBRN) as well as driverless cars, drones, and the insights of cybercrimes and globalization with privacy issues will be discussed.

A wide variety of developments that will impact forensic science have been identified within the Think Tank Committee of the Forensic Sciences Foundation, Inc. The goal of this presentation is to describe how new developments may impact forensic scientists in their work. Practical examples will be presented on national security globalization into privacy issues, driverless cars, drones, microbial forensic, nuclear forensics, cybercrime, and big data. This presentation will impact the forensic science community by providing an overview of some of the new developments in forensic science and by opening a forum for the discussion of issues that arise regarding such developments.

Digital cameras were invented in 1975. As with all exponential technologies, the 10,000 pixels were a disappointment until digital became superior and went mainstream in a matter of years. This will now happen with artificial intelligence, health, autonomous cars, education, 3D printing, agriculture, jobs, and … yes, forensics.

The amount of data that is available from digital investigation, and from sensors, is rising each year and the question is whether a statistical analysis of this data can be presented in court. Biometric algorithms are improving and analyzing large amounts of video and images in combination with location data and other data available provide the possibility of making summaries of the data that can be presented in court. When applying these methods, users should also be aware of the limitations and error rates of the algorithms used. Additionally, the use of Bayesian conclusion scales is under discussion and national security globalization appears to yield privacy issues.

We see the developments of Unmanned Aerial Vehicles (UAVs) and drones and the forensic issues with finding digital traces as one of the topics. The driverless car is also performing in the real world. Will we skip level 3 autonomous vehicles (human intervention) and go directly to level 4?

Another important topic is the investigation within a CBRN crime scene and the interrogation of CBR agents presenting a variety of problems. Primary among those at the scene is an intense degree of political scrutiny and a high thermal burden. How do you accurately take high-value samples when you are in a Level A "spacesuit," how do you know where the samples are, and what should you prioritize in the 20 minutes of air you have at the scene? The European Commission Generic Integrated Forensic Toolbox (GIFT) is answering these questions and can share some of this data.

The Chemical Forensics International Technical Working Group (CFITWG) was created in 2017 to address science and capability gaps for the source attribution of weaponized chemicals by chemical means (e.g., impurity profiling and stable isotope ratios). Source attribution can tell how and where a weaponized chemical was made to help find perpetrators or facilitators of chemical attacks or detect the illicit proliferation of chemical precursors. This presentation will provide a brief overview of chemical forensics research and review how the CFITWG will strive to prevent and deter chemical attacks through collaborative efforts among members and partners.

The use of microbial communities in entomology is important. Current research focuses on the structure and function of antemortem and postmortem microbial communities using microbiomes as spatial and temporal evidence. In the past year, developments have advanced in understanding the relationships between decomposing remains, microbial communities, and the environment.

How do we manage multiple terabytes of data, containing millions of traces? How can a case investigator obtain meaningful information from all the data in the case, in a quick and simple manner, without compromising on forensic validation of the methods, the various data, and privacy protection? Additionally, the amount of data in the average case is needed by more than one team, dispersed through the town or country, and as such can no longer be worked on by a single investigator. Furthermore, knowledge dissemination concerning new methods discovered is difficult and ineffectual. At the Netherlands Forensic Institute, a big data digital analytics platform has been developed that is in use by the Dutch police force. This presentation will focus on the lessons learned about scaling the platform, the cases, and enhancing the platform with newer analytic methods. Finally, this workshop will close with research examples of deep learning and forensic multimedia investigation.

To keep pace, laboratories need to be innovative in their approach to monitoring the market for peer-reviewed literature and markets, building in-house libraries and databases, and investigating many other channels of intelligence in anticipation of possible new threats as well as helpful techniques.

Driverless Cars and Drones, National Security, Microbial Forensics

A1 Investigating the Accuracy of Additive Manufacturing Skeletal Samples for Evidence Reconstruction

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After attending this presentation, attendees will be aware of the problems inherent within 3D modeling and additive manufacturing (3D printing) in forensic anthropology and will recognize that these issues are pertinent to forensic science reconstruction approaches and, in particular, the presentation of 3D prints as evidence in court.

This presentation will impact the forensic science community by alerting attendees to the uses and limitations of additive manufacturing from Computed Tomography (CT) scans in a forensic context. Recommendations and thought-provoking findings could initiate discussions and collaborations toward further exploration into the use of models for reconstruction purposes in anthropology and wider forensic disciplines.

This research investigates the metrology of 3D modeling and 3D printing osteological samples from CT scans. It is expected that such models will be sufficiently accurate for anthropological comparisons, but the extent of the effect of modeling parameters is currently unknown.

There are two documented cases in which 3D prints of human remains have been used in United Kingdom courts of law.[1,2] In one of these cases, lawyers cast doubt on the reliability of a 3D-printed cranium, as the process has not been validated in a forensic context.[2] This type of evidence can be utilized after a virtual postmortem (avoiding disruption of remains), from antemortem clinical CT data, or in cases in which the subject survives. Studies have begun to validate these techniques, such as the reliability of obtaining accurate anthropological measurements from CT reconstructions of certain skeletal elements and the accuracy of 3D printing in medicine/anatomy, but further research is needed.[3-6]

Three dry osteological samples (a cranium, clavicle, and first metatarsal) were CT scanned using a multi-detector Toshiba Acquillon™ ONE. 3D models were segmented and converted into Stereolithic (STL) files for printing. First, the parameters applied in the virtual model generation were investigated for quality. Second, two additive manufacturing methods, Selective Laser Sintering (SLS) and Fused Deposition Modeling (FDM), were tested on three printers, and a statistical comparison of anthropometric measurements taken from the original samples, the virtual models, and the 3D prints was undertaken.

The results of this study indicate that: (1) the threshold values (labels) used in virtual model segmentation affect the quality of the model, as does the amount of smoothing employed; (2) there was no statistically significant difference between the clavicle and first metatarsal 3D prints and the original dry bone (p values >0.05), except for one of the clavicle FDM prints; (3) the cranium 3D print was not statistically distinguishable from the virtual model (p values >0.05) following removal of inconsistent cranial measurements, but the cranium 3D print was statistically significantly different when compared to the source bone ($p < 0.05$); (4) the virtual models had mean Absolute Errors (AE) of 1.6mm±0.9mm for the cranium, 1.5mm±0.7mm for the clavicle, and 1.1mm±0.7mm for the first metatarsal; and, (5) the 3D prints produced using SLS technology had smaller respective AE, with 1.4mm±0.9mm for the cranium, 1.2mm±0.2mm for the clavicle, and 0.7mm±0.5mm for the first metatarsal.

This empirical research provides initial data to validate the process of additive manufacturing in forensic anthropology. The data demonstrated that accurate 3D prints can be produced from CT-scanned bones, but with limitations. Segmentation of the virtual model was found to be a crucial step for producing accurate models, and it is thought that applying additional smoothing could help. Further exploration of additive manufacturing and samples that exhibit trauma, pathology, and taphonomy will progress toward producing best practice guidelines and validation of the technique.

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Additive Manufacturing, Metrology, Evidence Reconstruction

A2 Radiographic Image Analysis and the Estimation of Age at Death in Adult Males

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After attending this presentation, attendees will understand how the analysis of digital radiographs of the pubic bone can be used to estimate age at death for male decedents.

This presentation will impact the forensic science community by providing data indicating that digital radiographic images of the male os pubis can be reliably used to place individuals within intervals of age at death comparable to those of more commonly employed methods based on morphological changes of the pubic bone.[1,2] The techniques developed in this research can be used to estimate age at death from pubic bones where details of the symphyseal face have been damaged or otherwise obscured and, with refinement, may eventually enable age-at-death estimation without excision and maceration of the pubic bone.

This research was conducted using the Hartnett-Fulginiti collection curated at the Forensic Science Center in Maricopa County, AZ. This collection is comprised of more than 600 specimens of pubic symphyses from decedents of known sex, age at death, and ancestry. A training sample, composed of 100 pubic bones from male decedents ranging in age from 18 to 91 years, was selected to provide roughly equal representation from each of the seven morphological phases of the Hartnett-Fulginiti method for the estimation of age at death.[2] Although preference was given to the left os pubis, the right was used when the left was damaged or otherwise unsuitable for analysis.

Digital radiographs of each specimen were taken from a fixed distance and with constant settings. The resulting images were subjected to analysis in which characteristics of the distribution of gray values (mean, standard deviation, minimum, maximum, median, mode, skew, and kurtosis) were recorded. In an attempt to control for individual variation in size and shape, image analysis was constrained to the triangular area defined by the upper and lower bounds of the symphyseal face and the most medial point on the margin of the obturator foramen. Recorded characteristics were evaluated for their correlation to age at death, and a subset of six variables (comprised of standard deviation, maximum value, skew, kurtosis, and the constructed metrics of range and signed difference between mean and median) was selected for a k-means clustering analysis. Although Hartigan's Rule suggested that 13 clusters was the optimal clustering solution for this data, this resulted in several clusters that were based around a small number of individuals, and a clustering solution of nine was adopted instead. Each of the nine clusters was defined by the location of its centroid and described by the mean, standard deviation, and range of the within-cluster age-at-death distribution.

To test whether radiographic image analysis could produce viable age-at-death estimates, the six variables listed above were recorded for a secondary sample of 57 randomly selected males ranging in age from 22 to 84 years. The six-dimensional Euclidean distance between each new individual and the centroids of the nine previously defined clusters was calculated, and individuals were assigned to the cluster whose centroid they were nearest to. The frequency with which the ages of the individuals in the secondary sample fell within intervals constructed around the mean age of each cluster was then recorded. Results indicate that overall, an individual's true age at death was within 17 years of the mean age of the cluster to which they were assigned in 75.4% of the trials. Although a 34-year age interval is large, it is nearly equivalent to those employed by widely used morphological techniques for the estimation of age at death.[1,2] Moreover, the majority of the individuals whose ages were not in the predicted interval were either very young or very old. For individuals whose age at death was between 25 and 75 years, true age at death was within 17 years of the assigned cluster's mean in 87.8% of cases and, for 81.5% of individuals between the ages of 40 and 60 years, true age at death was within ten years of the assigned cluster's mean.

Although preliminary, these results suggest that image analysis of radiographs of the male os pubis can be employed for the estimation of age at death. With further refinement, the utility of this technique for both younger and older individuals may be increased. Moreover, further exploration of the uses of radiographic image analysis may eventually obviate excision and maceration of the pubic bone for the development of a biological profile.

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Age-At-Death Estimation, Pubic Bones, Radiographs

A3 Landmark and Measurement-Based Data Assistant (LAMbDA): A Pedagogical Tool for Cranial Landmark Data Collection

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After attending this presentation, attendees will be familiar with the Landmark and Measurement-based Data Assistant (LAMbDA) interactive website, which can be used as a pedagogical and/or reference tool for aiding in the collection of cranial landmark data. Additionally, attendees will understand the need for standardized cranial landmark definitions and a centralized data repository of definitions and diagrams for cranial landmark data collection in forensic anthropological analyses.

This presentation will impact the forensic science community by addressing inconsistencies within standardized definitions for all cranial landmarks. This information will be located in an online repository that includes standardized cranial landmark definitions, their locations, exceptions to certain definitions, depictions and photographs, and currently available reference material. A document repository is especially useful for learning and/or practicing a skill by allowing the user to easily access all related material in one location.

Cranial landmark data is one of the most informative components of data collection in forensic anthropological analyses. This data is used in more refined analyses to estimate sex and ancestry as components of the biological profile. When learning data collection procedures for landmark digitization, users refer to various sources for standardized definitions and diagrams. Definitions of some landmarks can be found in the literature, but there is not an available definition for every cranial landmark. Therefore, word-of-mouth definitions are transmitted from instructors to students, further emphasizing the need for a comprehensive and standardized list of cranial landmarks. Additionally, discrepancies between landmark definitions across separate sources can contribute to interobserver error. Further, unclear or imprecise definitions in reference material can lead to differences in interpretation of a given definition. This error can lead to flawed reference data (e.g., via multiple contributors to reference data in FORDISC®) or incorrect measurements through misidentification of landmarks, ultimately impacting classification.

Currently, cranial landmarks are defined across at least ten references, yet a single complete reference for all landmarks used in forensic anthropology does not exist. LAMbDA serves as a repository for all cranial landmark definitions, with accompanying diagrams and depictions. The LAMbDA website (www.locatelambda.org) features a 3D, interactive digital model of the human cranium labeled with cranial landmarks. Pop-up definitions of each landmark appear when the cursor is scrolled over the landmark. These definitions come from their original references when definitions are clear and consistent. Cranial landmarks previously undefined, with inconsistent definitions across references, or those that lack precision, are defined by the authors and approved by a panel of experts on this topic. The website includes 2D photographs demonstrating proper placement of cranial landmarks on bone exhibiting anatomical variants, such as lambdoidal and bregmatic ossicles. LAMbDA is designed to be compatible with 3Skull, as this program uses the most comprehensive list of 108 cranial landmarks; however, the website can be used to aid with data collection using other software programs, such as CRANID. Each written definition includes the landmark's associated measurements to aid in the interpretation of landmark placement. This presentation's goal is to provide LAMbDA as a pedagogical tool both in the classroom and in practice to assist students and practitioners in collecting standardized cranial landmark data for forensic anthropological analyses.

Forensic Anthropology, Digital Reference Tool, Standardization

A4 A Test of Quantitative Age-At-Death Estimation of the Pubic Symphysis Using the forAge Program

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The goal of this presentation is to test the method of age-at-death estimation from the pubic symphysis proposed by Slice, Algee-Hewitt, Stoyanova, and colleagues on the Texas State Donated Skeletal Collection.[1-3] After attending this presentation, attendees will understand some of the implications of this novel age-at-death estimation technique to better interpret the results from forAge analysis.

This presentation will impact the forensic science community by helping shed light on avenues for amending or expanding on this novel age-at-death estimation technique.

This preliminary test of the objective age-at-death estimation methodology, as proposed by the aforementioned authors, examined the accuracy and bias of age-at-death estimation provided by the forAge program.[1-3] The forAge program performs shape analyses of 3D surface models, calculates surface parameters, and outputs an age-at-death estimation. After attending this presentation, attendees will understand some implications of this novel age-at-death estimation technique to better interpret the results from forAge analysis.

The left pubic symphysis of 50 modern White males and females from the Texas State Donated Skeletal Collection and 12 male casts from the original Suchey-Brooks method were scanned using the NextEngine® 3D Desktop Scanner following the methods of Stoyanova et al.[3] The individuals ranged in age from 18 to 94 years old, with a mean age of 60.88 years.

Excess data were removed to isolate the symphyseal surface in ScanStudio™ v2.0.2. The surfaces were divided into three groups for analysis: (1) original surface scans (with holes); (2) edited surface scans (holes filled); and, (3) Suchey-Brooks cast surface scans. Surfaces with holes were filled in MeshLab v2016.12 and saved as separate files. Scans were then exported as ASCII PLY files and uploaded into the forAge program.

Point estimates generated by forAge were analyzed for accuracy, bias, and correlation against reported age at death. Differences in age-at-death estimates between surface scans with and without holes were compared using paired *t*-tests. Lastly, paired *t* -tests compared the means of age-at-death estimates from the Suchey-Brooks casts scanned by this study and between this study and Stoyanova et al.[3]

Analysis of the Original Surface Scans exhibited high inaccuracy in age-at-death estimation, with a bias toward under-aging individuals more than 40 years old and over-aging individuals less than 40 years old. This study revealed greater levels of inaccuracy in age-at-death estimation compared to Stoyanova and colleagues.[1-3]

Surface scans with fluctuating surface topography tended to produce holes in the mesh. Analyses comparing age-at-death estimates between original surface scans with holes and edited surface scans with no holes resulted in no significant differences. Thus, this study advises against filling holes in the surface scans.

Scans of the Suchey-Brooks male pubic symphysis casts were used to test for interobserver error between the two contributors of this study and between the first contributor of this study and the Stoyanova study.[3] The age-at-death estimates between the two contributors of this study exhibit no significant differences except in one shape measure. The age-at-death estimates collected by the first contributor were significantly different for each shape measure compared to those published by Stoyanova et al.[3]

The disparities between this research and Slice, Algee-Hewitt, and Stoyanova et al. may best be explained by the differences in the sample distribution, which had more individuals more than 40 years of age, while the previous studies had more individuals less than 40 years of age.[1-3] High inaccuracy may be due to the inability of the shape measures to account for minute differences in the surface topography of older individuals, such as increased porosity and excess bony growth. Since the same standardized casts were scanned in these analyses, the significant differences observed in age-at-death estimation from the casts may be explained by differences in scan parameters and image processing (i.e., bracket vs. single scans). Overall, this research may shed light on avenues to amend or expand on the original research.

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Age-At-Death, Pubic Symphysis, Surface Scans

A5 The Glenoid Cavity in Sex Estimation Among Contemporary Filipinos: Preservation and Accuracy Rates

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The goal of this presentation is to demonstrate the utility of the scapula in determining sex among contemporary Filipino skeletons and to highlight the importance of population-specific methods for biological profile estimation.

This presentation will impact the forensic science community by presenting a method that can be applied to an underresearched yet important population. This presentation will also further validate the reliability of the scapula as a means for sex estimation.

Methods of biological profile estimation from skeletal remains are known to be population specific. The degree of sexual dimorphism, rate of senescence, and formulas for stature differ from population to population. Furthermore, the different components of the biological profile often rely on each other. Knowing the sex, for example, further calibrates later estimates of age and stature. Research into Filipino skeletal variation has been relatively scarce owing to the until-recent absence of appropriate reference collections.[1] Such a paucity of methods for estimating the Filipino biological profile is unfortunate, given the country's large population sizes, widespread diaspora, frequent exposure to natural disasters, and rampant violence.

The glenoid cavity of the scapula has previously been used to estimate the sex of unknown individuals.[2] In this study, the effectiveness of the glenoid cavity in estimating sex among contemporary Filipino skeletons was evaluated using metric measurements of the glenoid height and breadth. The performance of these measurements was then tested using discriminant functions developed from Thai, Greek, and Mexican populations.[3-5] Discriminant analysis was also conducted on the Filipino sample. The rate of preservation of the glenoid cavity was further qualified in order to assess the utility of this feature in forensic contexts.

The state of preservation of glenoid cavities from 124 individuals housed at the Archaeological Studies Program, University of the Philippines Diliman were observed. More than 71%-75% of glenoid cavities examined by sex and side were fully intact or had marginal erosion that did not affect measurement, often even when there was significant postmortem damage to the scapular body. From this larger sample, 70 adult individuals (35 males and 35 females) were selected and measured by three different observers. Statistically significant differences between Filipino males and females were found using a paired *t*-test at an alpha level of 0.05 (t=-5.44, p=0.00). Using multivariate discriminant functions from Mexican and Greek populations, correct classification of females was 100% and of males was 8.6%. Using the Thai discriminant function yielded classification success of 34.3% for females and 100% for males. Filipino-specific linear discriminant analysis produced a cross-validated overall correct classification rate of 82.9% (F=80.0%; M=85.7%).

These results indicate that the glenoid cavity is a robust skeletal feature that resists degradation. Further research into this feature is warranted, given the probability of its preservation within forensic contexts. Additionally, the degree of sexual dimorphism of the glenoid cavity is highly variable between populations. The poor success of Filipino males using Mexican and Greek formulas speaks to relatively reduced sexual dimorphism within this population. The Thai formula yielded better classification for Filipino males but not females; however, the successful performance of the Filipino discriminant analysis confirms the utility of the glenoid cavity in sex estimation, but is heavily population specific.

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Forensic Anthropology, Scapula, Philippines

A6 Reliability and Validity of the Walker and Klales, et al. Methods

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After attending this presentation, attendees will better understand the reliability and validity of two commonly used morphoscopic sex estimation methods.

This presentation will impact the forensic science community by reporting observer consistency in scoring commonly used skull and pelvis traits and investigating the role that experience level plays in obtaining accurate sex estimations.

To have forensic utility, methods must be both valid and reliable. Validity refers to how well a method measures what it is supposed to (i.e., accuracy) and reliability refers to the ability to produce consistent results. External validity and reliability of methods must be assessed prior to their incorporation into standard operating procedures, and, to comply with *Daubert* standards, forensic methods must be tested and potential error rates published. As a result, there has been a push to translate traditional qualitative methods into quantitative methods, in which morphoscopic traits are assigned ordinal scores; however, these scoring methods do not eliminate method subjectivity completely, and thus it is important to assess validity and reliability in these trait scoring methods.

Two popular sex estimation methods, Walker and Klales, et al., provide an ordinal scoring method for traits of the skull and pelvis, respectively.[1,2] Walker utilizes the nuchal crest, glabella, supraorbital margin, mental eminence, and mastoid process. Klales et al. utilizes the ventral arc, subpubic contour, and medial aspect of the ischio-pubic ramus.[1,2] The validity and reliability of these methods have been tested only a handful of times since their incorporation into forensic casework, and the results have varied. Recognizing the need for updated standards in the field, trait data have been collected from more than 2,500 individuals as a part of a National Institute of Justice (NIJ) grant to: (1) assess the reliability and validity of these methods; (2) evaluate the impacts of population differences, secular change, and asymmetry on sex estimation; and, (3) create a free morphological database for sex estimation using these traits/methods: MorphoPASSE. This presentation addresses the first of these goals: trait scoring validity and reliability.

Three observers with varying levels of experience (expert/grant Principal Investigator (PI), experienced, and inexperienced) collected ordinal score data using the Walker and Klales et al. traits. This interobserver sample consisted of 222 individuals from the Hamann-Todd (HTH) and Bass skeletal collections. The expert observer scored the individuals twice, with a year between scoring events for intraobserver analyses. Additional trait data were contributed by four other researchers, with various levels of experience, thereby facilitating additional tests of observer error. Their data were collected from the HTH (n=174), Bass (n=57), and the Operation Identification and donated collections at Texas State University (n=57) and included individuals also scored by the grant PI. Interobserver error was assessed using the Intraclass Correlation Coefficient (ICC), while intraobserver error was assessed with quadratic weighted Kappa (wK). Sex classification accuracy was evaluated using the logistic regression equations provided in the original publications.

Intraobserver agreement was nearly perfect for the pelvis and substantial for the skull. The ICC indicated excellent levels of agreement between the three observers that scored the entire sample of 222 individuals, with the exception of the orbital margin (good agreement). The expert and experienced observer achieved higher agreement than the inexperienced observer, indicating that experience does play a role to some degree. When the additional contributed trait data were included in analyses, excellent agreement was obtained between the three expert observers for all traits except the mental eminence. Classification accuracy was high for all three experience levels for the pelvis (96.6% expert, 93.6% experienced, and 78.2% inexperienced), but was generally lower, with a high sex bias, for the skull regardless of experience (73.5% expert, 61.4% experienced, and 70.7% inexperienced). In conclusion, the reliability results indicate general consistency in trait scoring among observers, while the validity results suggest that experience plays a larger role in the accurate application of the methods than has been previously reported; observers with a great deal of experience can expect much higher sex classification accuracy than observers with less experience.

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Walker Method, Klales et al. Method, Sex Estimation

A7 Dirt Matters: Case Studies in Forensic Archaeological Stratigraphy

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After attending this presentation, attendees will have a clearer understanding of the extent to which stratigraphy can be an important piece of evidence for the interpretation of forensic archaeological sites.

This presentation will impact the forensic science community by highlighting: (1) the frequent subtlety and complexity of stratigraphic information at forensic archaeological sites; and, (2) the importance of professional archaeological knowledge and experience to the successful recognition and correct interpretation of such stratigraphic cues. Further, it will emphasize that due to these two factors, forensic sites requiring mapping and excavation are best processed with the direct involvement of trained forensic archaeological professionals who have an understanding of how to record and interpret stratigraphic evidence.

Steno's Law of Superposition (1669) essentially states that older layers of soils and sediments underlie younger layers. A related principle, the Law of Cross-Cutting Relationships, states that sediment layers must necessarily be older than any deposit or intrusion that disturbs them. These are simple concepts that, when practically applied, can be extraordinarily complex. Though Steno's Laws are foundational principles of archaeology, they are not commonly associated with the forensic sciences; however, in the context of forensic archaeology, particularly when dealing with forensic scenes that have developed over an extended period of time, understanding these elementary principles of soil and sediment stratigraphy can be a crucial element of site interpretation.

The goal of this presentation is to illustrate the assertion that forensic anthropological professionals must have a strong knowledge of stratigraphic interpretation or risk being led astray and reaching incorrect conclusions during site excavation. To that end, this presentation will present six forensic archaeological excavation case studies in which astute interpretation of site stratigraphy constituted a significant line of evidence toward the successful excavation of the forensic scene and, in some cases, the recovery and identification of a missing individual. The discussed sites were excavated by Defense POW/MIA Accounting Agency teams searching for the remains of missing United States service members from three major wars. Each site is unique and necessitated a distinctive style of archaeology due to a number of factors. Soil and sediment types vary widely from one geographical location to another; no two sites are alike, and even within an individual site, formation processes can vary extensively from one area to another. Sites presented include those with very deep stratigraphic profiles, such as aircraft crash craters, others with little to no stratigraphic development, such as disturbed surface scatters, and unusual sites where the depositional environment is unclear or highly complex, such as ice fields or glaciers. Despite these differences, all of these sites share the distinctive aspect of having formed over long periods of time (typically 45 to 75 years). The comparatively protracted time depth of these sites often means more complex site formation processes are at work than would typically be found on more recent forensic archaeological sites. Relevant site formation processes, such as erosion, bioturbation, fluvial deposition, and human scavenging activities, will be examined.

A clear understanding of stratigraphy and the archaeological methods used to detect, interpret, and record formation and disturbance patterns in layers of soil and sediment is one of the many things that sets apart archaeological professionals from untrained amateurs. This is one of the primary reasons it is so important to have forensic sites excavated by experienced forensic archaeologists rather than by well-meaning but untrained and inexperienced non-archaeological professionals. An ability to detect, interpret, and record stratigraphy is a fundamental skill used by forensic archaeologists, and one that must be honed through a variety of field experiences; it is nearly impossible to learn in a classroom setting. Stratigraphy tells a story that can only be read through careful excavation.

Forensic Archaeology, Stratigraphy, Defense POW/MIA Accounting Age

A8 Analysis of Interobserver and Intraobserver Error Associated With the Use of 3D Laser Scan Data of the Pubic Symphysis

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After attending this presentation, attendees will understand the intraobserver and interobserver error related to collecting and editing 3D laser scans of skeletal material, as well as the repeatability of three new age estimation methods that use bone shape data extracted from the laser scans.

This presentation will impact the forensic science community by providing best practice guidelines for using laser scanners, scanned images, and coordinating data in forensic casework that will contribute to the standardization of 3D image processing procedures between different forensic practitioners and labs. It will also provide validation for the age estimation methods discussed here.

In age-at-death estimation based on visual assessment, objective evaluation and correct diagnosis of age-related skeletal traits are crucial, as these factors determine whether the aging methods can achieve their full potential — producing the most accurate and reliable age estimates. Nevertheless, the traditional, phase-based age estimation methods have been reported to yield inconsistent age estimates both within and between observers.[1,2] The reasons for these discrepancies lie in the fact that accurate macromorphoscopic analysis heavily depends on the correct interpretation of qualitative trait descriptions, conformity of the bone, and experience of the observer.

Recently, Slice and Algee-Hewitt and Stoyanova et al. have introduced three novel, fully computational aging methods using 3D laser scans of the pubic symphysis that minimize subjectivity in age estimation by reducing the effects of observer experience in the age-indicator/trait assessment and methodological bias; however, the reproducibility of these methods has not been fully explored or quantified.[3-5] This is of concern because there is potential for introducing error in the first two steps of data processing — when the scans are taken and edited at different times by different observers. In response to this concern, the current study evaluates the repeatability of these novel methods by assessing intra-scan variation, within, and between, observer differences in scan editing and its impact on age estimation.

The test data used in this study represent replicate scans of the Suchey-Brooks' (SB) male casts, taken using a 3D desktop laser scanner. The upper and lower stages of each of the six phases were scanned three times by a single observer (n=36). Four different observers with various experience levels and training backgrounds independently edited the triplicate of the SB scans using the scanner's accompanying software, such that the symphyseal face is extracted from the surrounding bones. From these isolated faces, x, y, and z coordinates were retrieved and analyzed via the Subarachnoid Hemorrhage (SAH) Score method, the Thin Plate Splines/Bending Energy (TPS/BE) method, and the Ventral Curvature (VC) method to compute shape measures.[3-5] These measures were subjected to single-variable and multivariate regression models to obtain age estimates for each replicate scan per observer. Finally, using the shape measures and final age estimates, a series of the Intraclass Correlation Coefficient (ICC) were calculated to evaluate within- and between-observer reliability in scan editing. Additionally, extra editing conditions were tested to simulate the situation in which the practitioner misidentifies age-related traits due to unfamiliarity with the scan editing protocol. A set of the SB casts was edited with different widths of the margin (2mm vs. 4mm vs. 1cm) left around the symphyseal face and with/without the pubic tubercle, which may impact the VC values as it protrudes ventrally. Possible effects of these conditions on age estimates were evaluated using the paired t-test.

This study produced high ICC values (0.75-1.0), demonstrating that the raw scans were edited consistently within and between observers and that the derived shape measures and age estimates were in excellent agreement among observers. Moreover, despite the simulated improper editing of the scans with various margin widths remaining, the methods were robust enough to self-correct and produce consistent and accurate age estimates ($p > 0.05$), with the exception of the faces with 1cm margin. Interestingly, the inclusion of the pubic tubercle for the shape analysis did not necessarily yield inaccurate age estimates for the VC method, while it produced statistically significant mean differences between the documented chronological age and age estimates of the SAH score method, TPS/BE method, and the two multivariate regression models ($p < 0.01$). These results demonstrate high repeatability of the computational methods regardless of the observer's level of experience or training background and support using a 3D laser scanner and scanned images to aid in resolving the issue of subjectivity.

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Age-At-Death Estimation, Observer Error, 3D Scans

A9 Analysis of Osteon Pull-Out and Collagen Degradation to Establish Fracture Timing

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After attending this presentation, attendees will understand the phenomenon of osteon pull-out, the effects of collagen degradation on pull-out morphology, and the utility of these morphological changes in distinguishing peri-mortem versus postmortem traumatic events.

This presentation will impact the forensic science community by documenting morphological differences between fracture-induced osteon pull-out during the peri-mortem and postmortem intervals.

It is hypothesized that the histological appearance of peri-mortem fractures exhibits greater degrees of osteon pull-out due to elasticity imparted by Type I collagen. As the organic collagen fibers degrade postmortem, the characteristics of pull-out differ due to the concomitant reduction in elasticity. This study provides a basis for further investigation into the use of osteon pull-out and histological morphology to distinguish between peri-mortem and postmortem fractures.

Fractures may exhibit characteristic features of peri-mortem injuries as long as they occur while the bone is reasonably elastic. The extended time during which bone maintains its elasticity after death creates an exaggerated peri-mortem interval in bone and limits the capacity of macroscopic fracture evaluation to delimit peri-mortem versus postmortem events. Fracture morphology associated with peri-mortem events is related to moisture content, mineral matrix ratios, and osteon fracture patterns.[1,2] This study evaluates fracture microstructure with Scanning Electron Microscopy (SEM) and correlates the histologic features with Accumulated Degree Days (ADD) and collagen degradation.

Thirty-two foot bones (16 metatarsals and 16 phalanges) from a non-reproducing pig (*Sus scrofa)* were obtained on the same day of slaughter. One metatarsal and one phalanx were selected to represent an ADD of 0. Cross-sectional samples were harvested from these two bones at mid-diaphysis with an autopsy saw. These samples were then washed in a phosphate buffer solution and fractured with a quick snapping force. Fractured pieces were fixed using osmium tetroxide, ethanol, and hexamethyldisilazane, then mounted on SEM stubs and sputter coated with gold. The remaining samples were catalogued, tied in porous cloth, and placed in a cage to decompose in an outdoor setting. ADD was recorded using daily temperature averages for Middlesboro, KY, with a minimum threshold temperature of 57°F. Samples spanning a postmortem interval of 0-1,043 ADD were fractured at regular intervals. Histologic morphology was examined with SEM, and postmortem collagen degradation was analyzed with mass spectrometry. Bone material was prepped according to established analytical platform protocols and analyzed using high-resolution (0.2ppm-3ppm mass error) data acquisition with an Orbitrap™ mass spectrometer.[3,4]

The mass spectrometry analysis of collagen revealed expected degradation with advancing ADD. The average OD_{550} of the two initial specimens resulted in an absorbance of 0.36 compared to the final specimen, which yielded an absorbance of 0.23, a difference of approximately 36.1%. Analysis of SEM images revealed significant differences in osteon appearance occurring at approximately 70% of initial collagen levels. Samples containing 70% or less of intact collagen demonstrated marked structural failure along canaliculi channels compared to samples with >70% of intact collagen. This preliminary data suggests that as collagen degrades with increasing ADD, the osteon pull-out mechanism becomes inadequate at dispersing force, and structural failure occurs along canaliculi channels, which may be a source of structural weakness. SEM analysis of fracture morphology coupled with mass spectrometry quantification of collagen degradation may offer a method for more precise interpretation of fracture timing.

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Bone Histology, Scanning Electron Microscopy, Skeletal Trauma

A10 Quantitative Population Differences in Anterior Zygomatic Projection (ZP)

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After attending this presentation, attendees will better understand the variation of anterior ZP within modern populations and its use in identifying unknown individuals of Asiatic/Native American ancestry.

This presentation will impact the forensic science community by expanding the knowledge of craniofacial trait variability used during ancestry estimation. Additionally, this presentation will demonstrate the value of updating traditional methods by incorporating contemporary technology, such as 3D virtual models.

Within forensic anthropology, qualitative methods have historically been favored for estimating the ancestry of unknown individuals. This leaves room for subjectivity when assessing traits and can potentially lead to misclassifications. Anteriorly projecting zygomas have traditionally been viewed as a trait indicative of Asiatic heritage (including Native Americans), but the trait is poorly defined and recent validation studies have demonstrated its vulnerability to high interobserver error.[1,2] In an effort to reduce subjectivity in describing ZP, two non-metric methods have been proposed to measure this projection, one commonly referenced to Rhine and the other from Bass.[3,4] In Rhine, the angle of projection is measured by dropping a line "from the middle of the upper margin of the orbit to the middle of the lower margin [that] produces an angle … with the Frankfort plane" using a 90° sectioning point to define projecting, vertical, or receding zygomas. In the Bass method, the observer holds the skull with the occipital region in their hand and the facial area up. A pencil is then placed across the nasal aperture and the observer inserts their index finger between the zygomatic and the pencil. If the observer cannot fit their finger in that space, the zygomatics are considered projecting. The Rhine method is qualitatively assessing an angle, while Bass is visually assessing an absolute distance, yet both use the terminology "zygomatic projection." The current study investigates the applicability of the available methods of assessing ZP and quantitatively examines the relationship between ancestry and ZP.

For both the Rhine and Bass techniques, two interpretations of each method were metrically assessed utilizing 3D virtual cranial models. All analyses were conducted via the use of Geomagic® studio software and ImageJ. This allowed for the crania to be automatically aligned to standardized orientations for viewing and facilitated the incorporation of inter-planar distance measurements, which are necessary to quantitatively test the original qualitative methods. The sample consists of 231 3D cranial models of United States White (n=73), United States Black (n=38), Arctic Native American (n=79), and Plains Native American (n=41) individuals.

Two interpretations of the ZP angle were tested following Rhine and two inter-planar distances between the nasal aperture and the zygoma were tested following the text and figures presented in Bass. To test for differences in group means, Analyses of Variance (ANOVAs) were run on all measurements of ZP, with two-way ANOVAs then conducted to examine the effects of ancestry and sex on each measurement. Tukey's post hoc tests were then performed to identify which groups displayed differences. Lastly, to provide more details on group differences and evaluate group classification rates, logistic regression was performed on all measurements of ZP that yielded significant ancestry differences ($p < 0.05$).

In each assessment, the Arctic Native Americans demonstrated the most anteriorly projecting zygomas, providing support to the general assumption that individuals of Asiatic ancestry have more anteriorly projecting zygomas than other populations, although significant differences were not always obtained. Of the interpretations tested, the inferior zygomatic inter-planar distance performed best at classifying between Arctic Native Americans and other groups when using binary logistic regression. This yielded correct classification rates between 70% and 98%. ZP was also found to be mildly sexually dimorphic, with females having more projecting zygomas than males within the same population. This study demonstrates that quantitatively modifying the current methods of ZP assessment through the use of 3D software can lead to information gains unobtainable through existent qualitative methodologies. Furthermore, this study examines the use of standardized terminology within the field of forensic anthropology and highlights the benefits of investigating alternative interpretations to traditional methodologies.

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Biological Profile, Ancestry Estimation, Cranial Traits

A11 The Use of the Mastoid Triangle for Sex Estimation

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After attending this presentation, attendees will better understand the use of the mastoid triangle for sex estimation in adult individuals.

This presentation will impact the forensic science community by providing insight into a method for sex estimation using metrics of the mastoid triangle. In addition, this presentation demonstrates the need for additional research into the use of the mastoid triangle for sex estimation.

Sex estimation is a key parameter of the biological profile because it allows for sex-specific methods to be employed in the estimation of other aspects of the biological profile. Previous studies utilizing the mastoid process for sex estimation have focused on both metric and non-metric traits of the mastoid process itself; however, Paiva and Segre measured the area of the mastoid triangle, defined as the area between asterion, mastoidale, and porion, and found significant sex differences.[1] Subsequent studies have found significant differences between males and females using dimensions of the mastoid triangle; however, no studies have examined the use of shape differences of the mastoid triangle for sex estimation. The goal of the present research is to metrically examine size and shape differences of the mastoid triangle between males and females.

A total of 200 adult individuals of known sex were sampled from the Hamann-Todd Collection housed at the Cleveland Museum of Natural History: 100 males and 100 females. Using a digitizer, three coordinates were collected from the left side of the cranium: Asterion (AST), Mastoidale (MS), and Porion (PO). Three Interlandmark Distances (ILDs) were calculated to explore size differences, and Geometric Morphometric Analyses (GMA) were conducted to explore shape differences. Classification accuracy was assessed using jackknifed Linear Discriminant Function Analysis (LDFA) of the ILDs, Procrustes Coordinates (PCoords) generated from the GMA, and Principal Components (PCs) generated from the principal component analysis.

Using LDFA of all three ILDs, correct classification between the sexes was 72.5% (females 73%, males 72%). Using separate ILDs, classification accuracy between the sexes was highest using LDFA of MS-PO (overall 71.35%; females 73.7%, males 69%), followed by AST-MS (overall 67.5%; 66% females, 69% males), and AST-PO (overall 63.3%; females 59.6%, males 67%). In addition, males were significantly larger ($p < 0.05$) than females for all three ILDs. Classification accuracy between the sexes using LDFA of the PCoords was 70.5% (females 67%, males 74%), which is similar to and slightly less than the size analysis. The first two PCs accounted for 99.9% of the variance, and classification accuracy between males and females using LDFA and these two PCs was 65.5% (females 68%, males 63%).

The results of this study demonstrate statistically significant differences between the mastoid triangle of males and females. Classification accuracy was highest using the ILDs; however, the results using the PCoords were comparable. The results of this study are similar to previous studies, which found significant differences between the mastoid triangle of males and females; however, classification accuracy using AST-PO differs from Jain et al.'s reported 45% classification accuracy.[2] This may be due to variability in the location of asterion between individuals and populations.[3] Combining the size and shape analyses from this preliminary study with the measurement of mastoid area or including measurements of both the left and right mastoid triangles in the analyses have the potential to produce even higher classification accuracies.

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Sex Estimation, Mastoid Triangle, Geometric Morphometrics

A12 The Impact of Asymmetrical Leg Lengths on Adult Stature Estimation

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After attending this presentation, attendees will have learned that left/right total leg length asymmetries are commonly encountered in forensic anthropological casework. Guidance will be provided on how best to estimate stature when asymmetries are present to avoid the production of inaccurate estimates and the erroneous exclusion of decedents as matches to their skeletal remains.

This presentation will impact the forensic science community by encouraging bilateral measurement taking whenever possible and selection of a 99% Prediction Interval (PI) when total leg length asymmetry reaches ≥0.7mm. This practice can prevent inaccurate stature estimates and the erroneous exclusion of decedents from their skeletal remains.

The skeletal elements required to execute Fully's anatomical method of stature estimation are often not present, are incomplete, or are damaged, precluding its use. Thus, forensic anthropologists often use regression formulas to estimate stature from limb bones. As previous research has revealed that: (1) stature estimates calculated from two or more long bones are superior to those calculated from one; (2) lower limb bones correlate more strongly with stature than upper limb bones; and, (3) asymmetry between sides is relatively minor, the left (by convention) femur and fibula (highest r-squared value combination in FORDISC®) are often used to estimate stature at the Defense POW/MIA Accounting Agency (DPAA) laboratory.

A review of recent DPAA identifications reveals that stature estimations using the left femur and left fibula are occasionally slightly inaccurate due to total length asymmetries between the left and right legs. In extreme cases, estimates calculated from one limb match an identified individual's reported stature, while estimates calculated from the opposite limb exclude the remains as a match. Realizing that conventional selection of the left leg could be resulting in inaccurate stature estimations in cases of asymmetry, this research was undertaken to investigate: (1) if statistically significant asymmetries exist between left and right total leg lengths; and, (2) if the longer total leg length more accurately reflects measured stature as it represents an individual's greatest height potential when standing up straight.

To address these questions, stature data was collected from adult individuals identified at the DPAA Laboratory between 2001 and 2017. Ultimately, this sample was composed of 70 males of European, African, Native American, and Hispanic descent ranging in ages at death from 18 to 40 years (average=21.9 years, standard deviation=3.66 years). Twenty-two individuals served in World War II and 48 in the Korean War. To be included, the remains must have had left and right femora and fibulae present for analysis, the remains must be atraumatic and complete, and both left and right sides must have been measured by the assigned forensic anthropologist.

Using element lengths documented in the case notes, each individual's stature was estimated using FORDISC® 3 and the appropriate Trotter MStats male database. First, the maximum lengths of the left femur and left fibula were used to calculate a 95% Prediction Interval (PI). Second, the maximum lengths of the right femur and right fibula were used to calculate a 95% PI. These PIs were then assessed for absolute difference and checked against the individual's antemortem stature (obtained by healthcare professionals during military medical evaluations) for accuracy. Stature estimates that did not include the identified individual's antemortem stature were run again using 99% PIs and checked to see if the identified individual's antemortem stature was captured.

Although left/right total leg length asymmetries up to 16mm were observed in 61/70 cases, results indicate that: (1) mean total leg lengths are not significantly different; (2) when there are left/right total leg length asymmetries, the taller leg does not consistently produce the more accurate stature estimate; and, (3) when a disparity between left and right legs ≥7.0mm is encountered, increasing the PI from 95% to 99% ensures the individual's living stature is captured. Implementing this practice among the current sample improved stature estimate accuracy (90% to 100%).

Overall, these findings reinforce the idea that although mean total leg lengths are not significantly different, bilateral asymmetry can be significant enough to affect stature estimates.

Biological Profile, Stature, Bilateral Asymmetry

A13 The Utility of Postcranial Non-Metric Traits in Ancestry Analysis

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After attending this presentation, attendees will be familiar with a novel approach to analyzing postcranial non-metric traits and how these variations may be used to estimate the ancestry of individuals in a forensic context.

This presentation will impact the forensic science community by addressing a notable gap in forensic anthropological literature regarding the usage of postcranial non-metric traits, which will assist in future approaches to ancestry estimation.

This research project sought to create a visual method for scoring a suite of postcranial non-metric traits, to define and illustrate the traits being used in the method, and to compare these postcranial non-metric traits between ancestral groups.

To begin, a categorical scoring method was created to analyze the usability and frequency of post-cranial non-metric variation between ancestral groups.[1] Based on a comprehensive survey of the literature, 11 different traits were used in this study. Due to discrepancies in the definitions and photographs of these traits throughout various studies, new definitions, trait states, and illustrations were created. Next, these 11 traits were observed and recorded on a sample of 210 specimens from the Robert J. Terry Anatomical Skeletal Collection, including American Black (n=105) and American White (n=105) individuals. Frequency distributions were calculated after data collection to depict the differences between the American Black and American White as different ancestral groups. After creating contingency tables for each trait, Chi-square tests were utilized to determine statistically significant differences between the two ancestral groups. Using these contingency tables, correspondence analysis was run using code modified from previous cranial non-metric trait analysis to create two-dimensional biplots visually demonstrating the relationships between American Blacks and American Whites.

Results indicate that, of the 11 traits analyzed, five had statistically significant differences between the two ancestral groups when running Pearson's Chi-square test with a significance level of $p < 0.05$. These traits include the spinous process bifurcation for both the third and fourth cervical vertebrae (C3: $x2$=60.9738, df=2, $P < 0.00001$; C4: $x2$=39.96, df=2, $P < 0.00001$), septal aperture ($x2$=13.6159, df=1, P=0.0035), third trochanter ($x2$=17.3744, df=1, P=0.000031), vastus notch ($x2$=4.3063, df=1, P=0.0379), and the anterior and middle calcaneal facets ($x2$=26.5157, df=3, $p < 0.00001$). These results were mirrored in both the frequency charts and, in turn, the correspondence analyses.

While the frequencies and Chi-square results of these traits are not enough to be used in isolation, this analysis of a non-metric postcranial trait list identifies the necessity for further research in these traits and their associations with ancestry estimation. This creation of the postcranial trait list will enhance the ability to visually ascertain ancestry. Further documentation of more populations paired with additional statistical analyses will allow forensic anthropologists to rely on scientifically tested data rather than relying on personal experience of visually estimating ancestry.

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Ancestry, Non-Metric Variation, Postcranial Skeleton

A14 Testing the Reliability of Ancestry-Specific Juvenile Age Estimation Methods Using the Diaphyseal Length of the Humerus

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After attending this presentation, attendees will have a better understanding of population variation in long bone length and how ancestry-specific age estimation methods based on humerus length do not necessarily improve the accuracy over other methods, particularly when the ontogenetic environment is not considered.

This presentation will impact the forensic science community by demonstrating that size is not ancestry specific, and that the reliability of juvenile age estimation methods based on long bone length is not necessarily conferred by matching the ancestry of the individual under investigation with the ancestry of the individuals in the sample used to develop the method.

Little work has been conducted on whether juvenile age estimation methods perform well beyond the population that was used as a reference. The accuracy of juvenile skeletal age-at-death estimation methods is known to be tied to the similarity between the method's reference sample and the target population. This similarity has been most often described in terms of "biological/genetic proximity" or ancestry and is the basis for advocating population-specific methods; however, similarity of growth environments has been less considered. The purpose of this presentation is to test two recently published juvenile skeletal age estimation methods (Stull et al., based on modern South African children; and Cardoso et al., based on 18th- to early 20th-century children from Portugal and England) on a diverse sample of known-age juvenile remains.[1,2]

Humerus diaphyseal length was collected from a sample of 81 known-age juvenile skeletons, aged between birth and 12 years. The data combines archaeological, anatomical, and forensic reference collections in the United States, Canada, and South Africa. Ages were estimated using Stull and colleagues and Cardoso and colleagues prediction models.[1,2] Mean residuals and mean absolute residuals were calculated to evaluate method accuracy and precision. To further evaluate reliability, 95% range inclusion frequencies were calculated as the percentage of individuals whose real age fell within the estimated 95% confidence interval.

Results do not lend support to the argument that ancestry-specific methods are more accurate, but seem more consistent with the ontogenetic environment hypothesis. Stull and co-worker's method is based largely on modern South African children, both "Black" and "Colored." It consistently performs best in the Forensic Data Bank sample, which is comprised largely of "Whites." Cardoso and co-workers' method is based on significantly stunted European children and consistently performs best in the Dart collection, which is comprised entirely of "Blacks." These findings have important implications for age estimation of juvenile skeletons in forensic contexts.

Reference(s):

1. Stull, Kyra E., Ericka N. L'Abbé, and Stephen D. Ousley. Using multivariate adaptive regression splines to estimate subadult age from diaphyseal dimensions. *American Journal of Physical Anthropology.* 154, no. 3 (2014): 376-386.
2. Cardoso, Hugo F.V., Joana Abrantes, and Louise T. Humphrey. Age estimation of immature human skeletal remains from the diaphyseal length of the long bones in the postnatal period. *International Journal of Legal Medicine.* 128, no. 5 (2014): 809-824.

Juvenile Skeletal Age, Inter-Population Variation, Ancestry

A15 A Revision of the Histological Age Estimation Formula From Stout for Sternal Rib Ends

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After attending this presentation, attendees will understand how forensic anthropologists use histological methods to estimate age at death and how the accuracy of these methods may be improved.

This presentation will impact the forensic science community by providing a revised regression equation to improve the accuracy of histological estimation of age at death from cross sections of sternal rib ends.

Given the lack of macroscopic features in highly fragmentary skeletal remains, histological methods may be employed to estimate age at death if complete skeletal elements are not available. These methods are based on the assumption that bone remodeling events accumulate throughout one's lifetime, meaning the bones of older individuals generally contain more osteons, the result of remodeling, than those of younger individuals.

Previous research by Stout and colleagues described a formula for histological age estimation based on Osteon Population Density (OPD) for cross sections of the sternal end of the fourth rib from an autopsy sample of 60 individuals (mean age=39.2 years, age range=11-88 years).[1] Though the original publication reported that age estimates produced by the histological prediction equation differed from known age at death by 8.8±0.98 years, reexamination of the original equation using individuals from the same collection revealed that age estimates differed from known age at death by 22.54±2.30 years.[1]

This current work seeks to revise the original formula, taking into consideration osteon area and osteon circularity in addition to OPD to create more accurate age estimates. OPD, defined by Stout and colleagues as the number of osteons per unit area, was calculated with the aid of a Merz counting reticle.[1] Osteon area is defined as the amount of bone in the boundary of an intact osteon in millimeters and osteon circularity is the quantification of how similar an osteon's shape is to a true circle, ranging from 0 to 1.[2,3] Osteon area and circularity were measured using ImageJ software to trace the cement line, or outside boundary, of each osteon. Data on OPD, osteon area, and osteon circularity were collected from the entire cross section of the rib. Given that information on known age at death for 14 individuals had been misplaced since the original publication, a subsample of the original autopsy sample was used to develop the revised equation, including 46 individuals (17 females, 29 males) of known age (mean age=43.3 years, age range=15-83 years). The formula was generated using multiple regression analysis in which the dependent variable was age and the independent variables were OPD, osteon area, and osteon circularity. OPD and osteon area were found to make statistically significant contributions to the age estimation formula ($p < 0.05$). Osteon circularity did not significantly contribute to the age estimates, and the final formula was developed using only OPD and osteon area as independent variables.

Age estimates produced by the revised equation differed from known age at death by 10.18±1.25 years, indicating that the revised formula was more accurate for estimating age than was the original formula. To test the accuracy of the revised equation, a "test set" approach was used in which a subset of individuals (*N*=16) was removed and the equation was calculated from those individuals who remained (*N*=30). Age at death was estimated for those individuals removed from the sample, and results revealed that age estimates produced by the "test set" equation differed from known age at death by 10.99±2.48 years, indicating accuracy comparable to the equation generated from all 46 individuals. Therefore, it is recommended that the revised formula be used for histological age estimation of rib cross sections in place of the previously described formula.

Reference(s):

1. Stout S.D., Dietze W.H., Işcan M.Y., Loth S.R. Estimation of age at death using cortical histomorphometry of the sternal end of the fourth rib. *J Forensic Sci.* 1994:39(3):778-784.
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Forensic Anthropology, Skeletal Histology, Age Estimation

A16 An Examination of Pelvic Scarring as a Determinant of Parturition Status

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The goals of this presentation are to: (1) develop a better understanding of the effects of parity and sex on pelvic bone scarring; and, (2) recognize the significant causal relationship between childbirth and dorsal pubic pitting.

This presentation will impact the forensic science community by providing a statistically rigorous study of pelvic scarring using a large modern skeletal sample and controlling for other relevant independent variables. This study helps to resolve the contradictory findings of prior studies, provides new guidelines for the determination of sex and parity from pelvic scars, and establishes a platform for future research on the processes that cause those scars.

Scars of parturition, or pelvic scars, have been examined frequently to explore their utility as indicators of childbirth and sex. Many studies found a significant association between the presence and/or severity of pelvic scars and parity, while others found no such relationship. The traits commonly considered to be indicators of parity are dorsal pubic pitting, the height of the pubic tubercle, the preauricular sulcus, and the interosseous groove. The goal of this current study is to examine these traits in a sample with known parity status, combining traditional qualitative scoring with quantitative measurements and using multivariate testing to separate the influence of parity from other independent variables. The null hypothesis is that parity does not independently affect the expression of pelvic scarring when sex, body size, and age at death are controlled.

Both male and female skeletons were assessed for the presence and degree of pelvic scarring. A sample of 530 identified, primarily Euroamerican individuals was drawn from the Texas State University Donated Skeletal Collection, the Maxwell Museum Documented Skeletal Collection, and the William M. Bass Donated Skeletal Collection. Sex, age at death, and ancestry was recorded for all specimens, and all females had known (self-reported) parity status. Coxa height was used as an indicator of overall body size. The presence and severity of dorsal pubic pitting, the preauricular sulcus, and the interosseous groove were scored traditionally using ordinal scales, and the width, depth, and length of pubic and preauricular pits were also measured with sliding calipers. The type of preauricular sulcus and interosseous groove present ("groove of pregnancy" or "groove of ligament") was recorded. The height of the pubic tubercle was measured using a contour gauge and sliding calipers, and retroauricular surface rugosity was scored ordinally to assess whether this area is also affected by childbirth.

Each of the examined pelvic traits was analyzed individually for its relationship to parity using IBM® Statistical Package for the Social Sciences (SPSS) Advanced Statistics 23.0. The independent effects of sex, age at death, and body size were assessed with binary logistic and ordinal regression for the non-metric traits and analysis of covariance for the metric traits. These powerful multivariate tests allow for a more reliable and precise interpretation of the effects of parity than has been attempted by previous works.

The results clearly demonstrate that only dorsal pubic pitting (presence, number, width, and volume) has a significant relationship with parity. Nulliparous females display a higher frequency of pitting than males, and the number and severity of pits increases in females with the number of births; however, parity's influence on pubic scarring is not as important as that of sex. Females with pitting are ~85% likely to have had at least one child, but females without pitting are only ~30% likely to be nulliparous. The preauricular sulcus and interosseous groove are strongly determined by sex and thus can serve as effective sex indicators, while variance in the height of the pubic tubercle can be attributed to body size and sexual dimorphism. The retroauricular surface increases in rugosity with age and body size and is more rugose in females, but is not influenced by parity.

These results challenge recent arguments for the lack of a causal relationship between dorsal pubic pitting and parity; however, because the effect of parity is secondary to that of sex, the practical applications for determining parity status from an unidentified decedent are limited. Furthermore, these results reinforce claims that the preauricular sulcus, interosseous groove, and pubic tubercle are caused or influenced by processes other than childbirth. All pelvic scars can be used effectively for sex determination.

Forensic Anthropology, Scars of Parturition, Dorsal Pubic Pitting

A17 Stature Estimation Using Measurements of the Cranium for Populations in the United States

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After attending this presentation, attendees will understand the accuracy and limitations of estimating stature for populations in the United States using only cranial measurements.

This presentation will impact the forensic science community by providing a method of estimating the stature of an unknown individual using cranial measurements if no other skeletal elements are present.

Stature estimation is part of the process of developing a biological profile for an unidentified individual. This is usually accomplished by measuring the lengths of the available long bones and calculating a stature range for the unidentified individual using published sex- and ancestry-specific regression equations. Stature can be estimated using measurements from other bones if long bone measurements are not available for an individual, but the estimated stature ranges generated from these equations are typically larger than those utilizing long bone measurements. Human crania are easily recognizable even to the untrained observer and are sometimes recovered in the absence of other skeletal material. The height of the cranium from points basion to bregma directly relates to stature, suggesting that this measurement and other cranial measurements could be used to estimate stature when no other skeletal elements are present.[1] Although stature estimation equations using various cranial measurements have been developed for several regional populations in various countries, no stature estimation equations utilizing standard cranial measurements exist for common populations in the United States. The purpose of this study is to develop and test stature estimation equations for four populations in the United States.

Seventeen standard cranial measurements were obtained from both a cranial Computed Tomography (CT) dataset and the Forensic Data Bank for Black, White, and Asian males and females and Hispanic males, for a total of 513 individuals.[2-4] Only individuals with either self-reported stature or stature measured at the time of the CT scan were included in this study, and all individuals were between the ages of 18 years and 60 years. Correlation coefficients and both simple and multiple linear regression equations were calculated for each of the 17 cranial measurements. Weak-to-moderate correlations with stature were observed for several cranial measurements in each population group, but none of the cranial measurements for any population demonstrated a strong correlation with stature.

The simple regression equations were tested using a separate dataset consisting of 94 individuals from the William M. Bass Donated Collection for which both cranial measurements and stature information (either self-reported or measured) are available. Stature ranges were developed from 95% confidence intervals calculated using the method by Giles and Klepinger.[5] Actual stature for these individuals fell within the calculated stature range for their respective regression equations 91%-97% of the time, but the calculated stature ranges for all individuals were very broad (±14-20cm). When individuals were tested using equations from groups other than their own, method accuracy dropped dramatically (41%-60%). The results of this study indicate that stature can be estimated using cranial measurements as long as the sex of the individual has been correctly assessed; however, this method produces broad stature ranges and should only be used if no other suitable skeletal elements are present.

Reference(s):

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2. Buikstra, Jane E. and Douglas H. Ubelaker. *Standards for Data Collection from Human Skeletal Remains* (Fayetteville, AR: Arkansas Archeological Survey, 1994).
3. Parks, Connie, Adam Richard, and Keith Monson. Preliminary Assessment of Facial Soft Tissue Thickness Utilizing Three-dimensional Computed Tomography Models of Living Individuals. *Forensic Science International.* 237 (2014): 146.e1-146.e10.
4. Jantz, Richard L. and Peer H. Moore-Jansen. *A Data Base for Forensic Anthropology: Structure Content and Analysis* (Knoxville, TN: Department of Anthropology, The University of Tennessee, 1988).
5. Giles, Eugene and Linda L. Klepinger. Confidence Intervals for Estimates Based on Linear Regression in Forensic Anthropology. *Journal of Forensic Sciences.* 33, no. 5 (1988): 1218-1222.

Stature Estimation, Cranial Measurements, Linear Regression

A18 Histological Variables at Multiple Locations and the Effect on Age Estimation

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After attending this presentation, attendees will: (1) appreciate the importance of sampling location in the human rib for accurate results utilizing histological age methods; and, (2) understand how differences in bone remodeling along the length of the rib may help or hinder successful application of rib aging methods.

This presentation will impact the forensic science community by demonstrating the need for in-depth analysis of the variance of histological structures. Increased understanding of the rib's bone biology will serve to improve sampling procedures and accuracy of histological aging methods.

Current histological methods for estimating adult age at death were developed exclusively from cross sections of the mid-shaft sixth rib; however, in forensic practice, it is not uncommon for histological samples to come from fragmented or previously segmented ribs, leading to uncertainty of sampling location.[1,2] The potential for error increases when the sampling location on the rib is uncertain and utilizing a section beyond the mid-shaft (either anterior or posterior) may result in erroneous age estimates.

Additionally, Frost recommended a minimum of 50mm^2 of cortical bone be read when assessing skeletal remodeling, which has resulted in the common practice of reading two to three serial sections in the ribs and averaging the results to account for local variation.[3] Problems have arisen when there is not enough bone to create or insufficient time to properly analyze multiple serial sections. This study sought to determine the importance of the mid-shaft distinction for age assessment by analyzing histological variables at multiple sampling locations along the length of the rib.

Histological sections were obtained from a single left or right sixth rib from five male postmortem human subjects (82-90 years, *Standard Deviation* (*SD)*=6.24 years). Three seriated sections were taken from each of three locations, posterior (25%), middle (50%), and anterior (75%), resulting in a total of nine sections from each individual (45 sections in total). All slides were imaged at 100x magnification and observed on the microscope using bright field and polarized light at 200x magnification. The following histological variables were quantified and analyzed: (1) Osteon Population Density (OPD): $[OPD_{(Intact)} + OPD_{(Fragmentary)}]$/Ct.Ar; and (2) Cortical Area (Ct.Ar.) per mm^2.

Ct.Ar was manually traced and measured from still images using a digitizing tablet in ImageJ software, while OPD was collected live from the microscope. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 24.

Serial sections were compared using a repeated measures Analysis Of Variance (ANOVA), which revealed no significant differences between Ct.Ar or OPD in any of the serial sections at the three locations. For the location comparisons, paired *t*-tests were used to examine OPD and Ct.Ar. at the anterior, middle, and posterior sites. Preliminary results indicate that OPD does not differ significantly between the anterior and middle locations (p=0.762) or between the anterior and posterior locations (p=0.131), although the middle and posterior locations are significantly different (p=0.05). Results for Ct.Ar revealed that there are significant differences between all three locations (anterior-middle: $p < 0.001$, anterior-posterior: $p < 0.001$, and middle-posterior: p=0.002).

Overall, results indicate that there is no significant difference in OPD and Ct.Ar between serial sections; however, significant differences exist between location sections for all variables analyzed. Due to histology's two-dimensional approach to bone, a three-dimensional structure, the absence of significant differences in the observed variables between serial sections is not surprising. Significant differences in Ct.Ar. at all locations reflect variation in the loading environment along the length of the rib. Though Ct.Ar. differences between the anterior and middle sites are significant, they are small by comparison to the differences between either of those locations and the posterior section. This may explain the observed trend of a lower OPD in the posterior sections than in the other locations.

These results suggest that it may be possible to obtain accurate age estimates using only one slide, rather than averaging multiple serial sections. Furthermore, these results indicate the importance of a mid-shaft location in histological age estimation; however, if the sampling location is uncertain, a more anterior section should be taken, as the histomorphology of the anterior rib is more consistent with that of mid-shaft. Future studies should explore Ct.Ar. and OPD in a more diverse sex and age sample to determine if the pattern observed in this study is consistent throughout the population.

Reference(s):

1. Stout S.D., Pain R.R. Brief communication: Histological age estimation using rib and clavicle. *American Journal of Physical Anthropology.* 87 (1992): 111–115.
2. Cho H., Stout S.D., Madsen R.W., Streeter M.A. Population-specific histological age-estimating method: A model for known African-American and European-American skeletal remains. *Journal Forensic Science.* 47 (2002): 12–18.
3. Frost H.M. Tetracyline-based histological analysis of bone remodeling. *Calcified Tissue Research.* 3 (1969): 211–237.

Age Estimation, Rib Histomorphometry, OPD

A19 Using Structure From Motion Photogrammetry to Quantify Volume Gain and Loss During the Human Decomposition Process

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After attending this presentation, attendees will better understand the basics of using photogrammetry to quantify volume gain and loss in human cadavers. Additionally, attendees will understand the typical decomposition process for human remains in central Texas.

This presentation will impact the forensic science community by presenting a new method that can be used to quantify volume gain and loss during human decomposition. Quantifying general trends (i.e., bloat) with respect to influential variables (i.e., body size and ambient temperature) will assist in producing more precise reconstructions of the progression of human decomposition, promoting accuracy and precision of postmortem interval estimates.

This research investigates the use of Structure from Motion (SfM), a type of digital photogrammetry that creates 3D models from photographs, for estimating the total body volume of individuals in the fresh, bloated, and post-bloated stages of decomposition. This project seeks to quantify parameters of volume gained during bloat and lost following collapse of the abdominal cavity to better understand how the progression of early decomposition events is affected by ambient temperature, as measured by Accumulated Degree Days (ADD), and body size.

This project utilized ten human cadavers procured through the Forensic Anthropology Center at Texas State's Willed Body Donation Program. Donor bodies were excluded from the study if the donor was being treated with chemotherapy prior to death, if the donation was not in the fresh stage of decomposition at the time of placement at the Forensic Anthropology Research Facility, if the donation was autopsied or presented with significant thoracic trauma, or if tissue samples were harvested from the donation for concurrent projects during the period of study. These criteria were selected so that the entire bloat process could be observed and to ensure that bloat proceeded "naturally" by minimizing potential antemortem disruption of the microbiome and preventing gas and fluid loss through autopsy or traumatic injury sites. After placement, donations were monitored daily. Flexible fiberglass tape measures placed under the body were used to monitor the circumference of the neck, chest, and abdomen. The presence of bloat indicators (e.g., protrusion of the tongue, inflation of the scrotum, and swelling of the head and neck) and purge occurrence and location were noted.

Three SfM models of the cadavers were created; one on the initial date of placement, one during the three days encompassing the period of maximum bloat as determined by the cadaveric measurements, and one after the collapse of the abdominal cavity. Photographs were taken using an Olympus® OM-D EM-5 Mark II 16-megapixel camera, on manual settings. Two different photograph collection methods were used to ensure proper camera calibration in Agisoft PhotoScan™. First, a "flat" project was completed by taking photos parallel to the ground surface and the body being photographed. Subsequently, a "dome" project was completed to ensure that the entire surface of the individual was collected completely. The photographs for the dome project were collected by moving around the individual, taking photos at four elevations. These elevations were approximately 6 feet, 5 feet, 3 feet, and .5 feet from the ground surface. Overlap of at least 66% between each photograph for the dome project was ensured by moving no more than 30 degrees between each photo station.

Photos were processed and optimized in Agisoft PhotoScan™ software, which was also used to measure the volume of each model. The model was scaled using ground control points shot in with a total station, which were placed near the head, hands, and feet of each individual. Percentage increase from the date of placement to the date of maximum bloat was calculated for all physical circumferences and model-generated volume for each donation to account for variation in initial body size. Tukey mean-difference plots were then constructed in R to assess agreement between methods, comparing the change in each circumference to the corresponding change in volume. ADD to maximum bloat and abdominal collapse, determined using physical measurements and volumes, were calculated using temperature data collected from an on-site weather station. Frequency distributions were constructed and descriptive statistics calculated for each group to describe the central tendency and dispersal of the data. ADD was also regressed against percentage change in physical measurements to determine how much variation in bloat is explained by temperature. Additional statistical methods may be employed as needed.

This project will contribute detailed quantitative data on variation in bloat, which is influenced by characteristics of both the individual and the immediate environment, and add to the body of knowledge about the decomposition of human remains. Postmortem interval estimation is a critical component of medicolegal death investigations, as it can aid in narrowing a list of missing persons and facilitating identification.

Photogrammetry, Decomposition, PMI

A20 The Use of the Mandibular Symphysis for Estimating Ancestry

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After attending this presentation, attendees will understand whether or not metric variation in the mandibular symphysis can be used to estimate ancestry in unidentified skeletal remains.

This presentation will impact the forensic science community by exploring a new metric method in the mandible for estimating ancestry from unidentified skeletal remains.

Maxillary prognathism is one of a suite of traits in the cranium that can be useful for differentiating between Sub-Saharan African and European ancestries in unidentified skeletal remains ("Black" and "White" individuals, respectively). While various metric and morphoscopic traits in the mandible also have been assessed for ancestry estimation, the projection of the mandibular symphysis that corresponds to maxillary prognathism has not been evaluated. Morphologically, this projection would include the area encompassing the anterior alveolar border, the incisive fossa, and the mental protuberance. This study assesses this projection for its potential in ancestry estimation using dimensions of the mandibular symphysis as a proxy.

Data were collected from 597 mandibles from the Terry Collection; 167 were measured twice to test for intraobserver error. Sliding calipers were used to take three measurements of the mandibular symphysis in the sagittal plane. Measurement endpoints included: infradentale prime, defined as the midpoint of a line tangential to the anterior-most projection of the alveolar processes of the central incisors; deep fossa, the posterior-most indention of the incisive fossa; and pogonion. Measurements consisted of: X, the distance from infradentale prime to deep fossa; Y, from infradentale prime to pogonion; and Z, from deep fossa to pogonion. Because endpoints are Type II landmarks prone to subjectivity, each measurement was taken three times per mandible, and the average was recorded.

A random sample of 75 individuals from each demographic group (Black and White, males and females) was selected for statistical analysis (N=300). Intraobserver error for each measurement was assessed on a random sample (n=63) using a paired t-test. None of the measurements exhibited significant differences (a=.05); therefore, all measurements were used in subsequent calculations and statistical analyses.

In addition to measurements, two indices and the incisive fossa angle were calculated to further explore symphyseal morphological variation. The indices included the "Symphyseal Index," derived by the formula $I_{symphysis}$=x/z, and the "Z index," derived by I_z=z/y. The incisive fossa angle was calculated using the inverse cosine function: A_{sulcus}=arccos $[(x^2+z^2-y^2)/2xz]$. Statistical analyses (Students' t-tests, linear regression) were used to assess variation in the measurements, indices, and incisive fossa angle between the sexes and ancestry groups. For all tests, significance was determined if p <05.

Results of the t-tests reveal significant differences between the sexes for two out of three measurements (Y: t=5.674, p=.000; Z: t=6.219, p=.000); therefore, subsequent statistical analyses were conducted with the sexes separated. For males, significant differences were found between Black and White individuals in four of six variables: X (t=-8.153, p=.000), Y (t=-4.626, p=.000), $I_{symphysis}$ (t=-6.793, p=.000), and I_z (t=7.665, p=.000). For females, all variables revealed significant ancestry differences: X (t=-6.843, p=.000), Y (t=-6.424, p=.000), Z (t=-2.389, p=.018), $I_{symphysis}$ (t=-4.958, p=.000), I_z (t=5.635, p=.000), and A_{sulcus} (t=2.416, p=.017).

Regression analyses show similar results. For males, the same four variables are significant (X, Y, $I_{symphysis}$, and I_z): adjusted R-squares are .305, .120, .233, and .279, respectively. For females, all variables are significant: adjusted R-squares are .235, .213, .031, .137, .171, .033 for X, Y, X, $I_{symphysis}$, I_z, and A_{sulcus}, respectively.

This study demonstrates that a relationship exists between the dimensions of the mandibular symphysis and both sex and ancestry. Sex differences are not unexpected. A larger mental protuberance impacts pogonion, thus affecting all variables except X, and possibly accounting for the stronger predictive values of these variables in males. For ancestry, the superior portion of the symphysis (X) shows the strongest relationship in both males and females, followed by I_z, which represents the proportion of the lower symphysis to the entire symphyseal length; however, despite the association to ancestry, results indicate that simple linear measurements do not adequately capture symphyseal variation to the extent needed for prediction. Future analyses that use geometric morphometrics to quantify symphyseal curvature may yield better predictive results that can be used in forensic contexts.

Ancestry Estimation, Mandible, Forensic Anthropology

A21 Scavenging Patterns in Hawaii: An Archaeological and Skeletal Case Study

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After attending this presentation, attendees will better understand the taphonomic processes associated with specific scavenging behavior.

This presentation will impact the forensic science community by providing vital insights into the complexities of scavenging patterns to assist in executing a forensic recovery and analysis of skeletal material.

The current state of taphonomic research in Hawaii, specifically related to mammalian fauna, is minimal, at best. The remains of an unidentified decedent were discovered on the surface of a forensic site, necessitating archaeological recovery methods on the island of Kauai. This investigation and recovery operation provided an opportunity to examine a specific case of scavenging activities and reconstruct the depositional history within the context of a natural setting.

An archaeological recovery was performed by conducting a pedestrian survey of the adjacent areas surrounding the majority of the skeletal remains. Azimuth mapping techniques were used to record the recovered skeletal remains and non-osseous evidence. Visible pig trails transecting the recovery site were also documented using Global Positioning System (GPS) track recording. All materials were collected in accordance with crime scene protocols. In a laboratory setting, peri-mortem and postmortem alterations to skeletal elements were observed and recorded by gross examination in conjunction with microscopic magnification of bone surfaces.

Skeletal elements from the lower spine, left lower limb, and sternum were found to be dispersed in a broad, patterned distribution, extending as far as 24m from the primary concentration of skeletal material. Skeletal analyses revealed evidence of pits, punctures, furrows, crushing, and scoring over many of the bone surfaces. Multiple areas of bone reduction were also observed, in conjunction with previously mentioned trauma. Peri-mortem and postmortem alterations were primarily concentrated at proximal and/or distal ends of long bones, in addition to peripheral margins of flat and/or irregular bones. The majority of the unrecovered skeletal elements consisted of ribs, small bones of the hands and feet, and some vertebral segments.

Feral, domesticated pig (*Sus scrofa*) trails transecting various areas of the recovery site correspond with the distribution pattern of recovered material, including both the main concentration and more broadly disbursed skeletal elements. Elements of unrecovered/missing skeletal material are consistent with previously recorded observations found in literature on mammalian scavenging patterns. Much of the peri-mortem and postmortem bone deformations analyzed were characteristic of tooth marks associated with both canid and suid (pig) scavenging. Pit, puncture, and scoring marks located on the superior region of the right scapula and anterior surfaces of the right calcaneus and cuboid are consistent with marks left by the canine and carnassial teeth of a canid. Crushing marks made by opposing teeth found on rib fragments, in addition to broad linear punctures and deep furrows found on the epiphyseal ends of the left femur, are consistent with scavenging patterns of suid. Other trauma, while consistent with tooth marks left by scavengers, could not be specifically assigned to an animal family. Recovery of a nearly complete piglet skeleton further suggests this area was important ground for local suid sounders.

Knowledge of the local fauna is often vital to the success of a forensic recovery. Knowing and understanding faunal scavenging patterns that potentially and often contribute to the formation of a site can aid the investigator in predicting and recognizing distribution patterns of material. In addition, this knowledge can be key in assisting the investigator with the forensic analysis of peri-mortem and postmortem changes to the skeletal elements.

Forensic Anthropology, Taphonomy, Hawaii

A22 Anatomy and Biological Anthropology: Time for a Family Reunion?

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The goal of this presentation is to acquaint attendees with the academic history of anatomy and biological anthropology in the United States and the nature of anatomy education among biological anthropology graduates. The results of a survey regarding anatomy education, training, and teaching experience among biological anthropology graduates will be discussed.

This presentation will impact the forensic science community by increasing awareness of biological anthropologists' educational training and competencies in the anatomical sciences (anatomy, histology, neuroanatomy, and osteology).

Biological anthropology and anatomy are separate degree programs today, but they share an integrated past. This academic history can be traced to Harvard, where George Dorsey graduated with the first PhD in anthropology in the United States, having received much of his training and mentorship from anatomists. In 1928, at the anthropology section meeting of the American Association for the Advancement of Science, Ales Hrdlička proposed the formation of a new professional society in physical anthropology, and the American Association of Physical Anthropologists (AAPA) was born. Two years later, the first meeting of the AAPA was held in Charlottesville, VA, in conjunction with the American Association of Anatomists annual meeting. More than 50% of founding members were anatomists and physicians, while only eight members were anthropologists. Biological anthropology programs gradually separated from anatomy departments to join the cultural, archaeological, and linguistic fields, following Boas' four-field approach. This movement of physical anthropology programs out of anatomy departments and into anthropology departments changed the foundational education of many physical anthropologists. Today, training varies depending on educational paths and research interests. Still, biological anthropologists increasingly fill faculty positions in non-anthropology departments, including anatomy faculties at medical schools and health sciences education programs, as well as applied positions in medical examiners' offices and government agencies. This study investigated anatomical sciences among biological anthropology master's and doctoral graduates and its implications for career paths.

A 22-question anonymous online survey was administered to biological anthropologists who obtained, or are in the process of obtaining, a graduate degree (Institutional Review Board (IRB) Application #17-001956, determined to be exempt from the requirement for IRB approval (45 CFR 46.101b, item 2) by the Mayo Clinic Internal Review Board). The survey was distributed to potential participants via several email listservs, including the Anthropology Section of the American Academy of Forensic Sciences. Questions surveyed anatomy education and training, graduate teaching experience in the anatomical sciences, the relevance of anatomy education to current and future career and research pursuits, and opinions regarding the need for anatomy training among biological anthropology graduates.

The 305 survey respondents were mostly anthropology graduates, and the majority had PhDs (88% anthropologists; 58% PhDs). The majority of the survey respondents obtained their degrees in the United States (65% of PhD and 75% of master's degree respondents). Europe, Canada, Central and South America, Asia, and Australia were also represented. Fifty-eight percent work/attend school in anthropology/social sciences departments, 16% in anatomy departments, and 5% in biology departments. Most were employed at research universities (65%); 24% reported teaching responsibilities in health sciences programs (medical, dental, and/or allied health professions programs). The majority (72%) of employed respondents consider anatomy knowledge essential to their current position. Sixty-seven percent report that it is relevant to their teaching load, and 72% consider it relevant to their research. Only 36% of PhDs reported that anatomy was/is a *required* course, but 72% of PhD graduates took an anatomy course (63% took gross anatomy with cadaveric dissection). Fewer graduates took histology, embryology, or neuroscience courses, as these are rarely required to obtain a graduate degree in anthropology and are not necessary for the career paths pursued by most graduates. Most respondents (76%) agreed that an anatomy course should be required in biological anthropology graduate programs; 49% agreed that anatomy should be offered as an elective, but not required. Nearly all (94%) respondents reported feeling that their educational training adequately prepared them to teach human osteology. Nearly half (49%) of the respondents reported feeling adequately prepared to teach gross anatomy with or without cadaveric dissection.

Responses indicate biological anthropologists still value, seek, and receive anatomy training, making these graduates uniquely suited to positions requiring an anatomical knowledge base and skillset; however, the nature and amount of anatomy training varies depending on educational paths and career interests.

Anatomy, Biological Anthropology, Survey

A23 The Forensic Application of Skeletal Stress Indicators: A Correlation Study of Linear Enamel Hypoplasia (LEH), Harris Lines, Cortical Bone Loss (CBL), and Stature

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After attending this presentation, attendees will understand how the analysis of multiple skeletal stress indicators can be simultaneously incorporated into forensic biological profiles.

This presentation will impact the forensic science community by expanding upon current studies of skeletal stress by addressing unresolved questions concerning the differences between manifestations of skeletal stress in recent, forensic populations versus in more traditional studies of prehistoric populations. Understanding the relationship between LEH and other postcranial stress pathologies in recent populations would help forensic anthropologists gain a more comprehensive foundation for incorporating skeletal stress into biological profiles. This research uses data obtained from the Hamann-Todd skeletal collection, which can be used as a proxy for recent, forensically relevant populations.

Skeletal stress indicators have provided important bioarchaeological insights into prehistoric populations, but are rarely used in forensic contexts. Incorporating LEH — a readily visible, non-specific indicator of stress — into current forensic biological profiles has proved a promising method for identifying and repatriating the remains of undocumented migrants in Southern Arizona; however, the relationship between LEH and other postcranial stress markers is unknown.[1] Harris lines, a postcranial indicator of stress associated with LEH in prehistoric remains, may be equally useful in this effort since both LEH and Harris lines can be linked to nutritional stress events during life; however, current studies focusing on correlations between Harris lines and LEH have been conducted on prehistoric populations rather than contemporary populations and yield mixed results.[2-7] Work on prehistoric samples also associates Harris lines with CBL and stunted stature within stressed populations.[4] In Neolithic contexts, these stress indicators have been associated with periods of agricultural transition, but analogous lifestyle changes are not seen in forensically relevant populations today.[8]

This study, therefore, considers the relationships between LEH, Harris lines, CBL, and stunted stature in the Hamann-Todd collection, a recent (1900s) skeletal population. One hundred twenty-five individuals (74 males and 51 females) ranging in age from 1-72 years were scored for LEH. Known statures provided by the Hamann-Todd collection were recorded, and tibiae from all individuals were radiographed and assessed for the presence of Harris lines. Cortical bone thickness was measured as a percentage of total bone width at the midshaft of each tibia using the same radiographs. All measurements were then recoded into binary to denote the presence ("1") and absence ("0") of skeletal stress. LEH and Harris lines were recoded based on the visual assessments. Stature and CBL were respectively recoded by plotting residuals for each age group and identifying outliers more than two standard deviations away from each group's mean. Multiple regression models and correlation analyses were run on the entire dataset as well as on each defined age group. Unlike previous studies on these indicators in prehistoric contexts, sex differences in trait correlations were also considered.

Results suggest that future studies should consider the interaction between these measures histologically at higher resolutions. Based on the intertrait correlations produced between Harris lines and cortical bone thinning (ranging across current age groups from -0.289 to -0.559), this study tentatively supports the hypothesis that Harris lines may be more indicative of periods of increased growth velocities rather than periods of malnourishment and arrested growth. Future forensic work on skeletal stress should incorporate histological measures of cortical bone. No significant differences in trait expression were found based on sex (p=0.260). Additional results from the same data condensed into new age groups (0-18 years, 19-34 years, 35-49 years, and 50+ years) are pending.

In conclusion, this study finds that incorporating non-specific stress indicators into forensic analyses requires the independent consideration of traits coupled with an in-depth analysis of life history events. While not practical for all forensic case work, this approach may aid the identification and repatriation of individuals with sparse antemortem data, such as undocumented migrants or long-unidentified individuals.

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Linear Enamel Hypoplasia, Skeletal Stress, Cortical Bone

A24 The Use of High-Resolution Micro-Computed Tomography (micro-CT) for Quantifying Vascular Pore Networks Across Whole Cross Sections of Human Cortical Bone

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After attending this presentation, attendees will appreciate how regional patterns of cortical bone loss may be visualized and quantified in 3D space using high-resolution micro-CT.

This presentation will impact the forensic science community by demonstrating that high-resolution micro-CT facilitates extraction of vascular pore networks from within complete, thick cross sections of human bone tissue. Tracking how vascular pore structures accumulate and expand across the human lifespan has future forensic applications, including: (1) assessing whether porosity-related bone fragility could have played a role in an accidental or non-accidental fracture; and, (2) distinguishing fragments of bone types based on pore structure, which appears to be sensitive to age, strain mode, fragility, and human/non-human origin.

Bone remodeling takes place throughout life but declines with age. Osteoclast cells tunnel into old or damaged bone and osteoblast cells fill this tunnel with new bone, leaving a central pore to conduct a blood vessel. Osteoblasts' capacity for bone formation slows with age. They cannot keep pace in filling the resorbed spaces, which accumulate as large pores. Lowered mechanical strains trigger further resorption to expand and connect existing pores. Vascular pores concentrate stress, providing a target for microscopic damage to initiate and propagate into a spontaneous fracture.

Research into pore structure has been limited in either axial or lateral dimensions. Two-dimensional histological sections of bone can describe how pore cross sections, but not their three-dimensional structures, vary between regions of bone tissue. High-resolution three-dimensional imaging often limits the specimen dimensions laterally (e.g., synchrotron X-ray imaging) or axially (e.g., confocal laser scanning microscopy). This research implements three-dimensional vascular pore visualization of a whole cross section of a human rib with high-resolution micro-CT.

The sample chosen for this pilot study was a fresh cadaveric fourth right-side rib from a 72-year-old female. In order to fit within a single field of view in the scanner at an approximate resolution of 5µm, a midshaft segment of the rib was cut to 1cm and further quartered along its medial, lateral, superior, and inferior long axes. These rib segments were scanned in air using a SkyScan 1172-D High Resolution Desktop Micro-CT with source voltage of 59kV, camera pixel size of 8.87µm, and voxel size of 4.88µm. In Avizo Fire 8.1, the four rib segments were geometrically transformed to their original positions, extracted and united using the Segmentation Editor, then exported as an image sequence of cross sections. In ImageJ, the Slice Geometry plugin of BoneJ was used to annotate each cross section with the major axis, which divides the cutaneous (skin-side, low-strain) and pleural (lung-side, high-strain) regions of the rib.[1,2] Cross sections were imported back into Avizo Fire 8.1, where the major axis guided the creation of masks for these regions to extract them independently. Vascular pore structures were extracted from each region in the Segmentation Editor, then exported as cross sections.

The Analyze Particles tool in ImageJ was used to calculate pore number and area for each cross section, mirroring analysis of two-dimensional cross sections. The pleural region (high strain) had fewer (mean=52+/-5.4, range=31 to 71) but larger pores (mean=0.0074+/-0.0029mm^2, range=0.0030 to 0.020mm^2). The cutaneous region (low strain) had more numerous (mean=85+/-6.7, range=66 to 103) but smaller pores (mean=0.0046+/-0.0022mm^2, range=0.002 to 0.011mm^2). Both regions had similar total pore areas (pleural mean=0.387+/-0.161mm^2, range=0.159 to 1.036mm^2; cutaneous mean=0.392+/-0.196mm^2, range=0.171 to 0.915mm^2). The Analyze Particles plugin of BoneJ was used to extract pore volumes.[1] Again, the pleural region had fewer (2,923) but larger volume (mean=0.00247+/-0.0272mm^3, range=2.79x10^{-6} to 0.956mm^3) pores compared to the cutaneous region's more numerous (5,841) but smaller volume pores (mean=0.00125+/-0.0222mm^3, range=2.79x10^{-6} to 1.343mm^3).

High-resolution micro-CT is an accessible and effective technology for three-dimensional imaging of vascular pore structures across whole, thick cross sectional regions. These preliminary results suggest using this technology for further investigation into trade-offs between pore volume and number that are sensitive to strain mode during life.

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Skeletal Histology, Three-Dimensional Imaging, High-Resolution MicroCT

A25 The Effect of Cranium Orientation on Positive Identification Using Frontal Sinus Radiographs

Alyssa R. Harrison, BS, Mercyhurst University, 501 E 38th Street, Erie, PA 16546*

After attending this presentation, attendees will understand the effect that variations in angle between antemortem and postmortem radiographs have on positive identification using the frontal sinus. This presentation will also outline the appropriate orientation for taking postmortem radiographs of the cranium for comparison to antemortem radiographs.

This presentation will impact the forensic science community by determining the margin of error for skull positioning to ensure positive identification using the frontal sinus, adding to the research previously conducted on courtroom admissibility. Furthermore, this presentation reinforces the standard orientation for postmortem radiographs of the skull as utilized by forensic radiologists and anthropologists.

Although many forensic disciplines have come to accept the qualitative analysis of frontal sinus outlines as an appropriate means of identification, these methods do not meet current evidence admissibility standards. Efforts have been made within recent years to update and improve frontal sinus methods to meet admissibility guidelines by empirically testing their uniqueness and developing more quantitative methods, but there are still many sources of potential error that have yet to be thoroughly examined. A study by Silva et al. began addressing the potential error that variations in angle between antemortem and postmortem radiographs have on the comparison of frontal sinus shapes; however, the quantitative methodology utilized by Christensen, using Elliptic Fourier Analysis (EFA), was not addressed in the Silva et al. study.[1-3] This study sought to evaluate how different angulations in cranial orientations during radiography may introduce variation in frontal sinus shape as interpreted from two-dimensional X-ray images and how these variations may affect positive identification results when Christensen's EFA methodology is applied.

This research utilized 16 crania from the Mercyhurst University Donated Skeletal Collection. The crania were radiographed 18 times each at five-degree increments in both the vertical and horizontal planes, resulting in a total sample of 288 radiographs. The frontal sinus outlines from these radiographs were then traced in Photoshop® and analyzed using EFA and principal components analysis. Euclidean distances were calculated from principal component scores within individuals and between individuals to test whether an individual's frontal sinus could be matched regardless of orientation during radiography. A logistic regression model was created to determine the probability of an individual's frontal sinus outlines matching.

Observability of frontal sinuses was affected by as little as five degrees in some individuals. The mean of the Euclidean distances from within individuals was smaller than the mean from between individuals with a statistically significant difference between the means ($p < 0.001$). The logistic regression model indicated that the highest posterior probability was only 80%, even when two of the sinus outlines matched exactly in shape space (Euclidean distance of 0). These results suggest the logistic regression model has a real, but limited, discrimination between matches and non-matches. Posterior probabilities greater than 0.5 were considered as matching; only 66% of the sample was correctly classified as matching or non-matching using the logistic regression equation.

Overall, these results suggest that orientation during radiography can have a significant impact on the accuracy and reliability of frontal sinus comparisons for positive identification. As such, it is important for both clinicians and forensic radiologists and anthropologists to pay close attention to the positioning of the skull during radiographs to ensure minimal variation in orientation. This orientation requires that postmortem radiographs be taken with the orbitomeatal line (from the upper margin of the external auditory meatus to nasion) perpendicular to the X-ray film in order to most accurately match the orientation utilized by clinicians during antemortem radiographs.

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Positive Identification, Frontal Sinus, Admissibility

A26 A Study on the Asymmetry Between the Left and Right Human Pubic Symphysis for Age-at-Death Estimation Based on 3D Laser Scans and Computational Methods

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The goal of this presentation is to investigate how asymmetry observed between the left and right pubic symphyseal surfaces of one individual affect the accuracy of age-at-death estimates produced by the use of novel techniques involving 3D laser scans and computational algorithms.

This presentation will impact the forensic science community by demonstrating that an accurate and reliable age-at-death estimation can be achieved with either the left or right human pubic symphysis, independent of individual factors that are potentially unknown to the forensic investigator, such as the weight or stature of the person in question. Attendees will be presented with a number of the many advantages that modern laser scanning technology has to offer to the field of forensic anthropology. Particularly, attendees will be familiarized with new computational techniques used for age-at-death estimation from the adult skeleton and show how, with these methods, they can produce accurate and reliable age estimates even in cases when one pubic symphyseal surface is missing or damaged.

There is a well-established tradition of using the human pubic symphysis for age-at-death estimation.[1] Therefore, anthropologists have devoted considerable effort to refining and improving the methodology.[2-4] In recent years, there has been a growing interest to better understand, capture, and quantify the link between the morphology of the symphyseal surface and age.[5,6] The currently accepted practice for age-at-death estimation using the pubic symphysis is based on the visual inspection of the skeletal element and its comparison to images, casts, and/or written descriptions that are associated with pre-defined age intervals. Such methodologies are relatively easy to apply but are prone to high interobserver and intraobserver error as they are highly dependent on the scientists' experience and expectations.[7] With the advancement of current data acquisition and analytic technologies, researchers have begun to look for new ways to study age indicator morphology and infer chronological age. Most recently, new methods for age-at-death estimation of the pubic symphysis have been developed that use 3D laser scan data and computational methods that seek to eliminate the element of subjectivity in estimation.[8-10] The methods include two surface analysis algorithms, one ventral outline measure, and two multivariate-regression models that combine each surface measure with the outline score. These models were calibrated on 3D scans of left or right pubic symphysis selected at random. These new computational methods have been shown to be at least as accurate and reliable as the traditional techniques and have the potential to allow for better understanding of the morphology of the symphyseal surface.

This study further investigates the utility of these methods by focusing on the issue of asymmetry. Data was generated from 88 White males with known ages at death, for whom both the left and right pubic symphysis were scanned; the three measures and five age estimates were produced using the aforementioned computational algorithms and the accuracy of the age estimate produced by each side was analyzed. The effect of the individuals' height, weight, and calculated body mass index must be taken into consideration. The results demonstrated that both sides are equally reliable in estimating the age at death of the individual. Neither side proved to be consistently less accurate. Further, regression models were built using only left or only right scans to demonstrate that neither side models the age-progressive changes better than the other. Overall, the difference in age estimates between the left and right estimates are less than five years for more than half of the data and nearly all estimates are less than 15 years apart. For individuals with notably asymmetric sides, the difference in estimates was not associated with advanced age, weight, height, or body mass index. The findings indicate that in situations in which one side of the pubic symphysis is damaged or missing, the side that is available can be used with the same level of confidence. Further, the fact that the computational algorithms produce consistent results regardless of the weight and stature of the individual means that scientists can apply these techniques to casework situations when no soft-tissue or personal life history information is available.

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Pubic Symphysis Asymmetry, Age-At-Death Estimation, Computational Methods

A27 Preauricular Sulcus (PAS) and Parity Status: A Possible Correlation? A Test on a Documented British Collection

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After attending this presentation, attendees will better understand the correlation between the PAS and parity status in humans.

This presentation will impact the forensic science community by providing further tests on the use of PAS morphology to examine parity status.

At previous AAFS Annual Scientific Meetings, a new grading system was presented to assess the morphology of the preauricular sulcus and examine its link to both parity status and sex. The grading system has five grades ranging from 0 (no PAS present) to a grade 4 (a deep, pitted sulcus). The grading system focuses on the changes in depth rather than the size or shape of the PAS. The system was developed using two British medieval populations. Although these populations were undocumented, they had previously been aged and sexed by both researchers and other forensic anthropologists using established methods and techniques.

This study presents a new test of the grading system on a documented modern British collection, the Spitalfields Collection at the Natural History Museum (London). A sample of 78 female individuals were selected; the parity statuses for all the individuals has been reconstructed using archival records and coffin plates.

The results confirmed the previous tests on the historical populations: the majority of the individuals (all female) present a PAS, 93%, supporting the use of the PAS as a sexual indicator. In addition, grade 1 is present in 16% of the sample, grade 2 in 29%, grade 3 in 33% and grade 4 in 15%.

The parity status of the individuals was also analyzed in correlation with the grading system; however, in this sample, no correlation between grade and parity status was found, despite previous analysis on another modern British collection of 35 individuals from St. Bride's Church, (curated through the Museum of London) where a correlation was observed.

The two populations are from a similar temporal and geographical period, and the differences between the results provide an interesting aspect to the discussion of the effect of parity status on the PAS.

Although the two collections present differences in the parity status/PAS correlation, they agreed on the sexual dimorphism of the sulcus, present in 98% of the females. For the St. Bride's collection, male individuals have also been examined (n=183), and it was demonstrated that only 51% had a PAS and no grade 3 or 4 sulci were found.

Although these two collections exhibit a discrepancy over the effects of parity status on the PAS, the study confirms that this anatomical trait is sexually dimorphic and can be used as an indicator of sex for biological profiling. Furthermore, this study demonstrated the reliability of the new PAS grading system as a tool for biological profiling in both forensic and archaeological cases.

Biological Anthropology, Parturition, Parity Status

A28 Forensic Fractography of Bone: A New Approach to Skeletal Trauma Analysis

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After attending this presentation, attendees will be familiar with how fractography can be used to assess skeletal fractures in forensic anthropological trauma analysis, increasing the accuracy of trauma interpretations.

This presentation will impact the forensic science community by enhancing the analysis of skeletal trauma using a new method of fracture examination that is reliable, easy, and inexpensive.

Fractography is the study of fracture surface characteristics and their relationship to crack propagation. This science is routinely used in forensic examinations of materials such as glass, ceramics, metals, and plastics to identify the initiation point and nature of the material's failure. Fracture surfaces of brittle materials typically display features reflecting the speed and stability of the propagating crack front. Generally, crack initiation sites are relatively featureless, becoming more featured with increasing crack speed and instability. These changes can be seen as arrangements of ridges and lines with specific orientations with respect to the moving crack front.

The fracture surfaces of bone also reveal information about fracture initiation and propagation, yet the science of fractography has rarely been applied to bone in forensic anthropological contexts. It is hypothesized that the science of fractography can be applied to forensic skeletal analysis to provide additional information about the bone's failure and the trauma event. Here, the application of fractographic principles to the analysis of fractured femoral cortical bone is tested to determine the utility of fractography in the forensic analysis of skeletal trauma. The bone samples consisted of 12 biomechanically fresh human femora that underwent controlled three-point bending as part of a previous study.

Several methods for enhancing visualization of the fracture surface were assessed, including magnification, oblique lighting, various contrast media (including powders, inks, and sputtered gold films), casting/molding, and Computed Tomography (CT) scanning. Such coatings and treatments are often used in other forensic fractography analyses to decrease reflection and increase contrast; in particular, bone's light color can result in reflections and low contrast that interfere with visualizing surface details. Dual-contrast fingerprint powder applied to the bone fracture surface combined with oblique lighting and low-magnification microscopy (1-4x) was determined to result in optimal visualization of the features. Moreover, this approach is relatively easy, inexpensive, and reversible.

The fractured femur specimens were then examined by seven assessors (three forensic anthropologists with no previous experience with fractography and four forensic fractographers with no previous experience examining bone). For each specimen, assessors documented the presence or absence of fractographic features, including bone mirror, arrest ridges, bone hackle, wake features, and cantilever curl. In addition, the assessors recorded their conclusions regarding the direction of crack propagation based on the presence, location, and orientation of these features.

The results strongly indicate that fractographic features observed in bone can be used to determine the location of fracture initiation and the direction of crack propagation. Interobserver error in identifying the presence of features was insignificant (p=0.244), and there was 100% agreement between all assessors for all specimens regarding crack propagation direction. Multiple correspondence analysis also revealed good agreement between the fractographic features and the fracture initiation site. Experience may play a role in identifying fractographic features, and greater precision is found in the identification of more features when the cortical area is greater.

Fractographic analysis of bone can be used to reliably determine the point of fracture initiation and the direction of fracture propagation by assessing the bone for the presence, location, and orientation of surface features. These features can generally be observed with the unaided eye, although they are further enhanced using oblique lighting, contrast medium application, and low-power microscopy. This approach is reliable and can be easily and inexpensively applied in forensic anthropological examinations. Fractographic analysis should be used in conjunction with the examination of other fracture characteristics to provide a more thorough and accurate analysis of the bone's failure and the trauma event.

Forensic Anthropology, Fractography, Trauma Analysis

A29 A Retrospective Study of Intentional Body Dismemberment in New York City: 1996-2016

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After attending this presentation, attendees will better understand various aspects of dismemberment cases in an urban setting.

This presentation will impact the forensic science community by providing a baseline for the comparison of dismemberment data originating from a large metropolitan area.

A detailed review of autopsy records, case photographs, and (when present) anthropology reports was conducted at the New York City Office of Chief Medical Examiner to locate cases of intentional body dismemberment. The initial list of cases was compiled based on database searches including terms "dismember(ed)," "dismemberment," "disarticulate(d)," "disarticulation," "amputate(d)," "amputation," "decapitate(d)," and "decapitation." More than 700 cases were reviewed based on these search parameters. Only cases involving intentional body dismemberment by another individual were included in the final dataset. In addition, there had to be complete separation of a major body part for the case to be included in the study (i.e., separation of bones without complete separation of the soft tissue was not counted). Additionally, the removal of ears, noses, and genitalia did not meet the criteria for inclusion in this study since these dismemberment locations involve only soft tissue/cartilage and would not be observable on remains in an advanced state of decomposition. After the vetting process, 75 different case numbers were found that pertained to intentionally dismembered bodies. Through DNA or anthropological re-association, 21 of these cases could be linked to other cases. After the consolidation of cases was completed, it was determined that a total of 54 dismembered bodies were discovered within New York City (NYC) between 1996 and 2016.

Data were collected on type of dismemberment: disarticulation, transection, or a combination of both. Disarticulation indicates that dismemberment was achieved by cutting between bones at joints. This is usually accomplished with a knife. Transection indicates that cuts were made through a bone. Transection usually occurred by sawing or chopping. In some cases, multiple implements were used and disarticulation and transection were observed on the same body. The results reveal that 54% of body dismemberment involved transection only (29/54), 35% involved disarticulation only (19/54), and 11% involved a combination of both (6/54).

Data were collected on the number of body parts represented after dismemberment. In 48% of the cases (26/54), the entirety of the body was never recovered. Due to missing elements, it was not possible to determine the total number of cuts or the full extent of the dismemberment locations in many of the cases. The total number of parts ranged from a low of 2 (e.g., decapitation only) to an extreme example in which the entire body was defleshed and nearly all of the individual bones were disarticulated. Frequent locations for dismemberment were the neck, shoulders, and hips.

Annual trends indicate that there was an average of 4 cases per year, with a high of 15 cases in 2005 and a low of 0 cases in 2016. These values indicate the year that the dismembered body parts were recovered. In most instances, the body parts were discovered soon after death, but, in several cases, the parts were not discovered until years after death. The frequency of body part discoveries by NYC borough reveals that Brooklyn has the highest percentage at 33% (25/75), followed by the Bronx at 27% (20/75), Queens at 23% (17/75), Manhattan at 13% (10/75), Staten Island at 3% (2/75), and 1 body part from outside NYC that linked to a NYC case (1/75). In some instances, these values represent the discovery of body parts from the same person that were spread between different boroughs.

Possible reasons for dismemberment include making the body more manageable for transport/disposal and attempting to hinder identification efforts. In a few NYC cases, the dismemberment was associated with body packing and intentional cutting of the decedent was conducted to retrieve drugs hidden inside their body. In other cases, the dismemberment may simply be an aggressive act to disfigure and mutilate the victim. It is theorized that NYC may experience a higher number of dismemberment cases than other large cities due to the density of the population and added difficulty of body transport and disposal, but currently there are no other United States studies available for comparison. Retrospective studies of dismemberment trends in other large metropolitan cities would provide an interesting comparison to the findings within NYC.

Dismemberment, Sharp Force Trauma, New York City

A30 Determining Common Skeletal Injury Locations Based on Manner of Death (MOD)

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The goal of this presentation is to inform attendees of common injury patterns associated with specific modes and causes of death.

This presentation will impact the forensic science community by discussing how these findings will assist attendees in better interpreting patterns of injury across the body and to help substantiate estimation of MOD in forensic casework.

Data was collected from the Washoe County Medical Regional Medical Examiner's Office (WCRMEO) to document specific injury locations based on the MOD and trauma class and explore which anatomical locations presented with the highest frequencies. This presentation will impact the forensic community by providing corroboration for trauma interpretations based on injury location, by increasing our understanding of the circumstances surrounding the death event, as well as informing scientists of influential future research directions.

The justification for the majority of trauma research is that it is assumed to be a common injury location and, therefore, will impact forensic anthropologists and other similar practitioners. For example, numerous studies explore cranial bone reactions to blunt force trauma, utilizing tools that resemble hammers, baseball bats, and other common weapons used in cases of homicide. The goal of the current study is to quantify the frequency of trauma on the skeleton based on MOD and trauma classes (i.e., blunt force, sharp force, or ballistic) as well as determine whether significant differences were observed.

Deceased individuals recorded in the WCRMEO VertiQ case-reporting database were included in the study if: (1) they have a documented MOD consistent with accident, homicide, or suicide; (2) they have a documented Cause Of Death (COD) consistent with some type of traumatic skeletal injury and not a drug, soft tissue disease state, or disease-related complication; and, (3) were more than 18 years of age. Only cases from January 2016 to July 2017 that had been designated "closed" at the time of data collection were included, which resulted in a total sample size of 300 individuals. Variables collected from the WCRMEO autopsy reports for each individual included age, sex, weight, height, body mass index, ancestry, MOD, COD, and specific skeletal trauma location. All fracture locations were recorded as present or absent for each bone and the general location on each bone (i.e., left/right, proximal/midshaft/distal). Frequency distributions of fracture location based on MOD were created as well as frequency distributions of fracture location based on trauma class, as interpreted from COD. Chi-square tests were conducted to explore the relationship between fracture location and MOD.

Interpretation of the frequency distributions of MODs suggested that cranial injuries were most common with suicides and accidental deaths. On average, greater than 50% of suicides resulted in fractures to the cranium. Ribs, the appendicular skeleton, and the innominates were most often affected by an accidental death. An MOD of homicide resulted in similar amounts of trauma (26%) appearing on both the cranium and thoracic area; however, unlike the aforementioned studies within the field, no MOD homicide reported a COD of blunt trauma. When examining frequencies of COD, sharp force trauma most frequently affected the ribs (62%) and ballistic trauma most frequently affected the cranium (70%). Blunt force trauma had a slightly higher occurrence on the ribs overall, where 55% of blunt force trauma cases resulted in lateral rib fractures; however, a substantial portion (28%) of blunt force trauma also appeared on the cranium. Chi-squared tests revealed that some of these location frequencies had a statistically significant relationship with MOD ($p < 0.05$).

The results from this research demonstrate that MOD has a significant correlation with the location of trauma. Recognizing common injury location does not directly influence the trauma interpretation, but rather provides information regarding the death event and corroborates any findings from the trauma analysis. These findings will assist forensic scientists to better interpret patterns of injury across the body and help substantiate estimation of MOD in forensic casework. Additionally, these findings will direct future forensic anthropological research by providing data on the anatomical areas presenting with the highest frequency of trauma per MOD and COD.

Trauma Analysis, Frequency Tables, Cause of Death

A31 Butterfly Fractures in Medicolegal Investigations

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After attending this presentation, attendees will have acquired knowledge regarding bending bone fracture patterns in adult long bones. Broken bones have been studied for more than 100 years, yet little research has applied and validated fracture patterns in medicolegal investigations.

This presentation will impact the forensic science community by contributing to the knowledge of fracture biomechanics and the utility of evaluating the direction of bending bone failure in a medicolegal environment as well as aiding attendees in the interpretation of long bone fractures. Blunt trauma is addressed as a whole, with an emphasis on: (1) the application of bone trauma interpretation in practice; (2) the significance of the identification of a blunt butterfly fracture pattern; and, (3) the recognition of biomechanical modes of failure. A Pedestrian Vehicle Accident (PVA), a common medicolegal occurrence in which the expertise of a forensic pathologist and anthropologist is needed, will be used as an exemplar for the discussion.

More than 30 years ago, anthropologists began to work hand-in-hand with forensic pathologists, which marked the beginning of a new relationship in forensic science that emphasized bone trauma diagnoses based in biomechanics. Autopsy became the ideal environment to apply and validate bone trauma research.[1] The identification of the modes of bone failure and the features of tension and compression of tubular bone fractures were shown to be accurate in determining bending direction of reconstructed bone and became standard practice in forensic anthropology.[2-4]

Recently, experimental research in three-point bending has exhibited considerable variation in butterfly fracture patterns and has urged caution in using these fractures in establishing bending direction:

"If practitioners consider only the presence and orientation of complete butterfly fractures, they may be unable to accurately interpret impact direction and all trauma cases."[5]

This introduces confusion in bone trauma analysis, whether blunt or ballistic, as all are based on biomechanical principles of bone failure. The recognition of these features in bone is usually based on visual patterns, such as blunt butterfly fractures, with the intent of immediately establishing bending direction. So where does the confusion lie in explaining and interpreting blunt butterfly fractures? And, how does this affect our current application and interpretation of long bone fractures in medicolegal investigations?

Obviously, interpretation of processed bone has limitations, as demonstrated by an exemplar of a broken leg from a PVA. In this case, it will be demonstrated how soft tissue and context makes the determination of bending and Point Of Impact (POI) possible. POI requires prior knowledge, despite claims in the historic literature. With prior knowledge and context, an analyst can reconstruct a bone fracture for pattern analysis and can further separate these bones for macroscopic examination of tensile and compressive failure. The above-mentioned experimental research on three-point impacts also concluded that surfaces of broken bone accurately indicated areas of tension and compression.[5]

This presentation contextualizes bending bone fractures within the framework of current knowledge and discusses the application of this knowledge to a medicolegal environment. This study contends that the variable morphology observed in a butterfly pattern is attributed to the continuous adjustments and failures of bone bending. With enough energy, tension failure forms in a bone shaft, with shear and compressive forces guiding or diverting fractures. Bone's ability to resist compression energy directs initial fractures longitudinally along the shaft. Eventually, complete fractures are formed as fractures approach the side of compression.

Therefore, the blunt butterfly fracture pattern indicates initial failure, which eventually progresses into or toward maximum compression failure. The overall morphology of the fracture pattern reveals general direction of bending, while discerning the actual contour of the bone in cross section assists in establishing bending direction of that bone at that location.[6]

An anthropologist must remain forensically conservative in his/her analytical approach to trauma analysis, particularly when prior knowledge or context is not available. A blunt butterfly fracture pattern can assist in establishing bending direction of the bone; however, an analyst must be cognizant of variation in fracture morphology and must not purely rely on these features for interpretation of an injury. Anthropologists need to be cognizant of the intrinsic features of bone, the biomechanical principles of bone failure, and the application of this body of knowledge to a medicolegal investigation.

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Blunt Butterfly Fractures, Biomechanics, Fracture Direction

A32 Early Signs of Direct Fracture Repair and Indirect Intramembranous Fracture Repair Without Indications of Endochondral Ossification in the Ribcage

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After attending this presentation, attendees will better understand the early stages of osseous healing and their macroscopic manifestation on dry skeletal material.

This presentation will impact the forensic science community by highlighting the need to carefully examine all fractures, including minute incomplete fractures associated with major fractures, for slight irregularities indicative of short-term survival after a traumatic event.

Partially skeletonized human remains were discovered beneath a structure that had collapsed several months prior. Associate Medical Examiner Dr. Kyle Shaw of Florida's District 5 Medical Examiner's Office evaluated the scene and remains; he referred the latter to the University of Florida C.A. Pound Human Identification Laboratory (CAPHIL) for additional analysis. Assessments at the CAPHIL indicated that the remains were those of a middle- to older-aged adult female of primarily European ancestry. Analyses also revealed the presence of several complete and incomplete fractures of the anterior rib cage. All fractures to right ribs 2-7 and left ribs 2-5 were consistent with an anterior loading (compression) event. These fractures were examined under 7-80x magnification using a stereoscope. Although most complete fractures revealed no indications of healing and were consistent with peri-mortem trauma, several incomplete fractures, including those directly associated with a complete fracture, revealed indications of very early osseous repair. Small spicules of new bone were present adjacent to larger incomplete fractures and bridge smaller incomplete fractures. In both cases, bony spicules were oriented perpendicularly to the long axis of fracture. The large majority of new bone present cannot be visualized on plain film radiography.

While there is extensive literature on the cellular and molecular changes underlying early fracture repair, there is a dearth of literature addressing the gross, anatomical appearance of early fracture healing in human skeletal material. Immobilized, highly mechanically stable fractures, including stress fractures and incomplete fractures, may demonstrate a high degree of direct healing and indirect intramembranous ossification.[1] For both direct repair and indirect intramembranous repair, bone formation at fracture sites begins prior to (and on rare occasions, in lieu of) endochondral ossification of a soft callus.[2] In direct healing, the cells in the local cortical bone build new lamellar bone and Haversian systems without intermediary woven bone.[3] This type of repair occurs only at fracture gaps of less than ~1mm with low interfragmentary strain (i.e., fractures that do not require calluses for stabilization).[3] In indirect healing, the intramembranous hard callus is deposited without a precursor onto the existing bone surface from the subperiosteal layer and forms adjacent to fracture margins before progressing toward the fracture line.[4] In non-human animal studies, intramembranous bone deposition usually begins by day three post-fracture.[5] In contrast, woven bone deposition in endochondral ossification may not begin until ~14 days post-fracture.[6]

Despite the mix of fractures with and without indications of healing in the rib cage of the forensic case examined by the CAPHIL (a mix observed even along the margins of a single fracture area), it is likely most of the fractures occurred as a result of a single, compressive event. Death occurred after the body initiated direct and intramembranous fracture healing at the incomplete (mechanically stable) fracture locations, but prior to any endochondral ossification at the complete (mechanically unstable) fracture sites. Although individual variation and a lack of studies on timing of these fracture repair stages in humans render an exact timetable difficult to establish, these findings suggest the individual died very shortly, but not immediately, after injury. In this particular case, these findings may have implications for cause of death. This case study illustrates the importance of careful examination of *all* fracture surfaces via light microscopy. Further, this case study highlights the need for future studies/autopsy series designed to elucidate macroscopic changes associated with fracture repair and the timing of ossification in human adults. Such findings would not only aid forensic scientists reconstructing the last days of someone's life, but would augment clinical understandings of human skeletal repair as well. In addition, because bone is one of the few truly regenerative tissues, better understanding of the initiation of bone regeneration in humans could have an important impact on tissue engineering.

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Fracture Healing, Trauma, Tissue Regeneration

A33 Initiation and Propagation of Fractures in Blunt Impacts to Unconstrained Human Cadaver Heads

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After attending this presentation, attendees will be informed about cranial fracture initiation and propagation in blunt impacts to upright, unconstrained cadaver heads.

This presentation impacts the forensic science community by adding to their current understanding of the relationship between location of fracture production, propagation of fracture, and implement type in cases involving blunt cranial trauma.

Previously, this research group presented the results of blunt cranial impacts performed on entrapped human cadaver heads at the American Academy of Forensic Sciences meetings.[1,2] High-speed footage of these experiments support Gurdjian's predictions that: (1) cranial fracture can initiate either at or peripheral to the impact site; and, (2) variables, including implement shape and impact energy, influence the location of fracture initiation and propagation.[3] Fenton et al. and Isa et al. report that fractures tend to initiate peripherally in impacts with larger, broader implements and at the point of impact with smaller, more focused implements.[1,2] As these experiments were performed on heads fully constrained within a rigid medium (plaster of Paris), it remains unclear how fractures would initiate and propagate in impacts to more realistically constrained heads.

The current study investigated fracture initiation and propagation in blunt cranial impact experiments designed to simulate a blow to the head of an upright individual. Nineteen unembalmed male cadaver heads were impacted using a new, custom-built pneumatic impact system. Three aluminum impactors were selected for this study to approximate the shapes of objects commonly implicated in forensic cases: a brick (3" diameter flat), a bat (2.5" diameter cylinder), and a hammer (1" diameter flat). Twelve impact experiments (n=4 for each implement) were performed at a base energy level: 91.8J±18.7J for brick impacts, 112.1J±3.7J for bat impacts, and 105.3J±19.5J for hammer impacts. Seven impact experiments (n=2 brick; n=2 bat; n=3 hammer) were performed at approximately 1.6-1.8 times the base energy level (153.6J±50.0J for brick impacts, 137.1J±39.3J for bat impacts, and 172.0J±10.6J for hammer impacts).

Impacts were delivered at the mid-parietal, inferior to the parietal boss, on heads placed in an upright position. Prior to impact, specimens were secured at the C4 vertebra to a mounting plate using an adjustable clamping mechanism. Heads were positioned for impact via breakaway tethers attached to a collar fastened around the neck. A high-speed camera captured fracture initiation and propagation at 10,000fps.

A key result of the base energy-level experiments was the observation of peripheral fracture initiation in impacts with all three implements. These results indicate peripheral initiation is not just possible, but likely following a blunt impact to an unconstrained head. A second key finding was that for all three implements, at least one of four experiments generated fractures that initiated peripherally and did not propagate back to the impact site. As a result, 4/12 experiments (2/4 brick, 1/4 bat, and 1/4 hammer impacts) produced fractures concentrated somewhere other than the impact site (primarily in the temporal and sphenoid).

Initial results also indicate that both implement shape and impact energy influence the location of fracture initiation and propagation in unconstrained heads. At the base energy level, experiments with brick and bat implements tended to generate peripherally initiating linear fractures that propagated back toward and/or away from the impact site. At a higher energy, brick and bat implements produced linear fractures that initiated at the point of impact and propagated away. In contrast, high-energy hammer impacts produced peripherally initiating linear fractures that propagated back toward the impact site.

The present study sought to investigate the issue of fracture initiation and propagation in blunt impacts to unconstrained adult heads. High-speed photography revealed fractures initiating peripherally with all three implements impacted at the base energy level. In some cases, peripherally initiating fractures also traveled away from the point of impact, resulting in fractures located distant from the point of impact. Practitioners, therefore, should be advised that the location of linear fractures does not necessarily correspond with the location of impact.

This project was supported by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.

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Blunt Force Trauma, Cranial Fracture, Trauma Analysis

A34 The Influence of Implement Shape on Fracture Pattern and Defect Size in Experimental Blunt Cranial Impacts

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After attending this presentation, attendees will better understand relationships between Point Of Impact (POI) -involved implement and fracture patterns from single, blunt cranial impacts to unconstrained human cadaver heads.

This presentation will impact the forensic science community by contributing to ground-truth data in support of assessing implement shape and POI in cases involving blunt cranial trauma.

Previous research has demonstrated that implement shape influences location and pattern of fractures in controlled impacts to fully constrained adult human heads and developing porcine specimens.[1-3] This current study further investigated the effects of implement shape on fracture patterns in experimental impacts to upright, unconstrained human heads. This study explored two major questions relevant to analyses of blunt cranial trauma: (1) Do different-shaped implements produce distinct fracture patterns?; and, (2) Can fracture patterns be used to estimate the POI? For 12 experimental cases in which implement and POI were known, these questions were explored through analyses of fracture patterns, defect size, and spatial relationship between fractures and the known POI.

Twelve adult male cadaver heads were impacted with a pneumatic impact system that allowed for controlled energy impacts to unconstrained specimens. Single impacts were administered to the mid-parietal, inferior to the parietal boss, with three implements that approximated a hammer (1"-diameter cylinder with a rounded surface; n=4), a baseball bat (2.5"-diameter cylinder with a curved surface; n=4), and a brick or broad, flat implement (3"-diameter flat disk; n=4).

Following single impacts, ectocranial fracture patterns were diagrammed and photographed. To observe endocranial outcomes, adjusted craniotomy cuts were conducted on the crania after maceration. Relevant data collected were: type of fractures present, spatial relationship between fractures and known POIs, and approximate size of any circular-type defects.

Energy to fracture and overall peak force were not statistically different between implements. For all three implements, the average fracture energy was 12.44J±6.04J, and the average overall peak force was 5221N±1936N.

The results of fracture patterns and their relationship with POI revealed trends by implement. In 3/4 impacts with the hammer implement, focal and circular depressed fractures circumscribed the POI. Endocranially, these impacts also generated corresponding internally beveled, delaminated “bone plugs” concentrated under the POI. Such endocranial defects were largely absent in the bat and brick impact experiments. In 3/4 impact experiments with the bat, curvilinear fractures occurred around the POI; however, they did not completely encompass the POI and exhibited an oval shape. The brick implement produced more variable fracture patterns. Half (2/4) of these impacts resulted only in linear fractures located remote from the POI in adjacent bones. In the other two brick impacts, large concentric fractures formed around the POI.

Circular-type defects were produced in 3/4 hammer, 3/4 bat, and 2/4 brick impact experiments. The hammer implement produced defects with the smallest average diameter (29mm±1.15mm.) These defects were of a consistent size, slightly larger than the implement diameter. The brick implement produced the largest defects (59mm±7.07mm); defects were typically smaller than the diameter of the implement. The bat produced defects of an intermediate size (34mm±15.72mm); however, defect sizes were inconsistent and overlapped in range with defects produced by the other two implements. These results indicate that defect size may assist in making a general distinction between small and large implements (i.e., hammer vs. brick), but it may not be possible to infer implement size based on defect size alone.

The results of this study reveal emerging trends in cranial fracture patterns associated with implement shape and suggest some baseline parameters for locating POI. In this experimental sample, an approximately circular defect, particularly in association with an endocranial bone plug, served as an effective indicator of POI. In contrast, when fracture patterns consisted only of linear fractures without the presence of round defects (1/4 hammer, 1/4 bat, 2/4 brick), impact location was obscured.

This project was supported by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.

Reference(s):

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Blunt Force Trauma, Cranial Fracture Patterns, Trauma Analysis

A35 Estimating Points of Impact in Multiple Blunt Force Cranial Trauma: Lessons From Experimental Impacts

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After attending this presentation, attendees will gain awareness of: (1) the influence of implement shape on fracture patterning in multiple, blunt cranial impact experiments; and, (2) the implications of this study for fracture pattern interpretation in a medicolegal setting.

This presentation will impact the forensic science community by presenting ground-truth data for multiple cranial impacts with known implements and known number and location of impacts.

The forensic literature is conspicuously lacking in guidelines for locating points of impact and, thus, accurately estimating number of impacts in cases involving blunt cranial trauma. The current study investigates the related issues of estimating locations and number of impacts in a series of multiple blunt force impact experiments on human cadaver heads. Research questions included: (1) Can fracture patterns be used to accurately locate all points of impact?; and, (2) How might implement shape influence practitioners' ability to make this assessment?

Controlled impact experiments were performed on 12 unembalmed, unconstrained human cadaver heads using a pneumatic impact system. Three aluminum impactors were selected to investigate implement effects on fracture patterns: a hammer (1"-diameter flat implement; n=4 specimens), a baseball bat (2.5"-diameter cylinder; n=4), and a brick (3"-diameter flat implement; n=4). Three impacts were delivered to each head: first to the mid-parietal, second to the anterior parietal, and third to the posterior parietal. After each impact, fractures were photographed and diagrammed. Following this series of three impacts, each cranium was macerated and a modified craniotomy cut was made to enable ectocranial and endocranial assessment of fracture.

Input energy for the impact experiments was 105.33J±19.48J for the hammer implement, 112.06J±3.70J for the bat, and 91.81J±18.7J for the brick. Energy differences between implements were non-significant.

The results indicated that sole reliance on ectocranial fractures may lead to an incorrect assessment of location and even a possible overestimation of the number of impacts. In contrast, assessment of only endocranial fracture, in this case internally beveled "bone plugs," may underestimate impact number.

Combined ectocranial and endocranial data provided clear indication of impact location for most impacts with the hammer implement. Nine of 12 impact sites exhibited circular fractures circumscribing the Point Of Impact (POI) ectocranially, 8/12 impact sites exhibited endocranial bone plugs, and 6/12 impact sites exhibited both. One or both features were present in association with known POI at 11/12 impact sites.

The bat produced semicircular fractures partially circumscribing the POI in 5/12 impacts, bone plugs in 3/12 impacts, and both features in 2/12 impacts. One or both features were observed in association with the known POI in 6/12 impacts. In all four specimens, at least one impact site was obscured due to the absence of circular fractures around the POI, presence of linear fractures distant from the POI, and/or lack of bone plugs associated with the POI.

The brick produced semicircular fractures surrounding the POI in 5/12 impacts, bone plugs in 1/12 impacts, and both features in 1/12 impacts. One or both features were observed in association with known POI in 5/12 impacts. In all four specimens, at least one impact site was obscured because: (1) fractures were linear and initiated at sutures adjacent to the POI (2/4 specimens); (2) new fractures intersected with fractures generated in previous impacts (4/4 specimens); and/or, (3) few endocranial defects were observed (4/4 specimens).

The results of this study suggest that practitioners should consider both endocranial and ectocranial data when assessing cranial blunt force trauma. In this experimental set, ectocranial circular defects and endocranial "bone plugs" were consistently observed in association with known points of impact. These features were observed in most hammer impacts, but only about half of the bat and brick impacts. This indicates that implement shape can affect assessment of impact location and, potentially, the number of impacts to an adult human head.

This project was supported by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.

Blunt Force Trauma, Cranial Fracture Patterns, Trauma Analysis

A36 Introducing MorphoPASSE: The Morphological Pelvis and Skull Sex Estimation Database

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After attending this presentation, attendees will be familiar with MorphoPASSE, a free interactive database for estimating sex in unidentified adults.

This presentation will impact the forensic science community by providing a new computer-based, statistical database program for sex estimation that is multivariate and combines two skeletal regions.

According to a recent survey administered to current American Academy of Forensic Sciences Anthropology Section members, most practitioners prefer to use both qualitative and quantitative methods when estimating sex from unknown skeletal remains; however, when only one method is used, qualitative methods are preferred nearly twice as often as metric methods.[1] Benefits of morphological methods include ease of use, efficiency, no need for specialized equipment, and applicability to fragmentary remains. Unfortunately, many of the qualitative methods used for sex estimation are based on subjective interpretations of skull and pelvis traits.

Studies by Walker and Klales et al. attempted to remedy the aforementioned shortcomings of morphological methods by: (1) providing modified descriptions of commonly used traits; (2) creating standardized illustrations with systematic ordinal scales; and, (3) including statistical analyses for a number of morphological skull and pelvis traits.[2,3] This was conducted to reduce the level of subjectivity and better conform to the scientific expectations presented in the *Daubert* proceedings and the National Academy of Sciences (NAS) Report. The Walker method utilizes glabella, nuchal crest, supraorbital margin, mastoid process, and the mental eminence, while the Klales et al. method utilizes the ventral arc, medial aspect of the ischio-pubic ramus, and the subpubic concavity/contour.[2,3] The eight traits included in these two methods continue to be the most popular morphoscopic traits for sex estimation, according to the Klales survey.[1] Both methods have been well received in the anthropological community and are currently being used nationally and internationally for forensic casework. To facilitate easier application of these methods and sex classification combining the methods, research was undertaken with the goal of developing a free database program.

Data were collected from more than 2,500 individuals from various United States and international collections to: (1) examine the reliability and validity of both methods; (2) examine the impact of experience in applying the methods; and, (3) determine the effects of population variation, secular change, and asymmetry on the methods. This research has culminated in the development of the free, interactive morphological database Morphological Pelvis and Skull Sex Estimation, known as MorphoPASSE. MorphoPASSE allows forensic practitioners to analyze sex in their unknown cases in a manner compliant with *Daubert*. Practitioners enter ordinal scores of an unidentified individual into the program based on the traits available for scoring (i.e., the program does not require complete remains or use of all traits). Then, these trait scores are compared to the known samples in the database for sex classification, either by selecting a specific population and/or temporal period in order to compare the unknown individual to the most appropriate reference sample or by using the generic logistic regressions equations created from the entire sample when the other parameters are unknown. MorphoPASSE then provides the user with posterior probabilities and associated error rates for sex classification that the user can then include in forensic case reports.

A publicly available user manual and website (www.MorphoPASSE.com) have been created to accompany the database. The first portion of the manual contains detailed descriptions of the traits, with reference to the original publications, and detailed instructions on scoring the traits in light of the results from this research. The second portion of the manual contains specific instructions for using the database and interpreting the sex classification results generated from the program. Since each forensic case presents with a different suite of traits, the statistical software package will provide a graphical user interface to simplify the entry of variables associated with each case. The package interfaces with R to conduct the statistical tests and the results are displayed in a user-friendly format. The score data from this research is available in numerous formats, including a CSV file and an R package.

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Walker Method, Klales Method, Sex Estimation

A37 Computational Anatomy: What Prospects for Forensic Anthropology?

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After attending this presentation, attendees will be able to consider the prospects of using computational anatomy in forensic anthropology.

This presentation will impact the forensic science community by drawing the attention to sex determination by computational anatomy and by developing an automatic method to simplify the anthropological approach.

The steady advances in medical imaging are the source of many complex images stored in hospitals. Unfortunately, after an image of the patient is obtained for a specific diagnosis, this image is usually discarded, without further analysis. Hence, there are many images stored in hospitals that could be further analyzed to extract knowledge on human anatomy. Computational anatomy is a recent research field which seeks to help practitioners in their daily practice by analyzing many individuals, which yields large-scale statistics on human anatomy.[1]

The purpose of this study is to assess the relevance of computational anatomy for forensic anthropology, in particular for sex determination.

Toward this goal, a novel groupwise registration algorithm was used, based on a keypoint detection, and was able to register several hundreds of full body images in a common space. This algorithm is fully automatic and can robustly register one hundred images within a few hours.

Preliminary results will be presented for 83 Computed Tomography (CT) scans of living individuals from the VISCERAL database.[2] The first results will focus on the hip bone for sex determination, which is known to be one of the most dimorphic regions between men and women. Experiments demonstrate that the well-known criteria for sex discrimination (e.g., the opening angle of the pubic symphysis or the greater sciatic notch) are well preserved in the mean images of men and women. Moreover, the results with the Probabilistic Sex Diagnosis (DSP: Diagnose Sexuelle Probabiliste) method (a tool using worldwide variability in hip bone measurements) will be shown by manually placing only 20 anatomical landmarks in the common space.[3] Sixty-two percent of individuals had been correctly estimated, 37% had been undetermined, and 1% of individuals had been determined with error of sex. The placing of landmarks manually is difficult and insufficiently accurate. Further analysis will address the skull and femur.

Currently, the limiting factor is the population size and a future goal will be to significantly increase the size to improve global accuracy. The long-term objective is to automatically computer generate local points of interest in the common space image. This new approach provides an automated profiling method to determine the sex of an individual and possibly her/his age, origins, and body measurements in future works.

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Computational Anatomy, Forensic Anthropology, Sex Determination

A38 Risen From the Ashes: An Exploratory Study for Developing New Methods of Analyzing Cremated Human Remains

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After attending this presentation, attendees will better understand potential new methods of recreating pertinent biological profile information, specifically the estimation of sex, of remains that have undergone cremation or burning.

This presentation will impact the forensic science community by providing results from an exploratory study of an area with little current or previous research. This presentation will add to research being conducted in the field of forensic anthropology by demonstrating how biological profile estimates are affected by a burning episode and how potential new methods may contribute to more accurate assessments of sex when human remains have undergone burning.

Reconstructing the biological profile of remains that have undergone burning or cremation often proves to be a difficult task due to the nature of damage that is incurred when bone is exposed to fire, such as warping, heat-induced fracturing, and alterations to bone shape.[1] These changes are compounded by the relatively small amount of literature available on the topic. This often forces the forensic anthropologist to rely on more traditional methods of assessing the biological profile since there are few cremation-specific methods available. While these methods can be applied successfully in many cases, it would be desirable to utilize methods designed specifically for cremated remains.

The basis of this research comes from data collected for a master's thesis in 2016 utilizing 49 cremations from the William M. Bass Donated Skeletal Collection housed at the University of Tennessee, Knoxville. Forty of the cremations were unprocessed (not pulverized after cremation) and the remaining nine cremations were processed. Each of the unprocessed cremations was carefully analyzed to determine which skeletal elements consistently survived cremation and whether they would be useful for identifying potential new methods of estimating biological profile. All skeletal elements deemed to be potentially useful were measured to determine if size sexual dimorphism was present. More traditional methods of estimating biological profile were also tested to determine which methods returned the most accurate estimates. Age was estimated for the unprocessed cremations using methods created by Brooks and Suchey, Buckberry and Chamberlain, Lovejoy et al., Meindl and Lovejoy, and Todd.[2-7] Sex was estimated using robusticity of the skeleton, the heads of the humeri and femora, as well as morphological traits of the skull and pelvis.[8] The weight of cremains was also used to estimate sex for both the processed and unprocessed remains using studies performed by Bass and Jantz, Van Deest et al., and Warren and Maples.[9-11] Stature was estimated using the Steele method of partial bone length reconstruction and Trotter's formulas for estimating stature from lone bone lengths.[12,13] The estimates were then compared with known demographic data of the individuals to assess which methods returned the most accurate estimates of the biological profile.

It was determined that a total of six skeletal elements/portions displayed size sexual dimorphism and may be useful for constructing new methods of estimating sex of cremated remains. These were the heads of the humerus, femur, and radius, the mandibular condyles, the first sacral vertebra, and the ischial tuberosities. Each of these elements/portions survived frequently in the cremations studied and could be measured and/or observed for size dimorphism. After assessing the results of the traditional methods of estimating biological profile, it was determined that sex was best able to be estimated using morphological traits of the skull and pelvis. Age at death was most accurately estimated using the Lovejoy et al. and Meindl and Lovejoy methods of assessing the auricular surface, and stature was best estimated using the Steele and Trotter methods paired together.[4,5,12,13] Ancestry was unable to be estimated for any of the cremations studied.

In conclusion, it was determined that it does appear feasible to develop new specific methods of estimating sex for remains which have undergone burning. While new methods of estimating age at death, ancestry, and stature could not be developed, it was determined that traditional methods can be used to accurately estimate age at death and stature for burned remains. This is important to the field of forensic anthropology as it demonstrates that there is potential to develop cremation-specific methods of estimating biological profile, which may lead to more accurate estimates of age at death, sex, and stature for remains which have undergone burning.

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Cremation, Human Remains, Forensic Anthropology

A39 Estimating Sex With Outline Shape Analysis of the Trochlear Constriction and the Olecranon Fossa

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The goal of this presentation is to demonstrate that using statistical modelling combined with outline shape analysis accomplishes a more accurate and unbiased sex estimation of the distal humerus than the non-quantitative visual assessment methods commonly used.

This presentation will impact the forensic science community by introducing attendees to a revised method for estimating the sex of an individual by using the distal humerus while turning the geometric morphometrics approach into a more accessible and easier-to-utilize interactive online application that allows users to control the principal component parameters.

Forensic anthropologists are often asked to establish a biological profile of human remains. Regularly, the skeletal remains are very damaged and the bones commonly used for sex estimation cannot be consulted. Fortunately, the distal aspect of the humerus is usually relatively well preserved due to its compact morphology. Therefore, this research will be particularly useful to researchers working with fragmented remains.

Several studies have investigated whether it is possible to estimate the sex of an individual by examining the distal humerus. A method using visual assessment of the morphology was developed by Rogers, with average accuracies ranging from 74%-91%.[1] Tests of this method in different populations have correctly classified 74%-82% of the individuals.[2-5] An attempt at a landmark-based geometric morphometrics reinterpretation of the method by Vance and Steyn reports accuracies between 78%-91%.[6]

A total of 80 female and 71 male humeri were analyzed using the 21st-Century Identified Skeletal Collection at the University of Coimbra (CEI/XXI) in Portugal. For this preliminary study, only the left humeri were evaluated. All specimens were photographed in a standardized position from the posterior view by placing the subject on a flat table and the camera directly above the olecranon fossa.

Using TPSdig2, a software program used for landmarks and outline digitization, an open curve and a closed outline were marked.[7] The first evaluated the constriction of the trochlea, with the curve reaching from the most medial inferior aspect to the most lateral inferior aspect of the trochlea. The second described the outline of the olecranon fossa and was recorded clockwise, starting from its most inferior aspect.

All the analyses were performed using the R programming language and the Momocs package developed by Bonhomme et al.[8,9] All coordinates were superimposed using Generalized Procrustes Analysis (GPA) as first proposed by Gower.[10] GPA allows multivariate statistical analyses on the shape of objects. This is possible, since GPA can superimpose shapes by iteratively rotating, translating, and scaling them until a consensus is reached.

Using 16 sampled points, natural six-degree polynomials fits were calculated for the open curve of the Trochlear Constriction (TC). An elliptical Fourier transformation was used to analyze the closed outline of the Olecranon Fossa (OF). This retained 99% of the harmonic power, which, in this case, resulted in six harmonics. Next, both sets of data were processed with the same statistical algorithms. Principal Component Analysis (PCA) was used to reduce the dimensionality of data and to visualize the morphospace.

Subsequently, Linear Discriminant Analyses (LDA) models were created using the principal components to estimate the sex from shape configurations. These classification models were trained on a $K=n$-1 cross-validation scheme to avoid overfitting.

After selecting the best predictive model, it was implemented into a web app, which was developed using the Shiny package for R.[11] This follows the recent trend in forensic anthropology, which attempts to make complicated statistical models more accessible and easier to utilize through interactive online applications.[12-14]

The two shape configurations exhibited strikingly different degrees of sexual dimorphism when evaluated using LDA with leave-one-out cross-validation. The trochlear construction performed poorly, with only 60.9% of the individuals being attributed to the correct sex; however, the olecranon fossa exhibited high sexual dimorphism, and the LDA model presented an accuracy rate of 92.1%. Thus, this character alone, using outline-based morphometrics, performs better than the combination of all four features of the visual assessment method proposed by Rogers.

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Outline Shape Analysis, Sex Estimation, Distal Humerus

A40 The Utility of Clavicular and Humeral Non-Metric Sex Assessment Methods in Japanese and Thai Individuals

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After attending this presentation, attendees will understand how select non-traditional, non-metric traits of the clavicle and humerus can be used in a statistical framework to ascertain the sex of Japanese and Thai individuals.

This presentation will impact the forensic science community by providing population-specific clavicular and humeral sex assessment methods for use when the traditional non-metric traits of the pelvis and cranium are absent.

Sex is one of the most important components of the biological profile as it can exclude roughly half of the population and it dictates the methods used in age, ancestry, and stature estimations. While the non-metric traits of the pelvis and cranium are used regularly and reliably in forensic and bioarchaeological sex assessment, trauma, taphonomic alterations, and incomplete recovery may preclude their inclusion in the biological profile. Therefore, it is necessary to develop, validate, and refine sex assessment methods on other sexually dimorphic regions of the skeleton that may be variably preserved, including the clavicle and humerus. The infero-medial clavicle serves as an attachment point for the costoclavicular ligament, and it is hypothesized that males more frequently show an excavation (rhomboid fossa) in this area. Sexually dimorphic differences in the distal humerus are attributed to differing female and male carrying angles; however, these non-traditional sex assessment methods were developed, validated, and refined in North America on individuals of African and European descent. Until relatively recently, Asian populations have remained understudied in forensic anthropological research, which is problematic in worldwide forensic contexts.

This study explores the expression of clavicular rhomboid fossae and distal humeral morphology in 1,397 Japanese and Thai individuals, 17 to 96 years of age. The Japanese sample is composed of 209 individuals from the late 19th to early 20th centuries (Chiba University) and 572 individuals from the modern era (Jikei University) (*f*=219; *m*=562). The Thai sample is composed of 616 individuals from the modern era (Khon Kaen and Chiang Mai Universities) (*f*=198; *m*=418). The rhomboid fossa of the medial clavicle was assigned an ordinal score based on the degree of expression and presence or absence. Similarly, the sexually dimorphic traits of the distal humerus (trochlear constriction, trochlear symmetry, olecranon fossa shape, and angle of the medial epicondyle) were assigned an ordinal score based on their "female-like" or "male-like" expression. Percentages, probabilities, and chi-square statistics were calculated to determine if differences exist between the sexes and populations. Subsequently, binary logistic regression equations and Chi Square Automatic Interaction Detection (CHAID) decision trees were calculated to identify the distal humeral traits that perform best in differentiating females and males. Additional non-parametric tests examined the effects of age and intraobserver error.

Overall, the results indicate that the methods developed on non-Asian populations exhibit reduced discriminatory power when applied to the Japanese and Thai populations. Concerning the clavicle, the majority of females and males (56.9%–97.4%) lack a rhomboid fossa; however, the presence of a fossa indicates male with a probability of 70%–91%. The left clavicle is more sexually dimorphic than the right. Concerning the distal humerus, 42%–85% of the individuals were correctly classified using the traits individually, while 68%–94% were correctly classified using a population-specific composite scoring method. Males were more correctly classified, and the angle of the medial epicondyle performed the best, while trochlear constriction performed worst. Further, the clavicular and distal humerus traits are influenced by age at death, population, and intraobserver error, thereby possibly complicating their use in sex assessment.

Though less accurate than the pelvis or cranium, this study demonstrates that clavicular and humeral morphology can be used in the absence of traditional non-metric traits to estimate the sex of Japanese and Thai individuals. Moreover, the findings underscore the importance of developing population-specific sex assessment methods for diverse Asian populations, as the Japanese and Thai are somewhat less sexually dimorphic than non-Asian populations and differ from each other.

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Sex Assessment, Non-Metric Traits, Asia

A41 Resolving Commingling and Past Accounting at Cabanatuan Prison Camp Cemetery

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After attending this presentation, attendees will understand the unique history of the World War II Cabanatuan Prison Camp assemblage and learn about some of the challenges in identifying individuals from this highly commingled context. In addition, using some specific examples within this assemblage, this presentation will inform attendees how identifications (and past mis-identifications) can be resolved with a multidisciplinary approach using anthropological, historical, and DNA analysis.

This presentation will impact the forensic science community by highlighting both the complications and the strategies used to solve commingled human remains cases.

Cabanatuan Prison Camp was one of several Japanese-run Prisoner Of War (POW) camps located on Luzon Island in the Philippines. It was occupied from June 1942 until the end of WWII in September of 1945. There were 2,763 confirmed casualties of American POWs at Cabanatuan. Several factors have influenced the degree of commingling present in the Cabanatuan skeletal remains and have created unique challenges to identifications.

A brief history of the Cabanatuan remains illustrates several different points in which commingling of the remains occurred. The initial burial system at Cabanatuan was a series of mass graves where all individuals who died within a 24-hour period were interred together. Each mass grave received a "Common Grave" (CG) number, and this number is still used today to reference the original provenience of the remains. After the war, the American Graves Registration Service (AGRS) exhumed the Cabanatuan Prison Camp cemetery. The remains were inventoried, preliminary anthropological analysis was performed, and many (300+) individuals were identified. The unidentified were interred temporarily at the United States Army Air Forces Cemetery in Manilla. Beginning in approximately 1947, the Cabanatuan remains at the United States Army Air Forces Cemetery were disinterred (again), processed, and more identifications were completed (1,000+). A review board in 1951 recommended that due to the commingling present, all remaining unknowns be buried in what is now the Manilla American Cemetery and Memorial (MACM) in the Philippines. Historians estimate between 990 and 1,006 unresolved casualties from Cabanatuan are currently at the MACM.

Common Grave 717 was originally associated with 14 individuals who died, according to Cabanatuan prison camp records, on November 19, 1942. Four of these individuals were identified in the late 1940s during the initial analysis of the Cabanatuan remains. The remaining ten individuals were buried as unknowns in the early 1950s at the MACM. In August of 2014, these ten caskets from CG 717 were disinterred from MACM and sent to the Defense POW/MIA Accounting Agency Laboratory in Hawaii for anthropological analysis and identification. The ten caskets from CG 717 are extremely commingled and, as of May 2017, there are 15 mitochondrial DNA (mtDNA) sequences represented in the ten caskets. These include sequences that are consistent with individuals who were previously identified out of CG 717 in the late 1940s, in addition to sequences that are consistent with the historical roster of individuals associated with CG 717.

The commingling present in the Cabanatuan assemblage includes individuals who were identified and resolved soon after WWII and individuals who were unresolved, or "unknown." This particular circumstance creates anthropological and historical complexity for these cases. For instance, given a set of remains from a resolved individual, how do we navigate an identification for those remains, and the set of (now unknown) remains that were buried after an erroneous identification several decades ago? Identifying the commingled remains of Cabanatuan entails revisiting identifications made under completely different operational and scientific standards of the late 1940s.

Using a specific example from an individual associated with CG 717, this presentation examines how commingling of both resolved and unresolved individuals requires an approach that uses anthropological, historical, and DNA data. In addition, it offers some lessons about past mis-identifications, and insight into how to best move forward with our current identification practices for commingled remains.

Commingled, Cabanatuan, Identification

A42 Procedures for Sorting Small-Scale Cases of Commingled Remains: An Integrative Approach Using Morphological, Metric, and Chemical Methods

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After attending this presentation, attendees will better understand the range and order of procedures for resolving commingled remains cases, including case studies that utilized morphological, osteometric, and non-destructive chemical methods.

This presentation will impact the forensic science community by demonstrating how using a range of methods for sorting commingled remains can produce optimal outcomes for medicolegal cases.

Methods for sorting commingled human remains have a long history in physical anthropology and include the determination of the minimum number of individuals and other quantitative measures for establishing how many individuals are represented within commingled and/or incomplete skeletal assemblages. Over the past decade, the Human Identification Laboratory at California State University, Chico (CSUC HIL) has developed a protocol for resolving small-scale commingling cases, including remains recovered from various scene contexts. This presentation discusses the CSUC HIL commingled remains protocol and presents four commingled case studies to highlight how the application of both low-tech and high-tech methods can successfully sort commingled remains prior to their return to next of kin for final disposition.

All methods for resolving commingled remains are based on the assumption that any two or more individuals vary on a number of different variables, including shape (morphology), size (osteometrics), taphonomic signatures, genetic markers, and chemical composition. Well-tested methods include physical matching and reconstruction of fragmented bones and teeth, visual pair matching of bilateral skeletal elements and tooth antimeres, physical matching of joint congruence of articulating skeletal elements and loose teeth with alveolar sockets, assessment of taphonomic patterns (e.g., staining, animal scavenging, and fragmentation patterns), and DNA profiles. More recently, the use of osteometric sorting and elemental analyses, such as X-ray fluorescence, has been introduced as a means for sorting commingled remains.

The CSUC HIL commingled protocol requires that analyses begin with a complete inventory of all remains and a sort by anatomical position. Where needed, analysis proceeds, beginning with skeletal reconstruction, followed by visual pair matching, osteometric sorting, and assessment of joint congruity. Assessment of taphonomic patterns can provide useful information to complement these methods; however, it can also be misleading if all remains were exposed to similar environments and, by extension, similar postmortem modifications. DNA results, if available, serve as a cross-check against the morphological and osteometric commingling assessment. For any remains that cannot be sorted through these methods, portable X-Ray Diffraction (pXRF) can be used to assign isolated skeletal elements to a particular individual. This application is most appropriate for two-person commingling scenarios. The most effective means for sorting commingled remains using pXRF is to scan a large sample of skeletal elements sorted to each individual through other means (e.g., reconstruction, visual pair matching, DNA), then to construct 95% confidence intervals for chemical concentration values. Concentration values for seven chemical elements (Silicon (Si), Phosphorus (P), Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), and Cobalt (Co)) provided the best discriminating power based on earlier research developed through the CSUC HIL. If the segregated bone groups from each individual do not overlap in their confidence intervals for at least one chemical element, unsorted skeletal elements can be assigned to the individual whose confidence interval includes the chemical concentration values. Thus, pXRF is a valuable tool for sorting commingled remains, but may not discriminate between individuals in all cases.

The use of multiple methods in the identification sequence helps to maximize the correct identifications of individual skeletal elements, especially in instances where a cross-check with DNA analysis is limited. Four recent case studies are used to highlight the utility of the CSUC HIL commingled remains protocol, including an outdoor surface context, a buried context, a fire scene, and a cold case representing several commingled individuals. Each of the case studies represents a different scene context, different length of time between recovery and identification, and different relationships between the individuals. They address issues associated with taphonomic damage, familial relationships, and detection of the number of individuals in group sizes larger than two. The goal of this presentation is to show how flexibility and diversity in the identification methods can aid in reducing the limitations often associated with commingled cases.

Forensic Anthropology, Commingled Remains, Portable X-Ray Fluorescence

A43 How Large Is Too Large? The Effect of Assemblage Size in Reassociating Commingled Remains

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The goal of this study is to examine osteometric reassociation accuracy as assessed through correct classification rates on commingled assemblages of varying sizes.

This presentation will impact the forensic science community by addressing a largely unexamined aspect of resolving commingling — size of the commingled assemblage.

Commingled assemblages present a common situation in osteological analysis in which discrete sets of remains are not readily apparent, thereby hindering biological profile construction and the identification process. Osteometric sorting has been shown to be a reliable and relatively objective method for resolving commingling.[1] Two statistical and probabilistic osteometric reassociation approaches have been successfully applied to resolving commingling: frequentist and Bayesian.[1,2] This study focuses on the latter approach.

To accomplish the goal of this study, linear measurements of the femur from 435 individuals were analyzed from the William M. Bass donated skeletal collection. Individuals were randomly removed from the total sample, acting as a closed-population commingled assemblage. The smallest commingled assemblage examined in this study was two individuals. The next smallest assemblage was five individuals and assemblage size was increased by increments of five to a maximum of 50 individuals. One femur was chosen from the commingled assemblage as the independent variable, with the possible matching femora representing the dependent variable. Using the remaining total sample, Bayesian regression via Hamiltonian Markov Chain Monte Carlo was used to estimate a range of possible dependent variable values. These values were smoothed into a probability density function using kernel density estimation, and the possible matches were evaluated against this distribution to calculate predictive probabilities. The femur with the highest predictive probability was considered the best match. This process was repeated 100 times for each commingled assemblage size.

As expected, there is a general inverse relationship between correct classification rates and commingled assemblage size. Accuracy decreased as the commingled assemblage size increased, with the two-individual assemblage showing the highest correct classification at 98%, and the fifty-individual assemblage showing the lowest at 76%. Interestingly, accuracy did not decrease markedly as the commingled assemblage size increased. A 50-individual commingled assemblage represents a 2,400% increase compared to a two-individual assemblage, with only a 22% decrease in classification accuracy. These results show that a Bayesian approach to resolving commingling is powerful across a large range of commingled assemblage sizes.

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Commingling, Osteometric Reassociation, Bayesian Modeling

A44 Commingling Among Unidentified Remains Stored at Mortuary Facilities in Bosnia and Herzegovina (BiH)

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After attending this presentation, attendees will be informed regarding the extent of commingling (and associated issues) among the unidentified human remains disinterred in the period of 1996-2016 and stored at mortuary facilities in Bosnia and Herzegovina.

This presentation will impact the forensic science community by providing information regarding commingling during exhumations as well as in mortuary facilities, particularly with regard to obtaining new identifications within the assemblage. The need for thorough analyses with regard to defining skeletal sets and establishing Minimum Number of Individuals (MNI) will be demonstrated, and the framework for re-association of skeletal elements based on DNA and skeletal morphology as assessed through standard osteological measurements will be discussed. Most importantly, the implemented system of meticulous case management will be discussed as a method for prevention of further commingling within the mortuary facilities.

In May 2013, the State Prosecutor of Bosnia and Herzegovina issued an order to commence the process of the anthropological examination of human remains stored within mortuaries and memorial ossuaries across Bosnia and Herzegovina. Through coordination of the International Commission on Missing Persons (ICMP) and state and regional participants, the No Name (NN) Project was created and implemented to ascertain the exact number of cases, review the history of each case, determine each case's current status, and issue recommendations for a national system for organization, storage, and management of all unidentified remains.

As of June 2017, a total of 2,548 cases containing a total of 5,673 skeletal sets have been reviewed by ICMP's anthropologists, indicating a pervasive level of commingling. Commingling in these cases was caused by a variety of factors: interment in caves or pits where numerous taphonomic forces were active; grave robbing and/or displacement (the repeated exhumation and reburial cycles intrinsic to secondary and tertiary mass graves); or through inadequate case management in the mortuary facility itself. In order to address the issue of commingling, the ICMP developed and implemented a thorough process of case review through the assessment of previously accumulated documentation and the use of scientific examination techniques for each case. Divided into several phases, ICMP's NN Project tackled the issue of commingling through determination of skeletal sets present in each case**,** defining them as a discrete set of remains belonging to one individual (ascertained through DNA matching, fracture matching, anthropological features, and visual pair matching) supported by additional findings. A total of 170 reviewed cases were determined to contain only skeletal elements designated as "ossuary," a category encompassing the commingled remains that cannot be associated with a skeletal set that has a successful or pending DNA sample and that will not be subject to DNA testing; these cases cannot be resolved currently, primarily due to the financial constraints of DNA testing.

Out of more than 2,000 DNA samples extracted from commingled or under-sampled skeletal remains during the project, 101 new matches with blood reference samples were obtained from 72 cases, out of which 39 cases had some degree of commingling. This number is likely to increase as many DNA samples are still pending extraction and processing. The examination phase of the project is scheduled to end in September 2017, when a complete summary of obtained results will be made available.

The NN project in Bosnia and Herzegovina illustrates the importance of a comprehensive and integrated approach to the analysis of commingled remains, fulfilling obligations to identify missing persons in the most meaningful and scientifically possible manner.

Commingling, Identification, Mortuary Review

A45 The Accuracy of Visual Pair Matching of the Humerus of a Large-Scale Commingled Assemblage

Carrie B. LeGarde, MA, Defense POW/MIA Accounting Agency, 106 Peacekeeper Drive, Bldg 301, Offutt Air Force Base, NE 68113*

After attending this presentation, attendees will understand the reliability and interobserver variability of visual pair matching of the humerus.

This presentation will impact the forensic science community by illustrating that visual pair matching by anthropologists can be confidently relied upon, particularly by those with experience with commingled human remains.

Commingled human remains pose a particularly difficult challenge for identification, particularly when the commingling is large-scale. DNA analysis is often heavily relied upon to segregate these remains into discrete individuals, but this can be time consuming and costly. Anthropological methods, such as pair matching, can be employed when possible to limit the number of elements that require DNA sampling. The purpose of this study was to determine the accuracy rates of visual pair matching for multiple observers with a variety of educational and experience levels.

The sample for this study involves left and right humeri (n=287 and n=293, respectively) from the commingled remains of the USS *Oklahoma,* which are currently being segregated and identified by the Defense POW/MIA Accounting Agency (DPAA). The humerus was chosen for this study for two reasons: (1) there is a relatively high degree of bilateral asymmetry exhibited as compared to other long bones, which could make visual pair matching more difficult; and, (2) all left and right humeri in the assemblage were sampled for DNA analysis, which could allow for the accuracy of pairs to be determined. Although less than half the DNA testing has been completed, accuracy was determined for those that have been tested. Five anthropologists completed this study with osteological experience ranging from 2 to 13 years, with the following education levels represented: post-BA (n=1), post MA (n=1), and post-PhD (n=3). Experience with segregating Commingled Human Remains (CHR) was also considered and separated into three categories: none (n=1), minimal (n=2), and experienced (n=2).

All left and right humeri were placed on ten tables for analysis. The observers were instructed to pair left and right humeri using four categories based on confidence level: (1) match (confident); (2) probable match (fairly confident); (3) possible match (not confident); and, (4) no match. Methodology for pair matching was not specified, but observers were instructed to document their process to examine differences in methodology. Only results for those categorized as "match" are given below. Additionally, matches between observers were compared and examined for congruency.

The amount of time required to complete the pair matching assessment ranged from 35 to 55 hours, but methodology was similar for all observers. The total number of visual pair matches ranged from 88 to 156. The observer with no CHR experience paired the least and all other observers paired between 144 and 156 humeri. The accuracy of pair matching for the five observers ranged from 80% to 100%, with the two experienced CHR observers having the highest accuracy (98% and 100%), followed by the observer with no CHR experience (96%). This reveals that the least experienced observer was more conservative by pairing the fewest humeri (by nearly half), but demonstrated high accuracy for the visual pair matches made. Accuracy was not found to correlate with level of education.

These preliminary results reveal that visual pair matching has relatively high accuracy, regardless of education or experience level; however, this accuracy increases with experience. Results suggest that visual pair matching by anthropologists experienced with commingled human remains are exceptionally accurate and can be relied upon with confidence. These results are preliminary and may change as additional DNA results are received.

Pair Matching, Humerus, Commingled Human Remains

A46 Osteometric Sorting of Commingled Upper Limb Bones

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After attending this presentation, attendees will understand the utility of using metric methods for sorting commingled human remains.

This presentation will impact the forensic science community by providing a statistically valid means of sorting commingled remains when traditional non-metric methods need to be supplemented.

Long bones of the arm and scapula are reported to be reliable for sex determination and stature calculation.[1,2] Therefore, in a commingled context, it is crucial to assess that these bones correspond to the same individual. In this framework, the present study sought to develop a number of functions for sorting commingled human scapulae, humeri, ulnae, and radii using linear measurements of these bones' articular surfaces.

For this purpose, a number of linear measurements was performed: the maximum height and breadth of the glenoid fossa, the maximum vertical head diameter and the maximum anterior-posterior head breadth of the humerus, the capitulum-trochlea breadth, the maximum olecranon breadth, the minimum olecranon breadth, the ulnar radial notch height, the maximum head diameter of the radius, and the vertical radial head height. All 222 individuals included in the study belong to the Athens Collection.[3] This skeletal collection consists of individuals of known sex, age, occupation, and cause of death. They lived in the second half of the 20th century in Athens, Greece, and their biological age ranged between 20 and 99 years.

Simple and multiple linear regression analyses produced a total of 11 equations (7 simple and 4 multiple regression equations) as the best statistical models for predicting measurements of one skeletal element using measurements of another. The standard error of the estimate ranged between 0.88mm-1.59mm for the simple regression formulas and 1.41mm-1.58mm for the multiple regression formulas. Pearson's correlation coefficient ranged between 0.69-0.93 (sig. <0.05) showing statistically significant strong positive correlations among measurements. The coefficient of determination (r2) scored overall higher in multiple regression analyses (0.71-0.86) compared to simple regression analyses (0.47-0.83). Sex and bilateral asymmetry did not have a statistically significant effect on the methods accuracy.

In conclusion, the regression equations developed in this study were found to be suitable for sorting commingled upper limb skeletal elements. The development of similar methods for other joints of the human skeleton would be beneficial for the anthropological analysis of commingled remains.

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Forensic Anthropology, Commingled Remains, Osteometric Sorting

A47 The Use of Portable X-Ray Fluorescence (pXRF) Spectrometry for a Large Commingled Assemblage

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After attending this presentation, attendees will understand the potential application of trace elemental concentrations in bone as measured by pXRF to large commingled skeletal collections when used in conjunction with other segregation techniques, as well as the pitfalls of its use stemming from taphonomic alteration.

This presentation will impact the forensic science community by serving as a further means of segregation in extensively commingled skeletal assemblages and will discuss approaches to combining elemental concentration data with other segregation techniques. These methods also have the advantage of being relatively inexpensive, rapid, and non-destructive.

Commingled skeletal assemblages present a challenge to the accurate segregation of skeletal remains, particularly when individuals from the loss incident share demographic similarities. PXRF has been shown to be useful in segregating individuals in small commingled assemblages, though its utility has not been established in larger scale cases. Developed methods use intraskeletal similarities in the trace elemental composition of bones (including some of the following: potassium, calcium, iron, magnesium, phosphorus, zinc, strontium, lead, sodium, silicon, titanium, vanadium, chromium, manganese, cobalt, nickel, and copper) to chemometrically group skeletal remains based on the relative concentrations of each element; however, many of these concentrations may be affected by taphonomic processes. This research compares elemental concentrations as measured by the pXRF at the bone surface to those measured on internal cortical bone exposed by previous DNA sampling. These data were also compared to trace element concentrations present in the surrounding burial environment. Trace elements with measurements that are much higher at the bone surface are likely to have been taphonomically affected, as are skeletal remains that exhibit an element concentration profile matching that of the burial environment. Additionally, pXRF analysis of these remains alongside standard reference materials with known elemental concentrations may be able to provide accurate measures of individual trace elements that can then be better compared across the skeleton. This research will use the resulting trace elemental concentrations in multivariate analysis to create groups that can be used in conjunction with other segregation methods.

The sample represents the remains of close to 400 men lost aboard the USS *Oklahoma* on December 7, 1941. After multiple identification attempts in the late 1940s, these remains were buried in the National Memorial Cemetery of the Pacific, Honolulu, HI. Their recent disinterment revealed extensive commingling with an estimated minimum number of 390 individuals, based on mitochondrial DNA profiles. Preliminary analysis of three bones, each from four individuals (a humerus, a femur, and an innominate) identified the potential for the use of specific trace elements in segregating remains. One individual displayed high concentrations of potassium and iron, another was characterized by relatively high values of titanium, and another by steady values in strontium, lead, and zinc. The fourth exhibited large fluctuations between measurements obtained at the bone surface and from exposed cortical bone. High concentrations of iron, the presence of vanadium and chromium, and variability in trace elements such as potassium and zinc imply that the composition of these skeletal remains may have been affected by taphonomic processes.

This study will present methods for integrating these chemometric data into visual examination and the use of DNA sequence information for a large commingled assemblage. This research will explore methods for increasing the accuracy of measurement for trace elements throughout this assemblage to better differentiate those useful for segregation of individuals and those indicative of taphonomic alteration.

The views herein are those of the authors and do not necessarily represent those of the Department of Defense or the United States government.

Commingled, X-Ray Fluorescence, Trace Elements

A48 Estimating the Number of Individuals in a Large Commingled Assemblage of Known Size

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After attending this presentation, attendees will understand the differences between methods that estimate the number of individuals in a skeletal assemblage and which method best estimates the true population size of a large commingled assemblage.

This presentation will impact the forensic science community by assessing the results of different methods that estimate population size in skeletal assemblages and highlighting a standardized method to inventory data in large commingled assemblages.

Estimating the number of individuals in a commingled assemblage is vital for planning purposes, including budgeting, resource allocation, analytical approaches, and the direction of future recovery efforts; however, the estimate is affected by factors inherent to the assemblage (e.g., fragmentation, preservation, and recovery rates) and the estimation methods themselves (e.g., variability of methods results in numerous estimates, lack of testing on assemblages of known size, and inconsistent inventory terminology).

Minimum Number of Individuals (MNI), Grand Minimum Total (GMT), and Most Likely Number of Individuals (MLNI) estimate the number of individuals in an assemblage. MNI determines the fewest possible individuals needed to account for the physical remains that are recovered and is traditionally estimated by the reported completeness of the most common element. GMT and MLNI use pair matching, with the former producing an MNI estimate based on counts of paired and unpaired bones and the latter using the maximum likelihood estimate of the total number of individuals that may have originally comprised an assemblage.[1] Previous assessments of MLNI typically have focused on smaller assemblages (<50 individuals) in which the original loss population was unknown; MLNI has not been tested on larger assemblages of known size. For all quantification methods, an accurate inventory of the assemblage is a crucial step, since this affects the accuracy of the estimate.

The December 7, 1941, attack on Pearl Harbor resulted in 429 casualties from the USS *Oklahoma*; 394 of these individuals were not identified at the time. The remains of these individuals are known to be severely commingled; the first disinterred casket, containing five "sets" of remains, yielded 95 unique mitochondrial DNA (mtDNA) sequences. The USS *Oklahoma* project, which seeks to individually identify the USS *Oklahoma* casualties, has an assemblage of nearly 13,000 skeletal elements with nearly 5,000 samples submitted for DNA analyses.

This study examines MNI, GMT, and MLNI estimates using inventoried elements and DNA results to date from the USS *Oklahoma* project. The inventory of the USS *Oklahoma* assemblage used both a descriptive system (e.g., distal portion present) and the zonation method of Knüsel and Outram, with slight modifications.[2] Part of the DNA strategy for the USS *Oklahoma* project included sampling all crania, humeri, and tibiae, enabling the calculation of both MNI and MLNI. MNI was calculated using the most frequent element, zones, and the number of duplicated elements per mtDNA sequence (n=308 mtDNA sequences to date). GMT and MLNI were calculated using visual pair matching of all humeri in the assemblage (*left*=287, *right*=293).

For the cranium, MNI is estimated as 356 individuals (Zone 5, occipital). For the postcranium, MNI is 336 (right femur, distal) and 354 (right femur, Zone 1). By duplicated element per mtDNA sequence, MNI is 394. Based on the humeri, MNI is: 289 (right humerus, distal); 298 (Zone 5); and 369 (GMT); and MLNI is calculated as 399±15 individuals.

In this assemblage, MNI by mtDNA and MLNI provide estimates that are identical or nearly identical to the original population size. GMT is the next most accurate method, followed by MNI based on zones. The least accurate estimator is MNI based on traditional descriptive inventory terms. While MNI by mtDNA sequence is currently very accurate, the sequencing is not yet complete for the project and it will likely take several years to process all 5,000 samples. Thus, this does not represent the timeliest or most cost-effective strategy. MLNI, while also very accurate in this study, has the potential to overestimate when the associated interval is considered, and both MLNI and GMT necessitate pair-matching across the entire assemblage. If this is not possible, the zonation method is more accurate than traditional MNI, and it provides a means to reliably assess fragmented remains, especially if recovery rates are poor or fragmentation is high. Finally, MLNI assessments corroborate previous findings on a larger scale than previously tested.

The views herein are those of the authors and do not represent those of the Defense POW/MIA Accounting Agency, Department of Defense, or United States government.

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2. Knüsel, Christopher J., and Alan K. Outram. Fragmentation: The Zonation Method Applied to Fragmented Human Remains from Archaeological Contexts. *Environmental Archaeology.* 9 (2004):85–97.

Commingled Skeletal Remains, MNI, Zonation Method

A49 Using Biological Data to Inform a DNA Sequencing Strategy

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After attending this presentation, attendees will be familiar with a successful approach using antemortem and postmortem biological data to prioritize DNA sequencing for a large commingled assemblage.

This presentation will impact the forensic science community by outlining a strategy to prioritize DNA sequencing of elements that fall >1 standard deviation from sample means for stature and age estimates, which can then be used to identify individuals prior to completion of all DNA analyses, reducing the missing person pool and the time families wait for notification.

Large commingled assemblages present unique challenges for identification. Unlike individual cases, the segregation of remains into discrete individuals can be difficult and time-consuming, even when DNA is incorporated. Using antemortem data and postmortem estimates from skeletal remains, this study outlines an effective and biologically informed strategy to prioritize DNA sequencing in a large commingled assemblage.

The assemblage is from the USS *Oklahoma* identification project and is comprised of nearly 13,000 skeletal elements; nearly 5,000 were sampled for DNA analyses. The remains are extensively commingled, with the first casket exhumed containing elements representing approximately 25% of the USS *Oklahoma* casualties unidentified following the incident (*n*=394). Analyses of the assemblage, including DNA sequencing, are concurrent with identifications being made.

All elements were inventoried and, as applicable, measured and epiphyseal fusion and pubic symphysis development recorded following McKern and Stewart.[1] The calculation of stature point estimates by element was automated for the entire assemblage using OsteoSort and linear regression equations from Trotter's Black and White male combined dataset in FORDISC® 3.[2] Only elements sampled for DNA analyses with a stature point estimate or age data were included. Antemortem data was drawn from military records of the unidentified USS *Oklahoma* casualties. Frequency distributions and descriptive statistics for antemortem data and postmortem estimates were produced using R, with the exception of epiphyseal fusion since mean ages per stage are not provided by McKern and Stewart.[1]

The frequency distributions for antemortem stature and postmortem stature point estimates are normal and nearly identical — in inches (mean, standard deviation, *n*): antemortem (68.56, 2.31, 374); humerus (68.26, 1.86, 507); radius (68.44, 1.47, 325); ulna (68.35, 1.49, 328); femur (68.17, 1.97, 642); tibia (67.08, 1.88, 573); and fibula (68.45, 1.97, 360). Antemortem age and pubic symphysis postmortem point estimates are skewed to the right, but also similar — in years: antemortem (24.49, 6.40, 394); and pubic symphysis point estimate (24.31, 5.21, 405). For casualty individuals, 160 have stature and/or age that is >1 standard deviation from the antemortem means.

Because of the demonstrated similarity between antemortem data and postmortem estimates, prioritization was defined in four tiers based on standard deviations of the point estimates from the means: Level 1 — stature estimate >2 standard deviations (*n*=89); Level 2 — age estimate $\leq$20 years and $\geq$30 years (*n*=247); Level 3 — Stage 3 fusion (~21 years, *n*=106); Level 4 — stature estimate 1-2 standard deviations from the mean (*n*=501); and total on priority list, *n*=943. Due to the antemortem age distribution, epiphyses that fuse in the mid- to late-20s were not prioritized (e.g., medial clavicle). When elements were found in multiple priority levels, the element was given the higher of the ranked priorities. It should be noted that all cranial and dental samples were sequenced prior to other elements in order to aid with Minimum Number of Individuals (MNI) estimation and dental identifications.

This prioritization strategy has thus far resulted in segregation from the large commingled assemblage of 20 of the 160 individuals whose biological data are >1 standard deviation from antemortem age and/or stature means. These individuals will be identified prior to the completion of DNA sequencing for the entire project, which would not be feasible if relying solely on processing samples in the order they were received. The ability to make identifications can be important for family members, especially when a large period of time exists from incident to identification, as well as the forward progress of the project, as each identification further reduces the pool of missing persons. This also can be applied when sampling elements for DNA. One limitation to this approach is the lack of or incomplete antemortem data, which will affect the ability to develop informed priors.

Reference(s):

1. McKern, Thomas W., and T.D. Stewart. *Skeletal Age Changes in Young American Males: Analyzed from the Standpoint of Age Identification.* Natick, MA: Quartermaster Research and Development Command Technical Report EP-45, 1957.
2. http://osteosort.net.

Commingled Skeletal Remains, DNA Sequencing Strategy, Biological Data

A50 A Multidisciplinary Approach to Identifying Unaccounted Service Members From the Battle of Tarawa in 1943

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After attending this presentation, attendees will better understand the interdisciplinary approach necessary to resolve unknown commingled remains associated with a World War II battlefield.

This presentation will impact the forensic science community by highlighting the collaborative efforts and multiple lines of evidence required to identify United States service members from the commingled Battle of Tarawa assemblage.

In November 1943, United States forces captured Betio Island, Tarawa Atoll, Republic of Kiribati (previously the Gilbert Islands), which contained a Japanese-controlled landing strip essential to the United States Pacific campaign. The battle resulted in more than 1,100 United States and more than 6,000 Japanese and Korean casualties on a ~381-acre island; many individuals were buried in temporary mass graves. Approximately ~48% (~500) of all United States burials were recovered after World War II, including Unknowns (Minimum Number of Individuals (MNI)=114) interred in 94 caskets at the National Memorial Cemetery of the Pacific (NMCP). Since World War II, local residents, Defense POW/MIA Accounting Agency (DPAA) recovery teams, and non-governmental organizations have regularly recovered human remains from Betio Island. The Tarawa commingled assemblage consists of remains from past and ongoing field recovery operations and disinterred Unknown Tarawa casualties from the NMCP. The actions of hasty, but necessary, interment after the battle with later disinterment, transport, and reinterment resulted in the loss of identity and commingling of the Tarawa casualties.

Commingled projects have largely relied on DNA analysis for segregation and identification of remains; however, the Schofield Central Identification Laboratory (Schofield-CIL) treated the Tarawa remains with embalming powder prior to their interment at the NMCP, which blocks primer-binding locations. Next Generation Sequencing (NGS) improves the ability to yield DNA sequence from chemically treated remains, but the average time for NGS results combined with the amount of samples required to rectify commingling can take years. In addition, low Family Reference Sample (FRS) coverage of ~68% for both mitochondrial and nuclear references complicates Tarawa identifications. In order to counter these limitations, dental record comparisons and historical records were used to create initial short lists of potential individuals in lieu of DNA as the first step.

These historical or dental short lists serve as the basis for all subsequent analyses (e.g., chest radiograph (CXR) comparisons, development of the biological profile, and osteometric sorting). Antemortem chest radiographs are available for ~25% of all Tarawa casualties, thus providing an additional line of evidence for identification. When a potential CXR match is made from the short list, DNA sequence data obtained from earlier field accessions, which are comprised of primarily small bones of the hands and feet, are examined for association with NMCP disinterments.

If there are elements that match the FRS on file for a potential service member from the dental short list or a chest radiograph match, these elements are compared to the disinterred remains via articulation, osteometric sorting, and pair-matching. These methods use osteometric data to compare elements statistically, enabling the association and segregation of field accessions and NMCP disinterred remains. Through the association/segregation process, a biological profile of the remains is developed for sex, age, ancestry, and stature, which are compared to antemortem records of the proposed individuals. This data provides a means of exclusion and inclusion between those individuals on the short list, thereby facilitating identification.

The Tarawa Project has successfully identified 18 of the NMCP Unknowns using this process. Seven of these cases involved matching remains from field excavations to remains disinterred from the NMCP using this strategy. The initial reliance on other, non-DNA driven methods for casualty associations has freed the NGS DNA sampling queue for cases in which an effective dental short list cannot be generated.

These efforts have allowed this study to determine possible relationships between disinterred remains and field recoveries, focus the DNA sampling strategy employed, and determine future recovery locations on Betio Island. Thus, identifications being made from the Tarawa Project rely on the totality of evidence and interdisciplinary collaborations from geneticists, historians, archaeologists, odontologists, and anthropologists; no one scientist works in a vacuum.

The views of these authors do not necessarily reflect those of the Department of Defense or the Unites States government

Commingled Human Remains, Tarawa, Anthropology

A51 A Comparison of Bullet Type on Cranial Gunshot Exit Wounds

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After attending this presentation, attendees will better understand that bullet type (full metal jacket vs. jacketed hollow point) has the potential to have a significant effect on the amount of resultant damage to human skulls.

This presentation will impact the forensic science community by discussing the importance of experimental research in trauma analysis, especially regarding gunshot trauma, while demonstrating that such research using human remains can be conducted respectfully and in a manner designed to obtain the maximum amount of data.

Gunshot wound interpretation, especially of the skull, has been extensively studied in forensic anthropology. Most studies have taken a retrospective approach, whereby information from autopsy reports or forensic anthropology reports are mined for data regarding predictive patterns. This approach is problematic due to the number of extrinsic variables present. These variables include factors such as velocity of the projectile, distance of the weapon to the victim, type of firearm, bullet caliber, and bullet construction. In retrospective studies, there is no way to know what many of these variables were, rendering the results less reliable. Further, while research attempts have been made at elucidating these variables for existing cranial gunshot wound cases, current recommendations advise against determining or estimating anything about weapon type, caliber, or distance when analyzing skeletonized remains with gunshot trauma due to the number of confounding variables.[1] An experimental approach is therefore recommended as such variables can be controlled for, with the goal of eventually enabling variable estimation.

Therefore, this project experimentally tested the effect that bullets of two different constructions (jacketed hollow point vs. full metal jacket) have on human skulls in terms of fracture pattern and amount of damage, as assessed by centimeters of total fracture and exit wound morphology. These different bullet types are designed to either fully penetrate with no fragmentation (full metal jacket) or penetrate and expand upon contact (hollow point). The hypothesis was that damage caused by hollow point ammunition will be greater, which has implications for fracture interpretation.

Forty-five donated human heads were obtained from an anatomical tissue supply company, specifically for the purpose of trauma research. A specialized shooting stand was designed to support each head at the height of an average adult male, and each head was shot once either in the frontal or temporal bone, using a revolver with a $1^{7}/_{8}$-inch barrel loaded with 0.38 caliber bullets. Bullet type (jacketed hollow point vs. full metal jacket) was distributed randomly yet evenly between individuals. Following the experiment, heads were autopsied and macerated using standard procedures.

The interaction between bullet type and exit wound for those individuals with entrance wounds in the frontal bone was tested using a chi-square analysis. Preliminary results reveal that there is a significant difference between impacts with jacketed hollow point bullets vs. full metal jacket bullets in that the former do not cause exit wounds, while the latter do (p=021).

This has implications for gunshot wound fracture analysis, in that these results reveal bullet construction can significantly affect exit wound morphology. Future research will include analysis of fracture pattern differences by bullet type and the interaction between bullet type and entrance wound location.

Reference(s):

1. Berryman, Hugh E., Lanfear, Alicia K., and Natalie R. Shirley. The biomechanics of gunshot trauma to bone: Research considerations within the present judicial climate. In: *A Companion to Forensic Anthropology*. Edited by Dennis C. Dirkmaat.

Gunshot Wounds, Fracture Analysis, Forensic Anthropology

A52 Ancestral Variation and Postcranial Metrics for Three United States Populations: Implications for Stature Estimation

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After attending this presentation, attendees will be aware of the influence of trihybrid ancestry on post-cranial metrics and stature within major United States populations and when group affiliation is unknown.

This presentation will impact the forensic science community by providing opportunities for improving stature estimation models for contemporary United States populations by using long bone measurements and estimated quantities of three-way continental ancestry.

The purpose of this study is to produce trihybrid estimates of continental ancestry for three of the major United States populations in order to: (1) test for associations between the proportions of ancestry, long bone measurements, and recorded stature; and, (2) determine if any of the three vectors of ancestry estimates contribute useful information to statistical models for the prediction of stature from upper and lower limb bone measurements. A sample of 934 males of recorded Black, Hispanic, and White identity, with matched cranial/postcranial metrics and forensic/cadaveric stature data, was selected from the Forensic Anthropology Data Bank. Of this total sample, 593 cases, with birth years ≥1930, were used in parallel analyses to address the issue of secular change in long bone measurements.[1,2] The proportions of African, European, and Native American ancestry for each individual were generated from the unsupervised model-based clustering of a standard set of craniometrics.[3] Four hypotheses are evaluated using these data: (1) estimates for each of the African, European, and Native American ancestry components are correlated with length measurements for the six limb bones and with recorded stature, when the sample is partitioned by population; (2) similar trends are observed when all cases are pooled and population affiliation is presumed unknown prior to analysis; (3) birth year, as a proxy for secular change, affects the outcome of these analyses; and, (4) stature estimation can be improved by including percent ancestry estimates in regression models using single bone measurements.

To investigate associations among ancestry composition and skeletal metrics, Pearson correlation coefficients, *r*, were calculated between each of the three vectors of ancestry estimates, the six limb bone lengths, and the recorded statures. Population-specific test results indicate that Black individuals showing less admixture with Europeans and Native Americans tend to have longer limb bones but shorter statures. Hispanic individuals with more Native American ancestry tend to have shorter limb bones and shorter statures, while those individuals with more African ancestry tend to have taller statures. Both larger quantities of African and European ancestry are associated with longer bone lengths for the Hispanic cases. White individuals in the Forensic Data Bank (FDB) are found to have low admixture (≈8%), as such correlation coefficients are small; however, their consistent direction suggests that greater metrics and taller stature are associated with European ancestry.

Results for the pooled data analysis find that all long bone metrics increase as African ancestry increases. The lengths of the humerus, femur, tibia, and fibula decrease with increased Native American ancestry just as the radius and ulna decrease with increased European ancestry. Stature is positively correlated with European ancestry and most negatively correlated with Native American ancestry. Trends similar to the individual population analyses are observed for the sample with birth years ≥1930. Yet, differences are noted in the ancestry proportions of the Black and Hispanic birth year subsets: in accordance with prior findings, the quantities of African and Native American ancestry are reduced, respectively.[4,5] This shift toward greater European admixture produces a change in magnitude, but not in direction, of the correlation coefficients.

To determine the effect of trihybrid ancestry on stature estimation, stepwise selection via Bayesian Information Criteria (BIC) and Akaike's Information Criteria (AIC) was applied to identify the best-fitting least squares regression models with significant ancestry component(s) and bone-ancestry interaction effects for each long bone measurement. Model improvement was gained with the inclusion of one ancestry component for all long bone regressions for the White sample, for all regressions except the fibula for the Black sample, and for regressions using the radius, ulna and fibula for the Hispanic sample. When populations were pooled, the best-fitting models include one (humerus, femur) or two (radius, ulna, tibia, fibula) ancestry component effects and one bone-ancestry interaction (ulna, radius) term.

These analyses demonstrate how: (1) quantifying variation in ancestral contributions is important to understanding within-population differences in both long bone measurements and statures; (2) population-specific trends are consistent when cases are pooled; (3) trends observed with the full sample are recapitulated with the birth year subset, indicating minimal influence of secular change on these trends; and, (4) the improvements gained in regression model fit suggest that future work on stature estimation equations may benefit from considering estimates of trihybrid ancestry, which can be computed from standard cranial measurement or forensic DNA data.

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1. Meadows Jantz L., Jantz R.L. Secular Change in Long Bone Length and Proportion in the United States, 1800–1970. *Am J Phys Anthropol.* 1999:110(1):57-67.
2. Wilson R.J., Herrmann N.P., Meadows Jantz L. Evaluation of Stature Estimation from the Database for Forensic Anthropology. *J Forensic Sci.* 2010: 55(3): 684–689.
3. Algee-Hewitt B.F.B. Population inference from contemporary American craniometrics. *Am J Phys Anthropol.* 2016:160(4):604-24.
4. Algee-Hewitt B.F.B. Temporal trends in craniometric estimates of admixture for a modern American sample. *Am J Phys Anthropol.* 2017:163(4):729-740
5. Algee-Hewitt B.F.B. Temporal, Geographic and Identification Trends in Craniometric Estimates of Ancestry for Persons of Latin American Origin. *Forensic Anthropology.* 2018: forthcoming.

Trihybrid Ancestry, Long Bone Measurements, Stature Estimation

A53 A Comparison of Historical and Present-Day Skeletal Analyses of Unidentified Remains Recovered From Europe During World War II

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After attending this presentation, attendees will be aware of accuracies and limitations of historical skeletal analyses of unidentified remains from World War II as they pertain to identification efforts, as well as the value of interdisciplinary collaboration between historians and anthropologists to interpret historical records.

This presentation will impact the forensic science community by identifying patterns in inaccuracies of historical skeletal inventories and biological profile estimations made using methods available in the 1940s.

The goal of this presentation is to compare historical documentation of remains recovery and analysis from World War II with current-day anthropological analyses of the same remains to determine possible sources of commingling and discrepancies in biological profile estimates, which may have originally rendered the remains as unidentifiable.

Defense POW/MIA Accounting Agency (DPAA) anthropologists and historians compared the historical records and current-day forensic anthropology reports of unidentified remains recovered in Europe during or shortly after World War II (n=34). Personnel from the American Graves Registration Command (AGRC) interred, disinterred, and processed the remains in the 1940s — sometimes multiple times — but could not establish identifications; the remains were subsequently buried as unknowns. Due to significant advances in DNA analysis and anthropological techniques, DPAA recently disinterred these unknowns for reanalysis in an attempt at identification. Variables examined in this study include degree of commingling and estimated biological profile.

Of the 34 cases examined, six involved some degree of commingling not documented in the historical record (18%). Commingling in these cases generally involved duplication in minor skeletal elements or fragments of elements. In some circumstances, these cases were buried in the same temporary cemetery or recovered in proximity to one another. Discrepancies between multiple historical dental and skeletal charts were also noted for four other cases (12%), suggesting possible commingling; however, upon accession at DPAA, anthropologists found no evidence of commingling with these cases.

DPAA anthropologists also compared historical biological profile estimates with modern reanalyses. AGRC technicians provided age estimates for 18 of the 34 sets of remains. Historical age estimates overlapped current estimates for 15 cases (83%), 10 of which indicated the method used for estimating age. For cases with discrepancies in age estimates, two of the three were historically overestimated while the other was underestimated. The two overestimations were made by the same analyst. For cases with historical stature estimates (n=28), 24 fell within current estimated stature ranges (86%). Stature was historically overestimated for individuals currently assessed to be of African ancestry and historically underestimated for shorter individuals. These variations arise from differences in formulas used to estimate stature, as historical skeletal measurements were similar to modern measurements. For remains with historical ancestry assessments (n=9), discrepancies occurred in three cases (33%) in which the current assessment was African or probable African, and the historical assessment was European.

Discrepancies between current and previous skeletal analyses may be due to limitations in facilities and personnel during the massive repatriation efforts of the late 1940s. During recovery operations in Europe, AGRC field teams frequently gathered and transported multiple sets of remains (sometimes dozens) from a battlefield at a time. These efforts taxed the capabilities of recovery teams, who often did not complete the necessary documentation of recoveries until days or weeks afterward. The first careful examination of the remains usually did not occur until delivery at a central identification point — some of which processed more than 2,000 sets of remains during the course of a year. Training and experience level for technicians performing the skeletal analyses also varied, as evidenced by two problematic historical age estimates made by the same technician.

Additionally, trends suggest limitations with anthropological methods used at the time. Stature equations used during the war appear problematic for shorter individuals, as well as non-European ancestry groups; however, historical stature estimates for Europeans of average height or taller were consistent with modern stature estimates. Further, historical age estimates that included a justification for the estimate were also consistent with modern age assessments. Although the identified discrepancies can complicate the use of historical analyses for identification and likely contributed to the initial inability to identify these remains, the identified patterns in consistencies and inconsistencies with current analyses can be used to aid in assessing possible candidates for identification for unidentified remains.

Historical Records, Commingling, Biological Profile

A54 An Assessment of Ancestry and Sex Estimation Using FORDISC® 3.1

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After attending this presentation, attendees will better understand the problems with estimating ancestry from skeletal remains and the problems resulting from the approach of linking sex estimation to ancestry estimation.

This presentation will impact the forensic science community by discussing: (1) how cranial measurements are used to illustrate problems with ancestry estimation methods; (2) how a change in terminology from race to ancestry has not solved any underlying problems with the methods; and, (3) how linking sex to ancestry severely compromises the accuracy of sex estimation.[1]

Thomas and colleagues found an "accuracy rate" of 90.9% for ancestry estimation for a sample derived from an historical review of Federal Bureau of Investigation (FBI) records.[2] One of their goals with the historical review was to assess how various methods have provided information that was potentially useful in a forensic investigation; however, as they noted, methodological issues with how they included/excluded cases for analysis (n=99) have likely resulted in the inflation of the true allocation accuracy. In the research presented here, an experimental approach was pursued using FORDISC® 3.1 to provide a better estimate of expected allocation accuracy. A realistic scenario was created for the Detroit-Windsor boarder area to address one question: How often would FORDISC® provide estimates of ancestry and sex that would be useful in a forensic investigation?

Nine standard cranial measurements that are resistant to taphonomic changes were used from a test sample of 105 documented cases from the Terry and Coimbra Collections that reflect the demographic composition of the region and potentially real forensics cases: Western European-born individuals, European Americans, and African Americans. One additional provenienced (Late Archaic) First Nations (Amerindian) cranium from this region was also assessed. Cases were selected by one researcher and analyzed blind by another. The estimated ancestry matched pre-mortem records only 48% of the time and sex was estimated correctly 75% of the time; however, FORDISC® 3.1 does not allow for the separation of ancestry from sex estimation and the software provided correct estimates of sex and ancestry in only 39% of the cases. Thus, in 61% of the cases, FORDISC® provided erroneous information that would have compromised efforts for identification. Furthermore, a well-known problem with discriminant function analysis, the statistical approach used by FORDISC® and other methods, is that it will force an allocation into one of the selected groups even if the unknown is not a member of any of those groups. Posterior and typicality probabilities are intended to deal with this lack of "none of the above" problem by flagging these cases with probability scores of less than 0.05. The results from this research indicated that in most of the erroneous cases, the variously calculated probabilities failed to flag these cases and instead suggested confidence in the erroneous allocations. For example, the 4,000-year-old Late Archaic cranium was classified as "Black Male" with a posterior probability of 0.314, and three variously calculated typicality probabilities ranging from 0.960 to 0.963.

The results from this research indicate that renaming of race as ancestry methods does not solve any problems with these methods and linking sex estimation to ancestry undermines sex estimation.

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Biological Profile, Ancestry Estimation, Sex Estimation

A55 A Multidisciplinary Approach to Identification and Repatriation at Mount Austen, Guadalcanal

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After attending this presentation, attendees will understand how historical, spatial, archaeological, anthropological, genetic, and isotopic analyses are synthesized by the Defense Prisoner of War/Missing in Action Accounting Agency (DPAA) personnel to streamline identifications and repatriations from an extensively commingled Solomon Islands battlefield site.

This presentation will impact the forensic science community by illustrating how a multidisciplinary approach can lead to identifications and repatriations within a Commingled Human Remains (CHR) project. This multidisciplinary approach specifically provides a useful method for sorting CHR originating from numerous nations.

From December 1942 to February 1943, Allied troops attempted to dislodge an entrenched Japanese position at Mount Austen, Guadalcanal. As a result, Japanese, American, Australian, New Zealander, Fijian, and Solomon Islander combatants overlapped spatially. Many of these individuals experienced fragmentation-causing skeletal trauma, and burials, which may have prevented further commingling, were not performed. Additionally, the Mount Austen site is now more than 75 years old and current occupants modify the site daily. The general area is also characterized by steep slopes, and downhill erosion exacerbates fragmentation and commingling of human remains.

While all large and fragmentary CHR projects endeavor to re-associate numerous portions of multiple individuals, the Solomon Islands Unidentified Project (SUP) specifically struggles to distinguish American from non-American remains, often without the use of morphological traits due to poor preservation. To efficiently and economically approach this challenge, a process was developed to manage the large quantities of evidence recovered from this battlefield, streamline American identifications, and repatriate the remains of combatants from other countries.

A detailed review of historical and archival documents pertaining to the Mount Austen battlefield loss was first completed by DPAA historians. From these efforts, a list of missing individuals was generated — 19 United States service members among at least 900 unaccounted-for Japanese. Battle timelines and troop movements were next georeferenced to better understand the context of the loss incident. DPAA archaeologists use this spatial data to target the most likely areas where missing United States members could be, and subsequent surveys and excavations have resulted in seven large accessions of human remains; there are also more than 25 additional accessions in the laboratory from unilateral turnovers allegedly related to the same loss incident.

Once evidentiary materials are received by the laboratory, the project anthropologist separates biological from material evidence, administratively removes non-evidence, designates group remains, marks remains with provenience data, sorts skeletal elements by size, reconstructs, makes pair-match determinations, nominates for DNA guided by the minimum number of individuals, builds individuals by genetic sequence, synthesizes provenience data by individual, and considers the haplogroup of each genetic sequence. An East Asian sequence is repatriated to Japan, while a sequence matching a United States Family Reference Sample (FRS) is processed for identification. A Caucasian or inconclusive sequence not matching an FRS is nominated for isotopic analysis as isotopic composition may aid in separating an American from a non-American service member.

After various results are consolidated, the project anthropologist provides feedback for the Mount Austen site archaeologists. Specifically, they identify exact locations where possible United States remains have been found to increase the likelihood of recovering additional American remains during the next excavation.

At present, all SUP remains currently in the DPAA Laboratory have been analyzed according to this method with numerous repatriations performed, several possible American sequences identified, and specific battlefield areas targeted for additional recovery operations. Through a combination of historical, spatial, archaeological, anthropological, genetic, and isotopic analyses, efforts to locate and recover United States remains from the large, commingled Mount Austen battlefield site are progressing.

The views of these authors do not reflect those of the Department of Defense or the Unites States government.

Commingled Human Remains, Fragmentary, Identification

A56 A Test of the (hu)MANid Classification Software on a Sample of United States White and Black Mandibles

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After attending this presentation, attendees will understand how the newly created (hu)MANid software performed when classifying sex and ancestry on a large sample of mandibular data.

This presentation will impact the forensic science community by testing the performance of this new software to evaluate its utility in forensic cases and by providing documented accuracy rates.

A recent publication by Berg and Kenyhercz describes a newly available statistical software program that can be utilized by practitioners to estimate sex and ancestry from the human mandible.[1] The software program, (hu)MANid, is available through the web or can be downloaded to be run with the statistical program R. It allows the researcher to input a combination of metric and morphoscopic variables from the mandible, from which either a Linear Discriminant Analysis (LDA) or Mixture Discriminant Analysis (MDA) can be performed to compare and classify the unknown mandible into one of the 15 reference groups within the program's database (total n~1750). Given the recent release of this software, there are not yet any published validation studies analyzing the accuracy of the assigned classifications.

In this study, morphoscopic and metric data were collected from a sample of 230 adult mandibles from United States White and Black males and females, and analyzed using the (hu)MANid web-based software program. Bigonial width, bicondylar width, and mandibular length had previously been collected from the physical specimens. The remaining metric variables (mandibular body height, chin height, minimum ramus breadth, maximum ramus height, and dental arcade width) and five morphoscopic variables (chin shape, shape of the lower border of the mandible, ascending ramus profile, gonial angle flare, and posterior ramus inversion) were collected following the descriptions provided in the "Definitions and Diagrams" tab of the software program from 3D virtual models of the specimens. The virtual mandibular models were created from 3D surface scans collected with a NextEngine® desktop scanner from individuals in the Hamann-Todd, Terry, and Bass Donated skeletal collections. All metric and morphoscopic data were collected using GeoMagic® Studio. The data collected from each specimen were run through the (hu)MANid program using the two different methods (LDA and MDA), first with all variables included, then using a Forward Wilks stepwise procedure, and both were compared to all 15 modern comparison groups and also to only the United States White and Black 20th-century samples. Altogether, eight separate analyses were run on each of the 230 specimens. The resultant group classifications were compared to the actual sex, ancestry, and combined ancestry/sex of the individual, and percentage of correct classifications was calculated.

In all cases, the MDA analyses outperformed the LDA analyses, and slightly better accuracy rates were obtained when all variables were entered (compared to using a Stepwise procedure). When performing an MDA on all available variables and comparing to all 15 modern reference groups, sex was assigned correctly in 76% of individuals, ancestry was correct in 52%, and both sex and ancestry were correctly estimated in 40%. Although these values may appear low, when considering that they were compared to 15 groups and the *a prior* classification rate would be less than 7%, 40% is a major improvement. An accuracy rate of 76% for sex estimation is also higher than most reports of sex estimation from the mandible, although lower than reported values for sex estimation from other regions of the skeleton. When the comparisons were limited to the United States White and Black reference groups, accuracy rates did not increase significantly: MDA on all variables resulted in 75% correct sex, 57% correct ancestry, and 41% correct sex/ancestry group. United States Blacks were much more likely to be assigned to the correct sex/ancestry group (77% correct, compared to only 7% correct for the United States White individuals). Overall, the (hu)MANid program performed relatively well, considering that all input variables are from the mandible. Although the program still needs to be tested on a sample of modern forensic cases, it exhibits great potential as a sex and ancestry classification tool.

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Sex Estimation, Ancestry Estimation, Mandible

A57 Comparing Socio-Economic and Population-Level Differences and Quantifying Their Impact on Subadult Age Estimations

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The goal of this presentation is to inform attendees on the impacts of socio-economic and population differences on growth and development markers and, subsequently, subadult age estimation. A comparison of diaphyseal dimensions and dental formation from individuals between birth and ten years of age from two economically and genetically diverse countries will advise forensic practitioners on the potential utility of global, rather than population-specific, models.

This presentation will impact the forensic science community by providing insight into the developmental onset of economic and population differences and at what age the differences need to be considered prior to estimating a subadult biological profile.

Subadult age estimation is usually the sole contribution to the subadult biological profile. It is regularly stated that there need to be population-specific methods; however, it is not known at what age the population differences become apparent, or at least influential, to age estimations. Similar to population differences, another concern is the socio-economic status of reference groups. Growth data is recognized as being heteroscedastic, meaning that as individuals increase in age, the variation also increases. Based on the nature of heteroscedastic data, if there are minimal differences in the first few years of life, it should be possible to build global models, or apply models derived from one population and to others. The purpose of this study is to compare the skeletal and dental formation of two economically and genetically diverse populations as a means to determine if differences in growth and development exist and, if so, to quantify their magnitude.

Long bone lengths and dental formation stages for the maxillary and mandibular first and second molar were collected from a modern sample of subadults from South Africa and the United States. Individuals were between the ages of birth and ten years of age. First, simple visualizations were used to display the growth and development trends for each age indicator and variable per population. Second, point estimates were created for each individual variable using multivariate adaptive regression splines and age as the response variable. Ranges and means of the differences between the fitted values and true chronological age provided insight into the accuracy of each variable. By visualizing the discrepancies between the estimated ages based on dental formation and long bone length, we can also obtain an idea of which variable is more or less sensitive to the environment and/or population differences.

Visual comparisons confirmed very similar trends for long bone lengths and molar development, especially in the youngest individuals (<5 years). Differences between the groups increase as age increases. In the youngest ages, South African subadults have longer diaphyseal dimensions than their United States counterparts. As age increases (~3-4 years) of age), the median values become more similar and eventually the United States subadults (>6 years) exceed the South African children. Mean differences for the estimated and true chronological age in the regression models is close to zero for both South African children and United States children for the femur diaphyseal lengths (-0.02, -0.003, respectively) and the first molar (-0.04, -0.03, respectively). No differences were found when dental age and skeletal age were visualized to explore differential sensitivities; there was an even spread of predicted age both below and above the ideal fit line.

The findings of this study prove that minimal skeletal and dental differences are expressed in economically and genetically diverse young, modern subadults, which suggests anthropologists could apply methods developed on one population to other populations. The results will substantially impact the field, considering the paucity of modern skeletal remains around the world from which to derive population-specific methods. Furthermore, by increasing the application of scientifically and statistically sound techniques, we can also increase the number of positive identifications.

Diaphyseal Dimensions, Dental Formation, MARS

A58 Age Estimation of Hispanic Children Using the London Atlas

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After attending this presentation, attendees will be familiar with the technique of estimating the age of children using dental radiographs. They will also be introduced to new population data for age estimation using The London Atlas of Tooth Development and Eruption.[1]

This presentation will impact the forensic science community by contributing a new data set to the bank of population-specific age estimation data. The accuracy of the results reinforces the principle that age estimation can be pragmatically accomplished using a universal tool rather than requiring population-specific methods.

Many different age estimation methods exist and studies have shown varying levels of accuracy, depending on the population group tested. The goal of this study was to test the accuracy of The London Atlas for age estimation of Hispanic children and to determine if there is any difference in age accuracy between males and females.

This study was a retrospective cross-sectional review of records of healthy Hispanic children from ages 6 to 15.99 years who had digital panoramic radiographs taken at the University of Illinois at Chicago College of Dentistry, Chicago, IL, between January 1, 2000, and January 15, 2016. A report of all patients marked as of Hispanic ethnicity or as Spanish speaking who had a panoramic radiograph taken between the ages of 6 and 16 years since January 1, 2000, was generated. After the list was generated, it was randomized to remove any order and was then screened by the primary investigator. The exclusion criteria were radiographs that were unclear and/or distorted, and patients who had hypodontia, hyperdontia, gross pathology (e.g., taurodontism, microdontia, amelogenesis imperfecta, dentinogenesis imperfecta, tumors, abscesses, fractures, etc.), previous orthodontic treatment, and/or severe malocclusion. Chronological age was blinded from the primary investigator and age estimation was performed using The London Atlas of Tooth Development and Eruption on the left side of both upper and lower jaws. Inter- and intra-examiner reliability tests were performed on 34 randomly selected radiographs.

There were 332 panoramic radiographs evaluated. In all age groups, 34 radiographs (from 17 males and 17 females) were reviewed, except for the age bracket 6–6.99 years, for which only 26 radiographs meeting the exclusion criteria were available. The intra-examiner comparison yielded a Cohen's Kappa of 0.793 and the inter-examiner comparison yielded a Cohen's Kappa of 0.764, which indicated good reliability in the use of The London Atlas. The mean age estimated of the entire sample by The London Atlas (11.44 years) was greater than the mean chronological age (11.09 years), which was statistically significant ($P < .001$). The mean difference between chronological and estimated ages for males was 0.30 years and for females was 0.40 years, but the difference between sexes was not significant ($P = .324$). One hundred sixty-two radiographs (49%) were estimated to the exact age interval, while 45 (14%) were under-estimated and 125 (38%) were over-estimated. Two hundred and forty radiographs (72%) were estimated to a value within one year of the actual age.

There was no difference in age estimation prediction accuracy between Hispanic males and females, but an age overestimation was seen. The London Atlas accuracy is suitable for use in forensic investigation.

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Age Estimation, Radiographs, Children

A59 A Geometric Morphometric Analysis of Contemporary Hispanic Populations From Mexico and Colombia

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After attending this presentation, attendees will have gained a more precise understanding of geometric morphometric analysis and its applicability to ancestry estimation in Hispanic populations.

This presentation will impact the forensic science community by illustrating that a greater degree of classification beyond Hispanic may be possible using geometric morphometric methods that provide a statistically grounded model to differentiate two Hispanic populations.

In contexts such as natural disasters, humanitarian efforts, and forensic investigations, the timely and accurate development of the biological profile is vital to the identification of decedents. An essential but problematic aspect of the biological profile is the estimation of ancestry. Geometric morphometric analyses are germane to forensic ancestry estimation because they employ a statistically sound approach, derived from W.W. Howells' well-defined landmarks that focus on craniometric shape differences independent of size; however, many of the current methods pose a challenge because the term "Hispanic" is frequently utilized to represent world-wide Hispanic populations with the untested assumption that all individuals from North, South, and Central America, and the Caribbean are skeletally homogenous.[1-3] Additionally, comprehensive studies of Hispanic populations are severely limited due to the lack of robust reference data. The continued use of unrefined methods is problematic when employed in areas where remains are likely to be of Hispanic origin. As such, this study tests the hypothesis that two contemporary populations from Latin America (Colombia and Mexico) will demonstrate significant craniometric shape variability and provides population-specific forensic ancestry estimation methods.

This study explores geometric morphometric population variation and differentiation in 422 Colombian and Arizona Migrant individuals, 18 to 102 years of age. The Colombian sample is composed of 191 individuals from the late 20th and early 21st century (University of Antioquia). The Migrant sample is composed of 231 border crossers from the Pima County Office of the Medical Examiner (PCOME) in Tucson, AZ. The PCOME reports that the majority of migrants who die along the United States-Mexico border are from Mexico, and, as such, this group will represent a contemporary Mexican population.[4] MorphoJ, a program written for geometric morphometric analyses, was used to interpret the data in order to understand shape variation between the samples.[5] A Generalized Procrustes Analysis (GPA) was performed to scale, rotate, and transform the data into a common coordinate system. Males and females were pooled together in order to maximize the sample size. Subsequently, a Canonical Variate Analysis (CVA) was run in order to maximize the differences among the groups and isolate the key features contributing to the variation.

Mahalanobis distances were produced from the CVA and indicated statistically significant differences between the Migrant group and the Colombian group ($p < .001$). The plot produced by the CVA graphically displays the separation between the Colombian and Migrant group. The results support the hypothesis that morphological differences exist between the Colombian and Migrant decedents, who are more likely Mexican nationals. Therefore, a greater level of classification accuracy beyond "Hispanic" is possible using geometric morphometric methods.

This study supports the notion that a higher level of forensic identification may be possible using a geometric morphometric approach for Hispanic populations. Both the Colombian and Migrant samples demonstrate variability in form that underscores the importance of introducing geometric morphometric methods into the forensic toolkit. Moreover, this study demonstrates that Hispanic populations are not skeletally homogenous due to unique admixtures from the three primary ancestral groups (European, African, and Native/Asian) and differing population histories. Therefore, within a statistical framework, geometric morphometric methods can derive accurate identifications by assessing ancestry in a meaningful way using the morphological variation present in the human cranium.

This research was funded by Boston University's Department of Anatomy and Neurobiology.

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Hispanic (Mexico and Colombia), Ancestry Estimation, Geometric Morphometrics

A60 The Frequency of Asymmetry in Non-Metric Craniofacial Trait Expression and Its Effect on Ancestry Assessment

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After attending this presentation, attendees will better understand the range of asymmetry within a forensic population.

This presentation will impact the forensic science community by emphasizing the importance of considering asymmetry when assessing ancestry and determining if the current method is the proper way to deal with asymmetry of traits.

The purpose of this study is to better understand the frequency of asymmetry in the craniofacial traits used to assess ancestry and to determine the effect of asymmetry, if any, on the results of the assessment.

Ancestry assessment is important in the creation of a biological profile of an unknown decedent. Non-metric trait frequencies have been compiled over time within forensic anthropology and are commonly used to analyze ancestry trait expression.[1] One of the most popular studies conducted on trait frequencies between and within groups was published by Hefner.[2,3] It has been established that many craniofacial traits are asymmetric.[4,5] Although Hefner briefly addresses asymmetry, noting that some traits (i.e., Inferior Nasal Aperture (INA)) exhibit it, and suggests scoring the left side in these cases; he does not go into the effects it may have on ancestry.[7]

Identified individuals curated at the C.A. Pound Human Identification Laboratory were assessed for the 11 non-metric traits described in Hefner. Of the 40 individuals, 15 were female and 25 were male. Ten of the 40 were of primarily African ancestry, 29 of primarily European ancestry, and one of primarily Asian ancestry. Six of the 11 traits (Anterior Nasal Spine (ANS), INA, Malar Tubercle (MT), Nasal Overgrowth (NO), Transverse Palatine Suture (TPS) and Zygomaticomaxillary Suture (ZS) have the possibility of asymmetry due to being bilateral. The right and the left of each of these six bilateral traits were scored independently. A final ancestry assessment was made for both the left and right sides of each individual independently and, based on a simple majority of ancestry group trait expressions, each expression was assigned to the group with the highest frequency of that trait expression; for the purpose of this research, MT was not used in the final ancestry assessment due to incorrect frequency data in the Hefner 2009 article.[6] The right- and the left-sided final ancestry for each individual was compared.

The results indicate that more than half of the individuals (22) had at least one asymmetric trait for a total of 26 asymmetric traits. Four individuals had two asymmetric traits; none had more than two. Thirteen of the 26 asymmetries (50.0%) were found in the ZSs, four in the INSs, (15.4%), three in the NOs (11.5%), three in the MTs (11.5%), two in the ANS (7.7%), and one case was found in the TPS (3.9%). Of the 22 individuals with asymmetry, only three (14%) were classified into different ancestry groups for the right versus the left. In each of those three cases, one side resulted in Asian ancestry classification and the other side as European classification. With two of the cases, the left side was the correct ancestry, and in the third, it was the right side. All three of these individuals self-identified as White. Using a z-test, no significant difference (p=115) in the frequency of asymmetry between the ancestry groups was found. Males and females exhibited asymmetry with approximately similar numbers (53% in females and 56% in males).

A tentative conclusion can be made that asymmetry occurs most often in the ZS and least often in the TPS. Earlier studies concluded that the ANS had only small deviations.[7] In this study, only two individuals had ANS deviations large enough to warrant a different trait expression in the right than in the left. This study suggests asymmetry can affect non-metric ancestry assessment, although this is uncommon. This finding is in line with other studies, which have noted that trait asymmetry is minimal.[4] Using the left side when analyzing bilateral traits worked two-thirds of the time and there does not appear to be a reason to discontinue using this methodology. Note that the sample size is small and that the ancestry assessment was simplified from what it would be in an actual forensic case. Further testing will be needed to fully understand the degree of asymmetry in craniofacial traits and their effect on ancestry.

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Asymmetry, Craniofacial Traits, Ancestry

A61 Non-Metric Traits of the Mandible in Ancestry Estimation

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After attending this presentation, attendees will be more knowledgeable on the subject of ancestry estimation via non-metric traits of the mandible, briefly reviewing similar research, a discussion of metric versus non-metric methods of assessment, and examining why the mandible holds potential as an ancestry-discriminating bone.

This presentation will impact the forensic science community by examining and discussing the usefulness of eight non-metric traits of the mandible in ancestry estimation and by suggesting traits to be considered in future research.

Ancestry represents a fundamental aspect of the biological profile. Therefore, when seeking to identify unknown human remains, ancestry plays a crucial role in minimizing the number of potential missing persons matches.[1,2] However, ancestry is routinely cited as the most difficult aspect of the biological profile.[2-6] As this assessment relies heavily upon the use of non-metric skeletal traits that are often clinal in nature, categorizing and standardizing these traits remains a challenge for forensic anthropologists.[4]

The traits most useful in ancestry estimation are housed within the mid-facial cranium.[1-3,7-9] This small and particularly fragile region of the skeleton has received substantial attention and research efforts with regard to ancestry estimation.[9] The mandible and post-cranial elements are less often used in ancestry estimation and, accordingly, the literature on these regions is less robust. As the mandible is more durable than many bones of the mid-face, it is more likely that this bone will be recovered in medicolegal investigations and can be assessed for ancestry.[9] The mandible also offers a plethora of traits, which can be helpful when the bone is found fragmented or incomplete. Furthermore, the mandible creates a functional unit with the mid-facial cranium, the most reliable region for ancestry estimation. As such, the mandible may also house traits fruitful in this assessment.

This study investigates the expression of eight non-metric traits of the mandible, including: pinching of ascending ramus, chin shape, chin profile, undulation of inferior border, shape of sigmoid notch, gonial eversion, height of coronoid process, and rocker jaw. These traits were scored among a sample of 470 3D surface scans, which originated from individuals of African, European, and Native American ancestries. Two-tailed *t*-tests were used to assess the significance of scores among pooled sexes, pooled ancestries, collections (within each ancestral group), and sex within each ancestry. A subset of these *t*-tests was supplemented with chi-square tests of independence to further assess the significance of these traits.

The results of these analyses suggest that pinching of ascending ramus, chin shape, chin profile, and undulation are useful ancestry discriminating traits (chi-square p-values: 0.045, <0.001, <0.001, <0.001, respectively). In this study, Europeans stood apart in regard to chin shape and chin profile, with a square/bilobate chin shape and protruding chin profile occurring most frequently among this population (53.9% and 54.6%, respectively). Similarly, Native Americans exhibited fewest occurrences of undulation (combined slightly undulated and undulated frequencies at 41%), supporting existing notions of Native Americans displaying very robust mandibles, and Africans far out-numbered Europeans and Native Americans in incidences of round chin shapes (68.9%) and displayed the greatest number of receding chin profiles (24.3%).

As pinching of ascending ramus, chin shape, chin profile, and undulation revealed significant results in *t*-tests and chi-square tests in this study, these variables should be investigated in future research. This study serves to indicate that morphological differences do exist between African, European, and Native American ancestral groups and encourages additional future research on non-metric traits of the mandible.

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Ancestry Estimation, Non-Metric Traits, Mandible

A62 Using Basicranial Landmarks to Estimate Ancestry in an American Sample

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After attending this presentation, attendees will appreciate the utility of cranial base landmarks in identifying human skeletal remains.

This presentation will impact the forensic science community by providing an alternate metric method for estimating ancestry from fragmentary crania.

The cranium is one of the most informative and widely used areas of the human skeleton in establishing the sex and ancestry of human remains. Standard methods of cranial morphometrics in forensic anthropology include the use of landmarks and features on the ectocranial surface of the vault and the face.[1] These methods largely require intact crania. In a forensic setting, the cranium is often damaged and the traditionally used landmarks are obscured or destroyed.[2] When fragmentary or unable to be reconstructed, the cranium is often not used for identification purposes in these cases.[3-5] As a result, potentially valuable information contained within the remaining unexamined portions of the cranium is discarded. For example, in the case of fatal fires, the cranium is often deemed unusable because the typically evaluated areas for anthropological analysis (vault, face, and mandible) are damaged or missing; however, the cranial base is protected by the neck musculature, and oftentimes presents with fragmentary remains. The cranial base is a relatively underused region of the cranium in forensic anthropological analysis and is only recently being rigorously evaluated for its utility in the prediction of ancestry and sex.[6-9] This project tests the hypothesis that there are differences in cranial base shape between American White and Black individuals by comparing cranial base landmark data with standard ectocranial landmark data.

A total of 73 landmarks of the endocranial and ectocranial surfaces were registered using a MicroScribe® G2X portable coordinate measuring machine from 245 adult crania in the Hamann-Todd Human Osteological Collection and the William M. Bass Donated Collection. Landmarks were divided into four subsets: endobasicranial (18 landmarks), ectobasicranial (18 landmarks), all basicranial (36 landmarks), and ectocranial (43 landmarks). The first three subsets include uncommon landmarks, while the ectocranial set includes landmarks commonly used in a FORDISC® analysis.[10] First, landmarks were subjected to a generalized Procrustes analysis to bring them to a common coordinate system. Second, a discriminant function analysis with cross-validation was performed to assess the efficacy of landmark subsets in accurately classifying the crania. Finally, sensitivity, specificity, negative predictive value, and positive predictive value were calculated to further assess individual model performance. All analyses were performed in MorphoJ v1.06d with α=0.05.[11]

All discriminant models exhibited statistically significant differences in mean landmark configuration between ancestral groups ($p < 0.001$). The ectocranial subset had the highest classification rate of 88.6%, followed by ectobasicranium (82.0%), basicranium (78.8%), and endobasicranium (77.9%). Overall, the models have higher specificity (range 83.9%-91.6%) than sensitivity (range 67.3%-83.3%) and are able to more accurately classify White individuals than Black individuals. Positive predictive values have a range of 71.6%-85.2% and negative predictive values have a range of 79.6%-90.4% with the ectocranial set performing best.

Overall, landmark configurations exhibit a longer and narrower base in the Black sample compared to the White sample. The most anterior cranial base landmark, the foramen cecum, is displaced posteriorly in White crania, and the internal occipital protuberance is displaced anteriorly, resulting in a shorter cranium in White compared to Black crania. The paired landmarks, including the stylomastoid foramen, endasterion, and jugular foramen, as well as the sigmoid sulcus point, which is the intersection between the posterior lip of the sigmoid sulcus and the occipitomastoid suture, all are more laterally displaced in White crania relative to Black.

These findings suggest that both midline and paired landmarks in the cranial base are useful in estimating ancestry, as corroborating evidence, or especially in cases in which the more commonly used areas of the cranium are not available for analysis. Although traditional ectocranial landmarks provide the highest classificatory rate, basicranial landmarks from the ectocranial and/or endocranial surfaces can be used to estimate ancestry and contribute to the construction of the biological profile. Forensic anthropologists should consider recording basicranial landmark coordinates when analyzing fragmentary cranial remains.

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Ancestry Estimation, Cranial Base, Geometric Morphometrics

A63 Craniometric Variation of Modern Asian and Hispanic Individuals Using Multivariate Analysis

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After attending this presentation, attendees will understand how cranial morphologies of certain modern Asian and Hispanic individuals compare to each other, and that population histories of different ancestral groups influence their cranial morphologies.

This presentation will impact the forensic science community by providing a deeper understanding of how Asian and Hispanic groups relate based on craniometric analysis.

According to Dudzik and Jantz, there have been a high number of misclassifications between Hispanic and Asian populations in the ancestry estimation software FORDISC®, as reported by forensic anthropologists.[1] In order to avoid mistaking an unknown Asian individual as Hispanic, or vice versa, it is beneficial to conduct further research on cranial morphological comparisons between these two groups with shared ancestry. This study primarily examines how modern Asian and Hispanic groups classify when compared with each other, as well as with other groups. Further, this study investigates any patterns of misclassification observed among and within the population groups used. Finally, this study examines how the samples used classify when ran with reference sample groups currently available on ancestry estimation programs such as FORDISC® 3.1.

The Asian samples included male and female individuals belonging to ten different population groups from East and Southeast Asia (*n*=575). All Asian data were either provided by Dr. Michael Pietrusewsky or obtained from the Howells craniometric database. The Hispanic samples in this study originated exclusively from Mexico (*n*=114) and, thus, are considered one population group. All Hispanic data were provided by Kate Spradley, PhD. Additional samples from the Forensic Anthropology Data Bank (FDB) were included in a second analysis with the initial sample pool to investigate how well Asian and Hispanic samples would classify when other ancestral groups were added for analysis. FDB samples (*n*=524) included American Whites, American Blacks, and Guatemalans. This study utilized 27 cranial Interlandmark Distances (ILDs), some of which are not standard distances used in FORDISC® 3.1; however, they were based on their overall representation of cranial morphology. The ILDs were analyzed using Discriminant Function Analysis (DFA) and canonical variates analysis to assess the similarities and differences in cranial morphologies of the samples, which were separated by sex. Last, 20 male samples were chosen randomly out of the original 11 Asian and Hispanic groups (*n*=588) and ran in FORDISC® 3.1. The purpose was to investigate how the samples from this study would classify when run with the Asian and Hispanic reference groups that are currently available to forensic anthropologists.

Results demonstrated that cranial morphologies of Asian and Hispanic groups are distinguishable. Hispanics represented by individuals originating from Mexico generally exhibit wider faces and nasal apertures compared to most East and Southeast Asians. Some Asian groups, such as North Japan, Ainu, and Philippines, also exhibit similar features to Mexicans. Overall, Mexicans display wider faces and nasal apertures than most Asian groups. Results from the second analysis yielded lower correct classification rates when run with the FDB samples (78.6% without and 71.9% with FDB groups).

Further, Hispanics are generally more similar to American Blacks and Whites than most Asian groups, which contradicts the findings of Dudzik and Jantz.[1] This may be attributed to the utilization of non-standard ILDs in this study, which is consistent with the findings of Spradley and Jantz that non-standard ILDs yield more accurate ancestry estimations, especially for complex groups.[2] Thus, it may be worthwhile to include non-standard ILDs when estimating ancestry for Asians and Hispanics in future research. Differences in cranial features help distinguish Asians and Hispanics from one another. Quantifying the influence that different cranial ILDs have on ancestral group assignment aids in understanding which facial and cranial features are important to differentiate Asians from Hispanics.

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Ancestry, Asian, Hispanic

A64 Age Estimation From the Measurement of Open Apices in the Developing Permanent Dentition

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After attending this presentation, attendees will better understand a relatively novel method of age estimation involving the measurement of apical width and tooth length of the developing permanent dentition.

This presentation will impact the forensic science community by providing novel Western Australian specific age prediction models.

This study involves establishing age estimation models for juvenile individuals (<18 years of age) from a Western Australian population based on the measurement of open root apices of the developing permanent dentition following the Cameriere method.[1] Current methods of juvenile age estimation primarily involve quantifying the timing of skeletal growth and dental development, which are known to occur in a predictable sequence; the latter is also not as readily affected by malnutrition and other environmental factors as bone growth and development. The specific goals are as follows: (1) statistically quantify intraobserver agreement of apical width measurements in Orthopantomograph (OPG) images; (2) determine the accuracy of the Cameriere method in a sample of Western Australian juveniles; and, (3) develop an age estimation model specific to a Western Australian population based on the Cameriere methodology.

The study sample comprises 187 OPGs of juveniles (97 male and 90 female) aged 3 to 14 years from a contemporary Western Australian population. The OPG scans were acquired from the Western Australia Department of Health Picture Archiving and Communication Systems (PACS) database. The OPG scans were visualized using ImageJ and OsiriX®; apical width and tooth length measurements were acquired from the developing permanent left mandibular teeth (except the third molars) in each OPG scan. These measurements were entered into the Italian Cameriere linear regression formula in order to estimate age.[1]

Prior to primary data collection, accuracy and precision were quantified to ensure all measurement data were repeatable and reliable. Measurement error was quantified using the Technical Error of Measurement (TEM), relative Technical Error of Measurement (rTEM), and coefficient of Reliability (R). The apical width and tooth length measurement data was analyzed with Statistical Package for Social Science (SPSS) and Excel®. The mean differences between actual and estimated age were calculated; paired sample *t*-tests were performed to assess the statistical significance of the differences. Subsequently, a series of stepwise and enter-method regression analyses were performed to establish which variables significantly contribute to accurate age prediction in the Western Australian sample.

Analysis of the accuracy of the Cameriere age prediction model in a Western Australian population revealed that the difference between actual and estimated age was statistically significant in both males and females (p <0.001); the mean difference between actual and estimated age was 0.803 years in males (Standard Error of the Estimate (SEE ±1.295 years) and 0.587 years in females (SEE ±1.312 years). Individual and pooled-sex Western Australian specific age prediction models were developed based on the quantification of apical closure and tooth length measurements. The individual-sex model had an R-value of 0.966 and SEE of ±0.875 years, whereas the pooled-sex model had an R-value of 0.963 and SEE of ±0.909 years. In the analysis of the accuracy of the individual-sex model when applied to the Western Australian holdout sample, the mean difference between actual and estimated age was 0.318 years in males (SEE ±1.458 years) and 0.636 years in females (SEE ±1.347 years). The associated SEE for the pooled-sex model was ±1.548 years. The accuracy of the age prediction models was thus deemed acceptable relative to extant methods for estimating age in the juvenile skeleton. The present study has contributed novel models of age estimation using the developing permanent dentition for Western Australian juvenile individuals and also provides a basis for further research in this area.

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Forensic Anthropology, Juvenile Age Estimation, Dentition

A65 Sensitivity Analysis of Craniometric Measurements and Modeling Techniques to Assess Impact of Measurement Error on FORDISC® Results

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After attending this presentation, attendees will better understand how the magnitude of variation in different craniometric variables affects the results of FORDISC® analyses.

This presentation will impact the forensic science community by challenging the current rule-of-thumb tolerance for measurement error in craniometrics and highlighting the use of mathematical modeling techniques in assessing the sensitivity of metric analyses.

This research uses iterative manipulation of craniometric measurements to assess the impact of measurement error on FORDISC® classification of sex and ancestry.[1]

Metric analysis of skeletal material is an important tool for forensic anthropologists assessing the biological profile of unknown decedents. As measurements constitute the fundamental data for these analyses, recent efforts have focused on standardizing definitions and procedures to produce the highest degree of precision and accuracy when taking measurements. Part of this undertaking has involved investigating which measurements are more problematic for repeatability, as assessed through both interobserver and intraobserver measurement error studies; however, the impact of the magnitude of measurement error on analytical results has not been well captured. Instead, the widely used rule of thumb for skeletal metrics is an arbitrary ±2mm tolerance level for all measurements, even though the size of standardized measurements from an individual can vary by an order of magnitude within both the skull and the postcrania. The discriminant function program FORDISC® 3.1 is routinely used to assess sex and ancestry from the cranium; it remains unclear how measurement error affects the results of these analyses.

Thirteen standard craniometric measurements were taken from 30 skeletal individuals of varying demographics curated at the C.A. Pound Human Identification Laboratory. To establish a baseline FORDISC® classification for each individual, all 13 original measurements for a given individual were entered into the program, and a discriminant function analysis was run without transformations against all groups in the Forensic Anthropology Data Bank. For this project, whether the baseline classification reflected the decedent's true demographic group was unimportant, as the goal was not to assess the accuracy of the program itself. For each individual, each original measurement was then varied in 1mm increments — up to 5mm greater than and less than the original measured value — and the FORDISC® analysis re-run in each iteration. Only one measurement was altered at a time, resulting in 131 FORDISC® runs per individual. The classification, Mahalanobis distance, posterior probability, and type F typicality results of all runs were recorded and compared. Deviations in classification from the baseline classification were recorded as "disparate classifications." To complement this analysis, Latin Hypercube Sampling (LHS), which varies all measurement values orthogonally in an N-dimensional hyperspace within the range permitted by FORDISC® and generates a new FORDISC® analysis for each iteration, was performed on one individual. The LHS method is widely used to test the general sensitivity of measurement variables to variation due to the few number of sample points needed to produce high-quality results.

Average disparate-classification rates by measurement when each measurement is varied by ±2mm (in accordance with the rule of thumb) ranges from 0%–33%, with cranial base length (basion-nasion length) creating the most deviation and the vault chord lengths creating the least deviation in classification. Additionally, the variance of the posterior probabilities and typicalities about the values generated using the original measurements is asymmetrical, but without a general, clearly defined magnitudinal or directional bias. The LHS sampling analysis revealed that midfacial measurements, especially when varied simultaneously, are the most sensitive to variation, even when sensitivity comparisons are scaled by size. Overall, the findings of these analyses suggest that accepting measurement error of 2mm is inappropriate for some craniometric measurements, particularly the relatively smaller breadths and heights measured in the midface. Conversely, the magnitude of within-demographic group variation for other measurements, particularly those in the vault, subsume even large amounts of measurement error, indicating that these are more tolerant measurements. Moving forward, the use of modeling techniques, such as those used above, will allow for the creation of empirically tested error ranges unique to each craniometric measurement, which satisfies *Daubert* criteria. FORDISC® is a powerful tool, and the more that can be understood concerning how different factors influence the outcomes of its analyses, the more successful and reliable forensic practitioners will be.

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Metric Analysis, Error Analysis, Latin Hypercube Sampling

A66 The Importance of Sitewide Taphonomic Assessments for Highly Fragmented, Comingled, and Heat-Altered Remains From Mass Graves

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The goal of this presentation is to illustrate the importance of pairing a careful archaeological recovery with a detailed taphonomic analysis in forensic anthropology. Specifically, the case of a mass grave from Mexico where burn patterns of highly fragmented human remains were used to map relative levels of heat alteration across the entire site will be addressed. This analysis allowed for questions of site formation to be addressed, not only through examination of the site itself, but also of the human remains.

This presentation will impact the forensic science community by explaining how this case and method of site analysis are important in that they exemplify the importance of human remains in assessing the formation of a forensic scene. Examination of remains in forensic settings typically takes place at the level of the fragment, skeletal element, or individual; however, when methodically collected and catalogued, human remains can contribute to our understanding of the larger site as a whole.

A detailed taphonomic analysis is critical to any forensic anthropological investigation. Unlike other characteristics typically assessed by the forensic anthropologist, such as the biological profile, the amount of information gleaned from a taphonomic analysis is directly related to the resolution of the preceding archaeological recovery. Cases in which the context and association of each fragment are maintained and proveniences are known offer a unique opportunity to examine site-wide patterns of postmortem alteration.

Taphonomy is a relatively new field of research, its name dating back less than a century.[1] In the time since, some aspects of postmortem skeletal changes have become relatively well understood. In the case of heat modification, clear patterns to alteration have emerged, and the beginnings of standardized characteristics to catalog and interpret are coalescing.[2] Data concerning the level of heat alteration is typically used to identify the pattern of burning on one individual. This cannot always be conducted in highly fragmented and comingled cases; however, the site can be treated as a distinct entity of its own, with different levels of burning based on local hot spots or areas of longer duration heat exposure.

The case presented here involved analysis of thousands of fragments of heat altered human bone from a minimum of 20 individuals. Due to the insurmountable number of specimens, differential ability to identify anatomical location and side of each, and the inability to re-associate remains to individuals, a means for the analysis of site-wide patterns was required. A methodological archaeological recovery had been performed, conserving context of fragments by 1m x 1m square. Fragments were sorted by square and broad anatomical region, when possible. Each collection of specimens was then scored as a group for its level of heat alteration. Scores were based on the overall percentage of fragments that displayed calcination by visual inspection. These scores were mapped across the site to examine any patterns to the burning that may have shed light on the formation of the scene.

Two interesting patterns were clearly visible when considering levels of heat alteration across the site as a whole. First, different anatomical regions revealed different overall levels of heat alteration. The anatomical region demonstrating the most calcination was the cranium, while the pelvic region demonstrated the least. This is interestingly consistent with a normal burning pattern of fleshed remains.[2] A second pattern emerged with the examination of anatomical regions across the site. When mapped, each region displayed a broad U-shaped area of more intense calcination. Inside this area, there appeared an area of relative sparing of remains, containing considerably less calcination.

While the exact cause of these patterns is not determinable based solely on the analysis of the skeletal remains, they add an additional layer of information that can support other indicators of site formation. By combining taphonomic information with other evidence, questions concerning how many areas of burning may have been active, whether or not the remains were altered before, during, or after burning, if multiple distinct burning events have taken place, and why some skeletal elements may be less represented than others can be explored.

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Taphonomy, Burning, Mass Graves

A67 The Occurrence of Osteon Banding in Adult Human Cortical Bone

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After attending this presentation, attendees will have an appreciation for the occurrence of osteon banding in adult human cortical bone.

This presentation will impact the forensic science community by providing key data supporting the presence of multiple osteon bands in single specimens, indicating that this phenomenon should not categorically be taken as evidence of non-human bone. This will result in a higher quality of forensic practice since human species origin should not be ruled out when a significant pattern of osteon banding is encountered in primary and/or secondary lamellar bone.

Differentiating human from non-human fragmented bone is often accomplished using histological methods if the observation of gross morphology proves insufficient. Linearly oriented primary and/or secondary osteonal systems, commonly referred to as osteon bands, are described in the literature as a strong indicator of non-human bone. This phenomenon has been conventionally documented using two-dimensional histology, but such analyses are destructive and typically limited to a single cross-section; however, progressive developments in high-resolution X-ray imaging allow for the non-destructive 3D visualization of bone microarchitecture.

In a previous survey of osteocyte lacunar variation in adult human skeletal elements using Synchrotron Radiation-based micro-Computed Tomography (SR micro-CT), the presence of osteon banding in cortical bone in various bone types was frequently observed.[1] As such, the primary objective of the current research was to visualize and document the occurrence of osteon banding in adult human cortical bone using high-resolution SR micro-CT.

SR micro-CT scanning was conducted at the Canadian Light Source national synchrotron facility. The presence or absence of osteon banding was visualized in human skeletal elements from three adult males with representative samples from all regions of the skeleton (n=129). Visualization of osteon boundaries were enhanced by applying z-projections through 90μm (100 adjacent slices at 0.9μm thickness) and by observation of osteocyte lacunar patterns. Additional projections (standard deviation and minimum intensity) were employed to enhance visualization of air-filled vascular spaces and high-density bone tissue. Subsequently, 3D renders of bone microarchitecture were created. All projections in each image stack were assessed for the presence of osteon banding. An osteon band was defined as a discrete row of five or more primary and/or secondary osteons, as described by Mulhern and Ubelaker.[2] The number of bands in each specimen and the number of osteons per band were documented.

Results indicated that 23 of 129 human cortical bone specimens exhibited osteon banding, representing 18% of the sample. Linear arrangements of primary and/or secondary osteons were observed in the following skeletal elements: temporal, parietal, frontal, occipital, clavicle, mandible, femur, tibia, ulna, second metatarsal, and sacrum. Multiple osteon bands were observed in the temporal, parietal, occipital, and mandible specimens, with three being the maximum number of bands observed. In these elements, osteon bands ranged from 5 to 12 osteons in length and were comprised mainly of primary osteons.

Per research, this work represents the first report of: (1) multiple osteon bands within a single adult human cortical bone specimen; and, (2) inter-element variation in osteon banding from cortical bone from various modern adult human skeletal elements. The frequent occurrence of osteon banding, and the presence of multiple osteon bands within a single specimen, indicate that this histomorphological feature is not solely diagnostic of non-human bone. This characteristic should not be used as a distinguishing feature if alternative non-human characteristics are clearly less visible. Overall, the analyst should not rule out a human species origin when a more significant pattern of osteon banding is encountered in primary and/or secondary lamellar bone.

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Cortical Bone, Osteon Banding, Micro-CT

A68 Estimation of the Postmortem Interval (PMI) of Skeletonized Human Remains Using Nile Blue and Indophenol Colorimetrics on Femoral Cortical Thin Sections

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After attending this presentation, attendees will understand the potential of colorimetrics for the estimation of a PMI when applied to femoral bone sections.

This presentation will impact the forensic science community by testing the staining reaction of Nile Blue (NBL) and Indophenol (IND) on thin sections of the femoral cortex and by quantifying the correlation between the coloration and the PMI.

PMI is an essential piece of information for estimation, especially when dealing with skeletonized dry human remains, as it determines whether the case is forensically relevant or historical/archaeological. Although the prescription period varies depending on the country's criminal code, it generally spans between 10 and 30 years (with no limit in homicidal cases for some contexts). PMI estimation is complex after complete skeletonization, and even with a thorough analysis of the taphonomy and surrounding environmental factors, there is no straightforward method available to clearly quantify a PMI. Following Berg and Specht's observations in 1958, the colorants NBL and Indophenol IND tend to reveal differential affinities with cortical bone depending on the PMI.[1] The variations in coloration were visually assessed but not discussed. It was noted that NBL was more sensitive to ancient bones and IND more accurate for coloring recent bones. The protocol was tested later by Knight and Lauder, but judged too unreliable for routine use.[2] As part of the research within the French National Forensic Institute (IRCGN), the PMI estimation through colorimetrics of NBL and IND was further investigated.

A sample of 32 femoral sections from individuals with a known PMI was collected from a Swiss osteological reference collection, a number of French archaeological collections, and a number of recent forensic cases. The coloration protocol followed the procedures described by Berg.[3] Cortical thin sections were sliced at 1.2mm, immersed in 2% NBL or IND for 10 to 20 minutes, rinsed, and differentiated for 12 hours with acetic acid or 70% alcohol (respectively, for NBL and IND). For a more objective assessment of the resulting color, the dried sections were measured using a Konica Minolta CM-2600d/2500d spectrocolorimeter, which quantifies the color using the L=lightness, a=ambiance, b=brightness (Lab) system. The consensus-measured color is the average Lab for 30 measurements of the same section. Some sections were measured on both sides to evaluate potential systematic variations. Additionally, some steps of the protocol for the NBL were tested (i.e., different concentrations of both the NBL solution and acetic acid and different immersion times).

The dispersion of PMI within the tested sample was not homogeneous as there were too few recent individuals (<30 years of age) versus old (30-100 years of age) and archaeological individuals (>100 years of age). Consequently, correlations and regressions did not produce results coherent enough to build estimation models; however, computing the raw Lab data in a principal component analysis allowed for an objective visualization of the distribution of the recent, old, and archaeological individuals. Although this preliminary study did not take into account the taphonomy factors (environmental influence on cortical bone composition and the coloration process), NBL demonstrated a moderate potential to distinguish individuals with a PMI inferior or superior to 100 years, and IND demonstrated a stronger potential to distinguish individuals with a PMI inferior or superior to 30 years. A larger sample that is taphonomically controlled and has a homogenous PMI distribution is required before building a robust estimation model using IND colorimetrics, but this rapid and cost-efficient technique is promising.

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Postmortem Interval, Human Remains, Colorimetrics

A69 Volumetric Histological Age Estimation Utilizing a Geographic Information Systems (GIS) -Based Analytical Approach

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After attending this presentation, attendees will appreciate the potential of using GIS software to visualize and analyze bone histological structures three-dimensionally.

This presentation will impact the forensic science community by introducing a new method of viewing and quantifying histological remodeling events that are used for age estimation.

If salient macroscopic elements are damaged or missing, adult human age can be estimated through histological examination of the remodeling events in cortical bone. Several techniques have been developed and refined but, to date, all analysis has focused on a single two-dimensional plane. Osteons are 3D structures; they are typically cylindrical in shape, round to ovoid in cross section, 200-250 micrometers (µm) in diameter, one to ten millimeters long, and oriented on average 11°-17° relative to the long-axis of the bone. They are also dynamic structures; they grow, age, and are resorbed. Utilizing the volume of osteons, or incorporating the stage of osteonal development into age estimation regression formulas, may minimize standard error estimates and improve age estimates when building the biological profile in forensic cases.

To date, the only way to view osteons in three dimensions is to use confocal microscopy, which provides a maximum z-axis depth of no more than 100µm, or to use micro-Computed Tomography (micro-CT) or synchrotron-assisted micro-CT, which is better suited for negative spaces, such as the pores or Haversian canals in bone tissue. The current study employed a GIS-based analytical approach to digitally map remodeling events on three serial cross-sections, thereby providing a 3D perspective of bone remodeling. The use of GIS techniques for bone histological analysis is novel, but researchers have previously utilized arcGIS® to manually delineate intracortical remodeling events as polygon feature classes across a single section of femoral midshaft. This study extends this approach in which osteonal systems are measured three-dimensionally, potentially allowing for a more realistic assessment of their features, but it necessitates several additional steps. First, vertical alignment grooves were cut into the cortex of an adult human femoral midshaft in order to maintain multi-planar spatial orientation. Three serial thin-sections of approximately 80µm were subsequently cut using a Leica® SP1600 microtome saw; they were separated by 300µm due to blade thickness, providing an overall height of approximately 840µm. Second, each sample was photographed under circularly polarized light and combined into a seamless image using free photo-stitching software. These entire cross-sections were then imported into arcGIS® v10.1 and aligned to overlay using the vertical grooves as georeferencing points, tying the layers to each other. Third, polygon features tracing the cement line were manually created to overlay each remodeling event.

To accommodate for the off-axis vertical path at which osteons may form, as well as the void created by the saw blade, buffers were produced in arcGIS® around the polygon features, allowing osteons found on multiple cross sections to be connected to each other. Once the osteons on the serial sections were identified as the same remodeling event, they were coded appropriately and excluded from other potential matches. Not all osteons could be followed through all three sections, and some osteons in one layer could potentially connect to multiple others in the next layer. In such cases, the relative size and shape of osteons were used to connect related osteons in different layers. Change detection algorithms to quantify differences in area and shape between remodeling events in the transverse sections provided a volumetric measurement of individual osteons based on the formula for a cylinder.

This study reinforces the value and utility of GIS-based analysis for identifying, comparing, and capturing patterns for histological mapping, as well as introduces the concept of volumetric osteonal measurement for histological age estimation.

Age Estimation, Skeletal Histology, Forensic Anthropology

A70 Validation, Verification, and Performance Checks of Anthropological Equipment and Software: The Importance of Quality Assurance in Forensic Anthropology Laboratories

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After attending this presentation, attendees will have a better understanding of the recent efforts undertaken at the Harris County Institute of Forensic Sciences (HCIFS) to address the pertinent issue of establishing a validation program and Standard Operating Procedures (SOPs) for anthropological equipment and software, specifically the MicroScribe® 3D digitizer and the 3Skull and FORDISC® software programs used to collect and analyze osteometric data.

This presentation will impact the forensic science community by providing best practices to mitigate potential issues of method and measurement error. The present research will illustrate the significance of validating anthropological equipment in every laboratory as a quality assurance measure, notwithstanding its general acceptance within the forensic anthropological community.

Establishing a quality assurance program and achieving laboratory accreditation has become paramount for forensic anthropology. It is through these processes that the field of forensic anthropology can demonstrate the level of surety associated with anthropological analyses. In August 2015, the HCIFS's Forensic Anthropology Division (FAD) became the first forensic anthropology laboratory to be accredited under the American National Standards Institute-American Society of Quality (ANSI-ASQ) National Accreditation Board's (ANAB's) International Organization for Standardization/ International Electrotechnical Commission (ISO/IEC) 17020 inspection body program. One challenge posed during the accreditation process was answering the question, "Do validation, verification, and performance checks of anthropological methods and activities follow a written procedure?"

Equipment and software programs, including the digitizer, mandibulometer, 3Skull, and FORDISC®, were in use prior to accreditation. Their use and data were assumed to be reliable since members of the forensic anthropology community deem them acceptable and references for their functionality are available in peer-reviewed literature; however, no internal validations were performed when the equipment and software were received. Plans were made to collaborate with the HCIFS's Quality Management Division (QMD) to properly validate the equipment and software in order to meet accreditation standards. All accredited laboratories, per ANAB's Guidance on Uncertainty for Testing Laboratories, must review significant factors that may contribute to the error or variability in measurements. Thus, accreditation standards require in-house validations for analytical equipment to demonstrate the level of surety associated with analyses.

To perform the internal validation of the digitizer, a reference standard was created by selecting an anatomical skull and pre-marking the osteometric landmarks. The elements were measured using calipers and standard collection protocols. Then, the analysts collected the landmarks following the newly-written digitizer SOP, checked that the coordinates were accurately captured in the 3Skull software, and compared the output to the caliper-derived measurements. In the course of establishing this procedure, measurement uncertainty calculations for 35 Interlandmark Distances (IDLs) and one angle were performed using Root Sum Squared (RSS) and following the National Institute of Standards and Technology (NIST) procedure for measurement uncertainty.

For the in-house FORDISC® validation, cranial and postcranial metric data were collected from four known, donated specimens typical of the Black and White groups within the Forensic Databank. These data were processed through FORDISC®, retaining a log of the osteometric measurements used and analytical iterations. The IDLs were then provided to the FAD analysts, who entered them into FORDISC® without viewing the previous results. Validation was considered successful when the FAD FORDISC® classification results were reasonably similar to the previous results when comparable analytical iterations were employed. While the FAD's equipment and software validation SOP is specific to the MicroScribe, 3Skull, and FORDISC®, forensic anthropologists will be able to identify the important elements that should be included in any anthropology software program validation (i.e., 3D-ID, (hu)MANid, Macromorphoscopics) or software update.

Formalizing a process for internal validation to the point where instructions can be documented in SOPs may seem unnecessary to practitioners, especially if anthropologists believe they are already employing validated methods/equipment; however, in-house verification is warranted to guarantee the appropriateness of their use for fulfilling the service needs of the laboratory. The goal is to advance analyses toward uniformity and higher quality. While this may be challenging for laboratories with only one forensic anthropologist, or offices without a QMD, validation, verification, and performance checks for *your* equipment in *your* laboratory is vital in this era of forensic science critique. Therefore, one of the primary deliverables for this project is the availability of the HCIFS's equipment and software validation plans and SOPs, which can be used as a template to meet the needs of any forensic anthropology laboratory.

Validation, Quality Assurance, Accreditation

A71 Accreditation of Forensic Anthropology and Practice in the United Kingdom

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After attending this presentation, attendees will have gained an understanding of the current state of professional practice in forensic anthropology in the United Kingdom, providing a valuable insight and opportunity for discussion in relation to the development of the discipline.

This presentation will impact the forensic science community by providing an update of the procedures, code of practice and ethics, and the certification of forensic anthropology practitioners in the United Kingdom. With the increasing focus on admissibility of evidence and the weight given to expert evidence, it is imperative that appropriate standards are set and maintained. By providing an update of the status of forensic anthropology in the United Kingdom, this presentation will provide an opportunity for discussion of best practices from an international perspective.

The establishment and maintenance of professional standards and codes of conduct and ethics are fundamental to the practice of forensic anthropology in the modern medicolegal landscape. The process of instigating such practices began in the United Kingdom in 2013, with the positioning of the Royal Anthropological Institute of Great Britain and Ireland as the professional body for forensic anthropology. Since that time, the process of certification of practitioners has completed a full three-year cycle, with practitioners certified at each of the three levels and recertification of practitioners ongoing.

With the increasing attention on standard setting and suitability of evidence and expert witnesses in the court room, the importance of professional certification and development of professional standards has never been higher. Cooperation between government offices, legal practitioners, professional bodies, forensic providers and individual forensic practitioners, and end users is vital if this is to be achieved and maintained. As this process is in its relative infancy in the United Kingdom, the opportunity to discuss these issues with international colleagues will provide wider insight into the process. It is hoped that establishing such dialogue will facilitate cross-fertilization of ideas and best practices in forensic anthropology, leading to a holistic appreciation of the position of certification and professionalization of the discipline, in addition to scientific expert witness evidence in general. This study will present experiences of this process in the United Kingdom, share the difficulties and benefits encountered, and establish knowledge exchanges with international colleagues who may have encountered similar or different challenges.

Forensic Anthropology, Certification, Professionalization

A72 Validation of the Acetabulum as a Skeletal Indicator of Age at Death

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After attending this presentation, attendees will better understand the biology underlying progressive acetabular changes.

This presentation will impact the forensic science community by demonstrating the utility of the acetabulum for age estimation; the changes occurring in this joint could prove particularly relevant to the development of future multifactorial aging methods.

This research investigated the nature of progressive changes in the acetabulum, with the goal of determining whether they are metamorphic or degenerative and ascertaining whether they are valid indicators of age at death.

The acetabulum has been a focus of age-estimation research during the past decade, and several acetabulum-based aging methods have become available to the forensic anthropological practitioner; however, the nature of the progressive changes that occur in the acetabulum remains poorly understood. These changes may constitute skeletal metamorphoses (akin to the formation of the ventral rampart of the pubic symphysis), which forensic anthropologists generally believe to be tightly linked with age. Alternately, acetabular changes may represent skeletal degeneration (osteoarthritis or OA) — generally viewed as less strongly correlated with age and more influenced by factors such as physical activity and obesity. If the former proves true, this would lend support for the use of acetabular changes in skeletal age-at-death estimation. If the latter proves true, this would suggest that use of the acetabulum for age estimation should be reevaluated.

In order to investigate these problems, acetabular changes and OA were analyzed in a sample of 409 European-American skeletal individuals from the W.M. Bass Donated Skeletal Collection (University of Tennessee, Knoxville). Acetabular changes were observed and scored.[1] In all major appendicular joints, OA was observed and scored.[2] These data were then compared with documented demographic data for the 409 individuals. In addition to age, sex, height, and weight (used to calculate Body Mass Index (BMI)), demographic data also included self-reported habitual and occupational activities, which were assigned values representing their Metabolic Intensity Level (MET) using a system developed for the coding of physical activity survey data.[3] Statistical tests assessed potential associations between acetabular scores and OA scores, ages at death, BMI, and MET values. Non-parametric tests and visualization techniques (e.g., Spearman's rank-order correlation, box-and-whisker plots) were used to assess associations between ordinal data such as acetabular and OA scores. Parametric tests and visualization techniques (e.g., linear regression, scatterplots) were used to assess associations between continuous data such as ages at death, BMI, and MET values. The alpha-level was set at 0.05, and the Bonferroni correction was applied in cases of multiple test iterations.

Acetabular changes were found to correlate statistically significantly positively with OA in all joints and body regions. This indicates that the changes occurring in the acetabulum are degenerative rather than metamorphic; however, these acetabular changes also correlated statistically significantly positively with age, indicating that they are still useful for age estimation. In contrast, acetabular changes showed no associations with BMI or MET values, indicating that these changes are resistant to the effects of obesity and habitual and occupational activities. This finding also supports their use in aging.

In summary, the degenerative changes of the acetabulum are valid skeletal age indicators that are relatively resistant to the effects of lifestyle variables (i.e., obesity, physical activity) in European-Americans. The strong age correlations of degenerative acetabular changes suggest that the metamorphic-degenerative dichotomy of skeletal age change is in need of revision. Further, the lack of association between acetabular degeneration and physical activity indicates that the oft-cited anthropological paradigm of activity-induced joint degeneration is overly simplistic. In a field where *science matters*, it is important not only to test and refine age-estimation methods but also to understand the biology underlying method success or failure. These findings advance an understanding of skeletal aging that can improve both identifications of the dead and health outcomes for the living.

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Age Estimation, Osteoarthritis, Skeletal Degeneration

A73 Exploring the Performance of a Global Subadult Age Estimation Model Using Unsupervised Machine Learning Techniques

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The goal of this presentation is to inform attendees of the possibility of developing a global multivariable, multi-indicator subadult age estimation model. Numerous variables from the three most frequently applied age indicators were collected from genetically and economically diverse populations and were used to develop and compare the performance of population-specific models and a pooled, global model.

This presentation will impact the forensic science community by providing information regarding the feasibility of a global application of subadult age estimation, which is crucial considering the increased migration events and subsequent admixture resulting in populations with greater levels of genetic variation and complexity.

Most subadult age estimation methods do not account for the variation that exists in the world, as they are tailored for application in a specific location. Unfortunately, there is a possibility of mass and natural disasters in any country around the world, in addition to increased population movements and subsequent increased genetic variation; subadult identification would be facilitated if models were global. Thus, anthropologists need to critically evaluate subadult age estimation and explore the feasibility of global models, specifically, if there are differences in age indicators between geographically diverse groups, how does a global multivariable, multi-indicator model compare to a population-specific multivariable, multi-indicator model?

Epiphyseal fusion, dental formation, and diaphyseal dimensions were collected from a modern sample of children aged between birth and 10 years of age from the United States and South Africa. Two population-specific and one global multivariable, multi-indicator models were constructed using feedforward Artificial Neural Networks (ANN). Unsupervised machine learning techniques make minimal assumptions about the underlying data and thus offer a very flexible prediction framework that can handle different types of data modes, such as continuous and ordinal variables. A test dataset was reserved for all models to independently assess the suitability of each final fit.

The age indicators were compared for each population and scatterplots depicted a complete and consistent overlap in the distributions of the diaphyseal dimensions across the entirety of the age range, though differences in length became apparent in the older ages. In terms of the developmental variables, older South African children expressed the same dental formation and epiphyseal fusion stage as younger children from the United States. The results of the multivariable, multi-indicator models reveal the data trained from the pooled samples provided better age estimations than the population-specific models, based on lower mean squared error. The test data revealed that the United States sample was more predictable than the South African sample, which may be due to the fact that the South African sample was, on average, slightly older than the United States sample.

Unsupervised machine learning techniques use pattern detection to build models. Therefore, if population-specific models are used, and thus account for less variation in the training sample, the error will always be higher. Using a larger sample to train with captures the true range of variation; therefore, the model will always be more precise. The results of this study are very exciting and provide a solid stepping stone to continue the research and address the needs for more rigorous subadult age estimation techniques. Population-specific models may perform just as well as global models, but until larger, more reflective samples of populations with equal age distributions are used, the global model can be applied.

Artificial Neural Networks, Computed Tomography, Juvenile

A74 A Multidisciplinary Protocol to Assess Chronological Age of Unidentified Migrants

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After attending this presentation, attendees will understand the role of forensic sciences in age estimation and the effectiveness of the scientific methods used to correlate biological and chronological age.

This presentation will impact the forensic science community by demonstrating the importance of a multidisciplinary approach in the age estimation of unidentified migrants to minimize errors and complaints.

In Italy, unidentified migrants usually claim to be underage because minors are processed through the juvenile system, where detention is not mandatory. In addition, unaccompanied minors will often have access to educational programs and may be granted a residency permit. In this scenario, the use of age estimation techniques is fundamental. There are several methods that correlate biological and chronological age. They are mostly based on the evaluation of the maturity of hands and wrist bones, teeth and clavicles.[1-5] In the forensic scientific community, there is widely held agreement regarding the use of a combination of these methods to reach the most accurate estimation.[6]

For these reasons, the city of Turin, the local health authority, and the prosecutor's office defined a formal multidisciplinary investigation protocol for age estimation. After the fingerprint pattern is captured as a digital image, a Unique Identification Code (UIC) is assigned; the acquisition of the informed consent is performed, then weight, height, dental formulas, and other clinical findings (secondary sex characteristics, scars, and tattoos) are recorded. Radiographs of the left hand and eventually the Orthopantomogram (OPG) are obtained. The age estimation is performed by the conventional methods for skeletal and dental formulas. The data, associated with the UIC, are reported in the final certification that is delivered to the officers and finally stored in a national database.

This study reports a retrospective analysis of the data collected during the employment of the protocol described above. From July 2014 to July 2017, 458 visits were conducted. Overall, 383 were male and 75 were female. The geographical origins were heterogeneous, mainly from sub-Saharan Africa (Nigeria 17.03%, Senegal 16.81%, Mali 8.3%, Guinea 8.3%, Gabon 7%, and Ivory Coast 6.55%). The mean declared age was 16.94 years (Standard Deviation (SD) ±2.05). The mean Body Mass Index (BMI) was 21.55 (SD±2.42). The languages used to obtain the consent were principally English, Italian, and French. In only one case did the person deny consent to a medical examination. There were 317 adults, and the remaining 141 were underage. In 195 cases, it was necessary to utilize the OPG. In 21 cases, the age estimation obtained with the evaluation of the left-hand X-ray was not consistent with the estimation obtained with methods based on tooth evaluation. In fact, at the same time, these 21 migrants were identified as adult using the evaluation of the left-hand X-ray and as underage considering the methods based on tooth examination. For this reason, in accordance with Italian law, they were considered underage.

The results reported above reveal that this diagnostic process provides accurate results because it is based on the combination of different expert opinions (medical examiner, dentist, and radiologist); however, in order to reach this result, it is necessary to employ well-trained clinicians. They should possess extensive knowledge of the different scientific methods widely used to correlate biological and chronological age in the forensic community. Besides, if the results derived by the application of different methods are not consistent, the clinicians should be capable of understanding and communicating them to the officers and prosecutor.

Finally, this protocol is widely accepted by unidentified migrants, since more than 99% of the subjects agreed to undergo the medical examination. It consists of low-cost procedures that are not harmful to health because the association between fingerprint patterns, the UIC, and the final certification (stored in a national database) determine the reduction of risks associated with exposing the subject to more X-rays and medical examinations, if arrested again. In fact, this presentation should serve as a stimulus to heighten the importance of using standardized procedures in age estimation in order to reduce risks and avoid errors.

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Age Estimation, Illegal Migration, Personal Identification

A75 Dental Morphological Ancestry Estimation in a Self-Identified Biracial Sample

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After attending this presentation, attendees will understand the potential for misclassification of Biracial individuals using current dental morphological quantitative methods.

This presentation will impact the forensic science community by showcasing the importance of reference samples and the necessity to understand admixed populations for forensic identification.

The importance of ancestry estimation to the biological profile is well documented in that it can aid medicolegal death investigations by narrowing missing persons lists.[1] Dental non-metric traits have been used successfully to estimate ancestry in American Black and White populations, but no method has examined the effect of mixed ancestry on classification rates. The goal of this study is to understand how a sample of Biracial individuals would ancestrally classify using current quantitative methods for dental morphology.

A sample of 13 (11 female, 2 male) living self-identified Biracial individuals were recruited to complete this study. For this study, the definition of Biracial includes those individuals who are a Black/White racial mix and simultaneously an African/African American and European/European American ancestral mixture. Approval for study on living individuals was granted by the Texas State University Internal Review Board (2016S173). Dental impressions were obtained for each Biracial individual by a licensed dentist. Dental casts were made from the impressions and analyzed using the Arizona State University Dental Anthropology System (ASUDAS) dental morphological traits and the expression count method.[2,3] Root traits were excluded from analysis because they cannot be scored on casts. The scores were used to estimate ancestry for the sample using two methodologies, Edgar and Scott et al. [4,5] The method proposed in Edgar uses discriminant function equations to distinguish between African American (AA), European American (EA), and Hispanic American (HA), while Scott et al. is an online system (http://apps.osteomics.com/rASUDAS/) that processes scores and compares them against sample groups from geographical regions (this study compared between Sub-Saharan Africa and Western Eurasia).[4,5] The system uses a naïve Bayes classifier algorithm to output posterior probabilities for the "Expected bio-geographical origin."

The majority of the 13 individuals classified as European. Using Edgar functions, five of the Biracial individuals classified as EA, seven were not classified as AA/EA and, therefore, HA, and one could not be classified.[4] The web-based rASUDAS system estimated ten of the Biracial individuals as Western Eurasian and three as Sub-Saharan African.[5] The posterior probabilities did not yield results that could indicate a mixture of two ancestries. These results indicate Biracial individuals have a high potential to be misclassified.

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Ancestry Estimation, Dental Morphology, Biracial Sample

A76 Improvement to the Estimation of Hispanic Ancestry Through the Combination of Cranial and Dental Traits

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After attending this presentation, attendees will understand how the simultaneous analysis of multiple datasets, in this case cranial morphoscopic and dental morphological traits, improves the accuracy of estimating ancestry in the forensic context.

This presentation will impact the forensic science community by highlighting the utility of combining data from different sources when estimating ancestry. Additionally, this presentation demonstrates the power of non-metric ancestry estimation methods in a statistical framework.

With respect to ancestry, "Hispanic" is an umbrella term that fails to account for population histories in different regions of the United States; however, in common usage, "Hispanic" refers to populations with a history of European and Native American admixture, with an added component of African ancestry in southeast and Caribbean populations.[1] The Hispanic population in the United States is growing, and forensic anthropologists increasingly encounter the remains of these individuals in their casework. Several methods have been developed that address the estimation of Hispanic ancestry, but each focuses on a single source of variation.[2-4] This presentation explores whether estimates are improved by combining data.

The present research is based on data from modern Hispanic individuals from a combination of medicolegal and research collections (n=154). It is important to note that many of these individuals are presumed migrants and have yet to be positively identified; however, an ancestry estimate of "Hispanic" has been established using other methods.[5] For each individual, cranial (n=11) and dental (n=23) variables were recorded based on published standards.[6-8] Ancestry estimates were produced using both random forests based on conditional inference trees and naïve Bayes classification on cranial data, dental data, or combined data, resulting in a total of six models for ancestry estimation. The models were tested on each of 30 randomly generated training and test sets to account for variability in correct classification rate. Differences between models were assessed using a paired sample t-test, since classification rates were generated from the same sample across models for each run.

Results reveal that models based on the combined data have higher Positive Predictive Values (PPV) for individuals of Hispanic ancestry ($p < 0.01$). The PPV is a measure of how often the models are correct when giving an estimate of "Hispanic;" because that is the only group considered here, it can be interpreted as a measure of model accuracy. Models based on both types of data have a mean PPV of 0.93 for random forests, and 0.81 for naïve Bayes. In contrast, dental variable-only models performed about 10% worse (0.82, Random Forest Modeling (RFM); 0.72, Bayes) and cranial-only models about 15% worse (0.78, RFM; 0.64, Bayes).

Previous research concluded that the improvement observed when simultaneously analyzing cranial and dental data was of practical, if not statistical, significance, especially in the estimation of Hispanic ancestry. The results presented here demonstrate a statistically significant improvement to ancestry estimation for Hispanic individuals. Future research that combines multiple types of data within a statistical framework will lead to improved estimates of ancestry.

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Hispanic Ancestry, Cranial Morphoscopic Traits, Dental Morphology

A77 SkullProfiler: A Simple New Capability for the Quantitative Estimation of Ancestry and Sex From Lateral Skull Photographs

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The goal of this presentation is to introduce attendees to the R capability SkullProfiler for quantitative estimation of ancestry and sex using outlines of skulls or crania extracted from photographs in norma lateralis.

This presentation will impact the forensic science community by providing a new and freely available computer capability for ancestry and sex estimation that is user-intuitive, field-friendly, accurate, and fast to implement.

SkullProfiler is designed to analyze left lateral skull or cranial photographs of unknown cases, taken with a specifically standardized protocol using a full-frame camera fitted with a 100mm macro lens and subject-to-camera distance of 1.2m. The photograph is imported into R using the SkullProfiler code, which then undertakes a series of semi-automated steps. First, outline coordinates are extracted from the case photograph and Elliptical Fourier Analysis (EFA) is conducted using 40 harmonics.

Group assignment for the unknown case is then achieved using linear discriminant function analysis of a truncated set of Principal Component (PC) scores. Mahalanobis square distances, posterior probabilities, and Chi-square typicality probabilities are reported for the unknown relative to samples selected from an inbuilt reference database. This database comprises skulls (>18 years of age) of known sex from the following collections: the Hamann-Todd Human Osteological Collection, William M. Bass Donated Skeletal Collection, Robert J. Terry Anatomical Skeletal Collection, Khon Kaen Osteological Collection, and Chiba Bone Collection. Groups for analysis are defined as United States Black female (n=87) and male (n=109), Japanese male (n=59), Thai female (n=39) and male (n=47), and United States White female (n=97) and male (n=134).

Five-fold cross validation results are provided in the data outputs to assist users in gauging classification accuracy. Tests using all seven reference samples produced cross-validated accuracies of 73%. This increased to 78% accuracy for reference samples of United States Black and White females and males only.

Tests using an out-of-group sample of nine individuals from the Defense POW/MIA Accounting Agency (DPAA) Laboratory, and all seven reference groups, yielded eight of the nine skulls correctly classifying as White male for both skull and cranial outline data (89%). All nine skulls were correctly classified in a four-way comparison of ancestry when sex was specified ahead of analysis — as would be possible in cases possessing infracranial elements, in which sex could be independently estimated from pelvis.

Both the cross validation and out-of-group validation test results indicated that SkullProfiler can successfully use lateral skull and cranial outlines to correctly estimate an individual's ancestry and sex with high degrees of accuracy, and at rates comparable to other computerized capabilities using linear measurements (namely FORDISC®). The reliance on a single, easy-to-take photograph, portable computer, and semi-automated segmentation program in SkullProfiler increases speed and flexibility of the method. This holds advantages for field use, where robust field assessments of basic bioprofile data can confirm or decline requirements for repatriation of skeletal remains. This becomes paramount in instances with large distances, expenses, and administrative bureaucracy, where unnecessary or incorrect repatriations are better avoided. SkullProfiler and supporting documentation is currently available for free download at CRANIOFACIALidentification.com.

Forensic Anthropology, Elliptical Fourier, Biological Profile

A78 Analyzing Morphometric Methods of Race Differentiation in the Human Pelvic Girdle

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After attending this presentation, attendees will understand the importance of replication studies in adequately assessing the reproducibility of scientific methods prior to implementation for casework.

This presentation will impact the forensic science community by providing results from a replication of previously published morphometric methods to differentiate race in the human pelvic girdle. This presentation will highlight the need for comprehensive validation of methods employed by forensic anthropologists and stricter adherence to current statistical standards.

Research has shown that measurements from the pelvic bones and femur can be used for race estimation when the skull is absent or damaged.[1,2] The literature reported up to 95% accuracy when utilizing discriminant function analysis to simultaneously classify race and sex.[1,2] This study attempted to replicate the findings of two previously published methods of race estimation from the pelvic girdle while evaluating them within the guidelines for admissible forensic evidence.[3] It also sought to update the methods where necessary to conform to current statistical standards.[4,5]

This study strove to keep as many variables as possible consistent with the prior research in an attempt to adequately assess the validity of the methods. Both DiBennardo and Taylor, and İşcan utilized the Robert J. Terry Skeletal Collection for the development of their discriminant functions; however, there are concerns that this collection, and collections like it, are no longer representative of populations in the United States and may not be useful for the development of forensic identification methods.[6] To address these concerns, the sample population from the Robert J. Terry Collection was supplemented with individuals from the more contemporary William M. Bass Donated Skeletal Collection, and descriptive statistics from the two skeletal collections were compared. The study sample consisted of 100 individuals each from the Robert J. Terry collection and the William M. Bass collection. A series of 19 metric measurements were recorded across the reassembled pelvic girdle, left innominate, sacrum, and left femur for each individual. The methods for reassembling the pelvis were derived from Bonneau et al. and Peleg et al, and four measurements described by İşcan were recorded.[2,7,8] The bones were then disassembled to record the remaining 15 measurements described by DiBennardo and Taylor.[1]

Statistical analysis was conducted using SPSS and Excel® software and included descriptive statistics, student *t*-tests, multiple discriminant function analysis, and Analysis Of Variance (ANOVA). Statistical significance for metric evaluation is typically set at 95% confidence, meaning there would be a 5% probability ($p < 0.05$) that the difference between groups is caused by chance alone.

Neither method sufficiently separated unknown skeletal remains by race. When the methods were modified to conform to current statistical standards, the overall accuracy fell considerably. The reproductions for DiBennardo's and Taylor's and İşcan's discriminant function analyses yielded accuracy rates of 85.8% and 60.4%, respectively, for the original grouped cases, and 80.7% and 58.9%, respectively, for cross-validated grouped cases.[1,2] These results were substantially lower than those reported in the literature. Thus, the results of the published literature were not reproduced in this study, nor did they adequately meet the standards for admissible evidence in a court of law. Furthermore, descriptive statistics revealed that more variations exist within African American and Caucasian American populations in the United States than among them.

The implications of this research demonstrate the need for more rigorous validation of published morphometric methods, emphasizing replication studies and comprehensive reviews of sample populations and statistical procedures prior to utilization for forensic anthropology casework.

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Anthropology, Morphometrics, Validation

A79 Masters of Our Own House: The Planning and Construction of the New Defense POW/MIA Accounting Agency (DPAA) Laboratory in Hawaii

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After attending this presentation, attendees will better understand processes related to planning, designing, and constructing a modern forensic human identification laboratory.

This presentation will impact the forensic science community by providing guidance, experience, and lessons learned from planning, designing, and constructing a modern forensic human identification laboratory. This presentation enables future planners to more easily plan and build similar laboratories.

The mission of the DPAA is to account for United States missing persons. These cases typically involve the identification of what forensic scientists often refer to as "residual remains." In November 2015, the DPAA occupied a new 52Kft^2 human identification laboratory on Joint Base Pearl Harbor-Hickam, HI. It joins the 35Kft^2 laboratory in Omaha, NE, forming the DPAA Laboratory system. The Laboratory, located on the third floor of a larger DPAA facility, supports a multitude of capabilities (e.g., DNA sampling, dental comparisons, and radiographic comparisons) needed to identify human skeletal remains. These capabilities translate into the following laboratory spaces: examination areas for large assemblages of skeletal and dental remains; morgue for decomposition and medical examiner-related cases (DPAA provides medical examiner support); maceration room; evidence transfer and long-term storage areas; radiographic facility including a Computed Tomography (CT) scanner; histology laboratory; DNA sampling facility; material evidence examination area; archeological laboratory; skull-photo superimposition laboratory; Scanning Electron Microscope (SEM) laboratory; synoptic and reference collections storage area; X-ray comparison/superimposition laboratory; photography studio; 3D printer laboratory; evidence cleaning, drying, and conservation areas; case file storage area; administrative areas and offices; visitor, tour, and education center; conference, meeting, and training rooms; family viewing room; and a locker room with showers.

The new Laboratory is the result of 14 years of collaboration of a multidisciplinary project team of forensic scientists, Quality Assurance (QA) experts, architects, and engineers. This improved facility meets the needs of DPAA by allowing rapid throughput of identifications involving the commingled human remains of hundreds of individuals. Already, the new Laboratory is showing dividends in the increased rate of identifications as well as a rise in morale of the Laboratory staff. The new Laboratory was accredited by the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD-LAB) in March 2016 on its first assessment and is a powerful asset overall for recruiting quality staff.

There were a multitude of lessons learned during this project. Foremost is the requirement that all users of the Laboratory are involved in all stages of the project and practice innovative and out-of-the-box thinking. In other words, a new laboratory cannot be a clone of the old facility. An architectural/engineering firm, experienced with forensic facilities, must be consulted from project inception to provide subject matter expertise and to keep the customer focused. The firm must have experience with QA standards (in particular the International Organization for Standardization (ISO) 17025) and work with the customer's QA staff to ensure the facility can be accredited. The team must work together, accept compromise, and balance a multitude of competing factors that influence laboratory design. These factors include, but are not limited to: facility layout and adjacencies of functional areas; evidence flow; project costs; energy efficiency; security; environmental impact; available real estate on which to build; long-term Operations and Maintenance (O&M) requirements and cost; public considerations; staff quality of life; future and evolving missions; various legislation and mandates (e.g., Americans with Disabilities Act (ADA)); evolution of QA programs; advances in forensic technology; and advances in building design and construction.

Government construction projects are protracted — in this case, 14 years. As such, the new Laboratory was designed with the future in mind. For example, the Laboratory was flexibly designed for expedient reconfiguration of space over time. Capabilities that were not required in the early 2000s were, in most instances, easily fitted into the design as they became needed.

Finally, maintenance staff must be hired and, as the move-in approaches, a plan formulated to move into the new facility.

In retrospect, there is little in the design that DPAA would change other than program more space for some functions. For example, there is a shortfall of examination space since the need to resolve commingled assemblages increased over time. More storage space and offices for key staff are also needed; however, the design is sound and used with great success in 2011 when planning the DPAA satellite laboratory in Nebraska.

DPAA, Laboratory Concept and Design, Laboratory Construction

A80 Learning From Our Casework: The Society of Forensic Anthropologists (SOFA) Case Database

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The goals of this presentation are to: (1) introduce the SOFA Case Database, including a review of the trends in method use for age, sex, ancestry, and stature, as well as biological profile accuracy statistics for the submitted cases; (2) better understand the scope and goals of the database, the user interface, current submitted case statistics, and research potential for users; and, (3) review the submission process, including case eligibility, appropriate case data, and permissions and responsibilities of SOFA Case Database users.

This presentation will impact the forensic science community by introducing a new tool and data resource for research on forensic anthropology case work trends, including method use, biological profile estimation accuracy, and decedent demographics.

SOFA is a United States-based, non-profit organization comprised of practicing forensic anthropologists throughout the country, mostly employed at law enforcement agencies or offices of medical examiners. SOFA's purpose is to promote collaboration of forensic anthropologists, and it seeks to improve the field of practice. In 2013, SOFA began developing the concept and platform for an online forensic anthropology case database tool to serve the broader forensic anthropology community of practitioners and researchers. The purpose for developing the database was to provide a comprehensive and centralized source of information on methods used in forensic anthropology casework. The database is comprised of only identified (positive or presumptive) cases, which then allows researchers to study how forensic anthropological estimations of the biological profile compare to the actual biological profile details of the identified decedent, as well as what methods are being employed in these cases.

The goal of such analyses is to provide insight into avenues for forensic anthropological method development and improvement, as well as decedent demographic trends. To meet this goal, SOFA is engaged in active recruitment of forensic anthropologists with past and present adjudicated cases. With the participation of practitioners and the submission of cases, the SOFA Case Database is the first forensic anthropological community-wide collective resource for case data that can be used to study: (1) the consistency of practitioner-generated estimates of the biological profile with the actual age, sex, stature, and ancestry of the individual, once identified; (2) the trends, accuracy, and precision in method use; and, (3) decedent demographics to address methods gaps. As case data continues to accumulate, interested forensic anthropologists/researchers can access and analyze the anonymous case data (electronically available for download at www.sofadb.com).

SOFA's Case Database fills a data gap in the field of forensic anthropology. At the time of its development, there existed no formal, organized space for the forensic anthropology community to share approaches to casework and casework outcomes. While informal peer interaction is ongoing and useful, a significant source of data was missing — that of casework outcomes (e.g., method-derived *versus* actual biological profile information), a vital aspect for the discipline's ability to self-assess its progress and success.

In the past months, SOFA members have begun actively submitting identified cases to the database, resulting in (as of August 1, 2017) a total of 82 cases representing a wide range of case data, including case year (1993-2017, μ=2012), sex (n_{female}=30, n_{male}=51), age (15-94 years, μ=43), ancestry (n_{White}=34, n_{Black}=23, $n_{Hispanic}$=15, n_{Other}=2), and stature (57-77 inches, μ=67 inches). Additionally, more than 30 forensic anthropological methods were employed by practitioners to assess the biological profile.

Case Report, Methods, Data

A81 Forensic Research Outdoor Station (FROST): The Implementation of a Cold-Climate Forensic Anthropology Research Facility

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After attending this presentation, attendees will better understand the importance of collaboration between university, community, law enforcement, and government entities in support of the successful implementation of a forensic anthropology research facility.

This presentation will impact the forensic science community by bringing attention to the processes that were implemented as part of the creation of the FROST at Northern Michigan University (NMU) and by highlighting some of the work that is expected to result from collaborations established between NMU and other research and law enforcement entities.

Casework and research have identified the need for climate-specific research that can inform the forensic community regarding the taphonomic processes associated with the postmortem deposition of human remains. Seven decomposition/taphonomy research facilities already exist in various sub-climates of the continental United States and one in Australia, making FROST the eighth facility of its kind in the world and the only one located in a cold climate. Particularly relevant to research at FROST will be the effects of deep snow and the freeze-thaw cycle on the decomposition of human remains. Additionally, the facility's location in Marquette, MI, with its proximity to Lake Superior and several cold-water inland lakes, has the potential to allow for research that will lead to a greater understanding of the effects of the cold, freshwater lacustrine environments on changes associated with decomposition and other taphonomic effects.

The early successes at FROST and its acceptance by the citizens in the local area, the university, and the law enforcement community can be attributed to extensive outreach, collaboration, and public communication about the facility and its potential benefits to the forensic sciences, law enforcement, and missing persons cases. Prior to conducting any research on the human donors at FROST, several baseline studies are being conducted that have developed collaborative relationships between NMU's Sociology and Anthropology Department and other departments, allowing for faculty and student engagement with the facility and increasing the sense of ownership and community within the university. Interdisciplinary baseline studies at the facility, all of which involve students, include entomological and zoological surveys, a soil chemistry/composition/pH analysis, a Ground Penetrating Radar (GPR) survey, a micro-climate/weather assessment, a Light Detection and Ranging (LIDAR) survey, and archaeological shovel test pits. Results from these baseline studies are included in this presentation.

Stewardship of the 2.5 acres of land that will be occupied by the facility and its parking and outbuildings was transferred to NMU from the Marquette Branch Prison property by the state of Michigan, a process that involved discussion and considerable cooperation on the parts of the university, local law enforcement, the prison wardens, the Michigan State Police (MSP), and the local community, where town hall-style meetings and public forums were held to ensure transparency and education of the various stakeholders. The land allocated to FROST is located approximately four miles from NMU's main campus; it is in an open field, non-residential area, screened from public view. The area (approximately 1.5 acres) includes a security fence with privacy screening and plans are in place to ensure limited access and 24-hour security, including some access oversight by prison personnel — a collaboration that is ideal for maintaining the integrity and security of the facility.

Although the program and the facility are in their infancy, FROST is already well-established as the product of extensive collaboration and communication between NMU and stakeholders of varying interests and backgrounds who have a common goal of advancing forensic science research in a cold climate and contributing to the improvement of methods and techniques used by forensic anthropologists, forensic scientists, and law enforcement

Forensic Anthropology, Taphonomy, Collaboration

A82 The New Revolution of Bone Collection and the Necessity for the International Digital Bone Collection Center (IDBCC)

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After attending this presentation, attendees will better understand the revolution of bone collection and the IDBCC. This presentation will also provide information on challenges faced by the Fourth Industrial Revolution era in the field of forensics.

This presentation will impact the forensic science community by suggesting mid- to long- term solutions for the facilitation of information sharing that can be achieved by the digitization of skeletal collections scattered in different parts of the world through 3D, Computed Tomography (CT), and Magnetic Resonance (MR) images, then sharing them through Memorandum Of Understanding (MOU) with research institutes and countries.

Modern society is evolving into the era of the Fourth Industrial Revolution -— the main theme at the World Economic Forum in 2016. The Fourth Industrial Revolution refers to the new industrial era of Information Technology (IT) convergence and infrastructure in which advanced information and communication technologies, such as Artificial Intelligence (AI), the Internet of Things (IoT), big data, and mobile, are converged into the economy and society as a whole.

With the emergence of the digital era, the field of forensic science has evolved into various areas through the introduction of digital forensics and precision scientific equipment. Bone collection is being pursued by universities and research institutes around the world and is very helpful for researchers in anthropology and forensic science; however, in order to conduct research on the collected bones, researchers have to physically travel around the world. It entails a substantial amount of time, manpower, and costs. Such limitations inevitably prolong the research period and increase the costs, thereby discouraging dynamic research activities.

The digital measurement method is compatible with the current manual measurement method and allows for easier access to the ethnic specification by population group, age, and gender. In addition, the level of reliability and accuracy of the judgments can be enhanced as various analytical methods can be employed through computers due to the development of digital technology.[1] Above all, as the information on bone collections can be accessed freely regardless of time and location, various methods can be developed and shared. In this regard, information sharing is revolutionary as it can eliminate aforementioned problems pertaining to workforce, time, expenses, and residence issues all at once. Also, by doing so, it will provide many young scholars and university students with abundant examples of digital data on the remains found in all different parts of the world.[2] The digital information will allow the analysis of not only human remains, but also others, such as animal bones and crime scenes around the world.

As such, The Ministry of National Defense Agency for KIA Recovery and Identification (MAKRI) of the Republic of Korea is in the process of collecting digital data of remains from around the world to establish the IDBCC. Ultimately, this is to build an environment where labs and organizations around the globe can freely access and contribute to the data collection for further research.

In order to achieve this, a system in which digital data can be donated and shared through cooperation from other nations and organizations is necessary. The center can either digitize collections of remains that are already stored around the world or can gather and centralize medical information such as CT from hospitals or medical institutions with patients' approvals. As the world's widely used medical digital video uses Digital Imaging and Communications in Medicine (DICOM), collections of remains can be managed if stored in the form of Stereolithography (STL), which is compatible worldwide. The data to be stored at the IDBCC will not only facilitate the study of measurement statistics but also characteristics of morphology, culture, era, physical, and biology.[3] A change in the research environment is necessary as we move into the digital era and MAKRI hopes that researchers and scientists around the world will join in the establishment of the IDBCC. This presentation will discuss the effectiveness of the digital bone collection and the necessity for IDBCC.

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Digital Bone Collection, Intl. Digital Bone Collection Center, Fourth Industrial Revolution

A83 The Ethics of Conducting Research on Human Subjects in Forensic Anthropology

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After attending this presentation, attendees will understand current legislation surrounding the use of human subjects in research and the ethical implications of conducting research when using deceased human remains.

This presentation will impact the forensic science community by informing practitioners of their obligations in research on human subjects and will establish an ethical means to accomplish this research.

Anthropologists are concerned with the study of humans and must carefully weigh the ethical treatment of human subjects in research. Forensic anthropologists are predominantly focused on the study of deceased individuals who are typically no longer considered human subjects; however, it is not uncommon for research topics or forensic analyses to require the direct involvement of the living or data (including medical records, such as radiographs) from individuals who are still alive. This presentation will outline the current legislation surrounding the use of human subjects in scientific research and its relevance to forensic anthropology. This discussion will also cover the legislation of working with deceased human subjects and ethical concerns surrounding the publication and use of these data for research.

This presentation will highlight the history of the use of living humans as research subjects (e.g., the Universal Declaration of Human Rights, the Nuremberg Trials, and the Declaration of Helsinki) with a focus on legislation within the United States (e.g., the National Research Act of 1974, the Belmont Report, and the "Common Rule"). Discussion will also include the upcoming changes to the Common Rule and the use of the Institutional Review Board (IRB).

This discussion will additionally center on the use of deceased individuals for research to whom the "common rule" does not apply. In the United States, a deceased body is recognized as "quasi-property," which provides the right of sepulcher, and ensures receipt of the decedent in a similar state as in life to the next of kin. This legal concept has ramifications for work conducted in a medicolegal context. Distinctions between forensic cases, donated collections of skeletal remains, and collections in which express consent was not given are also explored. Finally, it is important to consider how research results are presented and the implications for the next of kin, other living acquaintances, or the decedents themselves.

In light of this background, an ethical framework for research on human remains is outlined as relevant to forensic anthropologists. Such a set of guidelines could include obtaining permission from the next of kin or an individual (prior to death) for destructive tests, curation, data collection, and/or publication; however, it is not always possible to locate the next of kin to gain informed consent to conduct research on deceased individuals, and it is not suggested here that research should stop if consent cannot be obtained for such reasons. If permission cannot be obtained, the researcher should ensure the following: (1) the benefit of the research outweighs any harm to the next of kin or the skeletal remains; (2) the right to sepulcher is not hindered; (3) published results are not offensive or inflammatory; and, (4) data are anonymous and cannot be traced back to any living individual.

This proposed set of guidelines is broad in interpretation and scope, as ethical guidelines are typically adjudicated on a case-by-case basis, and what may be appropriate in one context, may not be in another. As such, the future of the discipline may require the adoption of a general ethical code for the practice of forensic anthropology or the implementation of a forensic review board (similar to the IRB process), which could provide oversight to individuals using human skeletal remains in research projects. In today's current scientific climate (e.g., the Native American Graves Protection and Repatriation Act (NAGPRA), the 2009 NSA Report on forensic sciences, the 2011 United States military mortuary scandal, and the 2016 President's Council of Advisors on Science and Technology (PCAST) report on forensic evidence), we cannot afford to appear callous in our treatment of human remains or forensic evidence. As anthropologists at the intersection of biological and social science, we must strike a balance between ethical treatment of human remains and the advancement of our field without alienating ourselves from future research opportunities.

Ethics, Research, Institutional Review Board

A84 Imaging Human Skeletal Remains: Ethical Considerations

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The goal of this presentation is to engage with practitioners in forensic anthropology, particularly those who are using 3D imaging, highlighting the recent ethical considerations that have arisen with the implementation of newer 3D imaging technology. After attending this presentation, attendees will better understand the ethical issues and debates that surround imaging human remains and have an opportunity to contribute and implement good practice guidelines in their future work.

This presentation will impact the forensic science community by making practitioners aware of the ethical issues that have recently become prominent due to the use of new imaging technology. This presentation will suggest competent practices when using 3D imaging, while proposing guidelines for good practice for the forensic science community to put into action.

The analysis of human remains via internal and surface documentation has demonstrated valuable benefits that include the digitizing, exposing, comparing, reconstructing, materializing, and sharing of remains. These digitizing techniques include laser, computed tomography, structured light scanning, photogrammetry, and structure from motion. Similarly, standard recording procedures, such as photography, have also been utilized in the same way. Researchers have a responsibility to justify the decision-making process, whether it is a method of presenting an image or manipulating the dataset. In addition, researchers have an ethical responsibility when dealing with the remains of the dead.

Debates and guidelines on ethical considerations with regard to human skeletal remains have previously been discussed and adopted, though, due to newer imaging techniques, some issues have become unaccounted for. It could be argued that many of the newer imaging techniques are respectful of human remains as they are non-invasive, limit handling, and thus reduce further destruction to the individual's remains; however, a key consideration is whether to also treat this digital representation with as much dignity and respect as the body itself. One issue is that the terminology of digitally documenting human skeletal remains is yet to be standardized. Therefore, certain terminology (such as representation, reproduction, and replica) may impact the interpretation of digital human remains, consequently resulting in differential legal status. Thankfully, scientists are becoming more open to discussing these new ethical concerns and practices, but there is a call for more communication due to the varying practices across multiple disciplines. Currently, there are no standard practice guidelines that refer to this technological progression.

Another consideration is that living in a digital age enhances the availability of the internet and the use of social media. Boundaries are constantly being pushed and digital data is becoming increasingly accessible. Not only can academics access this technology, but so too can the public. The impact of this may result in an increase of unnecessary and unethical images that can be accessed and created with one click of a button; however, this "shared data" may not have initially sought the owners' or relatives' permission to do so.

This presentation notes that everyone has an ethical responsibility, but it is up to academics to discuss these responsibilities with the uninformed public. Therefore, this presentation proposes a number of key points to consider prior to publicly sharing images of human skeletal remains. Considerations include whether a form of consent should be gained with regard to display, documentation, and data storage, increasing multidisciplinary discussions on ethical standpoints in the form of workshops and open forums, a standardization of terminology, and that a valid reason should always be sought prior to the undertaking of skeletal documentation.

Skeletal Remains, Ethics, Imaging

A85 The Current State of Forensic Anthropology as a Profession

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The goal of this presentation is to discuss the current state of forensic anthropology in terms of its qualification, certification, accreditation, and ethical processes, all of which are necessary when applying the criteria for a trade to be considered a profession.

This presentation will impact the forensic science community by defining what makes someone a professional, discussing how professional guidelines are, or are not, applied in the field of forensic anthropology, and what the greater implications of this are for the discipline.

Forensic anthropology is a relatively young field, having been formally established in the 1970s. Over the past several decades, the field has continued to evolve in various ways. For example, the definition of forensic anthropology has been expanded to include forensic archaeology, taphonomy, and even socio-cultural approaches to medicolegal issues. Further, the development of methods has shifted toward standardization and validation to meet the *Daubert* challenge. While these changes have served to broaden and standardize the discipline, the education, training, and the practice of forensic anthropology has remained largely static.

Pellegrino argues the practice of a trade can be considered a profession if the trade requires: (1) a body of specialized knowledge; (2) practice within an ethical framework; (3) fulfillment of some societal need; and, (4) some form of social mandate allowing for latitude in a discipline's ability to set standards for education and performance of its practitioners.[1]

To address these requirements within the context of forensic anthropology, this presentation will discuss how forensic anthropologists: (1) possess a unique knowledge base of archaeological methods, skeletal biology, cultural context, and analytical skills; (2) practice within an ethical framework, as long as the forensic anthropologists are certified by the American Board of Forensic Anthropology (ABFA) or are members of the American Academy of Forensic Sciences (AAFS); (3) provide a societal need via the medicolegal nature of the discipline; and, (4) possess a latitude for setting standards for both education and the performance of forensic anthropology, previously through the work of the Scientific Working Group for Forensic Anthropology (SWGAnth) and now of the National Institute of Standards and Technology (NIST) Organization of Scientific Area Committees (OSAC) and the AAFS Standards Board (ASB).

While forensic anthropology meets the structural requirements to be considered a profession, the field is lacking in several criteria, as clear standards for ethical behavior, education, and the performance of forensic anthropology have not been expressly delineated or widely accepted. The current processes for ethical practice, certification, and accreditation as stipulations for requirements to act as a forensic anthropologist extend to very few individuals. The reality is that analysts without the current requisite education, training, professional memberships, or Quality Assurance (QA) practices are performing forensic anthropological casework. To address these issues, this presentation further discusses the need: (1) to approach education and training in terms of qualifications and certification; (2) for an enforceable ethical code; and, (3) to approach the practice of forensic anthropology through processes for QA and traceability via accreditation and following published standards and guidelines (e.g., those published by the SWGAnth or NIST OSAC Anthropology Subcommittee).

In the present state of forensic anthropology as a profession, there is little consensus or acceptance of professional practices. As such, the current guidelines are not being widely adhered to and are sometimes completely ignored. The implications of this are that analysts may be acting unethically, either by performing analyses beyond their professional expertise (as determined via certification), misrepresenting their qualifications (i.e., whether or not they are certified to perform the work), or doing harm to the case/investigation (by practicing without QA and traceability protocols). Lacking agreement on ethical practices, qualifications, and accreditations weakens the credibility of forensic anthropology as a profession, and invites non-professionals to practice forensic anthropology without oversight. It cannot be expected that law enforcement agencies, attorneys, or medical examiners be trained in examining the nuances of forensic anthropology as a profession; thus, it is up to forensic anthropologists to collectively generate and follow standards for the practice of their profession. If the discipline is to grow and stay relevant, considerations need to be given to the standardization of ethics, education, training, certification, and accreditation in ways that are inclusive and enforceable.

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Professionalism, Ethics, Qualifications

A86 The Suitability of Digital Photographs to Evaluate Decomposition of Pig Carcasses in a Tropical Climate: A Preliminary Investigation

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After attending this presentation, attendees will understand the effects that methodology and the reliance on digital photographs have on the visual assessment of decomposition.

This presentation will impact the forensic science community by demonstrating that decomposition scores generated using photographs tend to overestimate decomposition in the early postmortem period and underestimate decomposition in the extended postmortem period. These scores were also affected by the method used, which did not always accurately reflect real-time decomposition.

The use of color photographs, including digital ones, is often encountered in medicolegal death investigations and retrospective research where the actual remains are not available; however, knowledge as to the suitability of photographs as substitutes for human remains in forensic reconstruction scenarios is currently limited.[1] Some studies demonstrated that the ability to observe and document postmortem changes can be compromised when photographs are relied upon under certain conditions.[2,3] This may hinder the ability to draw reliable postmortem conclusions, including the Postmortem Interval (PMI), from photographs.

To address this gap in the knowledge, the present study investigated the suitability of photographs as a proxy for pig carcasses that are commonly used in empirical research where human corpses are not available, using two published scoring methods to generate a Total Body Score (TBS).[1,4]

As a first step, the decomposition of three exposed pig carcasses (*Sus scrofa domesticus*) was evaluated in real-time at Chaminade University of Honolulu (CUH) in Hawaii (*n* observers=9), using both scoring methods. A month later, 2D digital color photographs of the same carcasses were evaluated by the same group of observers. As a second step, the scoring of the photographs was repeated with a second group of observers of similar composition and size (*n*=9) from University College London (UCL) in the United Kingdom, to investigate the replicability of the results and test the hypothesis that real-time TBS scores will not differ from photograph scores. Differences between real-time and photo-based TBS were identified using a two-way Analysis of Variance (ANOVA) with *post hoc* Tukey Honest Significant Difference (HSD) tests.

Among the CUH group, significant differences between the real-time and photo-based TBS were found with Megyesi et al.'s method ($F_{1,34}$=8.48, p=0.006).[1] Photo-based TBS were consistently greater than real-time TBS before 30 hours postmortem but were then consistently less until the end of the experiment (1,014 hours/42 days postmortem) (p <0.05). In contrast, no significant differences between the real-time and photo-based TBS were found with Keough et al.'s method ($F_{1,34}$=0.104, p=0.749); however, photo-based TBS were consistently greater than real-time TBS before 30 hours postmortem, but were then consistently less until the end of the experiment (p <0.05).[4]

Similar results were observed among the UCL group where significant differences between the real-time and photo-based TBS were found with Megyesi et al.'s method ($F_{1,34}$=13.3, p=0.001).[1] Photo-based TBS were consistently greater than real-time scores before 54 hours postmortem but were then consistently less until the end of the experiment (p <0.05). In contrast, no significant differences between the real-time and photo-based TBS were found with Keough et al.'s method ($F_{1,34}$=2.63, p=0.114) and no significant variations of the TBS according to PMI were observed (p>0.05).[4]

The results lead to the rejection of the research hypothesis because the scoring of real-time and photo-based TBS was found to be highly dependent on the method used and the PMI. Keough et al.'s method appears more suitable for photographs of decomposing pigs in a tropical climate. Overall, consistent overestimation in the early postmortem periods and underestimation in the extended postmortem periods of photo-based TBS demonstrates that the inability to access the remains in real-time can significantly impact the accuracy of TBS. This can have profound consequences on the estimation of PMI, which is a key element in medicolegal death investigations. These initial results indicate the need for further research to address the differences in the applicability of the two scoring methods to photographs and the development of ad hoc practices when photographs are used as substitutes for remains in a forensic setting.

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Forensic Taphonomy, Total Body Score, Decomposition

A87 Predicting the Postmortem Submersion Interval (PMSI) From the Microbiome of Bone in a Fresh Water Lake

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After attending this presentation, attendees will have a greater understanding of the use of microorganisms in estimating the PMSI for skeletal remains recovered from a freshwater lake.

This presentation will impact the forensic science community by providing information concerning a novel area of research, the use of longitudinal succession of microbiomes on skeletal remains submerged in a freshwater lake to predict PMSI.

When individuals die in or are disposed of in aquatic environments, the remains may not be recovered for a period of time, rendering identification difficult. Water turbulence, exposure to aquatic scavengers, as well as accumulated temperatures contribute to tissue decomposition. Once remains are skeletonized, microorganisms endogenous to both the environment and the skeletal remains themselves participate in microbioerosion of bone, increasing the rate of bone diagenesis in water.[1,2] Being able to estimate time since death or, in the case of water-related incidents, the PMSI is pertinent to medicolegal death investigations. Knowing the PMSI assists investigators in identifying remains, validating eye witness statements, and narrowing suspect pools. Because microorganisms are present throughout decomposition, this study proposes the use of longitudinal succession (including aspects of richness, diversity, and indicator taxa) of the microbiome of submerged skeletal remains to estimate PMSI. Advancements in metagenomics approaches (e.g., next-generation sequencing and pipeline analysis software) have demonstrated that bacterial communities can be a useful tool for estimating PMI and PMSI; however, none of these studies have focused on skeletal remains in aquatic environments.[2,3]

In this study, fresh pig (*Sus scrofa)* bones (*N*=100 rib and *N*=100 scapula samples) were purchased from a butcher. Beginning in November 2016 through November 2017, the bones were in cages attached to a flotation device and submerged in Henley's Lake in White Hall, VA (38° 05' 11.7" N, 78° 41' 02.8" W). Water temperature was recorded hourly using waterproof loggers. Every 250 Accumulated Degree Days (ADD), five scapulae, five ribs, and water samples were collected, photographed, and stored at -80°C until processed. Water samples were filtered; meanwhile, bone samples were cut and ground into a powder using liquid nitrogen in a mortar and pestle. DNA was extracted and purified using ChargeSwitch® gDNA Plant Kit. Following parameters set forth by Kozich et al., extracted samples were used to conduct sequencing-by-synthesis of microbial 16S recombinant DNA (rDNA) variable region 4 using the Illumina® MiSeq® 2X300 paired-end sequencing.[4] The resulting data were analyzed via MiSeq® SOP Mothur version 1.36.1.[5]

Preliminary Analysis of Molecular Variance (AMOVA) encompassing collections 0–1,250 ADD indicated a significant difference in the bacterial structure between rib-scapula, (p <0.0002), rib-water (p <0.0002), and scapula-water (p <0.0002). Therefore, samples were analyzed by sample type. Phylum and family level changes were observed for each sample type across ADD. In addition, rib (R^2=0.48) and scapula (R^2=0.64) samples demonstrated a positive relationship between Shannon species diversity and logADD, whereas the water samples showed a negative relationship (R^2=0.48). The decrease in diversity observed in water samples may be related to changes in temperature between the seasons, with bacteria able to survive well on a bone nutrient substrate, but not in colder water.

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PMSI, Waterlogged Bone, 16S rRNA Gene

A88 The Faunal Succession of Forensically Important Arthropods and Large Vertebrate Scavengers in Rural Northwest Florida

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After attending this presentation, attendees will better understand the biodiversity of forensically important arthropods and large vertebrate scavengers in rural northwest Florida where little is known about these taxa.

This presentation will impact the forensic science community by providing a survey of arthropods that are associated with decomposing pig carrion and large vertebrate animals that scavenge remains. Even though decomposition stages are the primary component to making Postmortem Interval (PMI) estimations, entomological evidence can provide more precise assessments.[1] Nevertheless, insect activity is dependent on factors such as temperature and habitat, which in turn will affect decomposition rates.[2] Since climactic conditions and insect assemblages vary around the country, and within different habitats, it is important to conduct research in a variety of ecological niches. While Florida is home to one of the most unique and diverse ecosystems, flatwoods and seepage bogs can be found along the Lower Gulf Coastal Plain of Florida and Alabama. Thus, the information garnered from this study may be used to understand arthropod succession and forensically important insect assemblages within Escambia County, FL, and surrounding areas with similar subtropical climates.

This study was conducted using pig carrion (*Sus scrofa* Linnaeus) as models representing human cadavers. One pig carcass was placed in dry flatwoods, while the other was placed in a seepage bog. Adult Calliphorid flies and beetles were collected and some blow fly larvae were reared to the adult stage and identified. Although the pig carcasses were enclosed in cages so large vertebrate animals would not disturb insect activity, game cameras provided evidence for local large vertebrate scavengers that presented themselves in the areas of the pig carcasses.

Based on previous research, it was hypothesized that insect assemblages would be similar but slightly different than those from northcentral Florida.[3] It was also hypothesized that habitat would result in varying insect assemblages, and that large vertebrate animals would be interested in pursuing decomposing remains.

Seven species of Calliphoridae were collected and identified from the carrion, including *Calliphora vicina* (Robineau-Desvoidy), *Chrysomya megacephela* (Fabricius), *Chrysomya rufifacies* (Macquart), *Cochliomyia macellaria* (Fabricius), *Lucilia coeruleiviridis* (Macquart), *Lucilia cuprina* (Wiedemann), and *Phormia regina* (Meigen).

P. regina was the predominant species collected and reared from both pig carcasses. There were also six Coleoptera species collected from the carrion, including *Saprinus pennsylvanicus* (Paykull), *Aphodius rufipes* (Linnaeus), *Trox suberosus* (Fabricius) *Oiceoptoma rugulosum* (Portevin), *Necrodes surinamensis* (Fabricius) *and Creophilus maxillosus* (Linnaeus). *O. rugulosum* and *S. pennsylvanicus* were the most common species collected. These observations reveal that species assemblages in northwest Florida are similar, but slightly different than those in northcentral Florida.[3] Many large vertebrate animals, including vultures (*Cathartes aura and Coragyps atratus)*, opossums (*Didelphis virginiana*), and feral dogs (*Canis familiaris*), showed interest in the carrion. The opossums and feral dogs accessed the carrion and scavenged the remains.

In conclusion, this project provides relevant information to the database of forensic literature regarding medicolegal entomology, decomposition, and postmortem interval. The results can be used to identify potentially forensically important fly and beetle species within Northwest Florida, as well as surrounding areas.

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Decomposition, Arthropods, Seepage Bog

A89 Analysis of the Interactions Between Taphonomic and Pathological Processes

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After attending this presentation, attendees will better understand how taphonomic events interact with different pathological processes and how these interactions affect overall skeletal preservation.

This presentation will impact the forensic science community by providing information regarding how the taphonomic environment of a burial can affect the preservation and presentation of pathology. As pathological conditions are often used along with the biological profile to create a positive identification and forensic remains are found in a variety of taphonomic circumstances, it is vital to understand how these factors interact.

Variation in pathological processes and damage associated with taphonomic processes can greatly affect observable skeletal material. Research into how these processes interact with each other is therefore important to combat biases that arise from incomplete or damaged skeletal material. Understanding the specific effects of taphonomy on pathology, and their cumulative relationship with preservation, is valuable to forensic practitioners. Pathological conditions are often used to help create a positive identification with a missing persons report. The National Missing and Unidentified Persons System (NamUs) often includes health information that can be used to identify unknown remains. As forensic remains are found in a variety of taphonomic circumstances, it is vital to understand how those factors influence the identification of pathology in skeletal remains.

This study tests the hypothesis that taphonomic events differentially affect bone exhibiting pathologies that increase or decrease bone density. Taking into account density-mediated attrition, taphonomic events are examined separately against pathologies that build or remove bone matrix.

Data were collected from three samples. The first was the Santa Clara Valley Medical Center (VMC) collection, a historic pauper cemetery currently housed at California State University, Chico (CSUC). The second sample was the forensic modern collection at the same location. The final sample included the New York City Office of the Chief Medical Examiner (NYC OCME) forensic anthropology cases. In total, 77 individuals were analyzed. Taphonomic processes were coded as present/absent based on skeletal presentation. Pathological indicators were also coded as present/absent. Attempts at diagnosis were not undertaken; instead, pathological indicators were considered based on the cellular response they elicited. Categories used included osteoblastic, osteoclastic, mixed response, mechanical destruction (e.g., eburnation), and healed trauma. Preservation was recorded in quartiles based on percent present. Chi-square and Cramer's V were conducted using R.

Of the 13 taphonomic events present in the sample, two have significant relationships with bone that exhibited no pathology: scavenging (p<=0.01, V=0.20) and weathering (p=0.04, V=0.08). Osteoblastic response is significantly associated with the same two processes: scavenging (p<=0.01, V=0.14) and weathering (p=0.04, V=0.08). Healed trauma is only significantly associated with overall skeletal preservation (p<=0.01, V= 0.14). Pathological processes that reduce bone density produce a greater number of significant associations. Osteoclastic response is associated with burning (p=0.04, V=0.08). Mixed reaction has significant relationships with staining (p=0.02, V=0.09), Postmortem Damage (PMD) (p=0.01, V=0.10), and acid corrosion (p<=0.01, V=0.11). Mechanical destruction is significantly associated with four taphonomic events: PMD (p=0.02, V=0.09); acid corrosion (p<=0.01, V= 0.12); adhered material (p=0.01, V=0.09); and exfoliation (p<=0.01, V=0.11).

The number of significant interactions demonstrates that taphonomy does differentially interact with pathologies that reduce or increase bone density. Pathological processes that build bone only interacted significantly with two taphonomic events, while pathological processes that remove bone interacted with six, including the more damaging taphonomic processes like acid corrosion and PMD; however, when the direction of the relationships is analyzed, pathological processes that remove bone have lower percentages of taphonomic damage than elements that do not exhibit those pathologies. This may be a byproduct of the extremely poor preservation found in the VMC collection. The high rates of acid corrosion and PMD at this site may have resulted in the removal of elements with reduced structural integrity before excavation, producing a sample with above-average density. While this result is possibly a product of the samples used and further analysis must be undertaken to assess these differences, it may indicate that the interaction between pathology and taphonomy encompasses more than just overall bone density.

Taphonomy, Pathology, Skeletal Preservation

A90 A Comparison of Two Methods for Estimating the Postmortem Interval (PMI) From Decomposed Human Remains

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After attending this presentation, attendees will understand how estimating the PMI, also known as the time since death, can differ between methods. Recommendations for future research and validation studies will be provided.

This presentation will impact the forensic science community by providing the results of a comparison of forensic anthropological and entomological methods of PMI estimation from medicolegal cases and reinforce the need for further studies using a multidisciplinary approach.

The goal of this study is not to test the accuracy of the methods, but rather to demonstrate the relationship between them. The two methods that are compared in this study include the Total Body Score (TBS) method used by anthropologists and the Time of Colonization (TOC) method used by entomologists.[1]

In 2005, Megyesi et al. published an anthropological method to estimate PMI by quantifying the stages of decomposition using a standardized scoring system.[1] The system involves scoring the head/neck, trunk, and limbs using four stages of visually assessed decompositional changes. The resulting score, the TBS, is the sum of the scores of all three body regions. TBS is converted into a PMI estimate via the use of Accumulated Degree Days (ADD). ADD is based on local average daily temperatures and provides an estimate of the number of days between the date of death and the date of recovery of the remains.

Entomologists often use the TOC method when estimating PMI based on the presence of insects found in and around the body of a decedent. Using this method, forensic entomologists identify and analyze the age of collected insect specimens to estimate TOC, which may then be used as an indicator of the PMI. Local weather station data is used to estimate the minimum Accumulated Degree Hours (ADH) or ADD for which the insect species present could have reached the maturity level at which they were collected. The relationship of the TOC estimate to the PMI is dependent on various assumptions that may vary depending upon the case.[2]

The present study is a retrospective examination of cases autopsied at the Harris County Institute of Forensic Sciences in Harris County, TX. The study sample was composed of 100 decedents in varying stages of decomposition. The PMI for each of the case had previously been estimated by the staff forensic entomologist using the TOC method. The inclusion criteria for the study sample was based on investigative reports of when the decedent was last known alive. TBS was calculated for each of these cases using autopsy photos and following the Megyesi protocol. The resulting TBS scores for the sample ranged from 4 to 33. Temperature data from a local weather station was used to calculate ADD. A non-parametric Wilcoxon paired-samples test was used to compare the PMI estimates from both methods. The results revealed a statistically significant difference between the PMI estimates derived from the two methods. TBS typically provided a higher PMI than TOC.

This study demonstrates a difference between the two methods for estimating PMI and provides a foundation for future studies. Since accuracy of the methods was not tested, additional research is required to address this issue. Although much research has been conducted on each method independently, there is a paucity of research addressing the comparative efficacy of the anthropology and entomology methods. Therefore, using controlled and context-appropriate validation studies to investigate which methods perform better in different conditions is recommended.

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Postmortem Interval, Total Body Score, Time of Colonization

A91 The Differential Effects of Environmental Factors on Immature and Mature Bone Degradation: A Controlled Experiment Using Pig Skeletal Remains

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After attending this presentation, attendees will understand the chemical changes in bone composition that occur as a result of the postmortem interval, the degradation environment, and the age at death of the individual.

This presentation will impact the forensic science community by not only advancing the knowledge of the dependency of bone weathering on postmortem environments, but also by providing a direct comparison between the relatively poorly studied chemical decomposition of juvenile bone and the more well-known breakdown of adult skeletal remains. This data provides a means for evaluating the current methods of estimating the time since death of juveniles in the forensic context.

Estimation of postmortem interval is an integral aspect of the forensic identification process. Such estimations provide investigators with a timeline, which can rule out suspects and help to narrow the pool of possible victims. In the case of skeletonized remains, time since death is approximated using trends in bone breakdown. These trends were developed using adult remains and have provided investigators with a relatively predictable timeline for adult skeletal decay; however, juveniles have not been sufficiently studied and, therefore, the applicability of time-since-death estimation methods to their remains is not well known.

This study uses a porcine model to explore the role of bone maturity with regard to: (1) overall susceptibility of the skeleton to chemical degradation; and, (2) the interaction of bone material with different burial environments. The ulnae of immature and mature pigs (*Sus scrofa)* were mechanically defleshed and used as a proxy for human bone of distinct infant and sexually mature age groups. Samples (n=200) from both age groups were left to degrade in a climate-controlled greenhouse, either buried or on the soil surface. These two varying environments provide the comparison of differing environmental impacts. Every month, four bones from each age group and environment were collected. Ash weight analysis was performed on each sample to determine the relative water, collagen, and mineral composition of the bones.

The results of this study indicate that, in the early postmortem interval, the degradation rates of collagen and mineral content of both immature and mature bone are relatively similar. While the collagen content of immature bone was initially higher, and the mineral content initially lower than the mature bone samples, the rate at which these values changed through time appears to be impacted solely by the environment. The buried environment resulted in a rapid destruction of collagen in bones from both age groups, with the values remaining relatively constant throughout the rest of the experimental period. The mineral content was inversely affected by the environment within the first month, then remained constant in both age groups until month six, when there was a noticeable decrease in the values, regardless of the environment. The water content, on the other hand, trended upward for both age groups in the buried environment; however, only the mature samples demonstrated a water content increase in the subaerial environment.

These results indicate that degradation environment plays a major role in the chemical decomposition of bone material, while the maturational stage of the bones only has an affect on the changes in water content throughout the postmortem interval. In particular, this study suggests that, unlike popular belief, immature bone does not seem to degrade faster than more mature bone, at least during the early postmortem period. These results have the potential to inform the forensic community of the behavior of juvenile bone in varying degradation environments, subsequently improving the accuracy of estimating time since death and identifying children in the forensic context.

Postmortem Interval, Juvenile, Degradation

A92 Science as a Human Right: Using DNA to Identify Missing Migrants

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After attending this presentation, attendees will understand how DNA science and technology can be used to support the identification efforts of undocumented migrants that die attempting to cross the United States-Mexico border.

This presentation will impact the forensic science community by highlighting alternative strategies for the positive identification of unidentified remains.

Since the year 2000, the Colibrí Center for Human Rights, in collaboration with the Pima County Office of the Medical Examiner (PCOME), has collected missing person reports from nearly 3,000 families of those who have disappeared attempting to cross the United States-Mexico border. Also during this time, the remains of more than 2,800 people have been discovered in southern Arizona. Despite the efforts of dedicated forensic professionals, consular officials, and others, 30% to 40% each year are not identified, adding up to a cumulative total of more than 1,000 unidentified human remains cases.

Due to the aridity of the Sonoran Desert and the remote paths migrants take to cross the border, the remains of the dead are difficult to identify without DNA. Even after a strong circumstantial comparison or identification hypothesis is made between an unidentified set of remains and records for a missing person, it is common for DNA to still be required to confirm or refute the match; however, many families of missing migrants cannot utilize the typical mechanism in the United States for investigating missing persons, which depends on reporting to United States law enforcement. Because families of missing migrants live in the United States as undocumented immigrants, or in Mexico or Central America, they are either afraid to contact police, or they are turned away when they attempt to file a report because their case is seen as out of jurisdiction. The more socially marginalized a family is, the more obstacles they face in obtaining information about their missing loved one.[1] The results of a genetic study of human remains examined at the PCOME indicated that those with indigenous backgrounds are less likely to be identified than those with more European ancestry.[2] This indicates that innovative approaches to human identification are needed that focus on macro-level structural conditions of access to the benefits of scientific progress rather than only on micro-level laboratory methods. Families are afraid of or unable to access these systems and wait for news for decades when the remains of their loved ones languish in cemeteries along the border.

The purpose of this presentation is to report the results of a new initiative by the Colibrí Center for Human Rights to bring the science and technology of DNA to the underserved population of Latin American immigrants in the United States. The program is designed to lower the obstacles to care for families of the missing by building sanctuary around the process of submitting DNA. Previous research in public health and medicine has demonstrated that undocumented Americans face challenges to accessing care due to a combination of fear of deportation, poverty, and isolation.[3-6]

The Colibrí Center's DNA Program addresses each of these challenges strategically to make the process of submitting DNA as easy as possible for families. Colibrí hosts DNA collection clinics throughout the United States. These are held in undisclosed sanctuary churches in major cities. Colibrí representatives partner with foreign consulates, a private genetic laboratory, and medicolegal officials, but do not allow United States law enforcement, press, or onlookers into the space during DNA collection clinics.

This presentation will report on two years of results for this initiative. Colibrí has collected Family Reference Samples (FRS) from 315 participants, representing 150 missing person reports. Results to date include 12 positive identifications achieved in collaboration with the PCOME and 26 additional strong genetic matches needing follow-up in the form of further FRS collection. Many of the identifications achieved through this project pertain to cold cases in which the individual died and was examined years ago. This initiative demonstrates the need for approaches to the application of DNA science and technology that are informed by public health literature relevant to the population being served.

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Missing Persons, United States-Mexico Border, DNA

A93 The Approach Toward Identification of Deceased Migrants in the United States and European Union: A Comparative Study Between LABANOF (Italy), OpID (Texas) and PCOME (Arizona) Experiences

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After attending this presentation, attendees will have acquired an overview of the complex topic of identifying migrants who either die in the United States or the European Union, with special emphasis on the differences and similarities existing in the projects currently conducted in Arizona, Texas, and Italy.

This presentation will impact the forensic science community by illustrating the importance of applying forensic anthropology to the emerging field of humanitarian sciences, which has become increasingly important in recent years and whose potential has yet to be fully recognized in other geographical regions and contexts in the world.

Due to the rise in migrant deaths in Europe, and the continuing crisis of migrant deaths in the United States, an assessment comparing and contrasting the approach of forensic anthropology in these humanitarian crises was conducted. The materials and methods for this research derive from an extensive period of participant observation, namely three years at the Laboratory of Forensic Anthropology and Odontology (LABANOF) in Italy working on the identification of migrants who have perished in the Mediterranean and three months in the United States embedded with anthropologists working at Operation Identification (OpID) at the Forensic Anthropology Center at Texas State University and the Pima County Office of the Medical Examiner (PCOME) in Arizona. Data was derived from systematic surveys of organizational and staffing structures, laboratory protocols and Standard Operating Procedures (SOPs), casework records, and experiences regarding the identification of migrant remains.

Results indicate there are many similarities regarding difficulties in the identification of deceased migrants around the world. The remoteness of migrant deaths often creates challenges in finding or recovering remains, with deaths in Arizona and Texas occurring in desolate, arid lands, while in the Mediterranean, deaths are often the result of a ship sinking and taking hundreds of lives with it at once. Often in the United States and European Union, migrant remains are not found with identification cards or visas that could provide an immediate identification hypothesis. Furthermore, the absence of missing persons reports and/or antemortem data is a universal difficulty.

Similarities and dissimilarities were also found in the way the three different organizations operate within their respective jurisdictions to attempt migrant identifications. Due to the high levels of deaths in Arizona for years, the PCOME is a state-funded agency with two full-time forensic anthropologists to help address the identification of unidentified skeletal remains. Since 2001, their agency has received 2,615 remains of presumed migrants and has helped facilitate the positive identification of 1,676 individuals. As part of a governmental agency, the PCOME also has a staff of death investigators who operate as case managers.

In contrast, the experiences of academic-based LABANOF and OpID have been largely volunteer-based, with OpID gaining a small paid staff only in recent years. This is, in part, due to the higher number of dead in these areas within the past five years, but also to the fragmented nature of migrant death investigations in Texas and the European Union, resulting in a smaller number of remains received by these organizations. Since its inception in 2013, OpID has received 238 sets of unidentified remains and has helped facilitate the positive identification of 24 individuals, while LABANOF has received approximately 1,000 remains/postmortem data coming from three different shipwrecks and has helped facilitate the positive identification of 21 individuals who died in the October 3, 2013, shipwreck that occurred in Lampedusa. The academic-based operations of LABANOF and OpID also lack a staff of death investigators, and therefore anthropologists have taken on the important role of case managers.

Within the United States, there are national databases, such as the National Missing and Unidentified Persons System (NamUs) and the Combined DNA Index System (CODIS), that offer a central repository for case information across jurisdictions, which are utilized by both the PCOME and OpID; however, in the European Union, there are no similar databases, resulting in a more fragmented system than in the United States.

In spite of their unique challenges, anthropologists in all three jurisdictions have implemented strategies and protocols for working with local, regional, and international members of law enforcement and non-governmental organizations to achieve identifications and move cases forward, though approaches differ based on locally available resources and legislative barriers; however, a general model of anthropological involvement has proven successful across the United States and European Union, and could be successfully implemented in other arenas of humanitarian need across the world.

Human Rights, Migrant Identification, Forensic Anthropology

A94 Humanitarian Science in the Texas Borderlands: Incorporating a Sociopolitical Perspective to the Forensic Investigation of Migrant Identification

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The goals of this presentation include an appreciation of the need for: (1) coordination, collaboration, and consistency among various stakeholders during large-scale humanitarian science efforts; (2) knowledge of how the local work of migrant identification connects local issues with global systems and history; and, (3) understanding that forensic science in a humanitarian crisis context requires balancing neutrality and engagement in novel ways.

This presentation will impact the forensic science community by illustrating how sociopolitical conditions and processes shape the work of forensic scientists in this context and how they navigate and transform social relationships and power dynamics circumscribing their work by highlighting identification efforts made by forensic anthropologists since 2013. This presentation proposes these forensic anthropologists are actors in the political economy of forensic science in the Texas Borderlands as they position themselves within a complex set of interactions. This allows them to garner the resources necessary to continue their work, as well as provides them the position to tell the stories of the dead. This presentation pulls from critical reflections of forensic anthropologists working in the Texas Borderlands to illustrate the complexities of operating within the current system, highlights the novelty of this approach, and contemplates how a sociopolitical approach to migrant death can be incorporated into the future of this work.

Forensic anthropologists are often consulted by law enforcement and medical examiners to assist in the recovery of human remains from various contexts and analyze those remains to address questions regarding the death and identity of the individual. While the majority of cases analyzed by forensic anthropologists involve a single individual, practitioners are increasingly becoming involved in mass disaster and mass death situations that unfold in relation to, or as a consequence of, processes of public policy and governance. For example, forensic anthropologists were first formally utilized in politically charged humanitarian efforts in 1984 in Argentina to assist in the identification of individuals “disappeared” during what became known as the Dirty War. The unique skillset of forensic anthropologists to locate and excavate graves as well as analyze skeletal remains positioned them to contribute to the work of identification, as well as the work of witnessing and legitimizing claims of human rights abuses.

In more recent years, forensic anthropologists have utilized their skills in humanitarian efforts in South Texas to exhume and identify migrants who perished crossing the southern United States border. With virtually no resources and deaths at mass disaster proportions, many South Texas counties made the decision to bury unidentified migrants in pauper’s areas of county cemeteries until they obtained the resources for costly forensic investigations. Unfortunately, little documentation survives detailing exactly where the burials are positioned and exactly how many burials are located in each cemetery. Local officials invited forensic anthropologists from several universities to locate, remove, and preserve the context of the burials through proper documentation. Once exhumed, scientific tools were utilized by forensic anthropologists during the investigation into personal identification, including osteological, histological, dental, isotopic, and genetic analyses; however, these scientific practices were implemented in a broader political economic context that formed and was shaped by politics and processes of social justice and human rights.

This presentation, from the view of a visiting volunteer forensic science team, illuminates migrant identification efforts in South Texas as a critical vantage point from which to understand the sociopolitically charged nature of humanitarian forensic science in a contemporary globalized society. Migrant identification requires cooperation, communication, information sharing, and resource coordination between a variety of local, national, and international stakeholders that may each have different missions, motivations, and goals. Thus, many forensic anthropologists working on migrant identification operate as part of a larger, sometimes fragmented and contentious, initiative toward social justice and human rights. As such, forensic investigations in this context are positioned in such a way as to spotlight particular public policies, practices, and social processes that lead to inhumane treatments in life and in death. Forensic scientific practice offers a window into the dynamic and global nature of this crisis and the response mechanisms currently in place and must be viewed through the interconnected lenses of history, power, and scientific practice.

Forensic Anthropology, Humanitarian Science, Migrant Death

A95 Assessing the Spatial Patterns of Undocumented Border Crosser (UBC) Deaths in the Southern Arizona Desert

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After attending this presentation, attendees will better understand the application of Geographic Information Systems (GIS) to the field of forensic anthropology through the analysis of spatial patterns of the recovery locations of deceased individuals over a large geographic area.

This presentation will impact the forensic science community by highlighting the utility of geospatial analyses for providing supplemental information to the investigation of UBCs recovered from the southern Arizona desert and potentially leading to more identifications.

Undocumented immigration into the United States via the Mexican border has been a topic of national debate for well over two decades, in part due to the continually high number of deaths as individuals attempt to enter the country via clandestine means. Since 2001, more than 2,600 UBCs have died in the Sonoran Desert and surrounding regions falling under the jurisdiction of the Pima County Office of the Medical Examiner (PCOME) in Tucson, AZ. The PCOME contracts with 11 of 15 Arizona counties, including the four bordering Mexico, averaging roughly 169 UBC deaths a year with an identification rate remaining steady at around 64%.[1]

Migration is an inherently patterned process, including a person's motivation for leaving, the routes they take, and the places they reside; so, presumably, patterns may emerge as to where they die. A directed analysis of the location and distribution of undocumented migrant deaths processed by the PCOME was warranted due to previous studies being only preliminary or limited to the geography of where people are dying in southern Arizona.[2] PCOME case recovery protocol includes the recording of GPS coordinates of the location of the remains, which is stored and mapped at both the PCOME internal database and on the Open GIS (OGIS) platform at www.humaneborders.info. Public data available via OGIS is limited and, therefore, was supplemented using PCOME records of 1,681 identified individuals to include nationality. The majority of identified individuals recovered at the PCOME are of Mexican origin (n=1,403, 84%), followed by the next two largest groups of Guatemalans (n=154, 9%) and Salvadorans (n=45, 3%).[1]

The purpose of this research was to examine the geospatial properties of deceased migrants recovered from southern Arizona to potentially aid in both the investigative and identification processes. Therefore, a combination of exploratory spatial data and GIS analyses was conducted on several demographic variables of identified UBCs (n=1,681). Spatial autocorrelation analyses — including Moran's I and Local Indicator of Spatial Association (LISA) cluster maps — for the variables of recovery year, sex, and nationality or country of origin revealed significant positive spatial relationships for each. As the majority of individuals examined at the PCOME are young, Mexican males, further tests were conducted on nationality, which also resulted in statistically significant positive spatial autocorrelation and areas of spatial clustering. Furthermore, clusters of recoveries from both the beginning and second half of the study period (2001–2016) were visualized using LISA cluster maps and express an east-west movement of case locations. These results may indicate that route choice, as seen through death and recovery location, is also patterned via a person's sex, country of origin, and when they crossed. This supplements other anthropological, sociological, and criminal justice research on the topic and provides further information to the study of international migration along the United States-Mexico border.

Future research will combine these geospatial data with the predictive powers of the biological data (craniometrics and morphometrics) on ancestral affiliation for identified individuals to potentially predict where an unknown individual is from and thus facilitate a more efficient identification.

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Undocumented Border Crossers, Geospatial Analysis, International Migration

A96 The Application of Stable Isotopes and Geostatistics to Infer Region of Geographic Residence for Undocumented Migrants

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The goals of this presentation are to: (1) determine how undocumented migrants fit into the established models by constructing coarse-grained baseline strontium and oxygen isoscape models using published data; and, (2) determine if using a probability assignment method for the dual-isotopes can aid in estimating the most likely regions of geographic residence for unidentified deceased migrants recovered along the Texas-Mexico border. Establishing residential history can reduce the potential matches for unknown cases within the National Missing and Unidentified Persons System (NamUs) database and potentially aid in generating positive identifications for deceased migrants.

This presentation will impact the forensic science community by shedding light on the issue occurring at the southern border and promoting interdisciplinary approaches to forensic and human rights problems.

Hypothesis: A dual-isotope isoscape and likelihood assignment method can be used to estimate region of origin for deceased migrants recovered along the Texas-Mexico border and improve the probability of making a positive identification.

Outcomes: This presentation will elucidate the humanitarian crisis occurring at the United States-Mexico border and promote interdisciplinary approaches to forensic identification and human rights issues. Specifically, applying methods from biogeochemistry and geostatistics to answer forensic anthropological questions will advance the field's foothold in the forensic sciences. An additional outcome is closure for the families that have lost loved ones and can finally have them returned home and laid to rest.

Synopsis/Methods: Recently developed bedrock, water catchment, and soil strontium isoscape models for Mexico, Central America, and the Caribbean are adjusted using bioavailable strontium ($^{87}Sr/^{86}Sr$) data collected from a variety of published sources. Oxygen (^{18}O) precipitation data are gathered from the Global Network of Isotopes in Precipitation online database. Digital Elevation Models)DEMs) are factored into the oxygen isoscape to account for isotopic changes in elevation for oxygen precipitation isotopes. Compiling the oxygen and strontium data in arcGIS® produced a multi-layered isoscape depicting isotopic variation in Central America, Mexico, and the Caribbean. Using the Operation Identification collection at Texas State University, dental samples (preference is given to maxillary premolars) are collected from five individuals (n=5) and analyzed for strontium and oxygen. Associated cultural material recovered with the deceased migrants are used as a predictor for region of geographic residence (e.g., an individual carrying quetzals is more likely to originate from Guatemala than Mexico). Strontium and oxygen isotope values extracted from the teeth are run through a likelihood assignment model established in previous publications to produce probability densities for the most probable regions of residence for each individual.

Results/Conclusions: The goal of the research is to map the isotopic variation. The likelihood assignment model in R studio uses probability densities to estimate most likely region of origin. After running the strontium and oxygen ratios through the model, the results consist of multiple heat maps displaying the probability densities for regions of most likely origin for each of the five individuals. Strontium isoscapes tend to be the more accurate model for provenancing migrants, while the oxygen isoscape for the region has a low accuracy rate due to the lack of precipitation data for the regions. Overall, the dual-isotope approach proves successful in narrowing the region of geographic residence for deceased migrants recovered near the Texas-Mexico border. Adding more strontium and oxygen data to each of the isoscapes will improve the method, allowing it to be applied to all migrants recovered along the United States-Mexico border and to be adapted for other regions of the world where deceased unidentified humans are recovered. The implementation of geostatistical and biogeochemical methods to investigations of unidentified human remains will improve existing techniques and increase the efficiency of current identifications.

Spatial Analysis, Forensic Anthropology, Stable Isotopes

A97 Digital Technologies and Forensic Archaeology: Reflections on the Experiences of the Committee of Missing Persons in Cyprus

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After attending this presentation, attendees will better understand the potential of digital forensic archaeological methods to: (1) improve search efficiency and accuracy; (2) ease access to various environments; (3) inform and create a more accurate record of elements recovered during subsequent excavations; and, (4) provide data outputs suitable for presentation to both experts and non-experts.

This presentation will impact the forensic science community by demonstrating how the combination of traditional investigation and excavation methodologies with digital forensic archaeological methods can affect the outcome of a missing person investigation and how new approaches to searching and documentation of unmarked clandestine burials may be applied around the world.

Using the example of the research undertaken in Cyprus, the objective of this presentation is to demonstrate the application of innovative digital technologies in a forensic archaeological environment and to highlight the pros and cons of interdisciplinary approaches compared with more traditional methods. Because this research was comprised of two distinctive areas of applied digital forensic technologies — those used for the identification of potential excavation sites by means of Non-Destructive Analysis (NDT), and those exploited for the documentation of the excavation process of exhumation sites — both will be included in this presentation. The combination of traditional investigation and excavation methodologies with digital sciences enables the project team to reflect upon the specific forensic needs of the Committee on Missing Persons (CMP) future strategical planning program and to highlight how such approaches may be applied at other unmarked clandestine burials around the world.

Since forensic archaeology became a recognized discipline in the mid-1990s, there have been considerable advances in the search for and recovery of human remains and other trace evidence. An increased appreciation of the role of archaeologists means that, in some countries, they are regularly engaged in forensic cases involving missing persons in the course of legal proceedings. Moreover, in many countries throughout the world, there have been pledges made to locate deceased and missing people for humanitarian purposes, with the main goal of ensuring that they receive the "basic dignity" of a formal burial and of providing answers for their families.

The CMP in Cyprus is a bi-communal body that was established in 1981 by the leaders of the Greek-Cypriot and Turkish-Cypriot communities, with the participation of the United Nations. The objective of the CMP is to determine the fate of 2,000 missing persons who have disappeared since the beginning of the inter-communal fighting of 1963-1964 and the events of 1974. So far, the remains of 1,192 individuals have been exhumed; 740 people have been identified. The locations of the burials of the remainder of these missing persons remains unidentified.

In 2017, a research project was launched between archaeologists, forensic experts, and digital technologists from the CMP, the Cyprus Institute, and the Centre of Archaeology at Staffordshire University (United Kingdom) to explore how tools from a range of disciplines could be utilized to detect and record individual and mass burials from the aforementioned periods of conflict. During this project, a wide range of methods were tested to identify appropriate emerging technologies and scientific applications that could be implemented in forensic scenarios now and in the future. A pyramidal approach was chosen, exploiting different devices mounted on aerial and terrestrial platforms. These included the use of Un-Manned Aerial Vehicles (UAV) coupled with image-based modeling techniques to create Digital Elevation Models (DEM), Digital Surface Models (DSM), and high-resolution ortho-photos. This methodology provided a first assessment of surviving anomalies, both in terms of surface geometry and vegetation growth, that may indicate the presence of burials. This approach was followed by geophysical surveys of selected areas to accurately scan the subsoil and identify and characterize potential underground targets (including potential burial sites). The fusion and visualization of this data was then undertaken alongside more traditional means of witness interviews and excavation.

Forensic Archaeology, Digital Technologies, Missing Persons

A98 Broken Link: The Role of Forensic Anthropology in Cultural Resources Management

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The goals of this presentation are to: (1) inform the medicolegal community, specifically medical examiners, coroners, and forensic anthropologists, of the lack of standardization and protocol faced by the archaeological and Native American communities when attempting to obtain a legal identification of human remains; (2) share the process developed in San Diego, CA, to attempt to mitigate for the missing link; and, (3) call on the forensic science community to adopt a standard definition for the identification of archaeological bone.

This presentation will impact the forensic science community by bringing awareness to a long-standing problem that has affected many people within the archaeological and Native American communities. After learning the obligations of the forensic community to archaeological human remains and the consequences the lack of a standardized process has on people, the community will want to adopt standardized identification language and try to help fix a broken system.

It is commonly stated that people who work in the medicolegal field "speak for the dead." There are many well-trained individuals who practice forensic anthropology within the defined space of legal jurisdiction; however, there is a place where many people are unspoken for, a place where forensic science, anthropology, the law, and human rights to bury the dead all intersect.

In the Cultural Resources Management (CRM) field, the chain is broken. The pathway from bone being discovered to the identification as human to the determination of Native American (or not) is a gray area that has little to no standardization. As one can imagine, circumstances in which archaeological bone is discovered is often treated as a nuisance and a potential hazard for the project budget. In practice, contractors look to archaeologists to make the identification and preferably identify as non-human. This high-pressure environment, the lack of experts in the field, and the lack of awareness of the medicolegal community may lead to human remains being labeled as other than human.

Archaeological bone is often passed over by the medicolegal community for a number of reasons, primarily because they are busy with forensic cases, understaffed, and/or assume it is not their jurisdiction; however, in many states and in federal policy, the coroner/medical examiner is the person or office that is responsible for the legal identification. Without guidelines, policy, or identification of a legal procedure, bones are not properly identified. Without proper identification by a skilled and legally authorized person, many Native American communities do not have any rights to claim or bury those remains as they see culturally fit.

Osteology, Archaeology, Identification Process

A99 Identifying Vulture Scavenging Locations Through Global Positioning Systems (GPS), Geographic Information Systems (GIS), and Remote Sensing

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After attending this presentation, attendees will be aware of how GPS, GIS, and remote sensing were used to identify when and where vultures scavenge.

This presentation will impact the forensic science community by demonstrating how GPS, GIS, and remote sensing were used to locate 2,104 vulture scavenging locations and to identify their predominant geographic, environmental, and temporal features. The information from this presentation derives from the only study that has tagged and released vultures from a forensic anthropology research facility. This study's findings can aid investigators and forensic anthropologists trying to determine if vultures were at a scene involving human remains.

Vultures have a profound impact on the taphonomic record because these obligate scavengers accelerate decay. As a result, forensic scientists need to account for vulture scavenging when estimating the postmortem interval; however, vultures' scavenging efficiency makes identifying their prior presence at a scene difficult to detect.

To determine the environments where vultures are likely to scavenge and outcompete necrophagous insects and other scavengers in the acquisition of decomposing remains, six vultures were intentionally trapped at the Texas State Forensic Anthropology Research Facility (FARF). Prior to their release on April 10, 2013, the vultures were fitted with 70g solar-powered GPS trackers (transmitters). The tagged vultures included two adult turkey vultures, two adult black vultures, and two sub-adult black vultures (approximately one year old), and they were released in good health and unharmed. The vultures were monitored through satellites for six months, and each transmitter collected hourly spatial point data for 19 hours per day.

More than 15,000 vulture GPS data points obtained between April 2013 and October 2013 revealed the vultures traveled throughout most of Texas and into Oklahoma. Moreover, each data point recorded by the transmitters included the vultures' location in latitude and longitude, altitude above sea level, flight speed, course direction, and a time stamp. The GPS point data had a spatial accuracy of ±18m and a vertical accuracy of ±22m.

To determine where the vultures were scavenging, a digital elevation model was generated for the Texas and Oklahoma study area. Ground elevation was determined for all 15,000 vulture GPS points, then subtracted from the vultures' height above sea level, which resulted in a dataset with the vultures' height above ground. From this new dataset, scavenging locations were defined as daytime points with heights above ground ranging from ±10m; negative values can result from normal variability in the GPS data.

A GIS was used to extract land cover values associated with each scavenging location and to calculate distances between vulture scavenging locations and water and roads.

Results reveal both turkey and black vultures prefer evergreen forests and shrub land for scavenging and normal daily movements. Additionally, vulture scavenging sites on average are located closer to a permanent water source than to a road. Turkey vulture scavenging locations (n=695) averaged 450m from permanent water sources and 609m from roads. Black vulture scavenging sites (n=1,409) averaged 361m from permanent water sources and 547m from roads. The isolation of scavenging locations within the GPS tracking data also revealed that turkey vultures prefer to scavenge in the afternoon, whereas black vultures prefer to scavenge the morning.

GPS tracking of vultures trapped and released from the FARF provided new insight on flight and scavenging behaviors of vultures in Texas and Oklahoma. GIS and remote sensing allowed for the identification and analysis of vulture scavenging locations. The results from this study can help forensic investigators decide which habitats and landscapes are associated with increased vulture scavenging when assessing human remains at crime scenes.

Support for this research was provided by the National Science Foundation (NSF) Doctoral Dissertation Research Improvement Grant (PD 98-1352) and the Louisiana State University (LSU) West-Russell Award. The findings and opinions to be presented are those of the authors and not necessarily those of either the NSF or LSU.

Vulture Scavenging, GPS, GIS

A100 Staged and Altered Homicide Scenes: An Analysis of Secondary Sites

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After attending this presentation, attendees will know how to use a mixed methods approach, including investigative methods, archaeological field methods, and forensic interviews, for locating clandestine burials. Attendees will also learn about homicide patterns involving dumped and buried remains.

This presentation will impact the forensic science community by offering research into an intelligence-based approach with best practice recommendations to provide law enforcement and forensic anthropologists tools and an understanding of the techniques and protocols utilized in each field to find buried bodies.

It is not uncommon that bodies and crime scenes are altered following a homicide, yet the number of cases resulting in dumped or buried remains in a secondary location are less frequent. Many long-term missing person cases are determined to be homicides with secondary sites. Locating human remains in a forensic context is a collaborative effort among law enforcement and forensic anthropologists who each have unique protocols for search and recovery. This presentation discusses an intelligence-based approach to developing search and interview strategies.

To investigate patterns of homicide and the factors that assist with locating clandestine graves and case solvability for cold cases, analyses of homicide cases from 1985 to 2010, in Hillsborough County, FL, were reviewed (n=421 closed criminal homicides). Only cases involving homicidal crimes were included, excluding cases of justifiable, lawful, or vehicular homicides. By analyzing location data and demographic information concerning the victims and offenders, such as whether they are known to each other or are strangers; we can understand modes of body deposition, the spatial patterning of various types of homicide, and the factors that contribute to case solvability through quantified, statistical analyses.

The frequency of bodies moved/tampered with/altered following the homicide represents 38.7% (119/414) of cases. It is expected that domestic killers will go to greater efforts to hide the murder than will strangers in an effort to conceal and distance their involvement with the crime; however, this did not always prove to be the case. Among cases in which bodies were moved/altered/tampered with, 37% were domestic, 27% were robberies, and 12% were associated with rape. It is further expected that bodies will be dumped close to the murder location, in areas of high concealment, more often with use of containers, and generally show highly predictable patterns. The patterns observed in this study generally support these assertions. The victims' bodies were moved to a secondary location or dumped in 40 cases. Among these, slightly more dump sites occurred in rural homicides (county n=51%) compared to city homicides (city n=37%). The victims typically know their killers (77%) and are most commonly friends (30%), followed by boyfriend/girlfriend (25%), and, less frequently, spouses (8%). In 28% of the cases, the homicide was victim-precipitated or robbery-related, whereas, in 25% of the cases, the homicide was sexually motivated. Note that this is a significantly higher concentration given their smaller proportion of cases overall.

Bodies are dumped on the ground surface rather than buried below the surface in a slight majority of cases (53%). Bodies are most often dumped in public spaces, along the road, or in wooded areas (74%). Some type of container was used in only about 10% of the cases; it may have been a blanket, tarp, barrel, trash bin, or other container. Among dumped cases, just over half (58%) were discovered while the remains were fresh or just beginning to decompose, whereas 42% of the victims were found in advanced stages in decomposition or skeletal. Most victims whose bodies were dumped were not killed in the primary residences of their homes or that of the offenders. Interestingly, only about 20% of victims were murdered in their home, then dumped, whereas victims murdered in the offender's home, then dumped, was slightly higher at 33%.

Buried Bodies, Dumped Remains, Staged Homicide

A101 The Application of Photogrammetry for Documenting Scenes With Skeletal Remains: Capabilities and Shortcomings for Use in Central Florida

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After attending this presentation, attendees will have a better understanding of the uses of Close Range Photogrammetry (CRP) for documenting the context of various outdoor forensic scenes. This presentation will focus on the differences in accuracy of 3D models containing faux skeletal material in three simulated outdoor forensic scenes.

This presentation will impact the forensic science community by describing how CRP has advantages over traditional photographic methods, as well as how the context and ground surface composition of outdoor forensic scenes can affect the output texture. The CRP models of the three scenes were created using Agisoft® PhotoScan® Professional.

The documentation of outdoor crime scenes with skeletal remains can pose a challenge to law enforcement professionals and forensic anthropologists because various taphonomic processes normally act upon the crime scene to alter and change context. Recent trends in forensic anthropology highlight the importance of applying more rigorous methodological approaches during forensic recoveries that emphasize proper documentation of context in crime scene scenarios.[1] The purpose of this presentation is to introduce photogrammetry methods, which can be invaluable tools in the preservation of contextual information, that were utilized to document three simulated forensic scenarios within the Central Florida pine flatwoods biome.

Photogrammetry is a data collection technique that relies on photographs and specialized software using a Structure-from-Motion (SfM) visual computing algorithm. The SfM software finds points of intersection among a series of images to compute a 3D model. The use of CRP techniques for the output of 3D models offers a novel approach to the collection and presentation of context at outdoor forensic scenes. While the use of 3D modelling techniques is currently utilized in archaeological settings, little research has focused on the potential use of CRP for comparing different forensic crime scene scenarios. The application of CRP to forensic archaeological settings offers a number of contextual advantages over traditional photographic methods, including documenting the overall context of a scene that may be obscured overhead, making drones and helicopters not a viable method in these instances.

A series of three simulated forensic scenarios utilizing faux human osteological material were constructed at the Deep Foundations Geotechnical Research Site at the University of Central Florida: a simulated, partially excavated burial, a small area scatter (in wooded/scrub), and a wide area scatter (in open field and wooded/scrub). The burial scenario was exposed between 20cmbs to 25cmbs and prepared for standard field documentation. The second scenario depicted an outdoor deposit with only minimal dispersal that was constructed under a pine tree canopy with the ground surface consisting of pine needles. Photographs for the first and second scenarios were collected using both a camera hand-held and attached to a tripod. The third scenario represented a widely scattered surface deposit that extended from under the pine tree canopy out into a grassy area. Photographs for the third scenario were collected using the camera hand-held and attached to an extension pole (camera height 780cm). All scenarios were photographed with and without ground control markers and models were rendered using Agisoft® PhotoScan® Professional.

Photogrammetry results indicate that the simulated burial scenario is the most accurate scene to model, even without ground control points. This is largely due to the flat, uniform surface of the soil in contrast to the faux osteological material in a defined space. Output texture imagery is the clearest, thus providing increased visual precision compared to the other scenarios. The scenarios involving ground scatter are modeled with less accuracy due to the intrusion and complexity of the surrounding surface vegetation and pine needles that decreased the visual precision when zooming in on features. Ground control points improve accuracy results in the final models of these scenarios. Images collected utilizing the extension pole provide models with good overviews of the scene, but at a loss of the vertical accuracy of the faux osteological material. Images captured using hand-held or tripod methods are both more precise visually and accurate to the shape and size of the faux osteological material. Overall, CRP can be a useful addition to forensic archaeological scene documentation protocols. While the scene outdoor surface composition can affect the accuracy of the models created, CRP should be a recommended method for the documentation of context when recovering skeletal remains from outdoor scenes because the output capabilities will be greatly increased over traditional photography.

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Photogrammetry, Forensic Archaeology, Scene Documentation

A102 An Examination of the Relationship Between Intrinsic Properties of Bone and Skeletal Element Recovery

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After attending this presentation, attendees will better understand which intrinsic skeletal factors have the greatest influence on the likelihood of skeletal recovery and how the relative importance of such factors varies across different taphonomic environments.

This presentation will impact the forensic science community by contributing to the development of methods for skeletal quantification and by informing field recovery of skeletal remains.

Skeletal assemblage recovery rates are important for estimating the number of individuals present and assessing the amount of skeletal attrition that has occurred, both for individual cases and larger assemblages. This presentation will examine the relationship between intrinsic skeletal properties and variation in skeletal element recovery rates.

A better understanding of the factors that influence recovery rates will assist the forensic science community in continuing to develop methods for skeletal quantification. Furthermore, evaluating the interaction between intrinsic skeletal properties and external taphonomic processes can assist in anticipating challenges in recovering certain elements in the field, as well as aid in selecting appropriate samples in research design.

This project addresses the hypothesis that skeletal attrition is non-random with respect to intrinsic properties of skeletal elements. Furthermore, the relationships between these intrinsic properties and skeletal recovery may be affected by variations in taphonomic processes.

Skeletal inventories were collected from the Forensic Data Bank (University of Tennessee), New York City Office of the Chief Medical Examiner forensic anthropology (NYC OCME) cases, and a subset of carnivore-scavenged cases from the California State University, Chico Human Identification Laboratory (CSUC HIL). Bone mass and length data were collected from the CSUC HIL skeletal teaching and donated cases collections and NYC OCME cases. Bone mineral density data for the six major long bones were obtained from the literature.[1] Inventories and bone mass and length data included 12 major appendicular skeletal elements.

Mass, length, and mineral densities were converted to ratios to account for inter-skeletal differences and to facilitate comparison. In cases in which a single element was bilaterally absent, imputation was performed using regression on the element with the highest correlation to the element missing. Mass and length data were not collected from cases with more than one element bilaterally absent. Counts for each element were converted to recovery rates using Perl; Pearson's correlations between recovery rates and property ratios were computed in R.

Comparison of mineral density, mass, and length for the long bone subset revealed significant correlation (p <0.05) of recovery probability with mineral density and mass for all three datasets. Correlations were higher for mass than mineral density for the CSUC HIL (r^2=0.95 vs. 0.93) and NYC OCME (r^2=0.92 vs. 0.88) datasets, while mineral density revealed the highest correlation for the Forensic Data Bank (r^2=0.95 vs. 0.86). Comparison of mass and length for all 12 elements demonstrated a significant correlation of recovery probability with mass for the NYC OCME dataset (p=0.046, r^2=0.58) and recovery probability with length for the Forensic Data Bank (p=0.0024, r^2=0.79). All other correlations were not significant.

The hypothesis that intrinsic properties of skeletal elements affect skeletal attrition in a non-random fashion was supported. The secondary hypothesis that variations in taphonomic environment will influence the relative impacts of these properties was not well supported. Bone mass was the predominant factor for both the NYC OCME and CSUC HIL datasets, which were affected by very different taphonomic processes. The results of this study suggest that durability may affect element attrition more than size, even when taphonomic processes are widely varied.

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Skeletal Quantification, Taphonomy, Commingled Remains

A103 The Use of Geographic Information Systems (GIS) to Identify Relationships Between Victim Dispersal Patterns and Skeletal Trauma After a Blast Event

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After attending this presentation, attendees will better understand the utility of GIS methods at a post-blast scene and the benefits to anthropological trauma analysis of blast victims.

This presentation will impact the forensic science community by providing the first data out of a series of controlled post-blast trauma experiments from an area with a scant amount of anthropological research to date. This study emphasizes the need for standardization of evidence collection at the scene so anthropological analyses of blast trauma can be effective.

Anthropological study benefits from the inclusion of geographic analysis by connecting observations to specific locations. Forensic anthropologists have recognized the importance of documenting the location of evidence by mapping victim remains found at scenes. The increase of blast-related incidents in areas of conflict worldwide as well as attacks on civilian populations in industrialized regions has necessitated research that simulates blast events to aid in the interpretation of blast trauma. Mapping post-blast evidence is a way to increase base knowledge of what is typical of post-blast scenes and to aid in identifying human remains onsite. As such, there is an immediate need for controlled experiments that test the accuracy of evidence collection methods at blast events and the relationships between victim trauma and geographic location at the post-blast scene. The current study uses GIS as a tool to understand the nature and scope of victim (and associated remains) displacement from their original location after a blast event.

Seventeen non-military grade, outdoor blast events with two porcine proxy victims (*Sus scrofa*) each (N=32) were used to test for the existence of commonality in victim dispersal patterns, fracture type and location on the skeleton, and accuracy of GIS post-blast scene mapping methods. Proxy victims were placed 1.5 meters away from, and facing, each device. Victims were positioned upright to simulate a standing human. After detonation, a Global Navigation Satellite System (GNSS) Receiver was paired with a Bluetooth® connection to a collector app to document all evidence, including victim remains. The GNSS Receiver was paired with a Satellite-Based Augmentation System (SBAS) to compensate for possible inaccuracies due to obstruction, data integrity, point anomalies, and signal availability. Evidence was also mapped using an azimuth board, a hand-mapping technique. Victim remains were decomposed at an outdoor facility, macerated, and analyzed for blast trauma to the skeleton. Seven blunt force trauma fracture types were identified and their frequencies were overlaid on GIS maps developed from scene data.

The remains of all 32 victims were dispersed in a cone-shaped pattern from the seat of the 17 blasts. Remains traveled as far as 57 meters, but the highest concentration of evidence was contained within a 30-meter radius of each blast. Anatomical portion (e.g., lower limb) and fracture type were significantly associated (p=0.036) with distance from the seat of the blast. When assessing accuracy of scene mapping, data points collected using this GIS procedure were not significantly different within 30 centimeters (p=0.081) of the same point collected using the hand-mapping method. Further, the use of the collector app allowed for photographic documentation simultaneously with the collection of geographic location.

The results of this study highlight the importance of collaboration between anthropologists and crime scene investigators at a post-blast scene. Mapping spatial data provides an additional analytical tool for the anthropologist during trauma analysis and scene recreation by investigators. Moreover, digital maps of this nature are often more easily interpreted by the public (i.e., juries) than charts or numerical data tables.

Post-Blast, GIS, Blunt Force Trauma

A104 Variety and Distribution of Orthopedic Devices in the Cyprus Research Reference Collection and Their Relationship to Skeletal Trauma: Preliminary Results

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After attending this presentation, attendees will better understand the variety, complexity, and distribution of orthopedic skeletal devices in a modern Cypriot skeletal population and appreciate how analysis of the bone associated with the devices can reveal information regarding the likely timescale of the medical intervention. The value of the manufacturer information combined with the skeletal evidence for positive identification will be examined.

This presentation will impact the forensic science community by evaluating the use of orthopedic devices, such as plates, rods, and femoral head replacements, for positive identification in Cyprus in the absence of a national registration database. Despite compliance with the European Medical Device Directive (equivalent to the Food and Drug Administration in the United States) after joining the European Union in 2004, implants are documented as items with all their manufacturing details by the distributor only. As a result, positive identification from medical implants and devices are problematic and reliant on the actions of individual doctors. This is the first detailed study of surgical implants and medical devices conducted on contemporary skeletal remains in Cyprus.

Surgically implanted devices are common in modern skeletal remains and may aid in positive identification, through comparison with antemortem medical records. Currently, positive identification of an individual exhibiting a medical device in Cyprus is dependent on the retention of relevant antemortem medical records by a participating doctor. There is no direct link between the manufacturer of the device and the patient. In the absence of a national registration database, Cyprus needs a platform whereby skeletal medical devices are logged in a way that links the individual with the device.

In a pilot study of contemporary skeletons, an assemblage of 150 individuals was examined from the Cyprus Research Reference Collection (CRRC) (dates of death: 1975-2010) for evidence of medical devices used in the prevention and treatment of trauma in the lower limb.

This study sought to assess and record: (a) the prevalence and distribution of orthopedic medical devices by sex of the individual; (b) to record the type of medical device used in relation to the types of trauma observed; and, (c) to create a catalog of orthopedic devices used in Cyprus between 1975 and 2010 for the purposes of positive human identification. An analysis of the skeletal changes associated with the surgical devices allowed an estimation of the time elapsed since trauma and, therefore, a timescale for the medical intervention.

A total of 49 medical devices were observed in 27% of the population, representing 18 individuals of both sexes (15.0% in males, 12.0% in females). Orthopedic devices such as plates, angled plates, rods, head implants, screws, and wire were recorded. The skeletal healing recorded ranged from <3 days to >1 year. Skeletal changes associated with medical intervention ranged from well-healed trauma to evidence of severe infection associated with the affected area. Peri-mortem injuries indicated mortality relating to post-operative complications. The medical devices were primarily used to stabilize fractures to the proximal femur and tibia and, less often, to the distal tibia. Femoral head implants were the most common of the devices observed.

The sheer number of medical devices observed in the CRRC suggests that their use is common in 20th-century Cyprus. The variety of devices identified offers insights into medical techniques and the evolution of devices over time. Device identifiers have been grouped for the period before and after Cyprus joined the European Union. Skeletal changes associated with the orthopedic device offer a relative chronology between medical intervention and time of death. The information highlights the challenges to forensic investigators in Cyprus attempting to use medical devices for positive identification.

Orthopedic Device, Medical Intervention, Skeletal Trauma

A105 The Application of Consolidation Materials to Burned Bone: A Comparative Approach

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The goal of this presentation is to provide attendees with a better understanding of the utility of archival-grade consolidants for recovering human remains from a fatal fire scene.

This presentation will impact the forensic science community by quantitatively and qualitatively elaborating on previous research by comparing four consolidants' (e.g., Acryloid™ B-72, Acrysol™ WS-24, Rhoplex™ B-60A, and Butvar® B-98) ability to increase the strength and toughness of thermally altered bone and to identify the most appropriate consolidant and recommend best practices for usage when encountering burned bone.[1,2] It is expected that all materials tested will reduce continued sample fragmentation, mirroring previous researchers' results.

Taphonomic agents and post-depositional factors can significantly influence a bone's ability to withstand the stress of scene removal and analysis. Fire-altered bone presents a particularly unique challenge owing to its inherent friability. The Bridgeville Fatal Fire Recovery Protocols were developed to maximize recovery and mitigate fragmentation of human remains; however, they do not address stabilization methods that could reduce fragmentation resulting from handling at the scene or in laboratory or analytical settings.[3] A possible solution is the standardized use of consolidation materials *in situ* during recovery efforts. Although consolidant materials have been employed on friable bone in numerous contexts, there is little-to-no consensus regarding the most appropriate consolidant for burned bone.

An open fire cell was constructed and subsequently ignited. The sample population, consisting of domestic pig (*Sus scrofa domesticus*) femora (n=58) and skulls (n=5) subdivided into five groups, including a control group, was placed on the structure floor and allowed to extinguish naturally. Consolidant materials were prepared at a 10% concentration and applied to all visible surfaces of the bone samples *in situ* using a polyethylene squeeze bottle for a total of four applications.

Variables investigated in this research include ease of solution preparation and application, dry time, changes to the appearance of the bone surface, and mode of deformation during a loading event, as well as ultimate strength and toughness of the sample after consolidation. Mode of deformation was assessed through load-displacement curves produced by a nanoindentor. Drop weight impact testing and exposure to forced vibration were used to assess strength and toughness. A stratified random sample of relatively smooth and flat fragments was selected for nanoindentation (n=18) and drop weight impact testing (cortical samples: n=176; femoral head: n=43) to ensure accuracy by keeping the sample perpendicular to the applied force. Proximal femora and condylar epiphyses were selected for forced vibration testing. Samples in color stages IV and V were preferentially chosen for mechanical testing as a means of controlling degree of calcination, while anatomical region and bone type were considered to control sample anisotropy.[4]

Results indicated all materials increased the strength and toughness of burned bone compared to the unconsolidated control sample. While Acryloid™ B-72 was not found to be as strong as Rhoplex™ B-60A or Butvar® B-98, the quicker dry time and the lack of alteration to the bone's appearance make Acryloid™ B-72 the most suitable choice for on-scene use. Alternatively, if dry time could be significantly reduced using a more volatile solvent, Rhoplex™ B-60A would be recommended since the alteration to the bone surface after application was minimal. Future analyses should be performed to better understand consolidant influence on modification to bone surfaces and impacts to DNA sampling, as well as the degree to which consolidant increases the ability to establish the biological profile. Results of this research indicate that the dentition, frontal sinus, and other regions displaying biological indicators of sex, age, and ancestry should preferentially be consolidated due to their utility in skeletal analysis toward the identification of the fatal fire victim.

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Burned Bone, Consolidant, Forensic Recovery

A106 The Effect of Scanner Performance on Capture Ability and Identification Success on Postmortem Biometric Data

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After attending this presentation, attendees will understand the applicability of alternative biometric scanning technologies to obtain postmortem fingerprint, iris, and facial biometrics for positive identification of unknown individuals, including the maximum number of days each biometric type can be successfully used for identification.

This presentation will impact the forensic science community by describing the advantages and limitations of different scanning technologies based upon the points of similarity and match scores for each identifier type.

Biometrics are measurable unique characteristics that are used to classify both living and deceased individuals. This study examines the effects of two different biometric scanners, representing different capture technologies, on the ability to capture fingerprints, facial photographs, and iris scans and successfully identify individuals in the postmortem period. This study examines the number of days in which usable biometric data can be successfully matched to an individual using two different digital technologies and how the image quality and ability to obtain sufficient match scores to make a positive identification differed with each instrument. For the purposes of this study, *usable data* refers to images that are able to correctly identify the individual through a digital biometric program that uses statistical algorithms to match the captured images with those images taken upon the initial receipt of the donated individual. This study was conducted in conjunction with Oak Ridge National Laboratory and the University of Tennessee Anthropological Research Facility between January 2016 and July 2017. Utilizing the SEEK II and the BioSled hand-held digital biometric capture devices, facial photographs (n=200), iris scans (n=172), and fingerprints (n=650) from the donated remains of 16 individuals were obtained daily until usable data could no longer be captured. The individuals were placed supine and uncovered. No interventions were made to the remains prior to data collection; however, specula were placed in the eye in order to enable iris capture during data collection.

Seasonality played a large part in the effectiveness of the instruments to obtain data. With daily high temperatures ranging between 59°F (15°C) and 84°F (28.89°C) during the spring trial (n=5), usable data was obtained for an average of 4.2 days for the SEEK II and 4.1 days for the BioSled; however, the early summer trial (n=6) included high temperatures between 8°F (27.22°C) and 91°F (32.77°C) and the number of days usable data could be captured was reduced to 3.8 for both the SEEK II and the BioSled. The winter trial (n=5) only had high temperatures between 18°F (-7.78°C) and 55°F (12.78°C) and demonstrated that useable data was available, on average, 5.5 days for the SEEK II and 5.9 days for the BioSled. Interestingly, while their overall averages are similar, the SEEK II outperformed the BioSled by an average of one day for fingerprints in the spring (6 days for SEEK II, 5 for BioSled). While both scanners performed similarly with regard to biometric capture longevity, each machine performed differentially in regard to usability. The BioSled excelled at non-contact data collection when capturing facial and iris biometrics on postmortem individuals, whereas the SEEK II required contact with the face for iris capture, which may not be practical in a forensic context. It was noted that both scanners had difficulty recognizing the iris for capture and focused more on objects in the proximity of the eye that resembled the iris (e.g., fly, specula, or dark spots on the face). In regard to scanner reliability, the SEEK II demonstrated a higher consistency than the BioSled in fingerprint capture, especially under inclement weather conditions.

While biometric capture longevity was similar between scanners, they each demonstrated unique advantages and limitations in terms of usability. The results of this study demonstrate that while biometrics do remain viable over time, the ability of scanners to successfully capture biometrics depended upon seasonality and environmental conditions. Temperature, precipitation, and insect activity were the primary factors affecting the retention of biometric information in decomposing human remains. This study builds upon previous work and continues to support the utility of physiological biometric identifiers during the decomposition process. Postmortem biometric research has the potential to make important contributions to forensic anthropology and the law enforcement, military, and medicolegal communities.

Biometrics, Human Decomposition, Positive Identification

A107 In Search of Jane Doe: An Analysis of Solvability Factors in Unidentified Remains

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The goal of this presentation is to investigate solvability factors in long-term unidentified persons cases with specific attention given to the methods employed in the Florida Cold Case Program model. Attendees will be presented with a model for cold case review that has led to seven identifications within the study period.

This presentation will impact the forensic science community by demonstrating the manners in which a comprehensive cold case model using new methods and technologies may provide fresh investigative leads on cases that are decades old.

In Florida, there are more than 10,000 unresolved open homicides, or "cold cases," dating to the 1960s, representing families who have been denied justice. There are also more than 850 individuals recovered in public places who have not been identified to date (including more than 50 juveniles), the majority of whom are homicide victims or who died of unknown circumstances. Many of the long-term unidentified cases remain unsolved because missing persons reports were not issued or not recorded in modern databases such as the National Crime Information Center (NCIC).

The Tampa Bay Cold Case Project started at the University of South Florida Forensic Anthropology Laboratory (USF-FAL) in 2007 and quickly grew in scope to a statewide initiative with cases routinely submitted for review and technical assistance. This project has led to many successful outcomes and positive identifications. As a result, missing persons cases are closed due to identification, and new investigative leads are now available for decades-old cases.

The purpose of this presentation is to conduct a study of the solved and unsolved cases analyzed in the cold case project to assess which methods and factors led to case solvability. To better shape protocols and policies surrounding cold cases, research into the reliability of effective methods is needed.

This study is a systematic assessment using qualitative and quantitative methods to investigate cold cases analyzed by the USF-FAL in the years 2015-2017 (n=58 cases). These cases have dates of discovery that range from the years 1967-2016 and had been previously analyzed at the time of discovery. They are predominately from Florida (n=36) but also represent cold cases from nine additional states. Nineteen individuals died from "homicidal violence" and 26 have an "undetermined" manner of death. The "undetermined" cases include numerous examples of disposal, such as dismemberment, concealment, burning, and the use of sulfuric acid and, in many cases, were reclassified as Homicide following the reanalysis.

The methods utilized in the reanalysis of cold cases are reviewed in the current study to assess their effectiveness and accuracy. These include: collaboration with law enforcement and Medical Examiners Offices (MEOs) to select cases for reanalysis; researching existing primary documents and photographs for leads; resubmission of fingerprints; locating and exhuming human remains; reassessing the biological profile using 3D-ID and other skeletal aging methods; chemical isotopic analysis; facial imaging; resubmission of DNA samples; and the use of the National Missing and Unidentified Persons System (NamUs).

Of the 58 cases investigated, seven were identified. In total, nine had fingerprint cards for resubmission. Eighteen cases were buried in John/Jane Doe graves and had to be exhumed. Although a total of 22 graves were unearthed in the search for the unidentified, three decedents were not located due to the loss of cemetery records. Skeletal analysis included reassessment of the biological profile for 41 cases, isotope sampling and georeferencing of 51 cases, and imaging for 34 cases. As a result, seven have been identified via fingerprint resubmission (n=4) and DNA analysis (n=3). The qualitative assessment of mapping of geochemical referencing and forensic imaging will be presented.

These findings demonstrate the utility for the use of a multidisciplinary approach for solving cold cases. Case reviews, fingerprint and DNA (re)submissions, updated biological profiles, chemical isotopes, and forensic imaging are shown to contribute to case solvability by providing new leads and increasing public interest in an otherwise stale case. In the face of seemingly impossible caseloads, multidisciplinary methods are shown to be of critical importance for addressing the issue of missing and unidentified persons.

Cold Case, Unidentified Persons, Forensic Anthropology

A108 Changes in DNA Quantity and Quality in the Human Tibia After Short-Term Surface or Subsurface Burial

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After attending this presentation, attendees will better understand how the quality and quantity of DNA varies along the length of the human tibia and how it is affected by surface and subsurface burial.

This presentation will impact the forensic science community by providing insight into the variation in DNA quantity and quality along the length of the human tibia, aiding forensic biologists in decisions regarding which bone regions to target for DNA-based identification.

Skeletal remains are a commonly recovered form of forensic evidence, and DNA retrieval from them has traditionally focused on dense cortical elements.[1,2] In contrast, Mundorf and Davoren concluded that smaller, primarily trabecular skeletal elements contain more DNA than larger, primarily cortical ones; however, several unexplored factors may contribute to which bones or bone regions contain the most DNA, and whether those regions change after burial.[3] In this regard, researchers in the Michigan State University Forensic Biology Laboratory have shown substantial intra-bone heterogeneity in DNA quality and quantity in bovine and porcine femora.[4] Additionally, Antinick observed some samples with higher DNA recovery following short-term burial.[4] The goal of the current research was to determine whether similar variation exists in human long bones and how it is influenced by short-term surface and subsurface burial.

Four pairs of unpreserved human tibias were obtained from female decedents aged 49–79 years, and soft tissue was manually removed prior to maceration in a 0.5% Terg-a-zyme® solution. Preceding burial, the proximal epiphysis and metaphysis, the distal epiphysis and metaphysis, and the mid-diaphysis were drilled to obtain ~30mg of bone powder. Alternating right and left tibias were buried one foot underground or placed on the surface above the other bone. Tibias were exhumed and drilled after one week and four weeks. Bone powder was digested as per Lorielle et al.[5] Extract volumes were measured, and DNAs were stored at -20°C.

Quantification of mitochondrial DNA (mtDNA) was performed using an in-house TaqMan® quantitative Polymerase Chain Reaction (qPCR) assay. Nuclear DNA was quantified using Quantifiler®. An in-house fluorescence-based quality assay that targets ~100bp, 200bp, 300bp, 400bp, and 500bp amplicons of the human mitochondrial genome was used to assess mtDNA quality. A degradation index was created based on peak height ratio of the 300bp and 400bp amplicons on a 0 to 1 scale in which a degradation index of 1 indicates no degradation.

Quantitative results indicate that the mid-diaphysis region contained the highest median quantity of mtDNA at week 0 and 1, which leveled off with the other locations by week 4. In contrast, all regions had similar quantities of nuclear DNA at week 0 and 1, yet the mid-diaphysis had higher nuclear DNA yields at week 4. Interestingly, approximately 33% of surface exposed and buried samples had higher mtDNA yields after one week, while 25% had higher mtDNA yields in week 4 compared to week 1. Nuclear DNA yields increased in 25% of buried and exposed bone regions in the first week, and approximately 10% increased from week 1 to week 4. No region increased during both timeframes, and no correlation existed between increasing DNA yields and bone region. This indicates that the bone likely softened during environmental exposure, making it easier to drill and releasing more DNA or the DNA sustained less mechanical damage from the drilling process.

The mitochondrial quality assay revealed the 100bp and 200bp amplicons were generated in all locations at all time points tested, while the 500bp amplicon was present in very few samples. The most variation occurred in the quantity of the 300bp and 400bp amplicons. In the distal metaphysis and epiphysis, the degradation index increased as mtDNA quantity decreased, indicating that the small amount of remaining mtDNA was of high quality. Potentially, this means that the remaining DNA was protected, possibly through binding to hydroxyapatite or collagen.

Overall, surface and subsurface burial impacted the quantity and quality of DNA along the length of the human tibia, and different bone regions responded differently to the burial conditions. The mid-diaphysis had the highest median quantity of mitochondrial and nuclear DNA after short-term surface or subsurface burial; however, it is unclear exactly why some DNA yields improve after short-term exposure or burial, which could be a factor in forensic investigations involving skeletal remains.

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DNA Degradation, Skeletal Remains, Short-Term Burial

A109 Long-Term Cocaine Use and Its Potential Effect on Bone Morphology

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After attending this presentation, attendees will understand that long-term cocaine use has effects on bone morphology at the macroscopic and microscopic level.

This presentation will impact the forensic science community by demonstrating that the effect of drugs on bone morphology is an area of study that should be expanded. With the findings of this study, it is apparent that cocaine use and substance abuse can potentially cause age-at-death estimation to be inaccurate. Other works and clinical analyses have proven other drugs to alter bone, but no research has examined the effect of cocaine on bone morphology.

The purpose of this study was to assess the bones of rats exposed to long-term *ad libitum* consumption of cocaine for physical evidence of cocaine use. While it is accepted that many drugs, both prescription and drugs of abuse, do have powerful metabolic effects, those changes have not been examined at the microscopic level.

Eleven male Wistar Rats (*Rattus norvegicus*) from the Laboratory of Behavioral Neuroscience at Boston University in Boston, MA, comprised the experimental group. The rats self-administered cocaine over four weeks at a 0.3mg/kg dosage and the concentration of the IV solution was 1.6mg/ml of cocaine. The control group included five female Sprague Dawley rats (*Rattus norvegicus*) from the Boston University Animal Science Center in Boston, MA. The specimens were exposed to a training protocol but were not given any drugs. All rats went through a dissection and maceration to obtain the femora and humeri. Mass, volume, and length measurements were taken for each element to be used for later analysis. One femur was chosen at random from each rodent to be used for histological analysis. Femora were embedded in a two-part epoxy resin, then cut in half using a diamond band saw. A Buehler® IsoMet™ low-speed saw was used to obtain thinner sections and a Buehler® MetaServ™ 250 grinder was used to achieve a thickness of 100µm-120µm. India ink was used for staining and all stained sections were put onto slides, covered with Permount™ and a cover slip, and labeled.

The outer circumferential lamellar layer of bone diaphyses were measured and compared between the experimental and control groups. Photographs were taken of each cross-section at 1x and 4x magnification through the NIS-Elements™ software. The ImageJ image-processing program was used for analysis. The thickness of the outer circumferential lamellar and the thickness of the total cross section was taken at four random locations of each 4x magnification photograph. The ratio of the thicknesses and the outer circumferential lamellar thickness alone were compared.

A significant difference was found between the density values calculated from the original mass and volume measurements between control and experimental groups. Samples that had been exposed to cocaine had lower density values than those not exposed to any drugs. The control group mean density equaled 1.492g/mL and the experimental group mean density equaled 1.082g/mL. A significant difference was also found between the ratio of the thicknesses and between the outer circumferential lamellar thickness alone. The experimental group had ratio values significantly higher than the control group. The control group's mean ratio equaled 0.2686, while the experimental group's mean ratio equaled 0.4427. This indicates that in the control group, the outer circumferential lamellar thickness, on average, covered approximately 25% of the total cross section, and the experimental groups outer circumferential lamellar thickness, on average, covered nearly 50% of the total cross section. These results were similar when comparing the outer circumferential lamellar thickness alone. The control group's thickness was significantly lower than the experimental group's thickness. The control group's thickness measurements had a mean of 189.7674µm and the experimental group's thickness measurements had a mean of 343.2753µm.

The data reveal that long-term exposure to cocaine has a detectable effect on bone morphology. Further exploration of this phenomenon may show that the changes in bone morphology may be diagnostic for drug abuse. That information could be of use in a medicolegal setting and may alter the accuracy of age-at-death estimations.

Forensic Anthropology, Histology, Substance Abuse

A110 Mismeasurement of theTibia and Femur Reconsidered: How Were Measurements Taken at the Central Identification Unit in Kokura, Japan?

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After attending this presentation, attendees will better understand historical changes in osteological measurement definitions and practices and how this impacts the reference samples used in the anthropological calculation of stature.

This presentation will impact the forensic science community by further illuminating change over time in measurement practices within the same forensic anthropological laboratory and how these may have affected reference standards.

In 1952, Trotter and Gleser published one of the baseline references for American forensic anthropology, using measurements from 1,200 World War II United States military casualties and 615 males from the Terry Collection to calculate a series of regression equations for the estimation of living stature from long bone lengths.[1] When Trotter and Gleser completed their 1952 study, the Central Identification Unit (CIU) in Kokura, Japan, conducted a test of how the new formulas compared to those of Krogman and Rollet (which the CIU had been using until that point) by applying both methods to 120 previously identified Korean War casualties. The average deviations between estimated and actual stature for the White males (*n*=100) were 1.24" (Krogman), 1.48" (Rollet), 1.14" (Trotter, averaging all bones), and 1.01" (Trotter, femur and tibia); for the limited sample of Black males (*n*=20), the parallel values were 1.61", 2.03", 1.19", and 1.19".[1-3] Based on these results, the CIU decided to adopt Trotter's formulas. Subsequently, Trotter and Gleser (1958) published a revised study using data from 5,517 United States casualties of the Korean War whose remains were processed at the CIU.[4]

In 1994, Jantz et al. analyzed the original data from Trotter and Gleser (1952) and demonstrated that the tibial lengths omitted the malleolus.[5] In 1995, they compared the mean lengths of the tibia and fibula in Trotter and Gleser (1958) and concluded that the tibiae had not been measured properly in that study either.[4,5] Although the Korean War data used by Trotter and Gleser has not been located, ongoing compilation of measurement data from Korean War casualties whose files are available to the DPAA has yielded a comparable reference set.

Between May and November 1953, the three anthropologists working at the CIU estimated stature in both ways to compare the results. In addition, they recognized that Trotter's measurement definitions differed from their previous ones, and on 35 measurement forms located to date, they recorded two distinct measurements of the femur and tibia. Because the forms only provided a single space for each long bone, whoever entered the data usually added a more specific designation. The first value entered is often unspecified, but several forms label it "KR," while others use "Bicondylar." The second, larger measurement is labeled "maximum" or "Trotter." Comparing the measurements recorded on these forms, the difference between the first and second measures of femoral length averages 4.5mm (*n*=44, Standard Deviation (SD) 2.40, range 1-11); for tibial length, the mean difference is 8.8mm (*n*=51, SD 5.62, range -1-21).

For a broader data set, measurements were compared from 316 skeletons that were analyzed at Kokura using both sets of stature formulas. The initial measurements were taken through May 1953, the second after September 1953. In 410 of 473 femoral length comparisons (87%) and 398 of 461 tibial length comparisons (87%), the later measurement was larger than the earlier one; the mean difference was 3.7mm for the femur (SD 3.17, range -6-15) and 4.5mm for the tibia (SD 3.91, range -6-16). By contrast, the fibula exhibited less change: the average increase in length was only 0.64mm (*n*=390, SD 2.13, range -8-11).

Tibia and fibula lengths were also compared. Until May 1953, the mean difference was 0.26mm (*n*=379, SD 5.09, range -15-14); after September 1953, the mean difference was 3.97mm (*n*=437, SD 5.12, range -13-23). While this increase does indicate that the post-1953 measurement standards were more comparable to those used by modern forensic anthropologists, they do not appear exactly the same.

It is clear that procedures for measuring the femur and tibia at Kokura changed over time. Before 1953, the (apparently unwritten) norm was to record the bicondylar length of the femur and anatomical length of the tibia; after the transition to Trotter and Gleser's formulas, the maximum lengths of both were standard; however, there is noticeable variation in measurements that leaves some uncertainty regarding how any particular bone was measured. In addition, the exact composition of the Korea data set provided to Trotter and Gleser is unknown, so we cannot be sure how many of the individuals within it were measured using each methodology. This introduces clear error into all of Trotter and Gleser's 1958 formulas using both femoral and tibial lengths.[4]

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Stature Determination, Skeletal Measurements, War Dead

A111 A Validation Study of the Langley Decision Tree Model for Sex Estimation

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After attending this presentation, attendees will be more knowledgeable regarding the performance of a zygomatic extension scoring method and sex estimation decision tree presented by Langley et. al.[1]

This presentation will impact the forensic science community by demonstrating if and how this method can be incorporated into forensic anthropological casework in light of the *Daubert* and National Academy of Sciences (NAS) Report recommendations.

Walker presented a sex classification method based on the ordinal scoring of five morphoscopic traits of the skull: glabella, mastoid, nuchal, supraorbital margin, and the mental eminence.[2] He provides six logistic regression equations that include combinations of two and three traits for sex estimation. The Walker method has been quickly incorporated into forensic casework both in the United States and internationally; however, studies have indicated high levels of intraobserver and interobserver disagreement for some traits (e.g., mental eminence).[2] Stevenson et al. modified the Walker method by incorporating the same five traits into a decision tree model developed using a chi-square automatic interaction detection.[2,3] Given the many reliability studies that indicate low levels of observer agreement for the mental eminence, the inclusion of this trait into these sex estimation methods is problematic. Seeking to eliminate this issue, among others, Langley et al. developed a decision tree for sex estimation that utilized only the two most reliable of the five cranial traits (glabella and mastoid scores) and included a new trait: zygomatic extension.[1] This study sought to test the reliability of the zygomatic extension scoring method presented in Langley et al. and validate their sex estimation decision tree method.[1]

Ordinal score data were collected from a total of 281 male and female United States White and Black individuals from the Bass Donated, Hamann-Todd, and Terry skeletal collections. Glabella, mastoid, nuchal, supraorbital margin, and mental eminence expressions were scored for each individual from the physical specimens following Walker as part of a previous study.[2] The zygomatic extension was scored from 3D surface models of the specimens following the descriptions, photographs, and diagrams provided by Langley et al.[1] For a subset of 30 individuals, zygomatic extension was scored, then re-scored by this study. Intraobserver agreement was assessed using Intraclass Correlations (ICC) and a weighted Kappa analysis was used to evaluate interobserver agreement. As Langley et al. appear to accidentally present two conflicting decision trees (Figure 2 and Figure 4) in their publication, both trees were tested.[1] These results were compared to results obtained using Walker and Stevenson et al.[2,3]

Intraobserver and interobserver agreement was high for the zygomatic extension (*ICC*=0.839, *wK*=0.716 and *ICC*=0.745, *wK*=0.585, respectively), suggesting that this trait can be reliably scored, although both observers noted some areas of subjectivity in the scoring procedure. The decision tree presented in Figure 2 resulted in only a 55.9% correct sex classification of the pooled sample.[1] If restricted to only the Bass sample (the same collection from which the method was derived), only 50.0% of the individuals were sexed correctly. In contrast, the decision tree presented in Figure 4 resulted in an overall 71.5% correct sex classification (77.4% for the Bass sample).[1] Based on these results and the fact that Langley et al. discuss details about the Figure 4 decision tree in the text, this is assumed to be the correct tree.[1] Still, results obtained were not as high as those reported by Langley et al. and a strong sex bias favoring female classification was observed (94.2% accuracy for pooled females; 49.3% accuracy for pooled males).[1] Male correct classifications ranged from 31.3% to 65.0%, depending on the sample. The method performed only slightly worse on the Black samples compared to the White samples, and similar results were obtained between historic and modern samples. The Walker mastoid/glabella equation and Stevenson et al. European-American decision tree produced higher accuracy rates (80.8% and 82.6%, respectively for the pooled sample), although sex bias remains an issue.[2,3] The results of this study indicate that there is room for further improvement both in cranial trait scoring methods and statistical methods of analysis.

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Sex Estimation, Cranial Traits, Decision Tree

A112 Age-Cohort Categorization and Multi-Factorial Age Estimation in Machine Learning Environments

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After attending this presentation, attendees will better understand the utility of age-cohort categorization using a multi-factorial approach to the estimation of age at death from adult human skeletal remains. As one part of a larger study into multi-factorial age estimation methods, this presentation addresses a straightforward approach to age estimates based on the combined data of three commonly used adult aging methods.

This presentation will impact the forensic science community by addressing the knowledge gap in multi-factorial age estimate strategies. The method proposed has the added benefits of quantification, verification, and robust statistical classification algorithms necessary for empirically based multi-factorial age estimates.

The traditional approach to producing a final age estimate from multiple skeletal indicators relies, in part, on heuristic cut-off points, minimum-to-maximum estimates, or non-statistical intervals developed using experience and expertise. This traditional approach is generally preferred over other, more robust methods because it is easier to apply during casework than current multi-factorial approaches, such as ADBOU, that provide age-at-death probabilities by means of transition analysis. Motivated by this knowledge gap and recent calls for research into multi-factorial age estimation strategies from the National Institute of Standards and Technology Overseas Security Advisory Council (NIST OSAC), this study tested the efficacy of an age-cohort categorization strategy using three commonly incorporated adult age indicators: the pubic symphysis, the sternal end of the fourth rib, and the auricular surface of the innominate. These age estimation methods were selected because: (1) they are commonly collected during forensic anthropological casework; (2) they constitute verified methods of age estimation; and, (3) as this research seeks to provide proof-of-concept results, these data represent current standards in the field.

Age data were collected following the Suchey-Brooks (pubic symphysis), Iscan et al. (rib), and Osborne et al. (auricular surface) methods. Data were collected for 358 (male, n=240; female, n=118) individuals from the William M. Bass Donated Skeletal Collection in Knoxville, TN, and the Hamann-Todd Collection Cleveland, OH. The sample represents modern American individuals comprising males aged 18 to 97 ($\bar{x}$=55.7, *Standard Deviation (SD)*=16.22) and females aged 20 to 94 ($\bar{x}$=62.4, *SD*=13.1). Age-cohort categorization was achieved using three strategies. First, a broad age class (young adult <39; middle adult 35-59; and old adult >60) was implemented. Next, the data was sub-divided into 10-year (e.g., 18-29, 30-39) and 5-year intervals (e.g., 18-24, 25-29). Male and female data were analyzed both separately and pooled. A two-part data analysis approach measured the ability of three classification algorithms to accurately predict the broad, 10-year, and 5-year age cohorts. To assess how well these data could be combined into a straightforward multi-factorial age estimator, quadratic Discriminant Function Analysis (qDFA) – Leave One Out (LOO) x-validation, Canonical Analysis of Principal coordinates (CAP – 4,999 permutations), and artificial Neural Networks (r-a-NN – hold-out samples for testing and validation) were conducted.

Variable importance measures indicate rib morphology was the single most important variable in every analysis, followed by pubic symphysis, then the auricular surface. This is not surprising given previously documented issues with auricular surfaces as an aging criteria. Classification accuracies for the broad age-cohort categorization were acceptable using qDFA (*F*=64%; *M*=67%; *Pooled*=66%), CAP (*F*=71%; *M*=71%; *Pooled*=68%), and r-a-NN (*F*=77%; *M*=75%; *Pooled*=77%). The qDFA and CAP performed well-below expectation for the 10-year and 5-year interval age-cohort categorizations; however, the regression analysis (with classification) developed using an artificial neural network performed promisingly well, correctly classifying the test samples (hold-out) for the 10-year (73%) and 5-year (74%) age cohorts. To improve network generalization and avoid overfitting, all successful networks were retrained using early stopping and regularization. Retraining generated age-cohort categorization models with mean squared errors lower than the individual performances and, as such, generalized even better to the testing and validation samples while avoiding overfitting.

Developing new multi-factorial approaches to age estimation may require novel applications of data mining and machine learning methods implementing current skeletal age-at-death indicators to predict "reportable" age estimates. The heuristic combination of age-at-death estimates may not properly weigh each indicator, placing emphasis on methods of very little import. In the end, age-cohort categorizations can provide accurate broad, 10-year, and 5-year interval age ranges derived from reliable and verifiable models.

Age Estimation, Machine Learning, Artificial Neural Networks

A113 On the Central Importance of Analysis of Covariance (ANCOVA) in Human Skeletal Research

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After attending this presentation, attendees will understand: (1) the power and flexibility of ANCOVA and the important role it can play in interpreting skeletal variability; (2) how basic errors in experimental design severely limit the utility of many forensic osteology studies; and, (3) how the choice of statistical procedures can lead to an over-emphasis on subgroup differences.

This presentation will impact the forensic science community by providing a new "best practice" standard for skeletal research that focuses on completing rigorous multivariate hypothesis testing before generating predictive equations.

The ability to construct a biological profile for the skeleton of an unidentified decedent is predicated on the availability of reliable comparative data, and the accuracy of that profile will vary directly with the quality and depth of prior skeletal research; however, a review of research published in the *Journal of Forensic Sciences* during the last two decades indicates that most studies suffer from one or more major flaws that limit their applicability in forensic settings. These flaws include errors of experimental design and inappropriate or incomplete statistical testing.

The following errors of experimental design were noted: (1) truncating or completely omitting fundamental hypothesis testing procedures. Many studies fail to properly isolate all relevant independent variables (sex, ancestry, body size, age at death) and thus cannot determine which ones actually affect the variance in a skeletal feature; (2) generating predictive regression or discriminant equations for subgroups based on variables that have no proven impact. If an independent variable is not significant in the variance equation, then there is no cause to generate separate equations for those subgroups. For example, stature equations are often provided for sex and ancestry subgroups without demonstrating that they substantively reduce estimation error over mixed-group equations; and, (3) inadequate sampling for the number of variables examined. To thoroughly test hypotheses, large samples are necessary because the addition of each new independent variable splits the sample across an increasing number of cells. For example, 200 specimens used in a typical 28-cell design (two sex groups vs. two ancestry groups spanning seven age decades) results in fewer than eight individuals per subgroup, reducing the power of subsequent statistical testing.

The following problems with statistical testing were noted: (1) over-reliance on simple univariate and bivariate procedures. Chi-squared tests, *t*-tests, and correlation cannot simultaneously control for the effects of multiple independent variables, and so the apparent effects of one variable may be due to the (masked) effects of a second (uncontrolled) variable; (2) ignoring interactions. Simple tests are susceptible to the confounding effects of interactions, which occur when the combination of two variables produces an added effect that cannot be attributed to either variable individually; (3) ignoring unbalanced sampling. Simple tests are influenced by uneven sampling, and since few death assemblages are balanced, most studies must overtly compensate; and, (4) assuming that other complex procedures actually test hypotheses. Principal components analysis and regression, for example, are not adequate for basic hypothesis testing.

A solution to these various problems is to employ ANCOVA as a first step in the experimental process. ANCOVA is a robust and sensitive hypothesis-testing procedure that partitions the variance across all independent variables simultaneously, separating the effects of interactions and controlling for unbalanced samples. The independent variables can be categorical (sex, ancestry) or continuous (body weight, age at death), and varieties of ANCOVA exist for categorical dependent variables (binary logistic regression, ordinal regression). Independent variables that are flagged by ANCOVA as significantly affecting the variance in a skeletal feature can then be explored further to construct predictive models for use in forensic settings.

The historical tendency to eschew thorough hypothesis testing and instead rely on simple statistical tests can explain many contradictory findings between different studies of the same skeletal feature. Furthermore, over time, these errors may have led researchers to emphasize the importance of subgroup differences (and thus the necessity of population-specific methods) at the expense of more robust, non-specific techniques with wider applicability. Until multivariate approaches such as ANCOVA are consistently applied, we will not be able to assess the true importance and relevance of subgroup differences in human skeletal variability and their practical effects on biological profile construction.

Biostatistics, Experimental Design, ANCOVA

A114 Fuzzy Inference System (FIS) as a Novel Statistical Method for Forensic Ancestry Estimation

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After attending this presentation, attendees will understand how fuzzy set theory may assist in ancestry estimation and the advantages of this type of analysis in the field of anthropology.

This presentation will impact the forensic science community by presenting a previously untested statistical technique for the process of forensic ancestry estimation that is built to accommodate such issues as multiple group affiliation and uncertainty in membership classification.

Fuzzy set theory, or fuzzy logic, is a statistical concept developed in the field of engineering.[1] Fuzzy logic is based on the premise that distinct groups are not an accurate reflection of reality due to inherent uncertainty and variation. Individuals may not perfectly belong to any delineated group, or they may identify with multiple groups. Individuals tested are given a membership value, or function, between 0.0 (no membership) to 1.0 (full membership), similar to posterior probabilities that are often presented as part of discriminant function analyses; however, fuzzy logic does not estimate the probability that an individual is part of a group, but rather estimates the degree of membership for each of the groups based on similarities.[2] Previous biological anthropological research has examined its utility in understanding group affiliation of paleoanthropology, primate behavior, and age estimation.[3-5] The accommodation of uncertainty and the analysis of membership to multiple categories makes this statistic an appealing choice for use in estimating the ancestry of individuals with diverse population histories. The objective of the present study is to examine this statistical technique's utility in producing an ancestry estimate for use in the biological profile.

Dental morphological data on eight traits collected by Tsuneiko Hanihara on global populations organized into three major geographic groups (i.e., African, European, Asian) commonly employed in forensic anthropology were used for the present analysis. These traits were not correlated with each other and previous research has shown utility of these traits in distinguishing between populations. This dataset was utilized as a starting point from which to create the system's rules and as a testing sample of the constructed equations. Data collected on Hispanic individuals from New Mexico and Mexico were also used to examine the method's efficacy in estimating individuals of admixed ancestry. A Mamdani FIS analysis in the MATLAB® (R2016a) Fuzzy Logic Designer package was utilized in the present analysis. This FIS uses a system of IF-THEN rules based on *a priori* knowledge to "fuzzify" the data and then "defuzzify" it around the centroid, producing a single membership score. These rules were based on population frequencies of traits gathered from the literature.[6,7] Each individual is then assigned a membership group output that corresponds to a particular ancestral group (e.g., 0.2 would suggest European-derived).

Preliminary results suggest potential utility of this statistic in estimating ancestry; however, the nature of the dataset used in the present analysis precludes a complete picture of its efficacy as the results tend toward a defuzzified membership score of 0.5. Patterns are seen that suggest the FIS is distinguishing between simple and complex dentitions. Any morphological complexity drives membership scores toward African- or Asian-derived estimates. There is also difficulty allocating between African- and Asian-derived populations due to crown complexity. These issues may be due to the use of dichotomized variables and overlapping frequencies of traits in multiple populations. The greatest success occurs when distinguishing between European and African/Asian-derived populations due to European-derived individuals typically having the least complex dentitions.

Breaking down each trait into separate grades, inclusion of more traits, such as enamel extensions and molar crenulations, and refinement of IF-THEN rules may improve the success of this method. Future tests will include a larger, more inclusive dataset to capture the range of variation present in the dentition as well as explore the effects of missing data. While this study represents an early use of fuzzy logic in forensic anthropology, there is great potential to explore this and other statistical methods to improve estimations of ancestry as part of the biological profile.

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Fuzzy Logic, Dental Morphology, Ancestry Estimation

A115 Body Mass Estimation: Preliminary Equations for the Undocumented South Texas Migrants Using Bayesian Inference

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After attending this presentation, attendees will understand the intricacies of body mass estimation in forensic anthropology and how using Bayesian inference can result in more representative body mass estimations of deceased undocumented migrants from the United States-Mexico border. The goals of this presentation are to generate body mass estimation equations specifically for undocumented migrants found along the United States-Mexico border using Bayesian inference and data from recent national health surveys as an informed prior and to evaluate the accuracy of the equations.

This presentation will impact the forensic science community by providing a new method that results in more accurate body mass estimations for Hispanic individuals.

Forensic anthropologists establish a biological profile for unidentified human remains to help narrow the number of possible missing persons matches. The inclusion of body mass provides additional information to aid in identification since weight (body mass) is often reported in missing persons reports. Previous research reveals there is a need for population-specific formulas for estimating components of the biological profile.[1,2] This conclusion is particularly true of the four currently established equations for body mass estimation from skeletal remains.[3] Operation Identification (OpID) at Texas State University is an initiative to identify migrants that die crossing the South Texas-Mexico border. The inclusion of body mass to the biological profile of the unidentified migrants can assist in the exclusion of many potential matches suggested for each individual in the National Missing and Unidentified Persons System (NamUs).

Reference data come from the National Health and Nutrition Examination Survey (NHANES) and the Encuesta Nacional de Salud y Nutrición (ENSANUT), which are nation-wide surveys that include anthropometric measurements to evaluate the health of the nation. Previous studies have shown that the Waist-to-Height Ratio (WtHR) is a better indicator of health risks associated with weight regardless of sex and ethnic differences.[4,5] This ratio is calculated by dividing waist circumference by height. Evaluation of the relationship between body mass and WtHR from the two health surveys demonstrates a moderately positive correlation (R=0.5757). The informed prior for this study includes the height, waist circumference, and weight of those who identified as United States-born Hispanics (from NHANES) and Mexican-born natives (from ENSANUT). The individuals used for this study (N=36377) include males (n=15,748) and females (n=20,629) between the ages of 20 to 80 years. Linear regression was used to estimate waist circumference from living bi-iliac breadth and again to generate body mass estimation equations for males and females using the WtHR. To test for accuracy of the equations, inverse calibration was conducted on each known body mass to calculate an expected WtHR. A Mann-Whitney Wilcoxon test revealed that there is no statistically significant difference (p=0.3984) between the mean expected WtHR value from the generated equations and mean observed WtHR value of the reference data. This means that WtHR can be used to accurately estimate body mass with a 95% confidence interval.

Skeletal data came from OpID and included an equal number of positively identified males and females (N=6). Fully stature and bi-iliac breadth were obtained for each individual when available. Stature was estimated using FORDISC® 3.1 when the Fully method could not be used. Waist circumference was estimated from bi-iliac breadth, and a WtHR value was calculated. Body mass estimations were generated using the aforementioned equations. The reported weights of the identified individuals from missing persons reports were compared to the estimated body mass ranges. Only one individual from each sex cohort (33.33%) was accurately estimated for body mass. The low accuracy rate may be due to small sample size, self-reporting error, or the individuals falling on the extreme ends of the body weight spectrum.[1,6]

Although the sample size of identified individuals is small, it represents the only remains that are positively identified with full skeletons and an estimated weight on a missing persons report.

Results demonstrate that given the distribution of body mass represented by NHANES and ENSANUT, the resulting equations produce promising body mass estimations when using a WtHR ratio generated from skeletal height and bi-iliac breadth for the unidentified South Texas migrants, but also demonstrates the limitations for cases of largely overweight and underweight individuals.

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Bayesian Statistics, Body Mass Estimation, Operation Identification

A116 Decomposition Rates: Autopsied vs. Non-Autopsied Human Remains

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After attending this presentation, attendees will better understand the impact of the bloat stage and the effect of penetrating trauma on the trajectory of human decomposition in an arid environment.

This presentation will impact the forensic science community by increasing sample size in future taphonomic studies while highlighting the importance of region-specific testing of the variables assumed to affect rate and timing of decomposition.

Forensic taphonomy research facilities accepting both autopsied and non-autopsied human remains must contend with subsequent sampling concerns. Autopsy incisions approximate penetrating trauma, while evisceration attendant to organ examination and sampling adds an additional layer of insult. Post-autopsy, organs are typically bagged and returned to the thoracic cavity, resulting in the removal of soft tissue structures and the associated necrobiome, both assumed critical impetus to decomposition. While the sum of these postmortem modifications may reflect the circumstances of case trauma, the appropriate use of autopsied remains in controlled research facilities is poorly understood, making assignment to research cohorts challenging and retrospective data difficult to apply.

The potential for differential decomposition between autopsied and non-autopsied human remains was tested at the Forensic Investigation Research Station (FIRS) in Grand Junction, CO.

A sample of 12 individuals, 6 autopsied and 6 non-autopsied, was used to assess the trajectory of decomposition within and between groups. Decomposition was measured using a Total Body Score (TBS), a qualitative measurement of decomposition derived from gross assessment of value-assigned categories of change within three anatomical regions.[1] The indices of time and temperature were combined as Accumulated Degree Days (ADD). ADD was calculated as the average of hourly temperatures within a 24-hour cycle using data collected from an onsite weather station. All data points collected from the time of body placement to the time of analysis were considered in this study. Maximum ADD presented a range of 6,073-11,916 ADD for autopsied donors and a range of 6,178-12,721 for non-autopsied donors.

To ensure there were no significant differences in autopsied and non-autopsied cohorts, *t*-tests were used to compare donor age, height, and weight (α=0.05). A Linear Mixed Model (LMM) using maximum likelihood estimates was used to determine if the rate of decomposition varied between the two groups. The dependent variable was TBS (TBS^2 transformation). The independent fixed variables were ADD ($Log_{10}(ADD+1)$ transformation), an indicator variable for autopsied (*indicator*=1) or non-autopsied (*indicator*=0) remains, and an interaction term ($Log_{10}(ADD+1)$ x Indicator). The random effects for both intercepts and slopes were ADD and donor. The interaction term was used to determine if the slopes were significantly different. The *p*-value for the interaction was determined by a likelihood test comparing the model with and without the interaction term. The analysis was conducted in Program R (version 3.4.0) using the lme4 package.[2,3]

There was no significant difference for age, height, or weight between the two groups. The decomposition rate did not differ between autopsied and non-autopsied donors based on a comparison of slopes $\chi^2_{(1)}$=0.576, p=0.448.

FIRS is located in a semi-arid, high-altitude region, which promotes prolonged periods of tissue retention following desiccation of dermal and visceral tissues. Bates and Wescott observed a similar pattern between autopsied and non-autopsied cohorts in west Texas and concluded that penetrating trauma affects the pattern of decomposition but not the rate.[4] Results at FIRS concur, but also suggest that the bloat stage may not influence the overall trajectory of decomposition as is generally assumed. Bloat is the byproduct of bacterial metabolism within confined organ structures. The removal of the constituent parts necessary for bloat did not have an appreciable effect on the rate of decomposition. This highlights the necessity for regional testing of the biological phenomenon (both direct and auxiliary) traditionally assumed to influence the rate of decomposition and suggests that while dramatic in gross presentation, bloat does not significantly affect decomposition rates in arid regions.

Understanding the variables that affect rate of decomposition is important in the evaluation of prior research studies, future research studies, and for forensic scientists estimating postmortem interval on remains presenting large penetrating wounds.

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Taphonomy, Autopsied/Non-Autopsied, Decomposition

A117 Testing the Accuracy of the Correlation Between the Condyles of the Distal Femur and Proximal Tibia: A Validation Study

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After attending this presentation, attendees will have an appreciation for the accuracy of predictive formulas correlating the distal femur and proximal tibia and the potential for this method to be used in mass fatality and/or commingled remains cases.

This presentation will impact the forensic science community by revisiting predictive formulas constructed by Waxenbaum and Linney and tests the accuracy and practicality of this methodology's use in the field on a novel sample.[1]

The knee is one of the most functionally important and largest joints in the body, prompting several researchers to explore sex and ancestral variation in the femur and tibia.[2-5] Assessing the relationship between distal femur and proximal tibia could prove itself useful when attempting to resolve commingled remains scenarios, including mass disasters, human rights violations, and archaeological assemblages, making this relationship both forensically and bioarchaeologically significant.[6,7] Not only do the distal femur and proximal tibia consistently preserve well, but the knee is also one of the last areas to burn when introduced to fire.[8] Due to the consistently strong survival rate of the knee in archaeological, mass disaster, and fatal fire scenarios, the distal femur and proximal tibia are a critical area that requires a more thorough anthropological examination. In 2011, Waxenbaum and Linney constructed predictive formulas, both general and specific to age, sex, and ancestry, on both a modern and archaeological sample through reduced major axis regression.[1] The present research will test the applicability of these equations on a novel sample.

Data were collected on a sex- and population-balanced sample of 103 individuals from the Hamann-Todd Collection housed at the Cleveland Museum of Natural History. Measurements of the left medial and lateral epi/condyles of the distal femur and proximal tibia were taken on all individuals and compared through analysis of variance and covariance as well as reduced major axis regression. The measurements were recorded using a digital sliding caliper and measured to the nearest +/- one millimeter. The effects of interobserver error to determine repeatability was also performed.

All analyses proved the equations to be accurate, yet not very precise. Interobserver error is likely too high for practical use. The medial condyle equations performed best for historic samples, while the potential for the use of the lateral condyle equations on modern samples needs further exploration. In the end, the pooled equations would be the only ones ever used in the field or laboratory. Despite the accuracy, unfortunately, the equations used in commingling situations is unrealistic.

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Knee, Condyles, Commingling

A118 Using Microbial Clocks in Human Cadaver Ribs as a Postmortem Tool

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After attending this presentation, attendees will have a basic knowledge of the microbiome (study of the genes of microbes) and its application in human decomposition studies. Attendees will be presented with the results of a pilot study, which sought to investigate the bacterial succession in human cadaver ribs during the advanced stages of decomposition.

This presentation will impact the forensic science community by exploring an area with little to no previous research. This pilot study reveals the changes seen in the bacterial communities of cadaver rib marrow, over time, during the later, drier stages of decomposition and its potential usefulness as a Postmortem Interval (PMI) tool.

Determining the PMI is an important aspect of forensic investigations, but accuracy in predicting the interval declines with time. Microbes are important players in the human decomposition process and have been studied on cadaver skin and gravesoil samples during the early, wet decay stages as a potential PMI "clock."[1-5] Bacterial succession on decomposing skin shows promising results, but it becomes less accurate as desiccation occurs and decomposition approaches the skeletonization stage.[1,2] This pilot study investigates the bacterial communities inside the human cadaver rib during the advanced decay stage and into skeletonization.

Twenty-four ribs were excised from three cadavers at the Southeast Texas Applied Forensic Science facility in Huntsville, TX. One rib was sampled from each cadaver every three weeks over a six-month period during the later decay stages (beginning May 2016), representing ~5,000 Accumulated Degree Days (ADD). DNA extracted from the rib samples was sequenced using the MiSeq® Illumina® platform, targeting the 16S recombinant DNA (rRNA) gene region.

Results indicate that bacterial communities shift in community membership with advancing time, with the biggest trend occurring between the first and last sampling periods (advanced decomposition stage and skeletonization stage, respectively). Statistics demonstrate a significant difference in the phylogenetic distance between these samples (first and last). Community composition, more than abundance, may play a role when determining a PMI. Similarly, rarefaction curves trend toward an increase in richness with increasing ADDs, although there were no significant differences found in diversity between samples; however, due to the study's small sample size and unique nature of cadaver research, only generalizations about the microbiome of cadaver bone can be made. A reliable PMI method cannot be formulated from this study at this time, but the results suggest that there is a trend in bacterial succession that could be useful and warrants further investigation.

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Decomposition, Microbiology, Bone

A119 Toward a Skeletal Atlas of Elder Abuse: A Pilot Study of Fracture Patterns in Documented Cases

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After attending this presentation, attendees will be aware there are skeletal fracture patterns associated with the abuse/neglect of elderly individuals distinguishable from those found in accidental falls.

This presentation will impact the forensic science community by introducing skeletal manifestations and patterning of elder abuse, contributing novel data to the ongoing effort to unmask elder abuse at a state and national level.

Increasing rates of elder abuse in the United States have produced an urgent need for improved diagnostic criteria.[1] Physical abuse of elders represents the most severe manifestation of this trend, yet is difficult to prove.[2] Skeletal expressions of elder abuse offer key indications of inflicted injuries, but often are masked by assignation to accidental falls. Currently, there is no standard for the diagnosis of elder abuse in the skeletal system; research in this field is "decades behind."[3-5] Here, results of a pilot project are presented identifying fracture patterns associated with suspected cases of elder abuse/neglect in contrast with those associated with accidental falls.

To establish skeletal patterns of abuse/neglect versus accidental falls, 300 investigative summaries, dating from January 1, 2014 through June 30, 2017, were reviewed. From these, 30 individuals above the age of 60 years were included in the pilot study population based on the following criteria. Decedent must: (1) have been admitted for full exam; (2) be associated with an Adult Protective Services and/or law enforcement investigation; (3) present with skeletal fractures; and, (4) have radiographs/samples available for review. Given these strict criteria, this sample size is known to be an underestimation of cases of abuse/neglect. To establish a comparative baseline of those fractures most likely to occur in accidental falls, 75 cases of witnessed falls from the same pool were reviewed.

A significant difference in the skeletal manifestation of injury was observed between those individuals involved in accidental falls and those for whom abuse/neglect is suspected. For individuals involved in a witnessed fall, fractures occurred most frequently in the hip (n=51; 68%), followed by vertebral compression fractures (n=14; 19%). Five individuals involved in an accidental fall presented with multiple fractured ribs (7%). In contrast, for those individuals identified as possible victims of abuse/neglect, fractures occurred most frequently in ribs 8-11 (n=8; 27%) and the arm (n=9; 30%), followed by fractures in the femur (n=7; 23%) and the tibia/fibula (n=3; 10%).

In cases of suspected abuse/neglect, femoral fractures occurred along the shaft or distal end, while femoral fractures in witnessed falls occurred at the proximal end. Rib fractures occurred in the posterolateral shafts of ribs 8-11 in cases of suspected abuse/neglect, but in varying locations in falls. When an accident involved fractures of the arm, the bones involved consistently displayed fracture types associated with a fall on an outstretched arm.[6-8] In cases of suspected abuse/neglect, arm fractures presented as chronic dislocation of the humeral head and/or healing fractures of the radius and/or ulna shaft. In several cases of suspected abuse/neglect in which the humeral head was involved, fractures of the lateral clavicle and acromion process of the scapula were also noted. This finding complicates reports that falls onto an outstretched hand account for the majority of proximal humeral fractures in the elderly and serves as a warning that cases of abuse/neglect may be masked by a tendency to over-assign arm fractures in the elderly to accidental falls.[8-11]

By exploring these patterns within their contexts, mechanisms that may account for observed differences can be proposed. In several cases of suspected abuse/neglect in this sample, fractures occurring in ribs 8-11 are correlated with reports of a "bear hug" restraint. Where fractures of the distal femur and tibia/fibula are associated with cases of suspected abuse/neglect, individuals were non-ambulatory and had been dropped, resulting in a "crushing" fracture. A rough "jerking" of the arms in the process of moving a non-ambulatory individual resulted in crushing and dislocation of the humeral head.

Fractures are the most common musculoskeletal condition requiring hospitalization among individuals aged 65 and older in the United States, and rigorous diagnostic criteria must be developed to differentiate accidental injury from abuse.[12] Results from this pilot project offer a first stage in the effort to develop and improve diagnostic criteria for a skeletal atlas of elder abuse.

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Elder Abuse, Fracture Patterns, Skeletal Atlas

A120 Results of Testing Interobserver/Intraobserver Error for "Planar" Proxy for Upper Facial Breadth and Novel Measurement of Interorbital Distance

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After attending this presentation, attendees will better understand the potential benefit of a proxy measurement for upper facial breadth and interorbital distance for analysis of fragmented remains. This study presents the analysis of data collected at the 2016 American Academy of Forensic Sciences (AAFS) Annual Scientific Meeting in Las Vegas, NV, to test for interobserver and intraobserver error in these measurements.

This presentation will impact the forensic science community by testing the accuracy and consistency of alternative methods for collecting cranial measurements. Cranial remains, specifically the facial component, are often fragmented due to the fragile nature of this area. This limits the collections of standard craniometrics, which are used to develop a biological profile necessary for the identification of skeletal remains. This could be rectified by the use of proxy measurements and the development of novel measurements to obtain the necessary information to make an identification. If found accurate and reproducible, these measurements would contribute to the resources and methods available for analyzing fragmented cranial remains when the standard points of reference are damaged. Previous research demonstrated that these measurements can aid in the identification of sex and ancestry of human skeletal remains.[1]

This study is a continuation of the trend in the discipline of forensic anthropology to re-evaluate standard craniometrics and add statistical rigor to the assessment of sex and ancestry. The previous study presented three Upper Facial Breadth (UFB) proxies and one alternative measurement for interorbital breath.[2] This research examined the "planar" UFB proxy and a new measurement, interorbital breadth at nasion, for interobserver and intraobserver error. These measurements are being tested for their utility on fragmentary remains in which the pristine standard craniometrics are unobtainable.

Cheramie and Kles found that "Planar" (a unilateral measurement from nasion to Frontomalartemporalis (FMT) measured in the same plane and multiplied by two) was not significantly different than UFB and produced the same results as UFB in discriminating sex and ancestry, suggesting it could be an effective proxy for UFB in damaged remains.[2] Testing also found the measurement of interorbital breadth at the height of nasion was significantly different than the standard interorbital breadth measurement taken at the height of dacryon, but it was also found to be useful in the assessment of sex and ancestry, suggesting it could be used instead of the standard measurement in damaged remains.

During the 2016 AAFS Annual Scientific Meeting, volunteers were solicitied to measure crania to assess interobserver and intraobserver error. Participants were asked to measure one of three crania with spreading and sliding calipers. They collected nine standard measurements, the Planar proxy for UFB, and the new interorbital distance at nasion measurement. Participants were asked to conduct the measurements twice and were asked a series of questions about their level of education and experience. Fifty-two individuals participated.

Results were inconclusive. The "Planar" measurement was found to be statistically different (p <0.05) in two of the three specimens. No significant difference (p <0.05) was found in interorbital breadth at nasion in two of the three specimens. Although the results were mixed, the findings suggest that these measurements have promise. Future testing will include examining the descriptions of the measurements for clarification, re-testing on larger samples, and collecting data on known samples to test the utility of interorbital distance at nasion.

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Craniometrics, Upper Facial Breadth, Interorbital Distance

A121 Challenges in Identifying United States Casualties From Past Conflicts: An Assessment of Lines of Evidence

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After attending this presentation, attendees will better understand the process of effecting individual identifications of United States service members associated with past conflicts (World War II, Korea, and Vietnam) and recovered by various methods (disinterment, field recovery, unilateral turnover, and a commingled turnover sample) using data from the Defense POW/MIA Accounting Agency (DPAA).

This presentation will impact the forensic science community by highlighting the importance of antemortem records, quality of evidence, and necessity of multiple lines of evidence to effect individual identifications. Differences in the number and/or quality of lines of evidence per identification are evaluated based on conflict and recovery type.

All identifications are guided by historical research and investigations; however, this is not included as a line of evidence in this study. Instead, this study emphasized the five lines of evidence that are analyzed or interpreted in the laboratory. DNA, dental (FOR), and chest radiograph (CXR) comparisons are used for individual identification purposes, as these analyses involve the comparison of skeletal remains against antemortem data. Identifications are supported by anthropological skeletal analyses (FAR), which are completed "in the blind," and analyses of material items (MER), which associate remains with an individual or a conflict.

Ideally, every line of evidence would be at the analysts' disposal; however, due to the effects of time and other taphonomic processes, evidence may be impacted or limited. For example, disinterments generally consist of more complete sets of remains that could not be identified in the past, while field recoveries often consist of incomplete or fragmented remains. Complicating matters, the disinterred remains of many unidentified service members were coated in a formaldehyde powder prior to burial, which presents challenges for DNA analysis. The type of DNA analysis depends on available Family Reference Samples, which may be limited or unavailable despite searches for appropriate relatives. Additionally, antemortem records may be lacking or of poor quality.

This study includes data from 166 recent identifications made by DPAA. Once evidence is received by the laboratory, analyses are completed based on the condition of the evidence. On average, identifications are supported by three different analyses. Only one case was identified with a single analysis, while eight cases were identified with all five types of analyses. Overall, DNA contributed to identification in 70% of the cases, followed by FOR (68%), then CXR (33%). FARs were completed in 98%, and MER in 42% of identified cases. FARs are critical because an "in-the-blind" analysis of skeletal remains provides an unbiased means of supporting other analyses; however, the level of detail possible in any analysis depends on the condition of the remains. Therefore, any analysis may indicate positive association or inability or exclude association with a historical candidate.

When assessed by conflict (World War II, n=67; Korea, n=87; Vietnam, n=12), DNA analyses contribute less to older cases (Vietnam, 83%, vs World War II, 58%); however, FORs corroborate more older cases (Vietnam, 25%, vs. World War II, 88%). CXRs predominantly support Korea cases (42%), which can be explained by the relative absence of antemortem radiographs for other conflicts.

Analyses are also affected by recovery type (disinterment, n=45; field, n=60; turnover, n=11, and turnover/commingled, n=50). Only World War II and Korea cases are represented by disinterments. Less than half of disinterments have DNA results (40%), while DNA is present in at least 65% of other cases. More than 80% of disinterments and field recoveries have FORs, compared to less than 45% of other cases. DNA is most integral for resolution of commingled cases (100%), while material evidence is most commonly used to support identifications from field recoveries (83%, compared to less than 36% of other cases), where identifying media helps confirm loss locations and direct site excavations.

Each case is unique regarding types and conditions of evidence and available records. The elapsed time since the conflict does not appear to limit identifications. Differences exist based on conflict and type of recovery method, which can be explained by antemortem record availability, postmortem processing, and other taphonomic processes. Often records, materials, and/or the remains are incomplete or fragmented, which emphasizes the inadequacy of a single line of evidence.

The views herein are those of the authors and do not represent those of the Department of Defense or the United States government.

Individual Identification, Lines of Evidence, Conflicts

A122 The Application of the Megyesi Method and Improved Total Body Scores (TBS) and Accumulated Degree Days (ADD) Equations to Pennsylvania, Ohio, and New York Cases

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After attending this presentation, attendees will better understand the applicability of the Megyesi method to other regions of the country, including Pennsylvania, Ohio, and New York. Attendees will also gain insight on the practicality and reliability of the Megyesi et al. method (Megyesi method hereafter) and the Moffat et al. method (Moffat method hereafter) for Postmortem Interval (PMI) estimation.[1,2]

This presentation will impact the forensic science community by demonstrating some current limitations of the Megyesi and Moffat methods, stimulating further research to refine and improve PMI estimation methods based on decomposition.

Techniques based on the Megyesi method have been criticized mainly for their allegedly limited applicability to regions different from that of the original sample. In this study, the methods presented in Megyesi et al. and Moffat et al. were applied to a set of forensic cases from the Northeast United States, with known PMIs, processed at Mercyhurst University.[1,2] Seventy-five cases from 1997 to 2016 were examined for this purpose, but only 10 fulfilled the strict criteria of the Megyesi method on context conditions, surroundings, and completeness of the remains. These were all outdoor ground surface deposits with all elements necessary to calculate the TBS according to the Megyesi method.[1] Megyesi TBS were calculated from scene recovery photographs and later recalculated using the Moffat method. Average daily temperatures to calculate the ADD were collected from records of the National Oceanic and Atmospheric Administration (NOAA) for the general area of each scene. Ninety-five percent confidence intervals for the ADDs were calculated based on the equations in each method and compared to establish which of the two methods rendered the closest estimate to the known PMI of each case.

In the areas under study, the Moffat method rendered only a slightly higher accuracy, although more precise (narrower) intervals than the Megyesi method. The Megyesi method incorrectly estimated the PMI in four cases, while the Moffat method estimated PMI incorrectly for three cases. The average error was 16 days for the Megyesi method. For the Moffat method, it increased to 78 days. The much higher error for the latter is mostly derived from a single outlier appearing when the TBS scale was recalculated according to the improved equations; however, after removal of this outlier, the average error of the Moffat method was still 28 days.[2]

These results suggest that, while perhaps providing higher accuracy, the Moffat method may be applicable to a narrower range of PMIs; narrower even than that represented in such a reduced sample as the one examined in this study. Deviations of the confidence intervals from the actual PMI appear to be less frequent in the Moffat method. When they occur, they appear to be potentially much wider than in the Megyesi method, with the narrower confidence intervals of the Moffat method providing only the illusion of precision. This indicates a need for further research, increasing sample sizes and the range of PMIs, and environments considered in future studies to improve TBS/ADD methods.

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Megyesi Method, Forensic Taphonomy, Postmortem Interval

A123 The Human Cadaver Decomposition Island (CDI) and the Vegetation Regrowth Interval

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After attending this presentation, attendees will have a clear understanding of what a surface human cadaver decomposition island looks like with and without scavenging interference and will understand that the CDI is present months after the remains are removed.

This presentation will impact the forensic science community by bringing awareness to those participating in search and rescue efforts to not only focus on looking for the skeletal remains of an individual, but to also look for the CDI, which is an identifiable and useful piece of evidence.

Typically, during the late phase of the Early stage of human decomposition, fluids of the body, formed during autolysis, hydrolysis, and by other chemical and bacterial actions, leach into the soil under and around the body, producing a CDI. The fluid contains high concentrations of organic matter and microorganisms, eradicating any vegetation adjacent to and under the body. When the soil pH and chemicals begin to return to a level similar to the original state, remaining nutrients in the soil cause native vegetation, and flora not indigenous to that area, to flourish. Previous research observing regrowth of vegetation has been conducted using *Sus scrofa*, although no literature to date has recorded the vegetation regrowth of a human CDI.[1]

In 2013, a pilot study was conducted at The Southeast Texas Applied Forensic Science (STAFS) Facility, Huntsville, TX. The pilot study revealed that the CDI is greatest under the trunk, reduced under the head, and further reduced under the limbs. The present study documented the timing of the formation of the CDI as well as the timing of the return of vegetation around and within the CDI. Three subjects were placed in similar environmental conditions; two subjects were inaccessible to scavenging and one was accessible. After the collapse of body cavities and the formation of the CDI, the subjects were removed from their CDI at predetermined intervals of zero and two weeks after leaching. Cages remained over the CDIs and daily observation occurred until regrowth within the CDI formed. For the subject removed at zero weeks (the day after CDI formation appeared complete), regrowth within the CDI occurred after 131 days. For the subject removed at two weeks after CDI formation, the regrowth occurred after 197 days. Most of the CDI for the subject accessible to scavenging had formed under the trunk of the body prior to being moved around by scavengers; however, the CDI of the limbs and head could not be clearly identified after movement of the body by scavenging had smeared the soil. The body was pulled away from the major part of the CDI by scavengers but remained at the periphery throughout the study. After 207 days, vegetation began to appear in the CDI of the scavenged subject. The accessible body that was not removed continued to cover portions of the CDI, thus prolonging the CDI's composition. Results demonstrate that in the piney woods region of Southeast Texas, if a decomposing body can leach the majority of its fluid in an area prior to extensive scavenging, vegetation regrowth may take 100-200 days to appear.

In search and rescue efforts for missing individuals presumed dead, a CDI may be detectable three to six months after death, even if the body has been displaced from its original location. Further studies using a larger sample are currently being conducted.

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Cadaver Decomposition Island, Postmortem Interval, Forensic Anthropology

A124 Modern Variation in Vertebral Column Segmentation and Transitions

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The goal of this presentation is to describe the variation observed in individuals accessioned at the University of Florida C.A. Pound Human Identification Laboratory (CAPHIL) from the modal human vertebral formula and discuss potential effects of this variation on forensic analyses. After attending this presentation, attendees will understand the value in assessing variation within the vertebral column.

This presentation will impact the forensic science community by highlighting the importance of understanding modern human biological variation and the potential of anomalous development to affect forensic identification processes.

The modal formula of the human vertebral column consists of 7 cervical, 12 bilaterally rib-bearing thoracic, 5 lumbar, 5 fused sacral, and 4 coccygeal vertebrae. Variation in the number of vertebrae and associated ribs can occur within each vertebral column region. These variations are generally attributed to embryological developmental irregularities, such as an amodal number of somites or abnormal patterns of genetic signaling.[1-3]

This study accessed skeletal inventory and descriptive data of 55 (*F*=14, *M*=41) identified and unidentified individuals analyzed at the CAPHIL between 2007 and 2017. The individuals ranged in age at death from late adolescence to older adult, and the sample varied in ancestry. Sample selection required the presence of at least one developmental vertebral or rib anomaly. Selection of developmental anomalies was limited to differences from the modal vertebral column expression, including: (1) regional segment count; (2) segmental cranialization or caudalization; and, (3) improper segmentation. This criteria allows for identification of variance in vertebra/rib count, transition locations, and segment separation errors. Thoracic and lumbar vertebrae are typically defined morphologically, with the orientation patterns of Superior and Inferior Articular Facets (SAFs/IAFs) or articulation to ribs as defining criteria. For this study, designation of a thoracic vertebra is determined by SAF/IAF orientation patterns and possession of costal facets; designation of a lumbar vertebra is determined by SAF/IAF orientation, regardless of rib-bearing status.

Forty-one of the studied individuals varied in segmental count in at least one region (74.5%). Of these individuals, three had 11 bilaterally rib-bearing thoracic vertebrae (7.3%), 2 had 13 bilaterally rib-bearing thoracic vertebrae (4.9%), 16 had 6 lumbar vertebrae (39.0%), 21 had 6 sacral segments (51.2%), and 4 had 3 coccygeal segments (9.8%).

Thirty-seven individuals exhibited variation in at least one transitional region (67.3%). Of these individuals, four exhibited variation in two regions (10.8%). In one male, C7 articulated unilaterally to a rib (2.7%). One male had a unilateral supernumerary rib of indeterminate anatomical origin in addition to the 24 thoracic ribs (2.7%). Sixteen individuals exhibited bilateral lumbar ribs (43.2%), one of whom exhibited a bilateral set on L1 and a unilateral rib on L2 and two of whom exhibited a unilateral rib on L1. For 13 individuals (35.1%), the thoracolumbar transition occurred at the level of T11; in ten of these cases (76.9%), the vertebra inferior to T11 had lumbarized SAFs/IAFs and was rib-bearing, followed inferiorly by five non-rib-bearing lumbar vertebrae. Thirteen individuals displayed sacralization (35.1%), with seven instances at the level of L5 and six at the level of L6. Of individuals with a sixth lumbar vertebra, 75.0% exhibited sacralization of the element. Four individuals (10.8%) exhibited S1 lumbarization.

Three individuals had segmentation defects (5.4%), of whom there was separation failure of C2/C3 in one female (33.3 %), of C7/T1/T2 in one male (33.3%), and of right ribs 3/4 in one male (33.3%).

In this study, the vast majority of developmental vertebral column anomalies were expressed in the caudal regions. This is likely due to abnormal genetic signaling during embryonic specification of individual vertebral identity in which the signaling code becomes caudally complex. Certain cervical anomalies can be associated with detrimental conditions that may result in perinatal or infantile death; thus, the cervical region has strong developmental constraints.[4,5] This constraint may contribute to the relatively low frequency of cervical anomalies observed in this study.

This study describes relative frequencies of developmental vertebral anomalies observed in a forensic sample. Vertebral anomalies may affect forensic stature estimations, as the vertebral column majority directly contributes to height, and standard stature calculation techniques assume modal vertebral frequencies. Further, deviance from modal expressions can affect the condition of the vertebral column, which may have clinical implications and serve as potentially individualizing pathological features.[6,7]

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Anomalous Variation, Vertebrae, Development

A125 Spatial Analysis and Animal Activity: A Taphonomic Study Using Geographic Information Systems (GIS) to Document Animal Modification to Human Bone at Outdoor Crime Scenes

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After attending this presentation, attendees will be familiar with the utility of a standardized approach to document and analyze taphonomy on forensic cases using GIS, including its usefulness in answering questions beyond forensic significance.

This presentation will impact the forensic science community by providing an experimentally derived protocol for documenting and answering taphonomic questions concerning outdoor crime scenes and a test of their applicability in order to broaden the community's understanding of scientifically derived answers to taphonomic questions.

Forensic anthropologists are commonly asked to assist with outdoor crime scenes that involve badly decomposed or skeletonized remains. Remains encountered at these scenes are generally exposed to numerous taphonomic agents, especially if the remains were deposited on the surface. Besides environmental variables, such as climate, wind, water, etc., animals are one of the more commonly encountered taphonomic agents. Carnivores are known to chew on remains, not only leaving signature marks on the bones, but also commonly scattering the bones or removing elements from the immediate scene. Rodents gnaw on the skeletal remains and can also move smaller elements. Although general patterns in carnivore and rodent modification have been described, these patterns have not been objectively analyzed.

In this study, GIS was used to analyze rodent and carnivore modification from a total of 51 Mercyhurst University Forensic Anthropology Laboratory (MFAL) forensic cases. The current practices of documenting taphonomic modification of human tissues is typically conducted through photographs and written notes. When collecting data from these cases, all the notes and photographs were standardized into a diagram format using a homunculus jpeg in Adobe® Photoshop® CC. A separate database was created to identify the case type, bone counts, and any information that was known about the Postmortem Interval (PMI). Individual layers representing carnivore modification and rodent modification were saved as jpegs, entered into GIS, and vectorized. The vectors give exact locations and quantities of taphonomic alteration on the skeleton. Density maps of locations of animal activity on the human skeleton were created. All of this was conducted following a prescribed and detailed protocol. For further analysis of these skeletal maps, four categories of modification based on location and animal type were created: epiphyses, diaphyses, rodent, and carnivore. This allowed the specific areas and timing of animal modification to be identified in order to test established taphonomic interpretations found in the forensic literature.

Using location and animal type categories, basic statistics were run on the data. Chi-square (<0.05) tests revealed there was a significant difference between animal gnathic modification location on the bones. With respect to the long bones, carnivores more frequently chewed on epiphyses, while rodents gnawed on diaphyses. For example, carnivore modification of the epiphysis of the humerus was noted 32.6% of the time and the diaphysis only 2.3% of the time. Rodent activity was noted on the fibula epiphyses 17.6% and the diaphysis 41.1% of the time. This provided a test of the accepted pattern in the forensic literature and illustrated through density maps and statistics that the identified patterns of animal modification on the skeleton are accurate: carnivores tend to modify the long bone epiphyses first while the rodents were more active on the diaphysis.[1-3] Additionally, forensic literature PMI interpretations were compared to the new findings. The average PMI for carnivore gnawing to be exhibited in the MFAL cases was 22 months, while the rodents was 76 months. Performing a *t*-test demonstrated that these means were significantly different. When trying to identify a specific timing of gnawing by animal type using logistic regression, no significant relationships were identified. These results are consistent with the accepted sequence of gnawing found in the forensic literature and indicate that carnivores modify remains while they are in an earlier state of decomposition, as compared to rodents.

This protocol used for mapping the animal modification can be expanded to analyze any taphonomic agent that results in physical alteration of skeletal material. If other variables were analyzed using this method, a larger regional expression of taphonomy could be created to better understand what happens at a crime scene from the time remains are deposited to when they are analyzed in a laboratory. A database of taphonomic human skeletal modification could be created in order to aid forensic archaeological recovery efforts and outdoor scene interpretations.

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GIS, Taphonomy, Documentation

A126 The Relative Compositional Changes of Buried Juvenile Porcine Ribs and Ulnae in the Early Postmortem Interval

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After attending this presentation, attendees will better understand the compositional changes that different bones of the juvenile skeleton undergo within the early postmortem period in a buried environment, as illustrated by the analysis of ribs and ulnae.

This presentation will impact the forensic science community by demonstrating that bone breakdown and time-since-death estimations from juvenile bone can potentially be influenced by, or vary according to, the skeletal element that is analyzed.

Estimation of postmortem interval is an important aspect of forensic investigations involving human skeletonized remains. Current methods are relatively poorly informed of the process in which juvenile bones degrade. Furthermore, the variation across different skeletal elements of a single individual has not been sufficiently explored. Experimentation with porcine bone as a proxy for humans provides one of the few opportunities to examine these issues.

This study uses a juvenile porcine model to examine the relationship between the length of the postmortem interval in buried environments and the changes in water, collagen, and mineral content of two distinct skeletal elements: ribs and ulnae.

Fifty-four suckling piglets (*Sus scrofa*) aged approximately between two and eight weeks were purchased from a local supplier. Ribcages and ulnae were disarticulated and manually defleshed, then left to decay in a controlled buried environment for 12 months. Each month, nine ribs and four ulnae were excavated, for a total of 108 ribs and 48 ulnae over the duration of the experiment. Each bone was sectioned to obtain a portion that was used for the quantification of water, collagen, and mineral content. This analysis was accomplished through a process of sequential controlled heating, designed specifically for this project. Each sample was weighed four times throughout the process, and the water, collagen, and mineral contents were expressed as a percentage of total weight.

Results indicate that the water, collagen, and mineral contents were approximately the same in both the ribs and ulnae at the beginning of the experiment. Water content diverged between the ribs and ulnae after the second month, with the values for ribs remaining relatively constant and ulnae increasing through time. Collagen appears to remain constant between the two skeletal elements throughout the experimental interval. An overlap in mineral content was observed between ribs and ulnae until month six, when a noticeable decrease in the ulnae values occurred.

These results suggest that the changes in the chemical composition of different skeletal elements may occur at different rates within the early postmortem interval. These varying rates can potentially impact the physical degradation of skeletal elements to differing degrees, thus influencing the reliability of time-since-death estimations from juvenile bone.

Decomposition, Collagen, Mineral

A127 Seasonal and Spatial Variation in Local Weather Station Data From Knoxville, Tennessee

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After attending this presentation, attendees will understand the seasonal and spatial variation in ambient temperatures at the Anthropological Research Facility (ARF) in Knoxville, TN.

This presentation will impact the forensic science community in terms of competency and performance by supporting research that National Weather Service (NWS) weather stations may be an unreliable source of temperature information for use in Postmortem Interval (PMI) estimation.

Estimation of the PMI is crucial to medicolegal death investigation. Some PMI estimation methods rely on the quantification of biological processes driving human decomposition, of which ambient temperature is considered an important factor. Furthermore, anthropological research in human decomposition increasingly utilizes Accumulated Degree Days (ADD) as a way to incorporate ambient temperature into predictive models designed to translate this information into estimates of PMI. An underlying assumption of these models is that the practitioner has access to accurate and reliable temperature data. Dabbs addressed this assumption by examining temperature data from multiple NWS weather stations.[1] She found that differences in recorded temperatures between weather stations translated into potentially meaningful differences in PMI estimation. Additional studies have explored the underlying accuracy and reliability of NWS data through comparisons with localized ambient temperature data loggers.

This study expands upon Dabbs and others by investigating seasonal and spatial variation in temperature data retrieved from NWS weather stations and local data loggers.[1] Specifically, temperature data were collected between 2014-2017 from an NWS weather station located at the University of Tennessee Institute of Agriculture (UTIA), less than 0.5km from the ARF, and were compared to a digital thermometer permanently installed within the ARF. Maximum and minimum temperatures (°C) were compared between both temperature data sources using a Welch's two sample *t*-test in R (version 3.1).[2] Then, daily temperatures were grouped by season (i.e., spring, summer, fall, and winter) and compared between those same two sources. Results were considered statistically significant at the $p < 0.05$ ($\alpha = 0.05$) level.

Overall, the results of this study indicated that maximum and minimum daily temperatures collected across data sources differed by less than one degree. The results of the Welch's two sample *t*-test indicated no statistically significant difference between data sources when all data were pooled. When the data were separated by season, significant differences emerged. Spring (mean ARF 26.6°C, mean UTIA 25.0°C), summer (mean ARF 29.9°C, mean UTIA 31.0°C), and winter (mean ARF 12.9°C, mean UTIA 9.2°C) maximum temperatures were significantly different between sources, as were the minimum temperatures in the summer (mean ARF 20.9°C, mean UTIA 19.7°C). These results clarify earlier studies examining the correspondence between the NWS station data and data collected at specific sites of human decomposition by finding a seasonal link in the variability in temperature data across sources. These results not only have regional implications for decomposition research conducted in East Tennessee, but also have wide-reaching importance for future anthropological and entomological studies of human decomposition. It is strongly recommended that researchers and practitioners independently validate the reliability of their temperature data sources, especially in longitudinal studies encompassing multiple seasons.

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Time Since Death, Accumulated Degree Days, Human Decomposition

A128 The Influence of Three-Layered Cranial Architecture Development on Non-Accidental Pediatric Cranial Blunt Force Trauma (BFT) Outcomes

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The goal of this presentation is to document ontogenetic development of the three-layered (i.e., diploe, inner, and outer table) architecture of the juvenile cranium and investigate the effect of this development on pediatric BFT outcomes.

This presentation will impact the forensic science community by providing forensic anthropologists and pathologists with an enhanced understanding of the spatial and temporal variability in the emergence of a mature cranial architecture and its response to (and, thus, risk associated with) fracture across varying regions and age groups, with important scientific applications for non-accidental BFT trauma prevention.

Approximately 75%-80% of all non-accidental pediatric deaths involve cranial BFT, disproportionately affecting decedents less than the age of one year. These typically involve subdural and retinal hemorrhage and diffuse traumatic axonal injuries, often associated with cranial fracture.[1] A number of extrinsic and intrinsic factors influence pediatric cranial fracture vulnerability, although prior research has focused on biomechanical factors associated with injury, less attention has been paid to the influence of intrinsic features of bone structure on BFT outcomes.

The neonatal cranium is unilaminar — a three-layered cranial architecture does not develop until sometime during the first or second year and a mature structure may not be present until later in childhood; however, the exact timing and mechanism of this process have not been studied on dry bone.[2] Also unknown is the effect of three-layer development on non-accidental (including repetitive) episodes of pediatric BFT.

This study has two goals. First, temporal and spatial development of the three-layered architecture in the juvenile cranium is chronicled. Second, implications of this development for cranial fracture outcomes are explored through testing of the hypothesis that the absence or lesser development of a three-layered architecture in very young subadults leaves their cranial bones thin and vulnerable to the effects of BFT compared to older subadults. Thus, regions of the pediatric cranium manifesting delayed or inadequate development of the three-layer architecture will exhibit greater vulnerability to (and thus, higher risk associated with) the mal-effects of BFT than regions with a developed three-layer structure.

Development of the three-layered cranial architecture is chronicled through macroscopic and microscopic (5x-40x) analysis of a sample of 50 juvenile crania from the Scheuer collection, ranging in age from perinatal to 17 years; 37 of the crania were below the age of 5 years. Variables recorded across the sample included age, bone, bone location, bone maximum, mean and minimum thickness, inner and outer table differentiation, and diploe presence and morphology (including thickness and pattern of diploe distribution, measured with the aid of the digital microscope). These data, including more than 300 micrographs of internal bone structure, were used to illustrate the progression and character of diploe development and generate a topographic map of cranial and diploe thickness and development across age groups. Digital macroscopic and microscopic images of cranial fracture locations from seven Radford University Forensic Science Institute cases involving non-accidental pediatric BFT were compared to the topographic map data to test the above hypothesis.

Results indicate the absence of a three-layered cranial architecture until 4-6 months of age, when initial development of diploe combined with differentiation of inner and outer tables can be noted in the posterior parietal/superior occipital. By 9-12 months, this has expanded to include other buttressed areas of the frontal and occipital crest and pterion. Development of the three-layered structure lags behind at fontanelles and sutures as well as lateral vault walls. A more mature (adult) cranial architecture pattern is not seen until 8 years of age. Although based on a small sample, comparison of forensic case fracture locations with mapped cranial fracture high-risk BFT impact regions across the growing juvenile cranium shows a concordance, supporting the above hypothesis.

These findings illustrate the importance of the growing juvenile cranium's bony architecture on BFT risk and outcome, add to the understanding of intrinsic variables which influence fracture from pediatric BFT, and offer avenues for possible preventive-based education regarding pediatric mortality.

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Cranial Architecture, Pediatric, Blunt Force Trauma

A129 Reexamining Differences in the Rate of Decomposition Between Previously Frozen and Never Frozen Human Remains Using the Accumulated Decomposition Score (ADS)

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After attending this presentation, attendees will have a greater understanding of how freezing affects the rate of decomposition in human remains in an outdoor setting.

This presentation will impact the forensic science community by adding to the ongoing research being conducted involving the post-thaw effect on human decomposition.

Understanding how the freezing of human remains prior to decomposition affects the rate of decay is important in a forensic setting. Bodies can be frozen due to low outside ambient temperatures or intentionally frozen before being relocated in homicide cases. There are also instances in which taphonomic research facilities need to freeze remains prior to decomposition.

There have been mixed results in studies that examine the differences in decomposition between remains that were previously frozen, then thawed, and remains that had never been frozen. A previous study by Roberts and Dabbs using the Total Body Score (TBS) method found significant differences in the rate of decomposition between previously frozen and never frozen pigs (*Sus scrofa*), with previously frozen subjects decomposing at a slower rate.[1,2] A study recently conducted using human remains and TBS found no significant difference in the rate of decomposition between previously frozen and never frozen subjects.[3] The purpose of this study is to reexamine the difference in decomposition between human remains that had been frozen, then thawed, and human remains that had never been frozen using a newly developed gross morphological method known as the ADS.[4]

For this study, a total of 20 human remains that had been left to decompose in an outdoor setting at the Forensic Anthropology Center at Texas State were used. Ten of the donations had previously been frozen and were pair-matched to remains that had never been frozen using Body Mass Index (BMI) and season of placement. All remains were placed unclothed and on the ground surface under a wire cage to prevent scavenging. An ADS was calculated for each subject at approximately 100, 300, and 500 Accumulated Degree Days (ADD).

An *F*-Test was conducted to explore the homogeneity of variance between groups. Results for both 100 and 300 ADD indicated no statistical difference in variance of ADS between groups ($F(1, 18)$=0.354, p=0.069; $F(1, 18)$=0.567, p=0.205); however, results for 500 ADD indicate there is a statistical difference in variance between groups ($F(1, 18)$=0.295, p=0.042). A two-tailed *t*-test of equal variance was run to test for significance in 100 and 300 ADD, while a two-tailed *t*-test of unequal variance was run for 500 ADD. The P value states that there is no significant difference between groups at 100, 300, and 500 ADD.

This research validates the previously documented results that there is no significant difference in the rate of decomposition between human remains that had previously been frozen, then thawed, and human remains that had never been frozen.

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Decomposition, Frozen, ADS

A130 Assessing the Utility of Total Body Score (TBS) and Accumulated Degree Days (ADD) for Estimating Postmortem Interval (PMI) in Clothed Vulture-Scavenged Human Remains

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After attending this presentation, attendees will have a better understanding of how clothing and vulture scavenging can affect the estimation of PMI.

This presentation will impact the forensic science community by demonstrating the accuracy of employing TBS and ADD to estimate PMI for clothed vulture-scavenged remains.

As taphonomic agents, animal scavengers have the potential to affect the discovery, recovery rate, and estimations of PMI in medicolegal death investigations; however, very little is known about the effects of vulture scavenging on the estimation of PMI when using the TBS to calculate the ADD.[2] The performed study observed clothed human bodies that were exposed to vulture scavenging to examine the effects of vulture scavenging on the estimation of the PMI. It is hypothesized that vulture scavenging of clothed human remains would cause a significant overestimate of the PMI when using TBS.

Five clothed individuals were dressed in white cotton T-shirts, blue denim jeans, white socks, and black tennis shoes. These clothing items are representative of "common" clothing found on deceased individuals from medicolegal investigations, including border crossers.[1] The bodies were placed in an open field for a period of approximately six weeks. Vulture scavenging was verified using motion detector cameras. After completion of the study, data related to estimating the PMI was collected by calculating ADD and determining TBS using the Megyesi et al. method.[2] Local temperature data was gathered from Weather Underground to calculate known ADD from the day of placement to the end of the study period. TBS scores of the head, torso, and limbs were subsequently assessed through photographs. The legs were scored by removing the jeans, if still on the body. The TBS was used to calculate estimated ADD using the Megyesi et al. formula.[2] Both bias and inaccuracy were examined. Bias provided information regarding whether the Megyesi et al. equation systematically under- or over-estimated the ADD/PMI for each individual.[2] The examination of inaccuracy provided information regarding how accurately the Megyesi et al. equation estimates the actual PMI that has elapsed for clothed and vulture-scavenged individuals.[2] A Pearson correlation test and Spearman's rank correlation test were used to evaluate the relationship between estimated and known ADD/PMI.

Results of the Pearson correlation test yielded a correlation coefficient of -0.47, and results of the Spearman's rank correlation test yielded a correlation coefficient of 0.50. Bias was determined to be -1,318.8, while inaccuracy was determined to be 1,318.8 These results suggest that the Megyesi et al. method greatly overestimates ADD, and therefore is not a reliable method for assessing PMI in clothed vulture-scavenged individuals.[2] There was also a low correlation between the estimated and known ADD for these five individuals. Future studies should look at developing a correction in order to properly estimate PMI in clothed, vulture-scavenged remains.

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Forensic Sciences, Forensic Anthropology, Taphonomy

A131 A Quantitative Approach to Estimating the Postmortem Interval (PMI) Using Histotaphonomy

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After attending this presentation, attendees will be better acquainted with how the specific depositions studied influence histotaphonomy.

This presentation will impact the forensic science community by presenting a model to quantify PMI.

Thirty-one *Sus scrofa* (16 juvenile and 15 fetal) fresh remains were obtained from the North Carolina State University (NCSU) swine farm in the summer, fall, winter, and spring months for two years (2013-2015). The traditional calendar for the start of each season was used as the initial day of placement. Juvenile pigs were used as a proxy for human children up to 9 years of age (35-50 pounds) and fetal pigs were used as a proxy for human neonatal remains (4-6 pounds). Each season, one juvenile pig was placed on the surface and one was buried, and one fetal pig was placed in a plastic bag and one fetal pig was wrapped in a baby blanket. All surface remains were enclosed in cages to prevent scavenging.

Histological thick sections were prepared from a femur midshaft from each of the pigs (n=31). Preparation of the histological samples followed published methods. One-millimeter-thick sections were produced using a Buehler™ IsoMet™ 1000 saw with a 15 HC diamond-edged blade. Each thick-section wafer was ground to a final thickness of 75µm-50µm on a Buehler™ variable-speed grinding unit with a diamond disc. Each thin-section was mounted on a glass slide with cover slip using SECUREMOUNT® mounting media. Histological sections were evaluated using a standard brightfield light as it produced better results than the recommended polarized light in order to assess the degree of diagenetic change and the Histological Index (HI) was employed as described by Hedges and Millard.[1]

Accumulated Degree Days (ADD) were calculated from daily maximum and minimum temperatures with data obtained from the State Climate Office of North Carolina Lake Wheeler Road Field Lab weather station located one-half mile from the open-air site. For the buried remains, ADD was calculated by summing soil temperature as minimum and maximum soil temperatures were not collected.

A destructive degradation model, which is used to model product deterioration over time, was applied using a loglogistic distribution (with the lowest Bayesian Information Criterion or BIC), which is more appropriate for decomposition studies that exhibit logistic patterns, to examine the relationship between the response or degradation measure (HI) and time variable (ADD). Statistical analysis was performed using JMP® Pro 12.1. Results reveal that there is a positive linear relationship between HI and ADD for all depositions. For the bagged fetal remains, there is a 67% probability that the HI score will be 1.5 at 2153.85 ADD, and for the blanketed fetal remains, there is a 70% probability that the HI score will be 2 at 2153.85 ADD. For the buried juvenile remains, there is a 69% probability that the HI score will be 2 at 7153.78 ADD, and for the juvenile surface remains, there is a 47% probability that the HI score will be 2.5 at 2153.85 ADD.

This project was supported by a National Institute of Justice grant.

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PMI, Histology, Taphonomy

A132 Microbiome of Forensically Important Flies (Diptera) Associated With Human Cadavers

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After attending this presentation, attendees will better understand how insects influence the tempo and mode of decomposition by introducing the fly-borne bacteria genera *Ignatzschineria* and *Wohlfahrtiimona* to a cadaver ecosystem.

This presentation will impact the forensic science community by providing information to a very poorly understood microbial ecology. Ultimately, bacterial data such as these can be incorporated to develop a model of microbial succession that can be used to more precisely determine the Postmortem Interval (PMI).

The tempo and mode of human decomposition depends on the conditions of the body at death as well as conditions of the biotic and abiotic environment.[1,2] Decomposition is greatly influenced by microbial interactions, but very little is understood of the bacterial biodiversity of a human cadaver.[3-6]

Flies (Diptera) arrive during the earliest stages of decomposition and have been recorded colonizing the corpse within minutes of death.[2] Therefore, they may be significant in establishing a cadaver-specific microbiome.[5] Understanding the ecology of flies and bacteria may allow for a more precise estimation of the PMI. Flies that colonize cadavers during decomposition may contribute to tempo and mode of decomposition by introducing bacteria to the ecosystem.[5] This study investigates the fly/cadaver microbiome by targeting specific organs of the fly that come in contact with cadavers.

Human cadavers were placed outdoors to decompose under natural conditions at the Southeast Texas Applied Forensic Science (STAFS) facility (a willed body-donation facility) at the Center for Biological Field Studies (CBFS), Sam Houston State University, Huntsville, TX. The first 40 flies visiting each cadaver were collected, identified, and dissected. The tarsi, labellum, and ovipositor were targeted to assess bacterial diversity. Sample processing, 16S recombinant DNA (rRNA) gene amplification, and Illumina® sequencing were performed following protocols benchmarked as part of the Human Microbiome Project.[7] The 16S data were processed and analyzed using the QIIME™ analysis package version 1.9.1.[8] Samples were grouped according to body site, cadaver of origin, and accumulated degree days. Special attention was paid to bacteria that had only been recorded in association with flies previously.

Site samples reveal an abundance of bacteria on the labellum, tarsi, and oocytes. Bacteria community structure seems to be most influenced by season of collection and not by fly organ (site of collection). Bacteria in the genera *Ignatzschineria* and *Wohlfahrtiimonas* were found on all three fly organs. These bacteria have been previously isolated from maggots found infesting live human tissues (myiasis) and recorded during decomposition of humans, particularly during the wet stages. These bacteria may contribute significantly to wet stages of decomposition. Additionally, there is a difference in diversity and abundance for seasons and fly sites sampled, indicating there is a unique microbiome on flies, changing across seasons and addition to years.

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Human Decomposition, Forensic Entomology, Microbiome

A133 The Effects of Hydrochloric Acid on Fleshed Porcine Ribs

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The goal of this study was to examine the effects of two concentrations of hydrochloric acid (14.50% and 31.45%) on fleshed porcine ribs and determine if environmental factors such as access to oxygen, agitation, availability of fresh acid, and ambient temperature affect the rate of dissolution.

This presentation will impact the forensic science community by providing a discussion of the importance of understanding the effects of hydrochloric acid and the factors that impact the dissolution of human remains to anticipate how the presence of corrosive chemicals will impact an investigation and to recognize the skeletal changes associated with exposure to hydrochloric acid as a taphonomic agent versus other potential postmortem agents.

Over the years, anthropologists and other forensic experts have been asked to assist with cases in which the perpetrator(s) used corrosive chemicals in an attempt to dissolve a body and other evidence of a crime. Previous studies indicate there are a number of corrosive chemicals capable of dissolving organic tissues, but hydrochloric acid is by far one of the most detrimental chemicals capable of dissolving organic tissue.[1-3] Although it is not a frequently used method, cases in which remains have either been severely damaged or completely destroyed by corrosive chemicals in an attempt to prevent the identification of the victim present unusual challenges for forensic experts.[1,4]

A total of 104 rib samples two to three inches long were placed in 100mL of acid for six days with controls in air and water. Eight samples were placed into an incubator that remained at an average temperature of 85°F throughout the experiment. Ambient and liquid temperatures were monitored and recorded in addition to observations at set intervals throughout the experiment, and the percentage of mass lost was calculated after six days.

The results from this study reveal that hydrochloric acid with a concentration of 31.45% was capable of dissolving macerated remains in less than 24 hours; however, in the case of the lower concentration of 14.50% hydrochloric acid, the acid was not capable of fully dissolving the macerated remains in less than 24 hours. When the remains were fleshed, this delayed bone dissolution by anywhere from 12 (31.45% HCl) to 24 (14.50% HCl) hours. Remains in a sealed container dissolved at a slower rate than those in an open container, indicating that access to oxygen affects the rate of dissolution. Agitating the sample quickened dissolution, and refilling the container with fresh acid delayed dissolution. In addition, it appears that temperature has the potential to impact the degree of dissolution, since samples that were placed in a heated environment were more decimated than those left at room temperature.

One thing is certain: the process of successfully dissolving a body in acid is a very complicated matter. It is important for anthropologists and other forensic experts to understand the effects of hydrochloric acid and the factors that impact the dissolution of human remains to anticipate how the presence of corrosive chemicals will impact their investigations. The effects of specific corrosive chemicals on bone need to be standardized using larger samples over longer durations in order to understand the skeletal changes associated with exposure to hydrochloric acid as a taphonomic agent versus other potential postmortem agents.[3] In addition, studying the differences between agitated and unagitated samples and the differences between refilled and unrefilled samples is important for reconstructing postmortem events and may help investigators understand the amount of effort a perpetrator(s) invested in concealing their crime. Future studies should involve a more thorough examination of the effects of temperature and involve the use of larger, completely fleshed body segments, if not complete pigs.

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Taphonomy, Hydrochloric Acid, Muriatic Acid

A134 Sex Estimation Based on Analysis of the Enamel Proteome

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After attending this presentation, attendees will better understand the use of the tooth enamel proteome in human sex estimation that could be applied to a broad range of samples.

This presentation will impact the forensic science community by offering an alternate approach to estimate sex in a statistically rigorous and scientifically quantifiable manner. This approach can be applied to deciduous teeth and to teeth from degraded skeletal remains.

Sex estimation is necessary to place skeletal remains in forensic context. The current field of forensic anthropology relies on two basic methods to estimate sex in skeletal remains: analysis of sex-specific osteological markers and detection of DNA markers specific to the X- and Y-chromosomes; however, sexually dimorphic osteological markers may be missing when not developed in sub-adult skeletons or degraded by environmental processes. Detection of DNA markers from X- and Y-chromosomes is more direct and predictive; however, the DNA backbone contains phosphodiester bonds and can easily degrade below the point at which it can be amplified. Therefore, this analysis is not often available.

Amelogenin genes on the X- and Y-chromosomes are expressed as protein in teeth and play a major role in the biosynthesis of enamel. Protein is more stable than DNA and can persist in skeletal elements well after DNA degrades. Enamel is also the most robust and archaeologically persistent tissue in the body. Detection of peptides unique to the Y-chromosome form of amelogenin protein (AMELY_ Human) in the enamel proteome is an unambiguous signal for the presence of Y-chromosome in the sample.

In this study, archaeological and modern teeth samples were processed. The enamel was demineralized in 1.2M hydrochloric acid, pH neutralized, alkylated, and treated with trypsin and mass-spectrometry compatible detergent. The resulting data collected from the mass spectrometer was analyzed using PEAKS™ analytical software.

This study measured the peptide ion signals that were specific to the AMELX_HUMAN and AMELY_HUMAN protein. After being normalized for enamel mass, the specific ion signals were plotted onto a Cartesian graph and a calibration curve was established. The curve revealed a clear male cluster, a female cluster, and a cluster of male false negatives. The range of values ranged widely across different samples, up to three orders of magnitude. Nevertheless, the male values for AMELY_HUMAN protein had a linear correlation with AMELX_HUMAN values, the R squared value was 0.88 (p >0.001) and a co-efficient of 0.17. To validate the calibration curve, this study processed 1,000-year-old archaeological deciduous teeth and was able to see clustering with male samples. Detection of the Y-chromosome AMELY_HUMAN is an unambiguous indicator of male sex; however, the lack of detectable AMELY_HUMAN protein could be either a male false negative or due to female sex. The likelihood of false negative assignment will reduce as the AMELX_HUMAN signal increases. This approach has the potential to evaluate sex estimation in a statistically rigorous and a scientifically quantifiable manner. It also has the potential to be applied to skeletons with no osteological markers for sex, such as degraded or juvenile skeletal assemblages.

Sex Estimation, Enamel, Proteomics

A135 Visualizing Commingling in the Korean War Project Assemblage

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After attending this presentation, attendees will be able to visualize the distinct form of large-scale commingling represented by the Korean War Project.

This presentation will impact the forensic science community by providing a novel manner to comprehend extensive commingling and discern patterns within data. A greater understanding of the commingling represented by identified individuals will provide important information for future identification efforts.

Commingling is the intermixing of human remains from multiple individuals and is often seen with the mixing of graves and mass fatality incidents.[1] The inherent challenges of commingling are compounded as the number of individuals and the degree of fragmentation increase; additional actions that result in disarticulation of elements further complicate forensic analysis.[2] Current anthropological methods to resolve commingling include the use of DNA and osteometric sorting.[3-6] Additionally, spatial mapping has been used to visualize the relative proximity of remains to determine associated elements.[7] At the Defense POW/MIA Accounting Agency (DPAA), it is critical to accurately resolve commingling to fulfill the mission to provide the fullest possible accounting of service members to their families and the nation.

The Korean War (1950-1953) resulted in 103,284 wounded and 36,574 killed; as of July 2017, there are approximately 7,800 unaccounted-for servicemen.[8] The Korean War Project consists of 208 boxes unilaterally turned over by the North Korean government between 1990 and 1994 and a further 124 boxes from Joint Recovery Operations (JRO) obtained between 1996 to 2005. The North Koreans provided an original location of each of the K208 boxes. The majority of the accessions originated in four areas: two POW camps (Suan, 97 boxes, and Camp 5, 21 boxes) and two battlefield losses (East Chosin, 47 boxes, and Unsan, 23 boxes). The remaining 20 boxes were purported to be from the demilitarized zone (DMZ) and North Korean coastal locations.[4] The JRO boxes originated mainly from Unsan (84 boxes) and East Chosin (28 boxes) locations; 12 boxes were from other locations. Initially, it was believed that each K208 box represented a single serviceman, but early analysis revealed that the majority of the boxes contained multiple individuals. Of the identifications made to date, the most commingled box has a Minimum Number of Individuals (MNI) of 24; the average number of individuals per box is 8.28 for the K208 accessions and 3.05 for the JRO boxes. For the assemblage as a whole, it is estimated MNI is approximately 600.[4]

Although the commingling of the Korean War Project is extensive, it is hypothesized that patterns would be present. It was expected that most of the commingling would be seen within the village/location. Where commingling occurred between K208 boxes and JRO accessions, it was anticipated that this would follow along location lines.

Identified individuals from the Korean War Project were assessed to identify commingling. Accession numbers were noted and tallies were made by purported village. A table was created and input into Circos software package to produce a circular representation of the data.[9] Analysis revealed somewhat unexpected results. For the majority of locations, commingling was higher within the village as expected; however, Suan Mining Camp and Okchi-ri revealed higher levels of commingling with boxes reported as coming from other localities (although still within the general Suan POW camp system). The commingling between the K208 boxes and the JRO accessions was seen across all regions. With the Unsan JRO boxes, anticipated commingling was seen with the Unsan K208 accessions, but also with accessions from Suan POW camp, East Chosin, Camp 5, the DMZ, and coastal regions.

The commingling observed in the Korea War Project directly affects and complicates the DPAA's mission to identify and return United States service members to their families. This presentation reports on the application of Circos software to anthropological research, demonstrating it is a useful tool to visualize large-scale commingling. Gaining a greater understanding of commingling present in the Korea War Project assemblage will aid the DPAA with further identifications.

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Korea War Project, Commingling, Circos

A136 Microbial Ecology and Soil Geochemistry in a Multi-Individual Grave

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After attending this presentation, attendees will have learned the differences in soil chemistry resulting from human decomposition. This presentation will increase attendees' knowledge of human decomposition processes, particularly as they pertain to the soil environment. This presentation will allow attendees to understand the differences in soil biology and biogeochemistry at different depth points in a human grave.

This presentation will impact the forensic science community by increasing understanding of variables related to postmortem interval estimation, burial location/site disturbance, and human skeletal DNA degradation. Therefore, this research is expected to inform models or practices relating to such areas.

Soil is a dynamic environment with multiple interacting physiochemical (pH, moisture, ion exchange capacities, redox potentials, and oxygen content) and biological processes (microbial communities).[1] These interacting features constrain cadaver decomposition within a burial environment; however, it is unknown how soil chemistry and biology differ at different depths within a human grave. Results may have significant implications for assessing skeletal degradation (or preservation) within a burial environment.

A burial containing three individuals was interred for four years prior to initiating this research. Soil samples were collected during disinterment at four depths surrounding the bodies within the grave: 0-5cm (surface; above interred individuals); 30-35cm (level with the shallowest bones); 70cm-75cm (base/floor of the grave); and 85-90cm (below the grave). Control samples were collected from undisturbed ground 5m away from the grave as well as from a control grave that had been excavated and backfilled without bodies simultaneous to interment. All soils were analyzed for microbial respiration, pH, conductivity, nitrate (NO_3^-), nitrification potential, NH_4^+, Dissolved Organic Nitrogen (DON) and Dissolved Organic Carbon (DOC), as well as extracellular enzymes (Leucine Aminopeptidase (LAP); N-Acetyl-β-Glucosaminidase (NAG); Phosphodiesterase (PDE); β-D-Cellulobiosidase (CB)). Bacterial (16S recombinant DNA (rRNA) gene) and fungal (ITS gene) abundances were quantified using quantitative Polymerase Chain Reaction (qPCR) and universal primers. Nematodes were also extracted from soil samples to assess population dynamics.

The soil at the base of the grave (70cm-75cm) exhibited significantly elevated microbial respiration rates, elevated DOC, DON, NH_4^+, soil gravimetric moisture, conductivity, and pH compared to surface and 30cm-35cm samples. Nitrification potential and NO_3^- were elevated at 30cm-35cm compared to surface or base-of-grave samples. There was a significant change in NAG enzymatic activity throughout the grave, with the greatest values measured at the base of the grave, indicative of microbial turnover. A mixed soil and adipocere sample collected from within the ribcage of an individual contained the highest activity of LAP and PDE enzymes, indicating that within heterogeneous regions of the grave, protein and cell wall degradation persists after four years.

Mean 16S gene abundances, reflective of bacterial presence, decreased with depth from 5.22×10^9 16S rRNA gene abundances at a depth of 0cm-5cm to 5.78×10^7 16S rRNA gene abundances at a depth of 85m-90cm. The presence of fungi initially increased from an average of 7.14×10^8 ITS gene abundances at 0cm-5cm to 2.56×10^9 ITS gene abundances at 70cm-75cm, then proceeded to decrease to 2.01×10^6 ITS gene abundances at 85cm-90cm. Bacterial and fungal gene abundances were highest in the mixed soil/adipocere sample described above (at ~40cm), consistent with persistent biogenic degradation of interred individuals. In addition to changes in bacterial and fungal communities with depth, nematodes demonstrated changes in community diversity, evenness, and richness with depth. Nematode community richness declined with depth, with no detectable nematodes at a depth of 85cm. Nematode evenness was variable within the grave compared with transect soils, and there was a marked shift in community composition toward bacterial and fungal feeders in the mixed soil/adipocere sample.

This study provides a characterization of soil biology and chemistry at different depths within a multi-person grave. Results provide novel insights into environmental changes within a grave that may inform our understanding of postmortem interval estimation, burial location/site disturbance, and human skeletal DNA degradation.

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Burial, Microbial Ecology, Taphonomy

A137 The Effects of Household Corrosive Acids on Restored and Non-Restored Teeth

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The goals of this presentation are to: (1) better understand how various types of household products affect restored and non-restored human dentition; and, (2) better understand how positive identification utilizing radiography is possible after exposure to certain acids and acid concentrations.

This presentation will impact the forensic science community by identifying the various changes to restored and non-restored teeth after exposure to acids, including changes to mass, crown width and length, color, and overall qualitative features. Additionally, this presentation will provide statistical analyses that identify the significant changes to dentition exposed to different household acidic solutions and concentrations.

A gap in the literature exists regarding the chemical effects of household acids at different concentrations on restored and non-restored dentition. While previous studies have focused primarily on how bone, hair, and teeth are affected by acids through quantitative and qualitative changes over 24-hour time periods, few studies utilize radiographic imaging for identification purposes subsequent to acid exposure or extend the acid exposure beyond 24 hours.[1,2] The paucity of information on acid effects is problematic in forensic contexts, as human remains are frequently exposed to acids for concealment purposes, thereby potentially complicating the identification process.

This study utilizes 105 adult human premolars (n=46) and molars (n=59) consisting of restorations composed of silver amalgam (n=62), porcelain fused-to-metal restorations (n=25), and teeth with no restorative material (n=18). All samples were collected from the Body Donation program cadavers at Boston University's Division of Graduate Medical Sciences. The household corrosive chemical agents consisted of hydrochloric acid (Clorox® Bleach Cleaner and The Works®) and sulfuric acid (Drano® Drain Opener and Watchdog® Battery Acid), in addition to one base (Biz® Detergent) as a control. The teeth were radiographed before and after exposure to the various household products to mimic antemortem and postmortem radiographs. Twenty-one teeth were placed in 20mL of each solution and were removed from the solutions throughout the experimental process after 1, 2, 4, 8, 24, 72, 120, and 264 hours. Documentation included mass, Mesiodistal (MD) and Buccolingual (BL) crown measurements, and photography. Additionally, an ordinal scoring system was developed to assess the visual changes after exposure to the acids.

The results indicate that 86 (82%) of the teeth could be positively identified by radiographs after exposure to the acids. The Works®, which is 20% concentrated hydrochloric acid, resulted in the most destruction and deteriorated 68% of the teeth (mainly the silver amalgam and non-restored dentition). The enamel, dentin, and pulp cavity of these teeth suffered liquefaction. The only dentition that was positively identified after exposure to the Works® were those of porcelain fused-to-metal. Only 28% of this sample were positively identified by radiographs. The Watchdog® Battery Acid, which is 51% concentrated sulfuric acid, was the next most-destructive solution and deteriorated 8% of the teeth (mainly silver amalgam and non-restored dentition). Most of the enamel and parts of the dentin were affected, while the pulp cavity remained intact. Further, 75% of the teeth were positively identified after exposure to the battery acid. The mass, MD, and BL measurements all decreased dramatically for the teeth that were exposed to hydrochloric and sulfuric products. Exposure to Clorox® Bleach Cleaner, which is 8.25% concentrated hydrochloric acid, and Drano® Drain Opener, which is 93.2% sulfuric acid, resulted in minimal damage to the teeth, with 100% of the teeth positively identified by radiographs after exposure. Only the outermost enamel was affected by these two solutions. The mass, MD, and BL lengths slightly decreased in size after exposure to Clorox® and Drano®. Exposure to Biz® Detergent, which is commonly used in maceration, had no effect on the teeth, with 100% positively identified by radiographs, and minimal mass, BL, and MD size decreases.

The results of this study demonstrate that various household corrosive substances can affect the morphology of teeth, and in some cases, destroy teeth, which could mask the identification of an individual; however, the restorations were minimally affected by corrosive agents and can therefore be used for positive identifications. Thus, the quantitative and qualitative data produced from this study can aid forensic cases that display evidence of acidic modification.

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Dentition, Household Acid Exposure, Identity Concealment

A138 Differential Recovery Rates of Skeletonized Human Remains

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After attending this presentation, attendees will better understand the differential recovery rates of human skeletonized remains encountered in outdoor forensic contexts with and without the assistance of forensic anthropologists.

This presentation will impact the forensic science community by demonstrating the utility of incorporating forensic anthropologists into the recovery phase of outdoor forensic scenes.

The field of forensic anthropology has historically been a lab-based discipline, often with law enforcement dropping off a box of bones at the forensic anthropologist's laboratory. The anthropologist's sole role was typically restricted to estimating a biological profile for identification. The field remained largely lab-based until the 1990s, when the rise of forensic archaeology and forensic taphonomy changed the role of forensic anthropologists. Dirkmaat and colleagues suggest that these two developments, along with DNA Polymerase Chain Reaction (PCR), the *Daubert* ruling, forensic trauma analysis, and improvement in quantitative methods, have significantly altered the trajectory of forensic anthropology within the past three decades.[1] Dirkmaat et al. said it best when they noted that "today less than at any point in the past, forensic anthropologists cannot be considered forensic 'sidekicks,' who may be useful advisors when forensic pathologists or law enforcement step into an unusual case or situation, but the most appropriate, and most logical first choice professionals in cases involving all manner of outdoor crime scenes and commingled or severely altered human remains."[1] This study hypothesized that the higher rate of evidence recovery by these experts conducted in a timely fashion surpasses those efforts conducted of law enforcement and coroner/medical examiner officials and, ultimately, significantly benefits the investigation. The primary purpose of this research was to compare recovery rates of skeletonized human remains from outdoor contexts between forensic anthropologists, who are extensively trained in human osteology and in recognizing taphonomically altered bone, versus recovery rates by law enforcement and coroners/medical examiners. The ultimate goal of this research is to demonstrate the value of incorporating forensic anthropologists into the recovery phase, especially in outdoor scenes where remains are often heavily modified by taphonomic agents.

A total of 56 cases were identified from 40 years of forensic casework contained within the Mercyhurst Forensic Case Database and conducted by Dr. Dennis Dirkmaat. Only cases that were completely skeletonized and recovered from outdoor contexts were included in this research. Approximately 60% (n=33) of these cases were recovered by forensic anthropologists, Dr. Dirkmaat and his Mercyhurst Forensic Scene Recovery Team, while the remaining 40% (n=23) of cases were recovered by law enforcement or medical examiner/coroner's offices. The skeletal inventory for each of these cases were charted in homunculi diagrams of the anterior view of the body, then layered to generate density maps using a Geographic Information System (GIS). Each skeletal element was coded as present or absent and bones that were present were further coded into fragmentary versus complete elements to compare recovery rates. For the cases recovered by forensic anthropologists, the recovery rates of burial versus surface-scattered remains were also compared with consideration given to the Postmortem Interval (PMI).

When skeletal elements were analyzed by type or skeletal region (for example, long bones, hands/feet, ribs, vertebrae, skull, upper limb, and lower limb), the forensic anthropology team recovered a higher percentage of total elements than the police/coroner/medical examiner, with the exception of the lower limb and rib fragments. Of note, the forensic anthropology team recovered a higher percentage of the crania, mandible, and pelvis (innominates and sacrum), which is especially important given that these elements are the most informative for estimating ancestry, sex, and age from human skeletal remains. For the forensic anthropology cases, higher recovery rates were found for all regions and bone type in surface-scattered remains, with the exceptions of the small bones of the hands and feet, when compared to recoveries from burial features. The results are not surprising given that the average known PMI for burial cases was 9.3 years, while for surface scatters, the average PMI was only 3.2 years. In conclusion, the incorporation of the forensic anthropologists into the recovery phase of outdoor scenes results in a higher recovery rate of nearly all skeletal elements/regions and especially those areas of the body that are most informative for estimation of the biological profile.

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Forensic Archaeology, Recovery, Skeletal Remains

A139 Fracture Pattern Comparison Between Pig and Human Crania Cremated on an Open-Air Pyre

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After attending this presentation, attendees will understand the differences in fracture patterns between pig and human cranial cremains. Attendees will understand the need for future research on human analogues before further cremation studies using pigs are undertaken.

This presentation will impact the forensic science community by addressing a current problem in cremation studies using pigs (*sus scrofa domesticus*) as human analogues. It will assist future researchers in establishing suitable proxies for humans in cremation studies and hopefully promote increased usage of human remains in such experimental studies. These future studies will allow forensic scientists to understand the process of cremation to a fuller extent and help with forensic cases involving cremated or partially cremated remains.

While fully cremated remains are not frequently encountered by forensic specialists, it is highly common for burnt/partially cremated remains to be discovered as part of a crime investigation; when these fragmentary remains are discovered, they can be extremely difficult to interpret and analyze. Cremation (even partial cremation) can obscure a great deal of information that the forensic scientist is familiar with using for purposes of identification, such as estimation of Postmortem Interval (PMI) or Minimum Number of Individuals (MNI) present. For primarily ethical reasons, animal analogues are frequently used in cremation experiments wherein researchers attempt to develop methods for better interpreting cremated human remains.[1,2] The literature on cremations has yet to establish whether animals are appropriate proxies for humans, and whether the patterns observed on animals can be accurately and comprehensively applied to humans. To address the absence of research in this area, the present study attempted to identify fracture pattern differences between pig and human crania through a comparison of experimentally created pig cremains and archaeological human cremains.

This study used both archaeological cremains from Roman Britain and pig crania cremated on an open-air pyre, constructed to replicate a Roman pyre.[3,4] Two pig crania were cremated together over a 4-hour period, reaching temperatures exceeding 800ºC, therefore reaching the fusion stage of cremation.[5] The resulting cremains were left to cool and were photographed and weighed.

The archaeological human cremains and pig cremains were analyzed using the same protocol. The photos were uploaded to ImageJ, where the surface area of the fragments was measured along with areas of patina and delamination. Additionally, fractures were counted for each fragment. A total of 248 specimens of pigs and adult humans were analyzed. When measured, each feature (patina, delamination, fracture count) was divided by the total surface area, creating a ratio that allowed fragment size to not affect the results.

Ultimately, it was observed that the pig and human cranial bones were affected differently by the open-air pyre cremation. Pig cranial cremains demonstrated more delamination per mm^2 than the human cranial cremains. Human cranial cremains exhibited more patina than pig cranial cremains. A greater number of fractures were present in human cranial cremains than pig cranial cremains.

The results observed in this small study suggest that pig crania should not be used in experimental cremation studies as a human analogue. Further studies should be conducted to test the similarities of human and pig post-cranial cremains, as well as the usefulness of other animals as human analogues. The researchers suggest that future studies on cremation processes be conducted on human subjects in crematorium or taphonomic facility settings.

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Cremation, Fracture Analysis, Thermal Alteration

A140 Postmortem Interval (PMI) Estimation Using Bone Lipidomics

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After attending this presentation, attendees will understand the potential of lipids in marrow cavities and epiphyses for predicting PMI of skeletal remains.

This presentation will impact the forensic science community by providing preliminary data on lipidomic techniques for estimating PMI in skeletonized human remains that are more precise and accurate than gross observations of taphonomic processes.

Common methods for estimating PMI of skeletonized remains are qualitative observations of taphonomic processes, such as bone weathering, staining, sun bleaching, and cortical exfoliation.[1,2] Histological techniques are available but not well validated due to variation caused by microbes.[3,4] Bone degradation garners interest as a means of predicting time since death, yet controlled experimental studies on human remains are limited in the scientific literature.

Successful lipid fraction of bone has been reported in forensic toxicology and archaeology research, but few studies describe the capacity of this methodology for estimating PMI.[5] It has been demonstrated that glycerophospholipids and very long chain fatty acids in the membranes of skeletal muscle tissue accurately predict long-term PMIs up to 30,000 accumulated degree days.[6,7] Lipids are abundant biomolecules with powerful predictive capacity for PMI.

Bone marrow is a rich source of lipid mediators. N-acyl amino acids are one class of lipid mediator monitored in bone, and they also modulate bone metabolism in the case of oleoyl serine. A pilot study was conducted to investigate the diversity of N-acyl amino acids in human bone. Bone marrow was extracted from trabecular bone in a dry human calcaneus with a PMI of approximately seven years. Utilizing a high-resolution electrospray ionization lipidomics analytical platform, 76 potential N-acyl amino acids were identified in the bone marrow sample.[8] The structural identities of these lipids are undergoing validation utilizing tandem mass spectrometry. The structural identities of palmitoyl and oleoyl serine were validated via generation of the MS^2 product ion for serine (<1ppm mass error). The number of lipids identified in the extracted bone marrow support the findings of toxicological studies reporting lipid extraction in skeletonized remains with a PMI of up to five years.[9] The results of this pilot study are being used to evaluate the postmortem metabolism of lipid mediators on an autopsy sample of sternal rib ends and vertebral bodies from cases with known medical histories and PMI.

This study expands previous research on skeletal muscle metabolites to bone, providing a quantitative method for estimating PMI of skeletonized remains. Successful bone lipid extraction after an interval of seven years provides preliminary data that will be used to validate a methodology for predicting PMI of severely decomposed, mummified, and skeletal remains with greater precision and accuracy.

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Postmortem Interval, Forensic Anthropology, Lipidomics

A141 A Method for the 3D Restoration of Fragmented Human Crania for Trauma Analysis

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After attending this presentation, attendees will better understand how 3D technologies can be used to reconstruct and assess traumatic skull injuries in forensic cases.

This presentation will impact the forensic science community by providing a method for visualizing trauma in skeletal material that is fragile and heavily fragmented. This presentation will demonstrate how 3D scanning and polygon manipulation can be used to assist in skeletal analysis and to communicate anthropological findings in situations ranging from the classroom to the courtroom.

When a forensic anthropologist is confronted with heavily fragmented crania, the task of reconstructing the skull for trauma analysis can be difficult. Skulls that are fragmented due to trauma are weakened and susceptible to further damage when reassembled using traditional methods of reconstruction (i.e., glue). The recent prevalence of 3D scanning technology in forensic anthropology laboratories allows for greater access to digital technology to solve problems related to the condition and fragility of remains. The goal of this project is to develop a method to digitally restore a cranium that can be 3D printed or exported as an interactive digital model.

Two crania were selected that had experienced catastrophic blunt force trauma and were heavily fragmented due to fractures and separated sutures. A FARO® Design ScanArm high-definition laser scanner was used to obtain surface scans of the individual fragments. A point cloud and polygon editing software (Geomagic® Wrap) was used to reassemble the individual fragments of each skull. Individual fragments were merged using points of commonality along suture lines and unique points along fracture lines. Both crania exhibited areas of missing bone that were not reconstructed to maintain the accuracy of the models to the source material. Selected cranial measurements that were not affected by the fragmentation were used to test the accuracy of the restored crania. The skull measurements were obtained with calipers and the models were measured using the measurement tool with the 3D software. Each of the 3D cranial measurements tested were within an acceptable error threshold (<1mm) of the caliper measurements. The results revealed a small amount of variation in measurement; however, this may be due inaccuracies and inconsistencies in measuring the crania within the editing software as opposed to traditional calipers.

Both of the test crania had experienced isolated traumatic events that only became apparent after the original manual reconstructions were completed. If this method of 3D digital restoration had been available at the time these cases were being analyzed, the restorations could have been completed with minimal disturbance to the fragments, and a 3D print could have been made. The 3D scans allowed for viewing of the model in a uniform and neutral color for distraction-free texture visualization. The locations, patterns, and behavior of the fractures were easily seen on the bone and permitted the reconstruction of the traumatic events. This technology is a valuable tool that could be used for teaching future generations of forensic anthropologists about trauma analysis as well as demonstrating complex traumatic events in the courtroom.

3D Scanning, Trauma Analysis, Blunt Force Trauma

A142 Influence of a 1.5 Tesla (1.5T) Magnetic Resonance Imaging (MRI) on Ferromagnetic Microtraces

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The goal of this presentation is to determine the influence of the external magnetic field of a 1.5T MRI scanner on the location of ferromagnetic microtraces smaller than 0.5mm in and on bone and in soft tissue.

This presentation will impact the forensic science community by explaining how Postmortem Magnetic Resonance Imaging (PMMR) is increasingly used in forensic medical examinations of crime victims.[1] The findings of this study have important implications for the application of PMMR. During autopsy, perforating injuries are measured and the depth of stab wounds are probed. Injuries extending into the bone are excised and examined for the presence of foreign materials. These microtraces are used to reconstruct a crime event, and microtraces may be linked to a specific (class of) object or weapon.[2] The presence of the external magnetic field in PMMR may disturb evidence when human remains contain ferromagnetic microtraces. Knives consisting of steel or contaminated with steel are known to leave ferromagnetic microtraces smaller than 0.5mm in soft tissue, on bone, or even embedded in bone.[2] Translocation may lead to mistakes in reconstructing a crime event. After attending this presentation, attendees will understand the influence the external magnetic field of a 1.5T MRI can have on microtraces.

Hypothesis: Translocation of microtraces smaller than 0.5mm was not expected. Calculations made before the experiments indicated that the magnitude of forces due to friction and gravity would be greater than the magnetic force on the microtraces.

Methods: Pig feet were used to simulate human remains. The samples were cut with a saw that was contaminated with ferromagnetic steel. The samples contained ferromagnetic particles from 0.2mm to 0.7mm. Computed Tomography (CT) and micro-CT were used to determine the coordinates of the position of the ferromagnetic microtraces before and after exposing the specimens to a 1.5T MRI scanner. Paired *t*-tests were used to statistically assess the translocation. Translocation due to the external magnetic field of MRI and translocation due to transportation were determined separately to preclude the possibility that translocation was caused by transportation instead of the external magnetic field of MRI.

Results: No significant translocation of the ferromagnetic microtraces (with a size of 0.2mm to 0.7mm) due to the external magnetic field of a 1.5T MRI scanner was detected (*p*-values: X 0.31, Y 0.11, Z 0.11). Transport of the pig feet between institutions caused a significant movement in one dimension (X-axis) that was detected (*p*-values: X 0.03, Y 0.41, Z 0.88); however, this movement was not relevant for forensic examination.

Conclusion: The use of 1.5T MRI in forensic medical examinations is applicable in the presence of small ferromagnetic fragments no larger than 0.7mm.

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Magnetic Resonance Imaging, Microtraces, Translocation

A143 The Incorporation of 3D Photogrammetry and Geophysics in the Recovery of a Mass Grave: Six Years of Experiential Learning

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The goal of this presentation is to provide crime scene investigators and physical anthropologists exposure to innovative field techniques (incorporating 3D photographic survey and geophysical methods) to increase success in identifying and recovering mass graves. In addition to a review of the methods and processes involved, this presentation will provide tips and tricks for minimizing costs and for working in remote locations.

This presentation will impact the forensic science community by explicating cost-effective and efficient field methods for surveying and documenting mass grave recovery projects. The methods in this presentation have been field tested over several years in an applied setting. 3D survey can be prohibitive in the field for several reasons, but the photogrammetry methods presented here are fast, inexpensive, and simple enough so they do not require specialized field personnel. The geophysical procedures, while still requiring trained personnel, are developed in order to reduce time spent excavating "false positives" and for dealing with difficult subsurface matrices.

The forensic community has seen a steady increase in the use of technology as a means of quickly and accurately documenting crime scenes over the past several decades; however, for the recovery of human remains, timing, conditions, and budgetary constraints often preclude successful use of cutting-edge technology in applied field situations. This research focuses on the application of advanced photographic and geophysical surveys as a practical means to bolster documentation accuracy while still keeping costs to a minimum and reducing the need for specialized personnel onsite. Advances in 3D photogrammetry applications and field methods for geophysics are adapted specifically for the recovery of human remains, and are shown to be particularly useful for mass grave recovery.

This presentation details field- and lab-based processing methodologies that can be implemented for the recovery of human remains in a variety of environments and at various project scales. For this research, experimental graves were surveyed using Ground Penetrating Radar (GPR), soil resistivity, and 3D photogrammetry. Most notably, a planned mass grave was surveyed with geophysical techniques periodically over the course of six and one-half years. Established in May of 2010, the irregular-shaped pit is just under four meters across, and geophysical data were collected beginning two years post-interment, so the usual indicators of clandestine graves on the surface were generally obliterated through natural weathering processes.

The area was additionally surveyed for 3D reconstruction both before and after excavation. 3D reconstruction and photogrammetry methods detailed in this presentation utilize commercial-grade cameras that are inexpensive and do not require specialized photographic skills. Point-and-shoot cameras easily and successfully capture sufficient details for digital measurement of less than 1mm. Even higher levels of precision can be reached with cameras that have macro photography settings. Available commercial software and free and open source software allow quick and easy processing and measuring of digital 3D models. Photorealistic textures are generated that add additional precision and measurable values, such as color and texture. Additionally, the 3D reconstructions provide very compelling digital products that can be explored in Virtual Reality (VR) or Augmented Reality (AR) environments.

The photogrammetry procedures detailed in this presentation can be learned easily, quickly, and, in most cases, do not require any additional equipment or personnel; however, the methods do provide a level of detail and accuracy that is equivalent or surpasses what can be obtained through ground-based laser scanning.

Experimental stations are located at the outdoor Anthropological Research Facility at the University of Tennessee, allowing for the unique examination of human remains in a variety of contexts representative of the natural environment. In addition to providing loci for experimental stations, the facility provides opportunities for student engagement and the training of law enforcement officers, thus providing further insight into how well methodologies can be adopted by these communities and informing pedagogical approaches. This presentation will briefly discuss ways that the aforementioned procedures can be incorporated in an experiential learning environment.

3D Photogrammetry, Geophysics, Excavation

A144 An Analysis of Computerized Tomography (CT) -Derived Bone Density Values and Volumetrics for Age and Sex Estimation From the Proximal Femur

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After attending this presentation, attendees will understand how the application of Hounsfield Unit (HU) values and the 3D volume of the proximal femurs can assist in the estimation of age and sex. Attendees will also gain a deeper understanding of how 3D models can contribute to forensic identification, even in fragmentary remains.

This presentation will impact the forensic science community by: (1) providing the results of a methodology that will assist in the forensic analysis of age and sex estimation; (2) enhancing existing research of osteological materials by adding data of a living modern American sample of the proximal femur *in situ*; and, (3) providing insight into the age-related decline of bone density and explaining how the HU values can be used as a predictor for age with an average accuracy of ±10 years. The total volume of the proximal femur can be used with a 94% accuracy in estimating an individual's sex.

The confirmation of identification for an unknown individual is a critical part of forensic practice. The age and sex of an unknown individual are key components in establishing a biological profile. Previous studies utilizing the 2D projections of the proximal femur derived from Dual X-ray Absorptiometry (DXA) estimated sex with a 90%–91.7% accuracy; however, the usefulness of bone density as an age estimate was not examined.[1] The purpose of this study was to determine if the average HU value of the proximal femur derived from CT can be a reliable method to estimate age in an unknown individual. Additionally, this study examined the effectiveness of volumetric quantification of 3D CT-derived proximal femurs to capture sexual dimorphism for the estimation of sex.

The University of South Florida (USF) Health Department of Radiology maintains an anonymized research database of all patient image data collected from 2009 to the present. A series of 200 femurs were acquired from abdominal-pelvic CT scans from living patients. The dataset consisted of 100 males and 100 females. Ages ranged from 18-90 years and were distributed evenly between the sexes. Any individuals with documented pathologies, implants, or clear surgical intervention were excluded from the study. Models of the proximal femur were initially isolated in a modeling software package using a threshold of 226–1,577. The medullary cavity and spongey bone of the femoral head were filled in via hand segmentation. The region of interest was limited from the femoral head to just inferior to the greater trochanter, cut 90° to the long axis of the femur.

For the statistical analysis, a paired *t*-test was run comparing the left and right proximal hip average HU (p=0.2) and volume (mm^3) (p=0.57). No statistical differences between sides were found. A stepwise analysis was used to construct a linear regression model for age estimation. A stepwise analysis was also used to perform discriminant function analysis, which was used to assign sex. All measurements with a $P < 0.05$ were considered statistically significant.

The results of the stepwise linear regression model revealed only the HU average was significant in model creation for age estimation ($p < 0.001$), with an R^2 of 0.63. The resulting model can be used as a predictor for age with an average accuracy of ±10 years. The results for the discriminant function analysis for sex estimation determined the proximal volume was the only relevant predictor with an overall 94% grouping cross-validated accuracy. Females were placed with a 100% accuracy. Males were placed with an 88% accuracy.

With the increasingly widespread use of postmortem CT (PMCT), there is an opportunity to utilize 3D volumetrics and bone density as tools for quick identification. The use of HU values as an age estimator should be limited to freshly deceased or fragmentary remains with soft tissue; however, the use of the proximal femur as a sex estimator can be utilized in any setting from the living to dry bone. The accuracy of the sex estimation found in this study reinforces the distinct dimorphism between sexes while also providing forensic practitioners more tools of analyses. The use of proximal femoral volume as a sex estimator need not be limited to CT scans but can also be used in laser surface scanning or photogrammetry.

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CT, Age Estimation, Sex Estimation

A145 Using Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR) to Measure Bone Degradation and Crystallinity for Forensic Reconstruction

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After attending this presentation, attendees will understand: (1) how FTIR-ATR can be applied to determine bone diagenesis; and, (2) how the degree of diagenesis can aid in depositional and taphonomic reconstructions, particularly in a forensic context.

This presentation will impact the forensic science community by offering a new method for weathering estimation and reconstruction of diagenetic processes. This method for assessing the crystallinity of bone may provide a more accurate representation of diagenesis than established methods of weathering, such as the Behrensmeyer weathering estimation, Postmortem Interval (PMI), and Accumulated Degree Days (ADD), and can be used in recent contexts.

Recrystallization is directly correlated to the loss of the organic components of bone.[2-4,7,11] The relationship between bone degradation and recrystallization is important for forensic science because it focuses on the process of degradation and removes chronological time as a variable. Due to the current qualitative methods for assessing the degree of diagenesis, analyzing human remains can only provide a very broad understanding of depositional context. Quantitative approaches must be established to create a system of standardization. The goals are to provide quantitative data to support weathering observations by analyzing diagenesis and to determine if different stresses provide the same or different degrees of crystallinity and diagenesis.

Studies previously addressing the question of bone crystallinity have used the FTIR-Potassium Bromide (KBr) method, but often do not report the relationship between crystallinity and diagenetic stress. The Behrensmeyer technique also does not discern between alterations different depositional and taphonomic environments.[2] Using the degree of recrystallization to assess degree of diagenesis will provide a more detailed and empirical objective scale to be used in conjunction with other methods to reconstruct the full depositional history of any organic bone remains, which is important in forensic science reconstruction.

FTIR-ATR was used to examine the degree of recrystallization and the uptake of specific carbonates from various weathering processes to try to understand bone degradation better. In turn, the goal was to use this information to better categorize degrees of weathering and processing. The use of KBr pellets has been the historical method for FTIR preparation for anthropologists, but the limitations have often been documented in literature.[1,7-10] Benefits of KBr pellets include its ability to use smaller sample sizes, its ability to make quantitative analysis easier, and its ability to not produce interfering wavelength bands; however, serious drawbacks include extensive preparation, changes that may occur during the pressing process, possible water absorption, and possible "solid ion exchange with inorganic compounds."[6]

Preliminary data, analyzed through Multivariate Analysis of Variance (MANOVA) tests, demonstrate that there is no statistically significant relationship between the processing groups and their crystallinity index (Carbon/Phosphate ratio and Splitting Factor ratio). This suggests that the crystallinity index is not a useful measurement or factor for reconstruction, but FTIR-ATR is still a useful tool for measuring the crystallinity index.

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Forensic Anthropology, Diagenesis, Forensic Reconstruction

A146 The Biomechanics and Composition of Juvenile Pig Ribs in Relation to the Postmortem Interval in a Subaerial Environment

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After attending this presentation, attendees will better understand how change in the chemical composition of juvenile porcine rib bone correlates with change in the material properties of the same bone over the early postmortem period in a subaerial exposure environment.

This presentation will impact the forensic science community by providing experimental data that informs how changes in bone composition over the postmortem period affect its material properties and the biomechanics of bone response to localized loading, and thus informs regarding the factors that affect fracture timing determination.

When performing trauma analysis, forensic anthropologists are often faced with the challenge of determining fracture timing based on bone characteristics, a critical step in ascertaining the circumstances of death. Bone fracture characteristics used to differentiate between peri-mortem and postmortem fractures are discussed in terms of occurring in "fresh" bone versus "dry" bone, and yet it is still unclear how long into the postmortem period bone can retain its fresh fracture characteristics, particularly in juveniles. Rib fractures represent one of most common occurrences among the pediatric population being involved in a number of fatal circumstances; however, little experimental data have been generated about rib fracturing in juveniles.

This study uses a juvenile porcine model to examine the relationship between the length of the postmortem interval in subaerial exposure environments and the association between changes in: (1) the biomechanical properties of juvenile ribs in response to localized load; and, (2) the chemical composition of juvenile bone.

Twenty-seven suckling piglets (*Sus scrofa*) aged approximately between two and eight weeks were purchased from a local supplier. Their rib cages were manually disarticulated and defleshed, and each rib cage was divided into right and left halves. Three half rib cages (*sub-sample*=45 ribs) were placed on top of a soil-filled container, and a total of 16 soil-filled containers (*total sample*=720 ribs) were studied over a period of 12 months. The first subsample was removed from the container after one week, the subsequent three subsamples were removed one week apart, the following two subsamples two weeks apart, and the remaining ten subsamples four weeks apart, in a total of 16 trials. Sub-samples were randomly assigned to each container. Twenty to 27 ribs were selected from each subsample/trial and fractured experimentally using a three-point bending test. This was performed to quantify mean peak stress and mean tissue modulus for each subsample. Nine of the experimentally fractured ribs in each trial were sectioned to quantify water, collagen, and mineral content through a process of sequential controlled heating. Each sample was weighed four times throughout the process, and the water, collagen, and mineral contents were expressed as a percentage of total weight.

Peak stress and tissue modulus increase up to the fourth month, then level off. Results from the analysis of bone composition revealed a noticeable increase, then a significant decrease ($p \leq 0.01$) in collagen within the first month, and a noticeable decrease, then a significant increase ($p \leq 0.001$) in mineral within the same period of time. Both collagen and mineral were relatively unchanged from week four on. Results did not exhibit a clear trend for changes in water content throughout the postmortem period. No clear relationship was observed between changes in composition and material properties of bone over the postmortem period in this experiment. These results may be affected by residual desiccated soft tissue or environmental factors, such as variation in temperature and humidity.

Fracture Timing, Collagen, Bone Stiffness

A147 Mitochondrial DNA and Stable Isotope Analyses as Molecular and Chemical Signatures of Identity of Victims: A Combined Approach for Provenancing Unknown Skeletal Remains From India

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The goal of this presentation is to scrutinize the combined forensic suitability of molecular and chemical methods of identification for human skeletal remains recovered from an abandoned well situated beneath a religious structure in a North Indian suburb of Ajnala in an attempt to satisfy the major objective of establishing their identity and origin status (local or non-local to the site) from the mitochondrial DNA (mtDNA) and stable isotope analyses of the recovered teeth and bones.

This presentation will impact the forensic science community by illustrating how the combined approach of application of mtDNA and stable isotope analyses has unraveled the scientific truth about these non-scientifically excavated human skeletal remains from a well by amateur archaeologists. The forensic anthropological findings have been corroborated by the molecular and chemical analyses of the remains, which will help in arriving at a number of valid conclusions regarding the forensic identity of these remains.

Unknown human remains have been reported from a number of disaster or multiple burial sites of forensic or bioarchaeological nature, whose identity establishment is of prime concern for a forensic anthropologist. The bones and teeth serve as anthropological, molecular, and chemical signatures of identity of the deceased as their physical structures, molecular signatures, and chemical compositions retain a number of specific individual imprints. Teeth are considered storehouses of invaluable biological, physical, and chemical information, and are crucially required for various forensic anthropological purposes, such as provenance or the establishment of biological identity (age, sex, ethnicity, occupation, migration pattern), exposure to pollutants or taphonomic/traumatic insults, ancient DNA analyses, estimation of dietary practices and subsistence patterns, paleopathology, etc. The small size and mineralized status of human teeth imparts them with a resistance against all types of decompositions and degradations, thus making them suitable for answering a wide range of forensic questions. In addition to certain gender-, race-, and age-dependent variations in human teeth, certain culture- and occupation-specific features also play an important role in the identification process. The disruptive and challenging life events (illness, malnutrition, starvation), especially during childhood, are impregnated in tooth enamel to reveal such severe and stressful life events.

The small size and high copy number of mtDNA ensures a higher probability of intact DNA isolation from severely degraded human remains found in forensic situations, which can be further amplified and studied using DNA markers from nuclear DNA as well. The human molars (mandibular), petrous bone, and femur are comparatively better and thus preferred sources for mtDNA extractions, compared to other traditionally used DNA sources.1 The stable isotope analyses of unknown human skeletal remains have provided novel approaches for their provenancing in forensic or bioarchaeological contexts.2 The isotopic ratios of bones and teeth have proven to be fingerprints of locality, migration patterns, subsistence activities, and dietary habits, such as milk or meat consumption by the victims, infant breast-feeding practices and weaning patterns, prehistoric diets, etc.3-4 Stable isotopes of bone and teeth vary significantly among different geographical regions due to diverse cultural dietary patterns and environmental factors (such as aridity, elevation, and distance from large bodies of water). Thousands of bones and teeth were recovered from an unused well situated beneath a religious structure at Ajnala in April 2014, after being identified in a book.5 These remains were provided to this study in 2015 to establish their biological identity.

Two hundred-fifty-six mandibular molars were randomly selected for mtDNA extraction, quantification, and amplification, and whole genome sequencing was performed for few samples. An equal number of teeth and some bones (femur, humerus, vertebrae, clavicle, metatarsals, and metacarpals) were processed chemically for stable isotope analysis of carbon, hydrogen, oxygen, nitrogen, and strontium. The tooth sample was completely settled in silicone rubber, a dermal cut-off wheel and bids were used to cut the root and make a hole through it, respectively. Approximately 50mg of powder was taken and processed for DNA extraction and analysis at 108 variable positions to genotype the haplogroup-defining motifs of the entire mtDNA. The ancient DNA sequences and haplogroups were compared with individuals of geographical areas to which the victims supposedly belonged. Samples were prepared for stable isotope analysis according to standardized techniques and the isotopic compositions were estimated from different dental and skeletal remains using Isotope Ratio Mass Spectrometer (IRMS), and were then compared with the thresholds available for comparisons for different periods and regions.2

Keeping in mind the enormous diversity of the Indian population, the ethnicity and geographic area of specific mtDNA diagnostic markers (N=104) were used to assign the haplogroups, and it was observed that out of 49 samples, the haplogroup pattern of 35 samples were similar to North Indian populations, whereas 14 samples exhibited more similarity with present-day Pakistani and Iranian populations. The frequency distribution pattern of all haplogroups across Indian, Central Asian, and Middle East populations assigned the affinity of the Ajnala skeletal remains to the Punjab and Uttar Pradesh states of India. These findings were contrary to the written versions and anthropological observations regarding these remains.5,6 The stable isotopic interpretations and the whole genome studies further corroborated these observations, thus unravelling the scientific truth concerning these remains.

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A148 Evidence Recorded in Fingernails: Carbon, Oxygen, and Strontium Isotopes Reveal Diet and Travel Histories

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After attending this presentation, attendees will understand the basic principles of how carbon ($\delta^{13}C$), hydrogen ($\delta^{2}H$), oxygen ($\delta^{18}O$), and strontium isotope measurements of fingernails can be used in reconstructing recent diets and geographical movements of individuals.

This presentation will impact the forensic science community by increasing the competence of the law enforcement community on an analytical tool that can be used in travel history reconstructions of unidentified decedents or for homeland security-related matters.

Stable isotope analysis of keratin tissues (hair and fingernails) have been used to reconstruct diet and geographic location across multiple disciplines. The foods that humans consume are reflected in the $\delta^{13}C$ and nitrogen ($\delta^{15}N$) isotope signatures and are indicative of the plant and animal protein contributions of their diet. Beverages that are incorporated from tap water and water in foods contributes to the $\delta^{18}O$ and $\delta^{2}H$ signals that reflect particular geographic locations. Strontium isotope ratios (Sr^{87}/Sr^{86}) are incorporated from environmental sources and reflect geologic structures that waters interact with, food is grown in, or are input from environmental dust. These stable isotope combinations form unique signatures that can allow researchers to understand an individual's diet and geographic movement and can play vital roles in forensic and homeland security matters.

This study focuses on the stable isotope patterns of fingernail clippings from volunteer residents of the Salt Lake City (SLC), UT, region who traveled for varying times to locations outside the United States (Central and South America) before returning home. It was hypothesized that the dietary isotopes ($\delta^{13}C$ and $\delta^{15}N$) would not significantly differ as they moved, because both the United States and South and Central America rely heavily on corn inputs of food sources (animal feed, corn sugars, or syrups in processed foods); however, it was hypothesized that the $\delta^{18}O$, $\delta^{2}H$, and Sr^{87}/Sr^{86} would vary with their travels, as precipitation isotope values ($\delta^{18}O$ and $\delta^{2}H$) and the geologic formations (strontium) significantly differ between their region of travel and SLC.

As hypothesized, the $\delta^{13}C$ and $\delta^{15}N$ values did not change over the course of the study, nor did the diets of the volunteers. $\delta^{18}O$ and $\delta^{2}H$ values displayed patterns that were consistent with their reported travel history. It was discovered that $\delta^{18}O$ and $\delta^{2}H$ values were similar to SLC residents when the volunteers resided in the area and, as they moved to their new locations, there was a departure from these values. The incorporation of the new isotope signature happened rapidly, but it took several months before that portion of fingernail was clipped. These findings were consistent with those of $\delta^{18}O$ and $\delta^{2}H$ incorporation patterns in human scalp hair. In contrast, Sr^{87}/Sr^{86} of the fingernail clippings provided surprising results. It was initially hypothesized that the patterns would be consistent with those of $\delta^{18}O$ and $\delta^{2}H$; however, the Sr^{87}/Sr^{86} were indicative of the volunteers' location when they clipped their fingernails. For instance, one volunteer who went to Ecuador initially displayed $\delta^{18}O$ and $\delta^{2}H$ values that were consistent with SLC, but the Sr^{87}/Sr^{86} values did not match those of SLC residents. This difference was attributed to how the isotopes are incorporated into the keratin protein of the fingernail. $\delta^{18}O$ and $\delta^{2}H$ are incorporated during the protein formation and, once the protein is formed, the isotope signature does not change. Thus, as the fingernail grows from the nail plate forward, the signal does not change. $\delta^{18}O$ and $\delta^{2}H$ of clipped fingernails also represent a time from four to five months earlier, based on average human fingernail growth rates. Findings suggest that Sr^{87}/Sr^{86} are incorporated into the fingernail keratin through environmental or bathing waters and reflect an individual's most recent location.

As one of the first multi-isotope studies on human fingernails, this study reports on new travel-related isotope signals that can be obtained from $\delta^{18}O$, $\delta^{2}H$, and Sr^{87}/Sr^{86} measurements. A major conclusion of this study is that human fingernails (as well as other keratin tissues) can provide information on an individual's diet and travel history. This study has also allowed us a more detailed look at isotope incorporation into fingernails and how these values can vary among individuals.

Stable Isotopes, Human Fingernails, Travel History

A149 Taphonomic Effects on Isotope Ratios of Human Hair

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After attending this presentation, attendees will understand how isotope ratios of human hair can change throughout decomposition and continued outdoor exposure over a one-year period.

This presentation will impact the forensic science community by providing information regarding the first study conducted on human hair samples to evaluate the effects of taphonomic processes on isotope ratios of postmortem hair. The forensics community uses isotope ratios of postmortem hair for predicting diet and geographic origins of unknown human remains, and this presentation will provide an assessment of geospatial modeling accuracy using isotope ratios from individuals with known residence histories.

Isotope analyses of human remains have been conducted with growing frequency in forensic anthropology. Isotope ratios of elements, such as carbon, nitrogen, hydrogen, oxygen, and strontium from hair have provided information regarding individual diet and geographic origin. The isotope ratios of hair can be projected across landscapes using geospatial models (i.e., isoscapes). As hair grows at a known rate, these data provide a serial recording of diet and travel history for the weeks and months prior to death; however, these isoscapes have been developed using clean modern samples from salons and do not reflect the typical condition of hair found in forensic contexts. A critical knowledge gap has been whether exposure to the outdoor environment and postmortem decompositional fluids have an effect upon isotope ratios in hair. If the isotope ratios of human hair are to be used reliably in forensic casework, it is essential to understand the effects of taphonomy on isotope signatures, and whether these signatures persist and reflect those seen during life.

This research was conducted at the Anthropology Research Facility in Knoxville, TN, an outdoor laboratory for the study of human decomposition. Body donors with known residence histories (n=8) plus two additional donors at the Forensic Anthropology Research Facility in San Marcos, TX, were enrolled in the study, and hair samples were collected over a one-year period of environmental exposure. Two facilities with different climatic and soil environments were used to understand the ways in which temperature, humidity, geographic location, and underlying lithology affect isotope ratios of hair. Donors were placed in both surface and burial conditions to determine the effects of placement condition on isotope ratios of hair. Carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), hydrogen ($\delta^{2}H$), oxygen ($\delta^{18}O$), and strontium ($^{87}Sr/^{86}Sr$) isotope ratios from the human hair samples were analyzed. Environmental samples of soil and precipitation were also collected and analyzed. Carbon and nitrogen isotope ratios in human hair underwent little change over time and were more consistent than hydrogen, oxygen, and strontium isotope ratios, all of which were impacted by the depositional environment.

Oxygen and strontium ratios were compared to isoscape models to create geographic predictions for last residence for each of the donors. Oxygen isotope ratios predicted regions within 250 kilometers of the last known residence of the donors 81% of the time, and strontium predicted regions within 250 kilometers of last known residence 100% of the time. Combined, oxygen and strontium predictions fell within 250 kilometers of last known residence 80% of the time. This study revealed that isotope ratios of human hair can change postmortem and are influenced by placement location (i.e., Tennessee or Texas facility), surface or burial placement, and duration of exposure. Isotope ratios of human hair, despite these postmortem changes, still provided valuable information regarding geographic travel history. As these postmortem changes become better understood, they could be incorporated into predictive models to improve model accuracy. Nonetheless, when the isotope ratios of human hair are analyzed postmortem, it is important to consider how taphonomic processes impact isotope ratios.

Isotope Analysis, Human Decomposition, Isotope Ratios of Human Hair

A150 Trace Isotope Analysis of Dental Enamel for Micro Regional Geographic Attribution of Human Remains in Virginia

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The goal of this presentation is to highlight the utility of trace element isotope ratios in bulk and surface dental enamel as a means of enhancing the biological profiles of unidentified remains.

This presentation will impact the forensic science community by providing a method of geographic attribution of birthplace and recent residence of unidentified remains using the presence of certain trace chemical isotope ratios in bulk and surface dental enamel.

Ultimately, this methodology could contribute to the compilation of a database of chemical isotope ratios by locality within Virginia, which could be applied to unidentified remains in long-term storage in the many Offices of the Chief Medical Examiner (OCME) for the Commonwealth of Virginia.

There are currently ca. 165 cases of unidentified human remains in long-term storage in the four Virginia OCME regions. In many of these cases, the remains are severely decomposed or skeletal and do not match any reports of missing persons, and fingerprints, DNA profiles, dental conditions, and facial approximations of these individuals have generated no leads to identification. Dental enamel preserves well despite decomposition and holds promise for the expansion of new forensic identification methods. The bulk enamel composition ceases to change appreciably after a certain age and is thus indicative of an individual's birthplace (or early childhood residence), while the surface enamel composition continues to change due to surface ion exchange and diffusion and is indicative an individual's recent residence.[1]

This study examined the bulk and surface enamel samples of ca. 80 teeth from 44 donors obtained from the Mission of Mercy Project and the Remote Area Medical Project in Wise County and Emporia, VA, respectively, with the Virginia Commonwealth University Institutional Review Board approval. Patients scheduled for an extraction were approached by a researcher and asked for written informed consent; if consent was provided, the patient was asked questions concerning age, sex, city and state of birth, and city and state of residence. Individuals in this study who were born and currently resided in Virginia had a donor age range of 22 to 77 years. The average donor age was 49.5 years with an average donor age of 49.7 years for 24 females and an average donor age of 48.3 years for 20 males. These locations were selected to expand on a pool of 74 samples from 52 donors previously compiled, predominately from central and northern Virginia.[2] After extraction, samples were disinfected in 10% neutral buffered formalin for two weeks. Surface enamel was etched directly using a trace metal-free nitric acid and glycerin solution, while bulk enamel was dissolved in trace metal-free nitric acid after the enamel was ground into a fine powder using a mortar and pestle. Samples were analyzed for the following trace elements via Inductively Coupled Plasma/Mass Spectrometry (ICP/MS): ^{7}Li, ^{11}B, ^{25,26}Mg, ^{27}Al, ^{52}Cr 3^{+}, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{58,60}Ni, ^{63,65}Cu, ^{64,66,68}Zn, ^{69,71}Ga, ^{78}Se, ^{86,87,88}Sr, ^{204,206,207,208}Pb, and ^{209}Bi. Principal component analysis and discriminant function analysis were performed to examine multivariate relationships among samples and determine which trace elements drive compositional differences among the samples and the locality groups.

The results from a one-way Analysis of Similarities (ANOSIM) yielded significant differences ($p \leq 0.00420$) between bulk and surface enamel ratios of individuals by geographic locality. Tooth characteristics (e.g., restorations, caries, debris, discoloration, chipping, cracking, occlusal wear, etc.) did not significantly affect the isotope ratios of either the surface or the bulk enamel. This suggests that geographic determinations based on the isotope ratios of bulk and surface enamel are most likely neither influenced nor obscured by the tooth type and/or tooth characteristics. Significant correlations were found for bulk enamel ratios with the geographic location of an individual's birthplace and surface enamel ratios with recent residence.

In conclusion, trace isotope ratios are useful in determining where individuals were born and currently reside, adding information to the biological profiles of unidentified remains and generating additional leads to the identification of these individuals.

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Human Identification, Trace Isotope Ratios, Dental Enamel

A151 The Application of Isotope Analysis to Aid in the Investigation of Unidentified Remains From a Suspected World War II Mass Grave

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After attending this presentation, attendees will have an understanding of how isotope analysis is employed in efforts to differentiate the origin and context of unidentified remains, specifically suspected combatants from World War II.

This presentation will impact the forensic science community by providing researchers with a greater appreciation of how scientific endeavors may be utilized to both reinforce and undermine prevailing historical and ideological narratives concerning perpetrators and victims in the Balkans and Central Europe. Additionally, this study demonstrates that complex, multidisciplinary investigations collating all data are needed to resolve the origin and identification of such cases and that reliance on single strands of evidence, be it community narratives, associated artifacts, or isotope analysis, does not provide a threshold of evidence to define origins beyond reasonable doubt.

The massive scale of military casualties incurred in World War II made it impossible to recover many of the combatants' material remains, identified and repatriated; however, discovery of graves and remains of combatants and civilians from the conflict has continued, with the lack of information concerning these individuals bringing ambiguity regarding their identity, origin, and fate.

This study concerns a group of suspected World War II combatants (*Minimum Number of Individuals (MNI)*=26) found buried in a mass grave in Bosnia and Herzegovina. The remains were recovered as part of ongoing national investigations into the missing from the 1992-1995 conflict; however, associated artifact evidence suggests the remains are of World War II German Army origin. One objective of the national investigations led by Bosnia and Herzegovina's state prosecutor's office and the Missing Person's Institute is to differentiate remains from previous conflicts to define jurisdictional authority and subsequent activities needed to determine if cases are relevant to prosecutions, as well as identify and/or lay remains to rest.

After Yugoslavia was invaded by the German Army and their allies in 1941, Axis-affiliated military and paramilitary organizations and resistance forces fought for control of the region. Present-day Bosnia and Herzegovina was primarily encompassed within the newly formed Independent State of Croatia, and the Axis military units present were principally composed of German, Austrian, Italian, Croatian, and Bosniak Muslim combatants. That local ethnic groups allied themselves with the different antagonists has been cited as a source of animosity fueling the 1992-1995 conflicts.[1-5] Unidentified remains and unknown combatants leave questions unanswered and may be utilized to reinforce local narratives of victimization inflicted by one's neighbors.[2,3,5] Additionally, records of foreign war dead initiate actions of war graves commissions, such as the German *Volksbund,* which has recovered 40,000 European war dead a year since 1991. Determining the origin of remains may prompt nations of foreign combatants to address their own history and culpability, and, while Germany has spent many years fostering a reconciliatory narrative, other countries, such as Austria and Italy, have been less active in acknowledging the role their combatants played in the World War II Balkan conflict.

The complex history of the conflict, lack of records, limited investigative data, and the potential for unidentified remains to be from a number of armies, nationalities, regions, and conflicts provides scope for scientific investigation to contribute data that may aid in including or excluding remains from active investigations.

To investigate the origins of these individuals, permissions were provided for cases to be examined, and samples of molar teeth (n=24) were subjected to lead, strontium, oxygen, and carbon isotope analysis. Isotopes reflect the local geology and environment of an individual's place of origin. Isotopic analysis indicated there was variation in individual's $^{87}Sr/^{86}Sr$ (0.7080-0.7115) and $^{206}Pb/^{204}Pb$ (17.8-18.4) ratios. There was also variably in the oxygen results (-3.99‰ to -8.16‰), reflecting potential geographical origins from central and southern Europe — though the majority of individuals fall within a range indicative of the southern Mediterranean. It was hoped that the isotope analysis would provide a definitive regional identity for these combatants; however, the results do not provide enough definition to suggest that the remains can be claimed by any nation with a stake in their identity. Some remains can be defined as not of Balkan origin, adding scientific evidence for Bosnia and Herzegovina authorities that the assemblage dates to World War II and is not relevant to investigations of later conflicts. Refining the Balkan isotope baseline data, additional carbon dating and isotope sampling of World War II-era individuals should aid these investigative efforts.

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Isotopes, Warfare, The Balkans

A152 Spatial Distributions of Carbon and Nitrogen Isotope Ratios in Human Hair From Central and Southern Mexico — Another Indication of Geographical Origin

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The goal of this study is to: (1) explore the relationship between carbon ($\partial^{13}C$) and nitrogen ($\partial^{15}N$) isotopes in human hair from traditionally high immigrant sending areas in central and southern Mexico; and, (2) explore the relationship between carbon and nitrogen values in these hair samples and published values from 14 other countries.[1-3]

This presentation will impact the forensic science community by presenting data on regional dietary differences among Mexican states and between Mexico and other countries and will assess the utility of this relationship to act as a predictor of region of origin.

The use of carbon and nitrogen isotopes in human hair, bone, and fingernails has demonstrated the ability to assist in provenance analysis for modern populations.[1-3] In this study, the influence of regional diet to predict region of origin in modern Mexican populations by analyzing carbon and nitrogen isotopes from hair was tested.

The Mexican hair samples consist of 78 samples collected from 12 contiguous states in Central and Southern Mexico, plus 10 samples collected in southeastern North Carolina. Carbon and nitrogen isotopes were measured in duplicate for samples for all locations using an elemental analyzer connected to a Finnigan–Mat Mass Spectrometer at the University of Utah SIRFER laboratory.

All data for $\partial^{13}C$ values are presented on the Vienna Peedee Belemnite (VPDB) scale, and those for $\partial^{15}N$ are presented on the Atmospheric Air (AIR) scale. Analytical precision for $\partial^{13}C$ and $\partial^{15}N$ was 0.1‰ and 0.2‰, respectively. Stable isotope ratios are reported using the standard ∂ notation. Hair values spanned a range from 8.1‰ to 10.5‰ and -23.5‰ to -19.‰ for $\partial^{15}N$ and $\partial^{13}C$ for Mexican locations, respectively. Hair values from North Carolina spanned a range from 8.4‰ to 8.9‰ for $\partial^{15}N$ and -19.2‰ to -16.6‰ for $\partial^{13}C$. A one-way Analysis of Variance (ANOVA) was conducted to determine if the mean values for $\partial^{13}C$ and $\partial^{15}N$ were different for the 12 regions in Mexico and between the Mexican regions and North Carolina. Means for measured $\partial^{13}C$ and $\partial^{15}N$ were statistically significantly different $F\ (7.87)= 9.040$ ($P < 0.05$); $F\ (7.87)=9.04$ ($P=0.01$). A Tukey Kramer post hoc analysis revealed that $\partial^{15}N$ values between the Mexican samples were not statistically significantly different, but values between Veracruz and North Carolina were statistically significantly different. Carbon data demonstrated the most promising differentiation. A Tukey Kramer post hoc test for carbon demonstrated within country regional differences with means for Chiapas, Mexico, the Federal District, and Oaxaca all statistically significantly different than Morelos, and Chiapas, Mexico, Oaxaca, and the Federal District all significantly different than North Carolina. The mean value for all combined Mexican states was 9.3‰ for $\partial^{15}N$ and -16.1‰ for $\partial^{13}C$. When mean values for $\partial^{13}C$ for Mexico were compared to published mean values for other countries, there was no statistically significant difference with Brazil or Costa Rica, but there were statistically significant differences between Mexico and 12 other countries.

This study demonstrates both the limitations and potential of carbon and nitrogen isotopes in human hair to distinguish provenance. Carbon isotopes clearly demonstrate regional differences both within Mexico and between Mexico and other locations, albeit more broadly than water isotopes; however, the potential demonstrated here for additional indications of region of origin suggest that carbon and nitrogen isotopes in human hair from Mexico are a reasonable investment in the analysis of region of origin.

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Provenance, Hair, Isotope

A153 The Application of Multi-Isotope Analysis to Assist with Georeferencing Unidentified Decedents

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After attending this presentation, attendees will better understand how heavy and stable isotope analysis assists with the estimation of geographic origin and migration of unidentified decedents.

This presentation will impact the forensic science community by providing and highlighting results from several ongoing cold cases, successful identifications, and known Floridian individuals' isotope values for the Tampa Bay region.

Forensic anthropologists employ methods involving skeletal analysis to estimate age, sex, ancestry, trauma, and pathology assessment for the unidentified decedent. By collaborating with other scientists in the field of geochemistry, the biochemistry of human tissue (i.e., bone, enamel, hair, nails) elucidates evidence of geoprofiling for the unidentified decedent. This research has two objectives: (1) to demonstrate how isotope analysis can aid with the solvability of unsolved cases of the unidentified; and, (2) how Florida has a particularly unique demographic situation in terms of a transient population.

An additional goal of this study was to collect more modern enamel and bone samples for known individuals from Florida and throughout the United States to improve reference sample sizes for forensic investigations. Stable oxygen isotopes ($\delta^{18}O$) and carbon ($\delta^{13}C$) were also used to tighten constraints when estimating geographic origins for the unidentified decedent.

Strontium ($^{87/86}Sr$) and lead isotopes ($^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$) were analyzed from the teeth and bone of the unidentified individuals to infer either geographic origins or recent area of residence. Strontium is absorbed into the individual's biology via the food chain. The ultimate source is the local bedrock, soil, and water. Long-distance importation of food and water may affect the individual's strontium isotope ratios and may not be entirely controlled by the local environment. Similarly, the lead isotopic compositions of individuals can be linked back to local environmental sources of lead from the soil or anthropogenic sources, such as leaded gasoline. Over the individual's lifetime, lead is absorbed through dust inhalation at very minimal amounts.

In contrast to strontium, it is believed that lead is more directly absorbed through soil and/or dust ingestion or inhalation and, therefore, is not likely to be affected by importation of foods from other regions. Exposure to lead from lead paint or lead pipes can affect the lead isotopic signal in human teeth and bones. A comparison of the isotope ratios of the enamel and bone can yield a pattern of migration of when the individual moved from one geographic region to another region throughout their lifetime. The tooth enamel formation during early childhood provides a biochemical profile of the individual's early years, while bone will remodel over a course of seven to ten years continually as a person ages. The bone offers a biochemical profile of the individual's last years of life. Human teeth and bone are an archive of long-term strontium and lead exposure.

Analyses of oxygen, carbon, lead, and strontium isotopes were completed on selected current and cold cases, which included a sample of 53 individuals (n=27 males, n=21 females, and n=5 unknown) from 2010 to 2017. Additionally, donated teeth (n=20) with known demographic information were utilized to generate an increased reference sample for Florida. Other known United States samples are included as a comparison for the heavy isotope values.

A trend in the isotope results also reveals that a number of cases in the Tampa Bay region are foreigners or out-of-state individuals. After the isotope analysis was performed for these cases, investigators were able to redirect their investigations and reach out to other agencies with the new information concerning the case. Two interesting non-local cases, which were positively identified, will be highlighted in this presentation. Moving forward, collaborative and multidisciplinary research across the board will enhance the solvability and success in current and cold casework.

Isotope Analysis, Cold Cases, Geoprofiling

A154 Multi-Isotope Approaches for Region of Origin Predictions of Unidentified Border Crossers (UBCs) From South Texas

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The goal of this presentation is to highlight the value of using a multi-isotope approach for predicting the region of origin of UBCs found deceased in the United States. Attendees will gain a better understanding of how stable isotopes can be used as a geolocation tool for unidentified remains of suspected foreign nationals from Latin America.

This presentation will impact the forensic science community by presenting novel approaches that can assist with unidentified remains cases, especially for foreign nationals.

Since 1999, the remains of more than 6,000 UBCs have been recovered along the United States-Mexico border. While increases in border security during the past two decades have reduced the number of border crossings, this period has been marked by a sharp increase in UBC deaths as migrants are forced to travel through more inhospitable areas, often dying due to heat-related illness. In 2011, the number of deceased UBCs in south Texas exceeded that of Arizona, with the majority of the deceased representing Central American nationals. Most of the deaths in Texas occur near the Falfurrias checkpoint in Brooks County, approximately 80 miles north of the border. This high volume of deaths has resulted in a massive identification challenge. Many barriers to identification exist, including lack of documentation, decomposition of remains, lack of antemortem records, and difficulties obtaining family reference DNA samples. In 2013, forensic anthropologists began exhuming unidentified remains interred in a cemetery in Brooks County for analysis and identification. Several field seasons of exhumations have recovered hundreds of UBC remains, which are currently being analyzed and identified through the efforts of Texas State University and its collaborators.

Stable isotope analysis can aid in identification efforts of unknown decedents, including UBCs from south Texas. Carbon and nitrogen isotope ratios of human tissues reflect cultural dietary practices, which can be used to screen remains as being of likely United States vs. Latin American origin. Oxygen and strontium isotope ratios of bone and tooth enamel reflect the geographic origins of an individual based on the source inputs of water and food, respectively. Further, these isotopes can be used to predict possible regions of origin using geospatial mapping tools (e.g., isoscapes). Although baseline data are incomplete for many regions within Latin America, a multi-isotope approach can provide numerous lines of evidence for predicting region of origin of UBCs.

This study presents isotope results for 30 UBC bone-tooth pair samples from Brooks County, provided by Texas State University. Bones and teeth were prepared for mass spectrometry, including carbon and nitrogen isotope analysis of bone collagen, carbon and oxygen isotope analysis of bone bioapatite and tooth enamel, and strontium isotope analysis of tooth enamel.

The mean bone collagen $\delta^{13}C$ value is –13.3‰ (±2.4‰, 1 Standard Deviation (SD); *range*=10.2‰) and the mean $\delta^{15}N$ value is +9.1‰ (±1.1‰, 1 SD; *range*=4.4‰). The mean bone bioapatite $\delta^{13}C$ value is –7.8‰ (±2.6‰, 1 SD; *range*=9.3‰). Tooth enamel, which reflects childhood diet, exhibits an even greater range of variation, with a mean $\delta^{13}C$ value of –4.9‰ (±3.0‰, 1 SD; *range*=13‰). The mean $\delta^{18}O$ value is –6.7‰ (±1.2‰, 1 SD; *range*=5.0‰) for bone bioapatite and –5.7‰ for tooth enamel bioapatite (±1.3‰, 1SD; *range*=6.0‰). The mean $^{87}Sr/^{86}Sr$ ratio for enamel is 0.70705 (±0.00126, 1SD; *range*=0.00617).

Carbon isotope ratios of bone collagen as well as bone bioapatite and tooth enamel are consistent with previous data on UBCs from south Texas, which reveal a high dietary contribution from C_4 resources such as corn. The high degree of variation in $\delta^{13}C$ values is primarily influenced by three individuals with very low $\delta^{13}C$ values for their bone collagen and bone and tooth bioapatite. Prediction maps based on oxygen and strontium isotope ratios further suggest these individuals may be of United States origin as opposed to Latin American nationals. The other 27 individuals have $\delta^{13}C$ values consistent with a Latin American origin. Measured $\delta^{15}N$ values reveal low variation, consistent with heavy consumption of terrestrial herbivore meat by all individuals. For the majority of the 30 UBC samples, isotope prediction maps based on oxygen and strontium isotopes are consistent with a Latin American origin and often include areas within Mexico as well as portions of Central America. Future research will seek to further narrow possible regions of origin.

Stable Isotope Analysis, Undocumented Border Crosser, Forensic Anthropology

B1 A Comparative Analysis of Globally Used Forensic Semen Detection Methods and Their Differing Applications

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The goal of this presentation is to inform attendees of the effectiveness of differing applications of commonly utilized forensic semen detection methods employed globally.

This presentation will provide the forensic science community with insight into the wide variety of different methods of applying semen identification tests, such as the acid phosphatase test for screening and extraction methods for spermatozoa staining, that are utilized in forensic laboratories worldwide.

The ability to detect and identify the presence of seminal fluid can be crucial to an investigation. Methods used to detect seminal fluid are common across the world; however, the application of these methods varies greatly on a global level. For example, while some laboratories favor the Alternate Light Source (ALS) for locating potential stains, other laboratories favor the Acid Phosphatase (AP) press test, as the ALS has been shown to be unreliable and not specific. The AP press test is common in Europe; however, there is a lack of research into how this method is applied, with varying approaches taken in different laboratories. Further, for the confirmatory identification of semen, the most commonly utilized method is microscopic visualization of spermatozoa; however, it is essential to extract potential stains from substrates, such as fabric, in order to perform microscopic examination. A number of different methods of extraction have been identified but have not been compared to date. Therefore, the goal of this study was to first investigate the differing methods of applying AP for presumptive testing and, second, to compare and contrast the differing extraction methods from a variety of substrates.

Following Institutional Review Board (IRB) approval, semen was collected with informed consent from healthy volunteers. For each experiment, 100µL of semen was deposited onto white cotton fabric in dilutions from neat to 1:1,000. The differing AP application methods examined included: (1) wetting both the substrate and test paper versus just the test paper, including examination of the potential transfer of spermatozoa to the test paper; (2) application of the two AP reagents (sodium α-naphthyl phosphate and Fast Blue B) as a combined formulation versus sequential application; (3) application of the AP reagents directly onto the substrate versus indirect application using test paper; and, finally, (4) evaluation of the reliability of the two-minute cutoff for the AP reaction. The differing extraction methods examined include five methods used globally and were performed on five substrates (cotton, denim, polyester, wool, and cotton swabs) for each of five dilutions, neat to 1:1,000. To evaluate the extracted stains, the extract was seeded on a microscope slide, stained with Christmas tree stain, examined under a microscope, and scored.

The results of this study investigating the differing AP application methods revealed: (1) wetting both the test paper and the substrate greatly enhances the positive AP reactions obtained, particularly through the dilutions, with no observable transfer of spermatozoa to the test papers; (2) the sequential application of the AP reagents provides stronger and faster color reactions; (3) similarly, the direct application of the reagents onto the substrate provides greater sensitivity and faster/stronger reactions, when compared to the indirect application onto the test paper; and, finally, (4) the two-minute cutoff for the AP reaction was insufficient time for positive reactions to be observed with dilutions above 1:5,000. The results of this study investigating the differing extraction methods demonstrated one particular method — utilizing two stacked Eppendorf tubes — to extract the most spermatozoa from four of the five substrates, across all dilutions.

This research highlights the potential impact on results obtained when using differing semen screening and identification tests identified across the globe. These results emphasize the need for more research into the varying application methods used. It is crucial for forensic laboratories to be made aware of the variety of these methods and the potential to improve the effectiveness and sensitivity of their testing.

Acid Phosphatase, Extraction, Christmas Tree Stain

B2 A Powder-Free Approach to Extracting DNA From Environmentally Challenged Bone Samples

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After attending this presentation, attendees will appreciate some of the advantages and disadvantages of altering bone processing, tissue digestion, and DNA extraction methods in an attempt to increase DNA quantity and quality for more successful Short Tandem Repeat (STR) typing from environmentally challenged bone samples. This presentation proposes a method to efficiently extract DNA from bone samples without the need to pulverize the bone tissue into a fine powder prior to digestion.

This presentation will impact the forensic science community by providing insight into the benefits and limitations of demineralizing bone tissue without the traditional requirement for powdering. This sample-processing approach is coupled with a commercial DNA extraction kit performed with and without automation for downstream STR analysis.

Bone samples are often encountered in missing persons' cases and mass disasters for human identification purposes. In order to generate a high-quality STR profile, it is necessary to extract a sufficient quantity of high-quality DNA (>100pg) while simultaneously eliminating unwanted contaminants and Polymerase Chain Reaction (PCR) inhibitors. Traditional bone DNA extraction methods rely on cutting and crushing bone into a fine powder and a long demineralization step coupled with an organic or silica-based DNA purification method. An alternate approach may be to use a whole bone digestion buffer in combination with a commercial DNA extraction kit to avoid powdering bone samples prior to purification. This study compared the efficacy of both methods with and without automation.

Bone samples (N=6) from human cadavers exposed to various insults (fire, decomposition, sun exposure, burial, and embalming) were prepared, extracted, and STR-typed in triplicate. Compared to each of the other methods, the whole bone digestion buffer paired with the automated extraction consistently resulted in a significantly lower DNA yield per milligram of powdered bone (p<0.05). Regardless of the chemistry used, significantly less DNA (p<0.01) was obtained when the extraction process was automated, compared to performed manually. No statistical difference was observed in the average DNA yields when the commercial kit was performed with or without the whole bone digestion step. Although DNA yields were higher using the complete demineralization protocol, STR success rates and overall profile quality were comparable across all the manual methods tested.

The whole bone system is an alternative method to conventional complete demineralization methods which are more laborious and time consuming (~30hrs versus ~16hrs), require powdering of bone tissue, pose a higher risk of contamination, and consume the entire sample. Another advantage of the whole bone system is the provision for a second DNA extraction from the same sample. These multiple extracts may be used to perform additional analyses or combined and concentrated to increase the amount of input DNA for improved results.

Overall, this research has shown that eliminating the need to powder bone tissue can simplify the DNA extraction process without significantly reducing downstream STR success.

Bone, DNA Extraction, Short Tandem Repeats

B3 Qualitative and Quantitative Analysis of Minute Levels of Saliva in Expirated Blood

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The goal of this presentation is to inform attendees of the use of the solution SALIgAE®, which has quantitative and qualitative capabilities to detect minute levels of saliva in expirated bloodstains.

This presentation will impact the forensic science community by providing insight into the difficulties of distinguishing between expirated and impact spatter bloodstains and by informing attendees how the use of SALIgAE® can help minimize those difficulties.

A major challenge with Bloodstain Pattern Analysis (BPA) is the differentiation of expirated and impact blood spatter stains. Currently, the only accepted method of classifying an expirated bloodstain pattern is the presence of air bubbles in the stain; however, this is a very subjective approach and leaves the assessment open to much scrutiny. As expirated blood is expelled from the mouth, it is logical to assume there would be trace amounts of saliva mixed with the resultant blood droplets. To date, a method has not yet been identified that is adequately sensitive or specific enough to detect these minute traces of saliva in expirated bloodstains. SALIgAE® is a somewhat new reagent for saliva identification. This is a clear solution that, when mixed with saliva, turns a transparent yellow color, displaying a positive reaction for saliva. It is reportedly more accurate than other saliva detection tests, with a sensitivity of 1:1,000. Additionally, it has both qualitative and quantitative analyses that are rapid and easy to perform. The goal of this research, therefore, is to investigate the ability of SALIgAE® to accurately detect the presence of, and quantity of, trace amounts of saliva within expirated bloodstain patterns.

Following Institutional Review Board (IRB) approval and informed consent, venous blood was collected from a volunteer into sterile EDTA Vacutainer® tubes. Saliva was also collected from the volunteer into a sterile Falcon® tube. The sensitivity of the SALIgAE® solution was first tested with dilutions of saliva:ddH_20 and saliva:venous blood, ranging from 1:1 to 1:1,000,000. Expirated bloodstains were created by placing 1mL of blood into the volunteer's mouth for 30 seconds, followed by the volunteer coughing the blood onto white butcher paper placed approximately 12 inches in front (vertical) and below the volunteer's mouth (horizontal). Two stains were created on separate days when the volunteer had not drunk liquids or consumed any food for at least one hour. Individual blood spots/stains were chosen to be tested from both the vertical and horizontal planes. Each sample to be tested was incubated in ddH_20 for 30 minutes before addition to the SALIgAE® solution. Both a visual color change test and a spectrophotometric reading using the Nanodrop™ OneC Ultraviolet/Visible (UV-Vis) spectrophotometer were used to determine the color change and absorption of salivary amylase. The visual test exhibited a color change from clear to yellow with the presence of saliva, while the UV-Vis test exhibited a change in the absorbance of the saliva and SALIgAE® solution, allowing for the acquisition of quantitative data.

The sensitivity of SALIgAE® with dilutions of saliva:ddH_20 produced the required positive color change up to 1:1,000, as previously reported, with absorbance values ranging from 1.28 to 10.0, and salivary amylase concentrations ranging from 0.12µg/mL to 1.33µg/mL. The sensitivity with dilutions of saliva:venous blood produced the same required positive color change up to 1:1,000; however, the red color of the blood made a distinct color change difficult to observe. The first expirated stain created a large dispersed pattern on the vertical plane, with less abundant but larger drops on the horizontal plane. Forty-two individual stains were selected for testing, each ranging between 1mm and 5mm in diameter. Of the 42 stains, 8 produced a positive color change, with absorbance values ranging from 0.08 to 1.05 and salivary amylase concentrations ranging 0.00µg/mL to 0.09µg/mL. The second expirated stain created a visually similar pattern to the first, and 42 stains were again selected for testing. Of the 42 stains, 9 produced a positive color change, with absorbance values ranging from 0.05 to 1.46 and salivary amylase concentrations ranging from 0.00µg/mL to 0.15µg/mL. While the concentrations obtained from the expirated stains were low, a visible color change did occur.

The results of this study highlight the ability of SALIgAE® to detect the presence of minute quantities of saliva when mixed with blood. This reveals the SALIgAE® method to be an ideal candidate for the differentiation of expirated spatter and impact spatter, thereby overcoming a significant challenge facing bloodstain pattern analysts. This information will ultimately help guide forensic professionals to develop more effective strategies in their processing and analysis techniques.

Saliva, Expirated Blood, SALIgAE®

B4 An Assessment of the PreCR® Repair Mix as a Viable Repair Method for Soil-Degraded DNA

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After attending this presentation, attendees will understand some principles of DNA degradation, how the PreCR® repair mix functions, and the ability to repair DNA samples that have been exposed to degradation by substances in soil, such as humic acid.

This presentation will impact the forensic science community by providing an analysis approach for DNA profiling of buried bloodstained evidence that optimizes the recovery of a DNA profile in order to help provide investigative leads.

The ability to detect a DNA profile from a piece of evidence, such as clothing, can be crucial in a forensic investigation. In many cases, evidence is found outdoors, possibly buried. Buried evidence containing human DNA, such as bloodstains, can pose a challenge for profiling due to accelerated degradation processes in soil by its many components. Humic Acid (HA) in soil has been determined to be a contributor of accelerated DNA degradation, as well as a Polymerase Chain Reaction (PCR) inhibitor. Therefore, the goal of this study was to determine if the PreCR® repair mix can be used for the repair of soil-degraded DNA, potentially aiding with casework by establishing or providing potential suspect and/or victim leads in violent crimes in which evidence containing human blood was buried.

Following informed consent, venous blood was obtained from a single-source volunteer in sterile Vacutainer® EDTA tubes. Then, 50µL and 150µL aliquots of blood were deposited in quadruplicate on 2"x2" polyester swatches, for a total of 30 stained fabric swatches, and dried beneath a hood for 24 hours. Once dried, 18 of the stained swatches and 6 non-stained swatches were buried 1.5" deep in a ten-gallon fish tank containing approximately 3" of soil. Six remaining bloodstained swatches were left in petri dishes on the bench. Over the course of four weeks, four stained samples from each blood amount, in addition to two non-stained swatches that were used as negative controls, and two bloodstained swatches from the table were extracted with the QIAmp DNA Mini® Kit, quantified with the Investigator Quantiplex® Kit, then amplified and profiled using the Powerplex Fusion® Kit with GeneMarker® HID software to assess the level of degradation in the donor's original profile. At the time of each extraction, soil moisture, pH, and room temperature and humidity were monitored in an attempt to keep as many variables as possible to a minimum. Portions of the degraded samples from each blood-stained group that had already been quantified were repaired using the PreCR® repair mix prior to Fusion® amplification. Generated profiles aided in determining the extent of repair. The results of each extraction group were evaluated based on the extent of allelic dropout/recovery and corresponding peak height ratios.

After one week buried in soil, the samples with 50µL of blood showed little (one to two alleles) to no allelic activity. Every PreCR®-treated sample for week one showed a recovery of two or more alleles out of a total of 43 alleles in the known person's profile. Weeks two through four with 50µL of blood did not show any DNA prior to being treated with PreCR® for any sample and zero out of nine bloodstained samples did not show any improvement when treated with PreCR®. Studies are currently being conducted following the above-mentioned protocol with larger quantities of blood to examine repair possibilities when larger amounts of DNA are present, in addition to assessing direct HA inhibition with various amounts of HA to study the direct effect of HA on DNA analysis and repair.

This research has highlighted the challenges that come with severely degraded human DNA, but also brings attention to the possibility of repairing soil-degraded DNA from blood-stained fabric using commercially available methods, such as PreCR®. Additional studies will need to be conducted prior to any laboratory casework application. It is hoped that extensions of this work will enable the forensic science community the ability to explore DNA repair.

DNA Repair, STR Analysis, Humic Acid

B5 An Evaluation of a Novel Massively Parallel Sequencing (MPS) 74-Microhaplotype Panel for Biogeographic Ancestry Prediction

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After attending this presentation, attendees will understand the potential of using novel Microhaplotype (MH) markers in addition to Single Nucleotide Polymorphism (SNP) panels for predicting the biogeographic ancestry of individuals.

This presentation will impact the forensic science community by illustrating the usefulness of an MPS MH panel to enhance the prediction of individuals' ancestry and to extrapolate valuable information on the minor contributor's profile in mixed DNA samples.

MHs are loci of two or more SNPs within a short distance of each other (<300 nucleotides) with three or more allelic combinations. The standard Sanger sequencing method does not enable determining the cis/trans relationship between SNP alleles within the same amplicon (i.e., haplotype) whereas MPS methods, which allow specific clonal sequencing of each individual DNA strand, can distinguish the parental haplotypes at a given locus. The key features of MH markers, such as small amplicon size, multi-allelic nature, absence of stutter peaks, and lower mutation rate than conventional autosomal and sexual linked Short Tandem Repeats (STRs), also make them an additional candidate marker for forensic identification, mixture deconvolution, and ancestry prediction purposes.[1] The most common method for ancestry prediction utilized is based on SNPs, which are of little use in mixtures given their bi-allelic nature. The goal of this project was to evaluate whether MPS-based MH analysis could be effective in the prediction of the biogeographic ancestry of individuals in cases of mixed samples where the use of SNPs is more challenging.

A set of 91 European-Americans (EAs), 54 African Americans (AAs), 87 Hispanics (HISs), and 51 Native Americans (NAs) was selected and analyzed on the Ion Chef/Ion S5™ MPS platform using a 74 MH panel specifically developed for improving the accuracy of ancestry prediction. To calculate the Random Match Probability (RMP) of each MH profile from the specific population of interest, allele frequencies from numerous populations, previously inferred with PHASE software, were used.[2] In particular, the RMP calculated for the full population sample sets was observed to be higher in all populations in which individuals self-identified as such. In addition, Likelihood Ratio (LR) values were calculated by dividing the highest RMP obtained among the four populations by the second highest. The resulting LR value expresses how much more likely it is to observe the given profile if it originated from an individual belonging to the population at the numerator than if it originated from an individual belonging to the population at the denominator. The biogeographic ancestry of representative EA, AA, HIS, and NA population samples was correctly predicted and the related LR values were found to be at their highest in the corresponding population of origin. These results support the hypothesis that the novel MPS 74 MH panel is a useful forensic assay that enables effective biogeographic ancestry prediction complementing the accuracy of current binary and non-binary marker-based ancestry prediction tools.

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Microhaplotypes, Biogeographic Ancestry Predict, Massively Parallel Sequencing

B6 Assessing Alternative Polymerases for Amplifying Mitochondrial DNA From Shed Hairs

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After attending this presentation, attendees will better understand that the choice of DNA polymerase used in Polymerase Chain Reaction (PCR) can influence the yield of products substantially. Additionally, attendees should be able to identify the importance of selecting an efficient and robust polymerase for use in the PCR, so that yields necessary for downstream sequencing are routinely obtained from hairs representing a range of treatments and biogeographic ancestries.

This presentation will impact the forensic science community by identifying efficient and robust DNA polymerases that can be used to amplify DNA from forensic-type samples.

Forensic DNA analysis of hair evidence typically involves the amplification and sequencing of the Whole Control Region (WCR) of the mitochondrial (mt) genome. In compromised hair samples, such as shed hairs, the number of mt genome copies could be low; thus, it is imperative that the polymerase used in PCR is efficient to ensure the maximum recovery of information. Considering this, the first phase of this study compared the yields obtained from 12 polymerases (sourced from a range of commercial companies) when amplifying the WCR, Hypervariable Region II (HV2), and Hypervariable Region II-B (HV2B). This initial assessment was performed using total genomic DNA extracted from 2cm of hair adjacent to the root from three donors. Two polymerases were identified that consistently resulted in significantly higher yields ($p<0.05$) for all three regions, when compared to the currently used polymerase (6- and 4-fold increase in yield). The second phase of this project was focused on assessing the broad utility of these top two performing polymerases for amplifying the WCR and HV2B from hair samples representing diverse biogeographic ancestries (i.e., Caucasian, Hispanic, African American, Asian, and Native American), treatments (i.e., bleached, dyed, and chemically straightened), and anatomical locations (e.g., head and genitalia hairs) (n=41). The results indicated that regardless of sample type, the top two polymerases still significantly ($p<0.05$) outperformed the currently used polymerase (13- and 7-fold increase in yield). The results from this study highlight that novel commercially available polymerases could greatly assist with the analysis of mitochondrial DNA, especially from the challenging hair samples encountered in evidence.

Mitochondrial DNA, Polymerases, Shed Hairs

B7 Direct Amplification of Sperm Using Laser Microdissection (LMD) and Promega's® PowerPlex® Fusion 6C Amplification Kit

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After attending this presentation, attendees will be able to adapt this method to help streamline Short Tandem Repeat (STR) analysis of sperm from sexual assault casework samples when equipped with a laser microscope.

This presentation will impact the forensic science community by illustrating that sperm can be directly amplified using a Polymerase Chain Reaction (PCR) kit that captures the 20 Combined DNA Index System (CODIS) -core STR loci now accepted for use at National DNA Index System (NDIS).

Sexual assault cases comprise approximately 45% of all DNA casework and account for a significant amount of casework DNA extractions. The major problem encountered with traditional differential extraction is incomplete separation of male and female DNA, especially when epithelial cells greatly exceed the number of sperm, resulting in a mixture. This can produce difficult DNA profile interpretations, statistical calculations, and ultimately, explanations of the results to a jury. Additionally, the traditional differential extraction method is laborious and time consuming. To overcome these problems, the Arizona Department of Public Safety (AZDPS) developed a direct amplification protocol for sperm isolated via LMD.

LMD microscopy replaces the challenges of traditional sexual assault casework methodologies. It provides the ability to simultaneously identify, physically separate, count, and collect cells from a microscope slide for further DNA analysis. Using LMD with a direct amplification expedites DNA analysis of sex assault evidence by offering the following advantages: (1) efficient physical separation of sperm and epithelial cells; (2) greater precision in locating and collecting small amounts of sperm cells in the presence of large epithelial cell populations; (3) elimination of the traditional differential extraction technique; (4) elimination of traditional quantification; (5) reduction in analyst handling and sample manipulation; and, (6) reduction in time spent on analytical interpretations, all while maintaining the ability to obtain full DNA profiles.

LMD with the Leica™ LMD6500 Microsystem integrates microscope and laser functions. In a user-friendly fashion, images are captured by a camera and combined with image analysis software to allow a large sample image to be displayed on a computer screen. This simplifies the ability to locate and dissect cells into separate tubes. Slides are mounted upside down onto the microscope stage for capture of dissected cells via gravity into PCR tubes located in a substage tube holder.

The AZDPS current direct amplification protocol for sperm isolated via LMD was optimized for use with PowerPlex® Fusion 6C STR amplification kit. This evaluation included testing of the previously validated LMD protocol, as well as experimental variations in the pre-amplification lysis solution, amplification buffer, and sperm lysate input volume. Because of LMD implementation, the laboratory workflow was streamlined and the processing time for producing CODIS-eligible DNA profiles for sperm positive sexual assault casework was significantly decreased.

Laser Microdissection (LMD), Direct Amplification, Sperm

B8 The Evaluation and Implementation of the Promega® Casework Direct Kit for Y-Screening on Sexual Assault Samples

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After attending this presentation, attendees will better understand how to implement the Promega® Casework Direct Kit to screen for the presence of male DNA and the kit's use as an alternative to conducting differential extractions as the primary extraction method on sexual assault samples.

This presentation will impact the forensic science community by informing attendees, especially those in the field of DNA analysis, on the ability to screen for the presence of male DNA using a new extraction method.

Current serological screening of sexual assault samples is time consuming and provides the analyst with little predicative power as to the DNA profile that may be obtained. Recent legislation across the country has increased the number of sexual assault samples that are submitted to forensic laboratories for testing. With this new demand on laboratories, there is a need for the rapid detection of male DNA in a sample and a faster and more efficient DNA process to generate DNA typing results. Prior to the introduction of new robust Short Tandem Repeat (STR) megaplexes and the advent of probabilistic genotyping software, differential extractions and DNA purification methods were developed to separate epithelial and sperm cells in an attempt to produce single-source DNA profiles. With more laboratories turning to probabilistic genotyping software, laboratory personnel may consider using a fast and efficient extraction method that results in DNA mixtures. The Promega® Casework Direct Kit enables the rapid generation of lysates from casework samples and can be used directly in an STR amplification to generate DNA typing results.

Evaluation of the Promega® Casework Direct Kit was conducted by the Palm Beach County Sheriff's Office (PBSO) Forensic Biology Unit. Samples were extracted with the Casework Direct Kit and PBSO's in-house differential extraction method using the QIAGEN® EZ1® XL Advanced® Robot and quantified with the Promega® PowerQuant® System on the Applied Biosystems® 7500 Real-Time Polymerase Chain Reaction System. PCR amplification was achieved using the Promega® PowerPlex® Fusion 5-dye System with 30 PCR cycles and Promega® PowerPlex® Y23 System with 30 PCR cycles on the Applied Biosystems® GeneAmp® 9700 thermal cycler. Capillary electrophoresis was conducted on the Applied Biosystems® 3500xL Genetic Analyzer with a 24-second 15kV injection and data was analyzed using Applied Biosystems® GeneMapper® ID-X v 1.5.

The data generated was evaluated to determine if the Casework Direct Kit could be used to detect the presence of male DNA and as an alternative to differential extraction. Quantification results were evaluated to determine if the autosomal-to-Y ratio could be used as an indicator for downstream process decisions, such as taking the lysate directly to autosomal amplification, Y-chromosomal Short Tandem Repeat (Y-STR) amplification, or for re-extraction of a sample using a differential extraction method to optimize male DNA recovery.

Quantification results obtained for the male target show that the amount of a male DNA recovered from the Casework Direct Kit extraction was comparable to the differential extraction method. Lysates from the Casework Direct Kit with an autosomal to Y ratio of less than 200 were taken direct to STR amplification and produced male DNA profiles in the presence of high female DNA. A full Y-STR haplotype was obtained in a 96-hour post-coital sample. The presence of male DNA was detected and a full Y-STR haplotype was obtained from non-probative samples where sperm cells were not observed during microscopic examination.

The results indicate that the Casework Direct Kit can extract DNA from typical sexual assault evidence and, based on the quantification results, may assist the analyst in making decisions regarding downstream processing or re-extracting with a differential extraction method. Implementation of the Casework Direct Kit would replace conventional serology screening for sexual assault evidence as it would provide a more sensitive method to determine the presence of male DNA.

Y-Screening, Non-Differential Extraction, Sexual Assault Samples

B9 The Development of a Multianalyte Paper-Based Device for Serological Measurements

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After attending this presentation, attendees will understand how the development of a paper-based device can be used to obtain serological measurements from various biological samples. This multianalyte device permits a simultaneous analysis of various body fluids obtained from a variety of substrates. This device is rapid, simple, and permits a quick presumptive screening of body fluids collected at crime scenes.

This presentation will impact the forensic science community by demonstrating that multiplexed serological measurements can be made using a microfluidic device developed from paper and wax with colorimetric sensing pads. The design and developmental validation of the system will be discussed, including stability, interference testing, and reproducibility studies. Fieldable test kits are important in evidence screening as they aid in the collection of samples and improve efficiency. Properly designed, they permit simple, fast, and presumptive testing to occur at a lower cost. Unfortunately, many of these tests generally run one body fluid at a time, which can be lengthy in time and potentially destructive to critical samples. This presentation proposes a multianalyte serological screening procedure based on a paper microfluidic platform.

Multianalyte paper-based devices utilize sheets of chromatographic paper and thermal wax to create hydrophilic channels that direct a liquid sample to multiple test wells, each with a different colorimetric sensor. This multianalyte paper-based device can be used to detect various body fluids for field-presumptive testing. The device produces quick and easily distinguishable results without the need for external instrumentation.

In this project, the developmental validation of this multianalyte device will be discussed. Sensitivity studies, interference testing, and experiments involving aged and degraded samples were all performed as part of these validation studies. Colorimetric tests used in this device include modifications of the Kastle-Meyer test for blood, the Urease-Nessler's test for urine, the amylase test for saliva, and the acid phosphatase test for semen. All four tests were implemented on a single paper-based analytical device. Key issues in design included adjusting reagent concentrations for visibility and long-term stability. Tests of single and mixed analytes were performed.

For the determination of blood, a Kastle-Meyer test was used; however, hydrogen peroxide was not an appropriate reagent for this test due to problems with evaporation. Therefore, sodium perborate was used in this test to oxidize phenolphthalein. Although this test is not blood specific, it did provide a generalized test method for the presence of blood. Dilution factors as high as 1:750 still produced results. Interferences included certain acidic foods and bleach. For the determination of urine, urease-based decomposition of urea to ammonia was utilized. The release of ammonia was then detected using the Nessler's reagent. Dilutions of urine up to 1:100 provided a response and urine samples up to 30 days in age still produced results. Interferences included other substances containing urea. For the determination of saliva, the ability of amylase to hydrolyzed starch/iodine mixtures was used. This reaction produces color change from a black-purple color to a clear color. Sample dilutions up to 1:100 were still detectable, as were samples aged up to 30 days. Lastly for determination of semen, the acid phosphatase reaction was used. Sample dilutions up to 1:400 were detectable on the chip. Interferences included herbal drinking tea and vaginal secretion fluids.

Experimental results with mixtures and single-source samples demonstrate clear, distinct signals for each serological sample that was present. Results discussed will include the sensitivity of each test, the range of interferences, and how age affects the results. Overall, this presumptive testing method is rapid, reproducible, and easily used in the field for screening unknown fluids during a forensic investigation.

Multianalyte, Serological, Validation

B10 Botanical Evidence in a Case of Environmental Crime: The Application of Short Tandem Repeat (STR) DNA Markers and Tree Ring Analysis of *Eucalyptus Globulus* Disks

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After attending this presentation, attendees will understand the importance of using botanical evidence in casework.

This presentation will impact the forensic science community by increasing the fundamental understanding of the application of STR DNA markers and tree ring dating analysis as it applies to the discrimination of illegally cut forest timber.

Forensic botany is an emerging discipline that uses plant evidence in matters of law. Botanical evidence is usually found at crime scenes and is sometimes the only available element for criminal investigations. Currently, as supporting evidence, DNA analysis is nearly the only element that can be used as a reliable identification tool, due to the high variability of DNA across all species. One way to identify a distinctive DNA fragment for a species is to study the Polymerase Chain Reaction (PCR) products for microsatellite markers (Simple Sequence Repeats (SSRs), STRs, Simple Sequence Length Polymorphisms (SSLPs), and Variable Number of Tandem Repeats (VNTRs)). DNA regions with short repeat units (usually 2bp-6bp in length) are called STRs. In this case, the application of STR markers enabled the demonstration of the molecular traceability of *Eucalyptus globulus* seizures. On the other hand, dendrochronological techniques require the presence of annual growth rings; new growth in trees occurs in a layer of cells near the bark. A simple method for determining the periodic nature of growth in trees of unknown origin is counting its rings with computer assistance. An Image Analysis System has been specifically designed to look for a precise and efficient way to measure annual tree-ring widths from wood disks, providing an independent tool to confirm the accuracy of dating.

A robbery case in a timber forest was investigated using STR markers and dendrochronological analysis. A truck driver was accused of stealing several *Eucalyptus globulus* logs and transporting them in a truck. The truck was found by the police in a nearby forest that had been illegally logged, and wood disks were collected from the vehicle. Genetic and dendrochronological analyses of wood samples collected from the truck (evidence) were performed and results were compared to those obtained from *Eucalyptus globulus* tree disks collected at the forest (crime scene). Briefly, *Eucalyptus globulus* DNA was extracted from the wood using a commercial kit and quantified with a fluorometer; the genetic profile was obtained using EMCR 9, EMCR 10, and EMCR 11 microsatellite markers. Genetic analysis revealed different profiles for wood collected in the truck and for wood obtained at the forest. At the same time, *Eucalyptus globulus* disks were dried, sanded, and placed face down on the scanner for image acquisition, and software measured the rings in the image by making straight lines with a single mouse click from the center to the bark. A graphic of ring-widths identified by year was displayed during the analysis. Dendrochronology techniques determined a non-match of tree rings when the wood evidence was compared against the wood disks collected at the crime scene. Consequently, *Eucalyptus globulus* logs found in the truck did not come from the illegally logged forest.

This investigation demonstrates the potential for plant microsatellite markers and dendrochronology dating analysis for linking botanical evidence and plants growing at the crime scene. Following this analysis, the arrested truck driver was declared innocent.

DNA Typing of *Eucalyptus globulus*, Dendrochronology Dating, Botany Forensic

B11 An Evaluation of the Illumina® ForenSeq™ DNA Signature Prep Kit and Promega® PowerPlex® Fusion System in the Evaluation of Degraded Identical Twin Samples

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After attending this presentation, attendees will better understand the Illumina® ForenSeq™ DNA Signature Prep kit and Promega® PowerPlex® Fusion systems' abilities to analyze naturally degraded samples.

This presentation will impact the forensic science community by providing an understanding of both kits' limitations in evaluating degraded samples when degradation levels are considered.

A comparison of Short Tandem Repeat (STR) profile quality generated by both Massively Parallel Sequencing (MPS) and traditional Polymerase Chain Reaction-Capillary Electrophoresis (PCR-CE) will be presented. The analysis of phenotypic, ancestry-informative, and identity-informative Single Nucleotide Polymorphisms (SNPs) from degraded samples will also be discussed.

MPS enables an increased amount of forensically beneficial genetic information to be obtained over traditional CE. The ForenSeq™ DNA kit simultaneously amplifies up to 231 genetic markers, namely 27 autosomal STRs (aSTRs), 24 Y-chromosomal Short Tandem Repeats (Y-STRs), 7 X-chromosomal Short Tandem Repeats (X-STRs), 95 identity-informative SNPs (iiSNPs), 22 phenotypic-informative SNPs (pSNPs), and 56 ancestry-informative SNPs (aSNPs). MPS amplicon sequencing allows for small amplicon sizes, hypothetically making MPS technologies more beneficial when evaluating degraded samples; however, minimal research has been conducted on the efficiency of the ForenSeq™ DNA kit in conjunction with environmentally degraded samples to test this hypothesis. This research sought to explore this hypothesis by comparing the ForenSeq™ kit with that of a traditional forensic chemistry, Promega® PowerPlex® Fusion, in the evaluation of naturally degraded samples.

Seventy-six degraded buccal samples were quantified using Applied Biosystem's® Quantifiler® Trio DNA Quantification Kit to obtain information on the samples' degradation levels. The samples' Degradation Index (DI) values ranged between 2.93 and 110.12, with values greater than 10 indicating severe degradation. The samples were then processed using the PowerPlex® Fusion and ForenSeq™ kits with DNA inputs ranging between 0.250ng and 1ng. Comparative analysis of the PowerPlex® Fusion and ForenSeq™ DNA kits were performed for the overlapping loci, while the additional ForenSeq™ loci were assessed to evaluate the kit's ability to provide additional individualizing information.

As expected, the quality of the profiles decreased with increased DI values. With some exceptions, MPS generated higher average percent profiles, exhibited less allelic drop-out, and produced better heterozygous balance, while PCR-CE produced better inter-locus balance. There was no significant difference observed for profile uniqueness between the systems when average Probabilities Of Identity (POI) values were calculated. All samples generated biogeographical ancestry estimations with three observed discordances from donor self-reported ancestry. Hair color estimations were only possible for the least degraded sample. Several previously unreported STR sequence variants were observed in aSTRs, Y-STRs, and X-STRs when samples were processed with MPS; however, no genetic differentiation between identical twin siblings was observed in either system.

Overall, the ForenSeq™ kit produced more forensically beneficial information for the degraded samples in this study, demonstrating that it is highly valuable for the analysis of degraded samples; however, the kit is limited in its ability to produce investigative leads from degraded samples due to the number of pi-SNPs required to generate phenotypic estimations and the ForenSeq™ Universal Analysis Software's incorrect estimations of donor biogeographic ancestry groups.

Massively Parallel Sequencing, Degraded DNA, STR

B12 Oxidative Mitochondrial DNA (mtDNA) Damage and Repair: A Modeling Approach Compared to DNA Recovered From Bullet Cartridge Cases

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After attending this presentation, attendees will understand the practice in identifying, characterizing, and repairing oxidative DNA damage lesions and will be able to observe oxidative mtDNA damage in both modeled samples and forensic-type samples.

This presentation will impact the forensic science community by helping in the development of best practices for collection of DNA evidence from cartridge case evidence, as well as accurately identifying, characterizing, and repairing oxidative damage lesions when performing mtDNA sequence analysis in forensic casework.

DNA damage involves a change in the chemical structure of DNA through the introduction of strand breaks and lesions and is a well-known characteristic of forensic evidence and ancient samples. The type of damage observed will depend on the conditions under which the evidence is exposed, the length of exposure, and the type of evidence that is collected. There are several categories of DNA damage; for example, hydrolytic and oxidative. Damage can result in deamination, depurination, and oxidation of nitrogenous bases.

The first part of this study compared the modeling of oxidative damage in the Control Region (CR) of the mtDNA genome to the pattern of damage observed when pristine DNA was exposed to the surface of different types of metallic bullet cartridge cases. Previous studies have explored the ability to recover DNA from fired cartridge cases using Short Tandem Repeat (STR) analysis. A challenge has been the ability to effectively recover enough DNA for a partial or complete STR profile. In particular, there has been little success when attempting to recover DNA from copper and brass cartridge cases. One hypothesis is that the DNA is highly damaged due to the oxidative properties of copper. In the laboratory, three types of cartridge cases composed of different metals were analyzed: copper, brass (copper and zinc), and aluminum. Buccal DNA was deposited on the casings through liquid extracts and touch DNA through handling, was recovered with swabs moistened in molecular grade water or 0.5 M EDTA, and DNA extraction was performed using a low copy number approach. Damage to pristine DNA was accomplished through a Fenton reaction (an iron catalyst reacting with hydrogen peroxide to create hydroxyl radicals that inflict damage lesions on the DNA to effectively model oxidative damage). The second part of the study utilized NEBNext® Formalin-Fixed, Paraffin-Embedded (FFPE) DNA Repair Mix to repair the damaged DNA. Following Polymerase Chain Reaction (PCR) amplification and library preparation with the Promega® PowerSeq™ Mito Control Region Nested Kit, Massively Parallel Sequencing (MPS) on the Illumina® MiSeq® FGx benchtop sequencer was used to assess the damage, characterize the lesions, and observe the effects of the DNA repair mix. Donor haplotypes and heteroplasmy status were previously determined for comparison purposes.

A preferred approach was identified for lifting DNA from cartridge cases containing copper. Oxidative damage was characterized through active damage, compared to results for DNA samples recovered from bullet casings and repaired using FFPE DNA Repair Mix. The findings presented should aid the practitioner in developing best practices for collecting DNA evidence from cartridge case evidence, accurately identifying, characterizing, and repairing oxidative damage lesions, and applying the findings when performing mtDNA sequence analysis in forensic casework.

Mitochondrial DNA (mtDNA), DNA Damage/Repair, Massively Parallel Sequencing

B13 The Development and Optimization of a Direct Polymerase Chain Reaction (PCR) System of Mixtures of Sperm and Epithelial Lysates From Cotton Swabs

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After attending this presentation, attendees will better understand a novel method for differentiating, extracting, and amplifying epithelial and sperm mixture samples.

This presentation will impact the forensic science community by providing results for a method that can decrease the analysis time for mixture samples of sperm and epithelial cells. Attendees will observe the results of an experimental design-based method that aids in direct PCR for amplification of post-coital lysates. These results provide a method to minimize the long cell lysis and purification process by quickly extracting and directly amplifying cell lysates.

At present, there is increasing concern regarding case backlogs involving Sexual Assault Kits (SAK). The goal of this project is to improve the speed of extraction and to provide a method for quickly separating and screening mixed samples. It is common for epithelial and sperm cell mixtures to require labor-intensive processes and time to achieve differential extraction. The goal is to develop a rapid Short Tandem Repeat (STR) -based screening method that minimizes the DNA extraction, purification, and quantitation steps for a faster analysis.

In this study, alkaline lysis and direct PCR were used to extract and amplify epithelial and sperm DNA from simulated SAK samples, with various concentrations of epithelial and sperm cells. The direct PCR method was developed and optimized using an experimental design software program using mixture design methods. Cotton swabs were treated with 0.05N sodium hydroxide (NaOH) under ten cycles of 15 seconds at 20kpsi and 15 seconds at ambient pressure. This removed and lysed epithelial cells from the cotton swab. The swab then underwent incubation in 0.4N NaOH at 95°C for five minutes to remove and lyse sperm cells. After neutralization, the lysate was incubated at 50°C for ten minutes with 125mM dithiothreitol (DTT) to remove the protamines, isolating the male DNA. The samples underwent drop dialysis and were amplified using an inhibition-resistant polymerase. The PCR reaction mixture was optimized using experimental mixture designs. The experimental design considered salt concentration, buffer addition, deoxynucleotide triphosphate (dNTP) addition, primer addition, and PCR enhancers to develop experiments to maximize amplification of sperm and epithelial DNA. Output was measured by peak height of Amelogenin, D5, D13, D7, D16, CSF, and Penta D. The STR multiplex was analyzed using capillary electrophoresis and peak height was evaluated to determine the most effective reaction mix.

Results demonstrate the most effective amplification of sperm and epithelial DNA lysates without isolation and purification. The alkaline and pressure lysis effectively separate the epithelial fraction from the sperm, with the sperm being further lysed with heat, alkali, and reduction. The experimental runs demonstrate the enhancement of direct PCR using combinations of the reaction components. The analysis of the experimental runs is accomplished using mixture design analysis with experimental design software. This uses the results to model a contour plot demonstrating the expected ideal reaction mix to maximize peak heights. The model is tested with more experimental runs based off the components that lead to increased peak heights to determine the ideal conditions for maximized peak heights.

Direct PCR, Experimental Design Methods, Pressure Cycling

B14 WITHDRAWN

B15 Protein-Based Human Identification Using Hair Shafts From Different Body Sites

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After attending this presentation, attendees will better understand a new protein-based approach for analyzing human hair evidence that allows inference of Single Nucleotide Polymorphism (SNP) variation in DNA and is both statistically and scientifically rigorous.

This presentation will impact the forensic science community by informing attendees of a method of analyzing human hair proteins to infer non-synonymous SNP (nsSNP) alleles using hair shaft protein. This expands what can forensically and scientifically be achieved with hair trace evidence.

Nuclear DNA typing is not always an option due to degradation or low quantity of starting material. For example, sufficient nuclear DNA is difficult to obtain from hair shafts due to low copy number. Instead, mitochondrial DNA (mtDNA) is commonly used for analysis as it is present in higher copy numbers in the hair shaft; however, mtDNA cannot be used for individualization because it is maternally inherited and there is no recombination. Consequently, all maternally related individuals have the same mtDNA haplotype. In such cases, it would be helpful for the forensic science community to have an alternative, quantitative method for analyzing hair for identification purposes.

With advances in mass spectrometry technology, it has become possible to use proteomic approaches since proteins are more chemically stable than DNA and are found in greater abundance. Recent studies have demonstrated that is it possible to use protein expression profiling to distinguish hair shafts from different body sites and to differentiate between unrelated individuals.[1,2] Additionally, proteins contain genetic variation in the form of Single Amino acid Polymorphisms (SAPs) that are the result of nsSNPs in the genome. These SAPs can be detected as Genetically Variant Peptides (GVPs) in proteomic datasets. From these GVPs, it is possible to impute the nsSNPs and calculate frequency estimates of profiles. This approach has been demonstrated in recent studies in which GVPs from scalp hair shafts were used to differentiate between unrelated individuals; however, whether this approach can be used for hair shafts from other body sites has not yet been determined.[2,3]

This study tests the hypothesis that individuals are distinguishable by GVP analysis of hair shafts from any of four body sites: scalp, beard, axillary, or pubic. It is further hypothesized that the GVP profile will not significantly vary between body sites and that hair between different body sites of an individual will have more in common than hair from another individual. To test these hypotheses, hair samples from the four body sites were collected from five volunteer subjects. The hairs were processed using a sodium dodecanoate-based method, then digested with trypsin. The digested peptides were analyzed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), and the resulting proteomic datasets were analyzed using the Global Proteome Machine (http://www.thegpm.org/). The datasets were then screened for previously characterized and validated GVPs. Preliminary GVP analysis of type I keratins from these proteomic datasets exhibits evidence of clustering such that hair shafts derived from any of the four body sites of a single individual are more correlated to each other than to those of another unrelated individual. Future work will focus on analyzing additional keratin, keratin-associated, and other proteins, as well as exploiting new sample processing methods to increase detection of characterized GVPs.

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Proteomics, Hair, Genetically Variant Peptides

B16 Argon Direct Analysis in Real-Time Mass Spectrometry (Ar DART®-MS) for Forensic Analysis of Illicit Drugs

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After attending this presentation, attendees will understand a new mode of DART®/MS using Ar instead of the popularly used Helium (He) as the DART® ionization gas, its ionization mechanism toward polar compounds, its capability in positively identifying commonly abused drugs, and its application in successfully analyzing drug tablets.

This presentation will impact the forensic science community by introducing a novel Ar DART®/MS technique as an alternative to He DART®/MS for forensic analysis of illicit drugs, especially in consideration of the looming worldwide helium shortage.

Helium was originally selected as the DART® gas because its long-lived $^2S_3$ state has an Internal Energy (IE) of 19.8eV, which is the highest among inert gases; however, a helium shortage has been looming over the past ten years. Argon is the most abundant noble gas in the air at 9,340ppmv, third only to nitrogen and oxygen. To this point, dopant-assisted Ar DART®/MS has been investigated for the analysis of melamine, labile compounds, polycyclic aromatic hydrocarbons, diesel fuels, etc. In this study, Ar DART®/MS is explored as an alternative to He DART®/MS for the forensic analysis of illicit drugs, without the assistance of any dopant.

The ionization capability of Ar DART®/MS was first tested with polar solvents, including water, acetonitrile, methanol, ethanol, isopropanol, ethyl acetate, acetone, and tetrahydrofuran with respective ionization energy of 12.62eV, 12.20eV, 10.84eV, 10.50eV, 10.17eV, 10.01eV, 9.70eV and 9.40eV. It was found that Ar DART®/MS was unable to efficiently ionize water, acetonitrile, and methanol, but was able to ionize ethanol moderately and the rest of the polar solvents efficiently. Therefore, it was concluded that Ar DART®/MS was able to ionize all the organic compounds, including illicit drugs as they are molecularly larger and should have IE lower than ethanol.

Subsequently, ten commonly abused drugs (i.e., (±)-amphetamine, cocaine, diazepam, heroin, Lysergic Acid Diethylamide (LSD), (±)-3,4-Methylenedioxymethamphetamine (MDMA), Phencyclidine (PCP), psilocin, testosterone, and Δ^9-Tetrahydrocannabinol (THC)) were analyzed by Ar DART®/MS to test the ionizing capability of Ar DART® toward compounds with diverse functional groups, optimize the instrumental conditions for the analysis of illicit drugs, estimate the Limit Of Detection (LOD) of the analysis, and develop a general analytical protocol for the analysis. Under optimum conditions, the LOD of Ar DART®/MS was determined to be approximately 100μg/mL. Because the sampling volume was approximately 1μL, the LOD of Ar DART®/MS was approximately 100pg in quantities. The general analytical protocol took approximately three minutes in the analysis of each commonly abused drugs. All the commonly abused drugs were positively identified at 100μg/mL because the experimentally measured monoisotopic mass of the predominate $[M+H]^+$ ion, sometimes with the additional molecular ion, from each drug were within ±5mDa of the theoretically calculated monoisotopic mass. No time-consuming and labor-intensive sample preparation steps were required during the analysis.

Finally, the general analytical protocol was applied to tablet analysis of six prescriptions drugs (i.e., clonazepam 1mg, cyclobenzaprine 10mg, metaxalone 800mg, oxycodone/acetaminophen 5/325mg, tramadol/APAP 37.5/325mg, and zolpidem10 mg). It was found that while Ar DART® was able to efficiently ionize and subsequently identify the active ingredients, it practically omitted the inactive ingredients in the tablets.

The ionization mechanism of Ar DART® was likely the generation of protonated molecular ions through direct Penning ionization of polar compounds by metastable Ar followed by self-protonation of the analytes, which was also confirmed by the observation of molecular ions of some commonly abused drugs. In comparison with He DART®, Ar DART® generated much cleaner background mass spectrum and simpler mass spectra of tested drugs due to the lower IE of metastable Ar species, which was an advantage in data analysis; however, Ar DART® had an LOD approximately two orders of magnitude higher, though it should be still sensitive enough for the analysis of seized drug evidence.

Illicit Drugs, Ar DART®/MS, Tablet Analysis

B17 Forensic Analysis of Illicit Drugs by Nitrogen Direct Analysis in Real-Time Mass Spectrometry (N_2 DART®-MS)

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After attending this presentation, attendees will appreciate a new mode of DART®/MS using cheaper and more easily accessible N_2 instead of the popularly used Helium (He) as the DART® ionization gas, its great ionization capabilities for illicit drugs, and a general analytical protocol to use N_2 DART®/MS for the forensic analysis of illicit drugs with either a JEOL AccuTOF™ orthogonal Time-Of-Flight (TOF) MS or an Agilent® 6550 quadrupole TOF (qTOF) MS.

This presentation will impact the forensic science community by introducing a novel N_2 DART®/MS technique to effectively ionize seized drug evidence, which has the potential to be used with a miniature mass spectrometer in the future for the forensic analysis of illicit drugs at crime scenes.

Currently, helium gas is popularly used in DART®/MS. Theoretically, He DART®/MS should have the best sensitivity because He is an inert gas that occupies a front position in the periodic table, therefore producing metastable species with the highest internal energy (i.e., long-lived He 2^3S electronic excited state atoms with an internal energy of 19.8eV); however, when He gas is not readily available (e.g., during space missions or the forensic analysis of illicit drugs at the crime scene), nitrogen gas is the best option because it is the next inert gas behind He in the periodic table.

In this study, N_2 DART®/TOF/MS with a JEOL AccuTOF™ mass spectrometer was first used to analyze ten commonly abused drugs (i.e., (±)-amphetamine, cocaine, diazepam, heroin, Lysergic Acid Diethylamide (LSD), (±)-3,4-Methylenedioxymethamphetamine (MDMA), Phencyclidine (PCP), psilocin, testosterone, and Δ^9-Tetrahydrocannabinol (THC)) to test the ionizing capability of N_2 DART® toward compounds with diverse functional groups, optimize the instrumental conditions for forensic analysis of illicit drugs, estimate the Limit Of Detection (LOD) of the analysis, and develop a general analytical protocol for the analysis. Under optimum conditions, the LOD of N_2 DART®/TOF/MS was determined to be approximately 10μg/mL. Because the sampling volume was approximately 1μL, the LOD of N_2 DART®/TOF/MS was approximately 10pg in quantities. The general analytical protocol took approximately three minutes in the analysis of each commonly abused drug. All the commonly abused drugs were positively identified at 10μg/mL as the experimentally measured monoisotopic mass of multiple ions, predominately the $[M+H]^+$ ion, from each drug were within ±5mDa of the theoretically calculated monoisotopic mass. No time-consuming and labor-intensive sample preparation steps were required during the analysis. The general analytical protocol was then applied to the tablet analysis of six prescriptions drugs (i.e., clonazepam 1mg, cyclobenzaprine 10mg, metaxalone 800mg, oxycodone/acetaminophen 5/325mg, tramadol/APAP 37.5/325mg, and zolpidem 10mg). It was found that while N_2 DART® was able to efficiently ionize and subsequently identify the active ingredients in the tablets, it practically omitted the inactive ingredients. Therefore, it was concluded that the general analytical protocol can be utilized in the analysis of seized drugs because they are mixtures with similar complexity as the tablets.

N_2 DART®/qTOF/MS with an Agilent® 6550 mass spectrometer was further used to analyze ten commonly abused drugs at 10μg/mL in order to test the applicability of N_2 DART® on different MS platforms. First, all of the commonly abused drugs were positively identified by TOF/MS of the $[M+H]^+$ ions with their monoisotopic mass and isotopic pattern. Then, they were further positively identified by qTOF/MS/MS of the $[M+H]^+$ ions at Collision-Inducted Dissociation (CID) at 10.0V and 20.0V. The obtained mass spectra were automatically matched with the Agilent® Forensic Toxicology Personal Compound Database and Library (PCDL).

Illicit Drugs, N_2 DART®/MS, Seized Drug Analysis

B18 Structural Elucidation of Synthetic Opioids

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After attending this presentation, attendees will better understand the analytical strategy employed by the Contraband Drug Analysis Section of the Canada Border Services Agency for identifying new substances.

This presentation will impact the forensic science community by providing an overview of analytical challenges encountered during casework involving fentanyl analogues and related drugs. The impact of salt forms and mixtures on the analytical approach as well as the use of Nuclear Magnetic Resonance (NMR) methods for structural elucidation will be discussed. These challenges will be communicated with the drug analysis community in the hopes of spreading awareness and providing others with the ability to apply caution when presented with analysis of these substances and gaining confidence in structural determination.

With the emergence of powerful new opioid agonists (such as W-18 and U-47700) and a resurgence of fentanyl and its analogues, it is increasingly important for drug chemists to share analytical data and strategies for the identification of these compounds. The number of sites on the fentanyl structure that are easily substituted has led to a vast number of potential analogues. Identifying where substitutions have occurred on the molecule by structure elucidation can be challenging. As a result, NMR techniques are often required.

There has been a steady increase in both the number and diversity of synthetic opioid shipments intercepted by the Canada Border Services Agency (both importations and exportations). As of the beginning of 2017, the Contraband Drug Analysis Section has identified an average of one new fentanyl analogue per month. According to a recent United Nations Report, Canada consumes more prescription opioids per capita than any other country in the world. As a consequence, many users look to the illicit market to support their addictions. The emergence of fentanyl, its analogues, and other low-dose drugs in the illicit market has developed new concerns for public safety professionals and new challenges for forensic drug analysis.

When encountering a new drug substance, database references are not always available and literature references can be difficult to find. In these cases, full structural elucidation allows for the identification of the compound. The Contraband Drug Analysis Section is fortunate to have a number of instruments to assist in substance identification: Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR), FT-Raman, Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), Gas Chromatography/ Infrared Spectroscopy (GC/IR), and Nuclear Magnetic Resonance (NMR), among others. Upon analysis of fentanyl analogues, there have been circumstances in which typical analysis methods were not enough to confidently identify the compound. As an example, NMR methods beyond the routine ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HBMC, and Distortionless Enhancement by Polarization Transfer (DEPT) experiments were employed, including ^{1}H-^{15}N HMBC and Two-Dimensional Incredible Natural-Abundance Double-Quantum Transfer Experiment (2D INADEQUATE) in order to identify 2-methoxy-furanylfentanyl and rule out 3-methoxy-furanylfentanyl. Details of this case as well as structural elucidation of other compounds will be discussed.

Fentanyl, NMR, Elucidation

B19 The Detection of Gamma-Hydroxybutyric Acid (GHB) in Water and Mixed Drinks Without Sample Preparation Using Total Vaporization-Solid Phase Microextraction (TV-SPME) With On-Fiber Derivatization

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After attending this presentation, attendees will be aware of a new technique by which GHB can be effectively identified by Gas Chromatography/Mass Spectrometry (GC/MS) in aqueous or mixed drink samples with no sample preparation required.

This presentation will impact the forensic science community by introducing a new procedure for the detection and identification of GHB in drink samples using GC/MS without extensive sample preparation. The method is simple and effective and could be used to acquire evidence of drug-facilitated sexual assault involving the use of GHB.

GHB is often used in drug-facilitated sexual assaults because it incapacitates victims, induces memory loss, and does not persist in the human body.[1] The drug can be surreptitiously administered to a victim's drink, in which case a forensic scientist would receive a beverage suspected of containing GHB. The "workhorse" technique of forensic science laboratories is GC/MS. GHB is difficult to analyze by GC/MS due in part to its acidity (pKa 4.7), high polarity, and high solubility in aqueous solution. It is also well known that GHB readily dehydrates to Gamma-Butyrolactone (GBL) in the high heat of the GC inlet, further complicating its analysis.[2] One method used to make compounds more amenable to GC/MS analysis is derivatization, in which labile hydrogens are replaced with more stable groups, resulting in a product that has increased volatility and stability.[3] Derivatization in solutions followed by liquid injection GC/MS is already in use in forensic science laboratories, but this practice requires separate steps for solvent extraction, derivatization, and analysis.

Solid Phase Microextraction (SPME) is a technique in which the analytes are pre-concentrated onto a thin fiber coated in absorptive or adsorptive material. In TV-SPME, a small aliquot of analyte in solution is placed in a vial and heated until the sample completely vaporizes, resulting in a two-phase system. An SPME fiber is then introduced and the sample is absorbed onto the fiber coating. The maximum volume for total vaporization of a given solvent can be easily calculated given the solvent vapor pressure, molecular weight, vial volume, and temperature.[4] For example, the calculated maximum volume of methanol for total vaporization in a 20mL vial at 60°C is 24µL. The use of TV-SPME for sampling can streamline the derivatization process by allowing the derivatization to be done on-fiber. In this process, an SPME fiber is exposed to the headspace of a vial containing derivatization agent, then exposed to the heated headspace of a vial containing the sample. The reaction between the analyte and the derivatization agent then takes place directly on the SPME fiber. After sufficient time for reaction, the fiber is moved to the inlet of the GC for desorption. The use of a robotic autosampler can make this a fully automated process for which no sample preparation is necessary.

This method is ideal for the analysis of GHB in drink samples. GHB has been successfully identified in aqueous solution and in simulated mixed drinks by TV-SPME with on-fiber derivatization using N, O-Bis trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS).

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Solid Phase Microextraction, Derivatization, GHB

B20 The Detection of Phytocannabinoids From Buccal Swabs Using One Vial Headspace Vaporization Derivatization Coupled With Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS)

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After attending this presentation, attendees will better understand the application of Heated Headspace/Solid Phase Microextraction (HHS/SPME) for the extraction of phytocannabinoids from the headspace of air-dried buccal swabs.

This presentation will impact the forensic science community by providing a novel methodology for the detection of phytocannabinoids from buccal swabs. This HHS/SPME-GC/MS analytical platform potentially can be used to non-destructively extract phytocannabinoids from buccal swabs. Further chemical or biological testing of the HHS/SPME processed buccal swab evidence can still be conducted when needed.

Headspace derivatization of phytocannabinoids will also be discussed for the qualitative and quantitative phytocannabinoids analysis using GC/MS.

Marijuana is classified federally as a Schedule I controlled substance and is becoming a prevalent controlled substance reported in motor vehicle accidents. In order to screen for target drugs in evidence, samples undergo preparation to concentrate the drug of interest and remove interferences before instrumental analysis. SPME is a versatile alternative to Liquid-Liquid Extraction (LLE) and Solid Phase Extraction (SPE). This study combined the Total Vaporization Technique (TVT), high-temperature technique, and in-vial derivatization with SPME in one single step to facilitate the extraction and detection of phytocannabinoids from buccal swabs.

One vial headspace vaporization derivatization of phytocannabinoids was achieved by placing a 250uL glass insert containing a derivatization reagent inside a 20mL headspace sample vial. In order to determine the interferences level from buccal swabs using HHS/SPME, five different swab sources were tested. Each swab had 0.4µg of Δ9-THC added onto it for the test. Approximately 5mg of each swab material was placed into a 20mL headspace vial and sealed with a silicone septum and a magnetic cap for automated HHS/SPME-GC/MS. To determine the optimal headspace derivatization and extraction in one HHS/SPME step, varying amounts of derivatization reagent were evaluated. First, 4µL aliquots of Δ9-THC standard solution (100µg/mL) were placed in eight separate 20mL headspace vials. After drying the solvent, 1µL, 2.5µL, 5µL, 7.5µL, 12.5µL, 15µL, 20µL, and 25µL of N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) were added to the inserts inside headspace vials. HHS/SPME-SPME conditions had been optimized for the separation and detection of phytocannabinoids and their derivatized products.

This technique allowed for the detection of seven phytocannabinoids on buccal swabs, including Cannabichromene (CBC), Cannabidiol (CBD), Cannabigerol (CBG), Cannabinol (CBN), delta-8-Tetrahydrocannabinol (Δ-8-THC), delta-9-Tetrahydrocannabinol (Δ-9-THC), and Tetrahydrocannabivarin (THCV), at sub-microgram levels. This new approach not only improved sensitivity and selectivity through the successful derivatization of phytocannabinoids from sample headspace but also facilitated automation of HHS/SPME-GC/MS. This methodology has the potential for the forensic application to detect phytocannabinoids from swab samples.

Marijuana, Buccal Swab, HS/SPME

B21 The Determination of the Source of Origin for Methomyl in a Fatal Poisoning Case by Gas Chromatography/Isotope Ratio Mass Spectrometry (GC/IRMS)

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After attending this presentation, attendees will better understand how stable isotope analysis by GC/IRMS can apply for determining the source of origin for methomyl, a carbamate insecticide, from a fatal poisoning case.

This presentation will impact the forensic science community by demonstrating the application of GC/IRMS in a methomyl poisoning case. With the application of GC/IRMS, it was possible to correlate a potential perpetrator to a methomyl poisoning case.

On the night of March 9, 2016, six people who live in a small town were playing a Korean card game in a town community center. While playing cards, two men opened two bottles of Soju (one of the most popular alcoholic beverages in South Korea). While the two men were drinking the Soju, they felt nauseated, began vomiting, and were admitted to an emergency room. During medical treatment at the hospital, the 62-year-old man was pronounced dead due to fatal poisoning from methomyl and the 67-year-old man was hospitalized for further medical treatment.

The two bottles of Soju were seized at the crime scene and biological samples from the two victims were also submitted to the laboratory for analysis to determine their source. To determine who blended methomyl into the bottles of Soju, seven different methomyl products were seized from suspects who played a card game together and lived in the same town; however, it was found that ^{13}C isotope ratio values of seven methomyl products ($\delta^{13}C \approx -28‰ \sim -32‰$) were different from the values of methomyl contained in the two bottles of Soju ($\delta^{13}C \approx -22‰$).

Three weeks after the initial incident, the husband of one of the suspects committed suicide in his barn and an energy drink bottle containing methomyl was found next to his body. It was discovered that the cause of death was also methomyl poisoning. The empty energy drink bottle and biological samples of this third person were submitted for isotope analysis. It was determined that the stable isotope ratios of carbon and nitrogen ($\delta\,^{13}C$, $\delta\,^{15}N$, $\delta\,^{2}H$) for methomyl components from the Soju bottles found at the town community center were similar to the $\delta\,^{13}C$, $\delta\,^{15}N$ values of the bottle of energy drink, indicating that methomyl from the Soju bottles originated from the same source as the energy drink bottle. In order to identify a manufacturer of methomyl products, the stable isotope ratio ($\delta\,^{13}C$, $\delta\,^{15}N$, $\delta\,^{2}H$) of 11 types of methomyl products with different expiration dates were collected from suspects and were analyzed by GC/IRMS. As a result, it was determined that a Samgong methomyl product with an expiration date of October 31, 2011, had a similar stable isotope ratio.

This study illustrates that GC/IRMS can be readily applied to the determination of a source when it is not possible to identify a perpetrator due to lack of information.

GC/IRMS, Methomyl, Forensic Provenancing

B22 A Comparison of Portable Infrared (IR) Spectrometers and the Narcotic Identification Kit (NIK) Field Test for the On-Scene Analysis of Cocaine Hydrochloride (HCl)

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After attending this presentation, attendees will understand the benefits and limitations of both portable IR spectrometry and the NIK field tests for the on-scene analysis of cocaine HCl.

This presentation will impact the forensic science and law enforcement community by determining whether portable IR spectrometers or NIK field tests are more advantageous for the analysis of cocaine HCl at crime scenes.

The majority of inmates across the United States are incarcerated for drug-related offenses. It is important that the technology used to test for controlled substances is accurate and reliable. For on-scene presumptive testing, the NIK test is most often utilized to test for the presence of a controlled substance. The NIK tests use colorimetric reactions, which rely on a specific moiety of the drug molecule to react with the provided reagents to produce characteristic color changes. In the NIK test used to detect and identify cocaine, there are three chemical ampoules within a plastic pouch closed with a safety clip. To test a substance, a loading device is used to deliver the correct amount of sample to the pouch. A positive result for cocaine is indicated by blue or pink with blue speckles after the reaction in the first ampoule, pink after the reaction in the second ampoule, and a pink top layer with a blue bottom layer after the reaction in the third ampoule. Recently, it has been discovered that there have been numerous cases where false positive NIK tests at the scene were later disproven via confirmatory lab testing. As a result, there have been hundreds of wrongful convictions.[1] This has resulted in press coverage condemning the NIK tests, followed by significant public outrage. In addition, as of July 2017, the Houston, TX, police department has stopped using these tests at the scene, eliminating all on-scene drug testing. The possible dangerous exposure of officers to potentially lethal drugs such as fentanyl was also cited as a reason for stopping on-scene drug testing; however, this abandonment of on scene presumptive drug testing could be premature given the availability of portable IR spectrometers that can be used to provide accurate and reliable identifications at the scene, with minimal risk of exposure to law-enforcement personnel when appropriate levels of personal protective equipment are employed.[1] IR spectroscopy measures the absorption of IR radiation, specifically the vibrations of the bonds between atoms, to determine the structure of a molecule. This research compares the use of portable IR technology with NIK tests to determine which method is better suited for the on-scene analysis of illicit drugs, specifically cocaine HCl.

This research assessed important performance characteristics for each method, including a short- and long-term cost analysis, whether the method is destructive or non-destructive, the ease of use, knowledge and skill required of the operator, speed of analysis, limit of detection, susceptibility to false positives and false negatives, and the effect of common diluents on the ability to identify cocaine. The experimental determination of the limit of detection and the effect of common diluents on the recognition of cocaine HCl used common chemical diluents (e.g., lidocaine, mannitol, and caffeine) as well as common household diluents (e.g., artificial sweetener and baby formula). A positive result for cocaine HCl with the NIK test was indicated by the appropriate color changes and was documented with photographs. A positive result with the portable IR spectrometer was a "hit" for cocaine using the library search function for the instrument. Manual spectral analysis was conducted as well to identify any instrument false positive and false negative results that could be due to the search algorithm used for library matching. Replicates of each analysis were conducted to ensure reproducible results.

This research concluded that although portable IR spectrometers require a large initial financial investment, their high-performance characteristics (e.g., ease of use, rapid analysis, non-destructive, acceptable limit of detection, minimal false positives and negatives) makes them a more superior tool than the NIK tests for the on-scene presumptive analysis of cocaine HCl.

Reference(s):

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Infrared Spectroscopy, Controlled Substance, NIK Tests

B23 An Assessment of Drugs in Syringes From New York City Syringe Exchange Programs

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After attending this presentation, attendees will be able to describe the number and variety of substances in syringes collected from 11 Syringe Exchange Programs (SEPs) in New York, NY.

This presentation will impact the forensic science community by providing data on the use of syringe testing as a surrogate for evaluating the cutting agents and toxic adulterants present in injected drugs through the analysis of methanolic rinses and acid/basic extracts from syringes collected from intravenous drug users between May 2017 and July 2017.

Fentanyl is driving an increase in fatal overdose in New York City, and this study was conducted to estimate the prevalence of fentanyl in the drugs used by SEP participants. An SEP is a service that provides hypodermic syringes and associated injection equipment to injecting drug users at no cost. The goal of the program is a reduction in potential harms to the users, including risks of infection from non-sterile drug injection equipment and the transmission of blood-borne pathogens, such as HIV and HCV.

In addition to their pharmacoepidemiological value as an index of patterns of drug use, drug paraphernalia is frequently submitted to forensic science laboratories for analysis. These exhibits may contain important information regarding the presence of controlled substances, helping in the investigation of crime scenes, drug deaths, and drug possession or trafficking cases in addition to helping characterize the drug abuse scenario and the cutting agents in a specific population.

Three hundred and fifty-six syringes were submitted by the New York City Department of Health and Mental Hygiene for analysis of residue or drug content. Samples were submitted to NMS Labs for an initial screening for the presence of controlled substances. The internal surfaces of the syringes were washed with methanol, and a portion of the methanol wash was used to perform an acid/base extraction to clean up and concentrate the samples. The acid/base extracts were tested by Gas Chromatography/Mass Spectrometry (GC/MS) at NMS Labs. The instrument was operated in the electron impact mode and full scan acquisition (range 40-550m/z), using a ZB35HT column, injection volume of 1µL, splitless mode, injection and detection temperature of 265°C and 300°C, respectively. Total run time was 15 minutes.

The methanol rinses and the remaining acid/base extracts were transferred to the Center for Forensic Science Research and Education (CFSRE) for further analysis by Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). A reverse phase gradient was performed using ammonium formate (10mM, pH 3) and methanol/acetonitrile (50:50) for chromatographic separation on a Phenomenex® Kinetex C18 analytical column at a flow rate of 0.4mL/min for a total run time of 15.5 minutes. Precursor ions were acquired by TOF/MS scan (100-510m/z) via positive electrospray ionization. Precursor isolation was performed using SWATH™ acquisition, consisting of 27 overlapping isolation windows. Fragmentation was achieved using a rolling collision energy of 35±15eV. The acquisition total cycle time was 0.77 seconds. Data processing was performed using PeakView® with an extracted ion chromatogram (XIC) list containing 1,463 compounds, of which 382 had fragment and retention-time data, and accurate mass library containing 1,790 compounds.

Of the 356 syringes analyzed, 270 (75.8%) were positive for any substance. The most common drug identified was heroin (47%), followed by cocaine (40%), fentanyl (22%), methamphetamine (10%) and furanyl fentanyl (3.9%). Caffeine (34%) was the most common cutting agent detected, followed by quinine/quinidine (28%), lidocaine (21%), levamisole (16%), and phenacetin (12%).

The cutting agents used are constantly changing over time and may contribute to the toxic effects of the drugs on users. Levamisole and phenacetin are among the most-used cutting agents and are associated with neutropenia, agranulocytosis, skin necrosis, and nephrotoxicity. Some studies have already reported deaths as a result of complications secondary to levamisole-tainted cocaine.

Knowledge about the drugs used and cutting agents found in paraphernalia from a select drug using population can help to inform patterns of drug use and associated health risks to users, including the rates of exposure to toxic adulterants or highly risky substances, such as fentanyl. The presence of drug combinations, cutting agents, and adulterants may assist in determining common origin from drugs in the possession of different users. The analysis of drug residue in syringes from this population can contribute to a better informed public policy that helps reduce risk for people who inject drugs.

Syringe, GC/MS, LC/qTOF

B24 Narcotic Age and Working Dog Performance: Instrumental Perspectives on Training Aid Lifespan

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After attending this presentation, attendees will better understand the chemicals emitted from both pseudo and real narcotic K-9 training aids evaluated over periods of time and how these aids perform in routine canine training.

This presentation will impact the forensic science community by providing strong, scientific perspectives, implementing both chemical and behavioral studies, concerning the odor concentration levels of K-9 narcotic training aids over time. Canines are the front line of defense in detecting narcotics by police and military working units worldwide. Therefore, this study will further enhance optimum canine detection procedures for national security purposes. The optimal implementation of canine narcotic detection impacts the forensic field by providing a valuable, highly deployable tool in the war against drugs.

There has been little scientific research into the use of narcotic training aids in relation to K-9 performance, even though they are a pivotal part of the training regimen. Many different associations that certify canines as narcotic detector dogs have very few standards as to the optimal lifespan of their training aids. Emerging research is beginning to look into canine detection, but none is specifically looking at the age or lifespan of narcotic canine training aids and their subsequent impact on canine performance.

The goals of this study were to monitor and provide a calibration standard of the target odor vapors emanating from real and pseudo K-9 training aids based on age and to document their training aid alert performance during K-9 field testing. The odor evaluation process consisted of collaboration with the Lubbock Police Department (LPD) Canine Unit and the use of their narcotic training aids that range up to 10 years of age compared to fresh training aids. The study used nine certified narcotic detection K-9 teams for field testing purposes. Instrumental evaluation utilized Divinylbenzene/Carbon/Polydimethylsiloxane (DVB/CAR/PDMS) -coated Solid-Phase Microextraction (SPME) fibers that were injected into a Gas Chromatography/Mass Spectrometry (GC/MS) system for the identification of extracted narcotic headspace odor profiles of heroin, methamphetamine, and cocaine. The LPD narcotic training aids were sampled in individual mason jars for time increments of 15 minutes, 30 minutes, and 1 hour to allow for headspace extraction time optimization. The pseudo narcotic formulations were evaluated in a controlled laboratory setting of storage time ranges of 2 weeks, 4 weeks, 6 weeks, and up to 12 weeks. Evaluation of both abundance and type of target volatiles was performed at each extraction time to measure training aid condition for both real and pseudo narcotics. This was performed in order to document the emission of odor vapors at different time ranges. Field testing of both the real and the pseudo narcotic training aids were then run through routine K-9 detection narcotic training by a double-blind line-up test. The findings include an assortment of chemical compounds emitted from each narcotic exhibiting distinctive odor profiles as a factor of age.

Conclusion and Significance: The benefit this study provides is enhanced knowledge in the realm of optimal canine detection procedures for national security purposes and K-9 detection performance. This research will ultimately bridge a gap in knowledge regarding the odor concentration levels for canine narcotic training aids at various ages and how this age or lifespan impacts practical canine field work, an aspect which has not previously been evaluated.

Canine Detection, Narcotic Odor, SPME-GC/MS

B25 The Classification of Synthetic Phenethylamines According to Structural Subclass Using Multivariate Statistical Procedures

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The goal of this presentation is to demonstrate the application of multivariate statistical procedures for the classification of synthetic phenethylamines according to structural subclass.

This presentation will impact the forensic science community by increasing familiarity with the use of Principal Components Analysis (PCA) to identify ions characteristic of the phenethylamine subclasses and the subsequent use of Discriminant Analysis (DA) to develop classification models based on mass spectral data.

Recently, synthetic designer drugs have become a major concern in the United States. These drugs are synthesized with a slightly different molecular structure than already scheduled compounds to mimic the effects while avoiding legal ramifications; however, definitive identification of these rapidly emerging analogs is often challenging as no reference standard is immediately available to aid in identification.

The study presented describes the development of statistical models that can be used to classify a new analog to a known designer drug class or subclass. The initial work focused on three phenethylamine subclasses: Aminopropylbenzofuran (APB); 2,5-dimethoxy- (2C); and 2,5-dimethoxy-N-2-methoxybenzyl- (NBOMe) phenethylamines. A number of compounds from each subclass were selected to represent the variety of substitutions on the core structure.

Each phenethylamine was analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) and the resulting mass spectra were first subjected to PCA to identify ions that were most characteristic of each subclass. In the PCA scores plot, members of each subclass were grouped together, with clear distinction among the three phenethylamine subclasses. The loadings plot for each PC was then assessed to identify the ions contributing most to the variance described in the scores plot. For example, *m/z* 91, 121, and 150, which are dominant ions in the NBOMe-phenethylamines, were weighted positively on PC1 whereas, *m/z* 44, which is the base peak in the APB-phenethylamines, was weighted negatively. As a result, the NBOMe- and APB-phenethylamines were distinguished on the first PC. The same *m/z* values were weighted positively on PC2 while *m/z* 165, 180, and 197 were weighted negatively. These latter ions are present in many of the 2C-phenethylamines with the result that this subclass was distinguished from the other two subclasses on PC2. Additional PCs were investigated in a similar manner to identify additional characteristic ions.

The ions identified in the PC loadings were used as the variables to develop classification models using DA. To do this, a training set was defined that included phenethylamines representative of the three subclasses. Models were tested using an external test set that consisted of phenethylamines representative of the three subclasses that were analyzed on a different day.

The first model included the nine characteristic ions identified in the first two PCs, which accounted for 46% of the variance in the data set. With this model, the classification success was 79%, with two of the 2C-phenethylamines and two of the NBOMe-phenethylamines misclassified. The second model included 14 characteristic ions that were identified in the first three PCs, which accounted for 56% of the variance in the data set. With this model, classification success increased to 89%, although two of the NBOMe-phenethylamines were still incorrectly classified as 2C-phenethylamines. A third model was developed that included the 18 characteristic ions identified in the first four PCs, which accounted for 64.6% of the total variance; however, despite the increased number of variables, there was no improvement in the rate of successful classification.

This presentation will demonstrate the utility of multivariate statistical procedures for the classification of synthetic phenethylamines. While this research focuses on phenethylamines, the statistical methods can be applied to develop classification models for different classes of drugs to combat the growing problem of new emerging designer drugs.

Synthetic Phenethylamines, Classification, GC/MS

B26 The Development of a Multichannel Paper Microfluidic Device for the Detection of Drugs of Abuse Using Gold Nanoparticle/Aptamer Complexes

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The goal of this presentation is to describe a design of a multichannel paper microfluidic device that provides colorimetric detection based on the formation of a complex of gold nanoparticles and aptamers. Information provided will include the design of the multichannel paper microfluidic device, the multiplexed detection of different controlled substances, and the validation of the multichannel paper chip.

This presentation will impact the forensic science community by demonstrating an application of presumptive detection using a novel paper-based detection method. This new procedure is rapid, inexpensive, and applicable for the detection of multiple seized drugs, including cocaine, codeine, and methamphetamine.

Recently, new types of aptamers have been developed to bind specific drugs of abuse. Aptamers are oligonucleotides or peptide sequences that bind other molecules. This binding can be exploited for detection using various techniques such as colorimetry, amperometry, or surface plasmon resonance. An alternative platform for gold nanoparticle/ aptamer detection based on paper microfluidic devices has been investigated. To accomplish this, a paper microfluidic chip with a multiple-channel design has been created that combines gold nanoparticles and special aptamers into a ready-to-use format. To operate the procedure, samples are dissolved in a carrier solvent in vials, then applied to the paper just before analysis. The high specificity of aptamer binding can result in a useful presumptive test with minimal interferences. These devices are easy to prepare and inexpensive to operate.

Paper microfluidic devices were prepared with a wax-ink printer, thermal laminator, chromatography paper, gold nanoparticles, and aptamers. The melted wax ink creates hydrophilic channels on paper chips. Gold nanoparticles and the developed aptamers are then placed sequentially in each channel. Drug samples dissolved in ionic moving solutions migrate up the channel via capillary action, whereupon they reach a zone containing free aptamers followed by a zone containing gold nanoparticles. If the target drug is not present, the aptamers bind the nanoparticles in a non-specific fashion, and no color change occurs; however, if the target drug is present, the aptamers bind the drug and a salt-induced color change occurs since there are no aptamers left to prevent nanoparticle aggregation. The resultant color change from red to black indicates a positive response. The entire process takes five to ten minutes.

This new microfluidic device permits the development of rapid, inexpensive, and easily operated tests for drugs of abuse. The multi-channel design provides a safe and convenient presumptive tool for the detection of several drugs at once.

Presumptive Drug Testing, Aptamer, Gold Nanoparticles

B27 The Prevention of Occupational Exposure to Fentanyl and Fentanyl-Like Compounds: Elbow Grease and OxiClean™

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The goal of this presentation is to provide information on safely and effectively preventing occupational exposure to fentanyl through a decontamination procedure.

This presentation will impact the forensic science community by providing a method of preventing occupational exposures to fentanyl, which has not been addressed to date.

The current opiate epidemic not only affects the lives of those using the illicit substances; the lives of first responders and evidence custodians are also at risk. There have been multiple occupational exposures in the state of Ohio in the first seven months of 2017. Although the Centers for Disease Control and Prevention has provided useful information regarding personal protective equipment, there is very little information regarding appropriate and effective methods to clean a potential fentanyl spill. While there is one publication regarding the best oxidizers to degrade fentanyl, that study was not practical for various reasons.[1] The present study was designed to account for a working environment and supplies that can be purchased easily. This study also addresses the physical act of scrubbing the area with a standard paper towel in addition to chemical detergents.

Consistent amounts of fentanyl or acetylfentanyl were measured and placed in tape-defined squares on a laboratory benchtop surface. The powdered drugs were then subject to either tap water or a solution of tap water and OxiClean™ Versatile Stain Remover powdered detergent using 5mg in 500mL. This specific type of OxiClean™ uses oxidizers from the Qi study, where other versions may not.[1] Using time points of 0, 15, 30, and 60 minutes, samples were collected after allowing the water or solution to "soak" the drug or after "scrubbing" the area after soaking. The swabs were then soaked and agitated in methanol for 15 seconds and analyzed using a Gas Chromatograph coupled to a Mass Spectrometer (GC/MS). The maximum amount of drug detected by the instrument was calculated to be 200ng based on the amount seeded on the table, the volume injected into the instrument, and the volume split off during method acquisition. This nanogram level of fentanyl is less than the microgram levels that constitute a pharmaceutical dose of the prescription-only opiate. The evaluated spectra demonstrate that scrubbing the area is more effective than soaking at all time points evaluated. Further, OxiClean™ combined with scrubbing the area has more beneficial results compared to water, but both soaking and scrubbing conditions fail when evaporation occurs. For this reason, the area should be cleaned with scrubbing action within 15 minutes of spraying the OxiClean™ solution and the paper towel should be discarded in a biohazardous waste container along with personal protective equipment (e.g., double gloves, disposable laboratory coat, particulate mask) used for decontamination.

Reference(s):

1. Qi, Lihong et al. Oxidative Degradation of Fentanyl in Aqueous Solutions of Peroxides and Hypochlorites, *Defence Science Journal.* 2011, 61(1), pp.30-35, DOI:http://dx.doi.org/10.14429/dsj.61.68.

Fentanyl, Decontamination, Occupational Exposure

B28 Touch DNA in Forensic Science

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The goal of this presentation is to inform attendees about the use of touch DNA.

This presentation will impact the forensic science community by advancing the proposal that touch DNA should be accepted as routine evidence.

The relationship between fingerprint component topography and touch DNA retrieval will have an impact on the forensic science community by increasing the efficiency of touch DNA retrieval from fingerprints. Optimized touch DNA collection methods based on this knowledge will be presented in order to expand their application across the forensic science community.

Touch DNA is a trace amount of DNA left on a surface by a donor who touches said surface and can be found on everyday objects as well as on evidence left at a crime scene. Fingerprints may lead to the identification of a donor through their uniqueness in ridge detail, but they may also be used to identify the donor as touch DNA evidence. Skin cells, eccrine sweat, and sebum comprise fingerprint residue. Though a touched surface may retain cells from the donor individual, cell-free DNA can also contribute to the retrieval of human touch DNA. Microorganisms on the skin also transfer to surfaces. DNA analysts, with improvements in technology and techniques, have the potential to extract a full or partial DNA profile from a single fingerprint from a touched surface; however, several confounding factors can inhibit this potential, such as shedder status of the donor and degradation of the DNA present due to environmental factors.

A thorough survey of the location in which cell-free DNA, whole human cells, and microorganisms reside within a fingerprint was conducted to fully assess the impact of these topographical differences on touch DNA retrieval. To meet this goal, three research objectives were developed and executed: (1) develop a technique for fluorescent and bright field visualization of palmar keratinocytes, cell-free DNA, nuclear DNA, and microorganisms in true fingerprints; (2) assess fingerprint topography before and after DNA collection methods to determine where any lack of collection may be occurring; and, (3) optimize DNA collection techniques on various surfaces and with various collection media based on these results to minimize sample loss.

Due to the complex composition and unique donation of DNA from fingerprint to fingerprint, mock fingerprints containing a known quantity of DNA were developed from buccal epithelial cells to generate standard curves for quantification purposes. Standardizing these mock fingerprints as a positive control for collection alongside true fingerprints allowed for further examination into loss of DNA during collection and extraction. By determining the quantity of DNA retrieved from these mock fingerprints, estimations of extraction efficiencies as well as initial DNA deposited in a true fingerprint can be made.

Both true and mock fingerprints were visualized through staining of various biological components: whole cells, cell-free DNA, nuclear DNA, and microorganisms. The distribution of these components in true and mock fingerprints was gauged before and after collection of touch DNA from glass slides using a variety of collection methods and devices. Once the optimal collection method from glass was determined from these results, mock and true fingerprints were deposited onto/collected from various surfaces, such as wood and paper, and various objects one might receive as evidence, such as a steering wheel or piece of tile. The most successful retrieval methods and collection devices for each respective surface will be presented in order to encourage their use by other forensic analysts and, ultimately, lead to enhanced performance and efficiency in forensic DNA collection.

Touch DNA, Fingerprint, Low Template

B29 Mathematics Takes a Holiday — The Scientific Working Group on DNA Analysis Methods' (SWGDAM's) Imaginative Y Haplotype Guidelines

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After attending this presentation, attendees will understand that the formulas recommended by SWGDAM for Y haplotype evidence are not based on mathematics or on population genetic models, but rather represent confused analogies and cookbook statistics.[1]

This presentation will impact the forensic science community by raising the level of scepticism about trusting expert or committee authority rather than insisting on logic and data.

Mathematical treatment of forensic DNA evidence has some weak spots, the worst being Y haplotypes. SWGDAM's recommendations for computation rest on several confused ideas about mathematics and genetics.

Start with genetics. SWGDAM offers the claim, "It is recognized that population substructure exists for Y-STR haplotypes" to justify an "Eq 3," $Pr(A \mid A) = \theta + (1 - \theta)\, p_A$, adapted from a similar formula in autosomal practice. In the autosomal world θ comes into play to cater to the idea of population substructure from preferential mating — sexual reproduction. But Y haplotypes reproduce clonally, not sexually. There cannot be Y haplotype "population substructure," not in anything like the sense of the autosomal forerunner to Eq 3. The conclusion is that Eq 3 comes about not from clear thinking, but by a careless false analogy that confuses two different senses of "population substructure" — one based on sexual mating, the other on geography or on tribal identity.

What does the guideline mean by θ? The guidelines are vague on that, but reverse-engineering from tables of numbers in an appendix in the guideline reveals that θ is quite close to the average matching chance of two randomly selected haplotypes. The left-hand side, $Pr(A \mid A)$, of Eq 3 must also be on average close to the average matching chance. It would follow that p_A, which purports to be a probability, is negative about half the time!

The guidelines avoid confronting the embarrassment of negative probabilities by bad mathematics: though p_A is meant to be a probability — which is an inference from *data* ("the degree of expectation of its occurrence, which we are warranted in entertaining by our present *evidence*" as the 19th-century thinker JS Mill put it) — the guidelines insist on confusing it with population frequency and on estimating that frequency by a clumsy cookbook statistical procedure which is intended — both misguidedly and unreliably — to be generous.[1]

Hence, the entire process is founded on confusion. Nobody can possibly understand it because there is nothing there to understand. But the above-average analyst, who mistakenly trusts the expertise of the committee, is likely to imagine that the recommendations are fair, useful, and meaningful. The analyst thus gulled testifies in court, unfairly in a Native American context, that the involvement of θ means that the formula takes into account tribal structure. In another case, the pointless generosity means a big break to an offender for no principled reason. Confusion is awkward and expensive — and careless reasoning is bad precedent.

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Y Haplotypes, SWGDAM Guideline, Forensic Mathematics

B30 Using Computers to Overcome Forensic DNA Testing Bottlenecks and Improve Recovery From Complex Samples

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After attending this presentation, attendees will better understand the potential for automation of DNA mixture interpretation and genotype comparison.

This presentation will impact the forensic science community by demonstrating the advantages of automating DNA mixture interpretation with computer technology.

DNA testing has been utilized in criminal investigations for more than 30 years. In that time, technological advances have improved the ability of laboratories to provide information to investigators and the courts. These gains in technology have primarily come through an improved ability to recover and detect alleles present in the DNA sample, but additional gains have come through automation.

A recent survey of the National DNA Index System (NDIS) -participating laboratories demonstrated that nearly all respondents have implemented automation to some degree. In practice, automation tends to be limited to DNA extraction and setup of DNA quantification, amplification, and sequencing plates. While automation has reduced the time required for completions of these steps, the unintended effect is often a backlog in profile interpretation.

Technological improvements have also increased both the number of samples submitted to laboratories and the complexity of the DNA profiles obtained from these samples. Laboratories are now testing many more sample types than when forensic DNA testing was first implemented. While initial DNA testing was restricted to identifiable body fluids, such as semen, blood, and saliva, improvements have led to testing of various substrates, such as articles of clothing or items that have been handled or manipulated. Such items are often referred to as trace or touch DNA evidence. These samples tend to present mixed profiles showing varying numbers of contributors and mixture ratios.

Mixture interpretation in general can be time consuming, and interpretation of mixtures from trace DNA samples may be very difficult and, at times, too complex for accurate interpretation by human analysts. This complexity has in the past led to many results being reported as inconclusive. Those mixtures that can be interpreted by the human analyst may require a significant amount of time for the analyst to capture all the possible genotype combinations for each contributor and even more time for technical review. This bottleneck leads to an overall increase in case turnaround time.

Contamination assessment is another problem. While some software (e.g., Genemapper® ID-X, the Combined DNA Index System (CODIS)) allows for comparison to reference profiles such as laboratory staff or crime scene investigators, these searches are typically restricted to simple allele-to-allele comparisons. Depending on the number of alleles at each locus, the size of the comparison database, and the search parameters, a simple allele-to-allele comparison can lead to a large number of candidate matches, which must all be reviewed by the human analyst. Conversely, if the search is restricted to reduce the number of adventitious matches, then a contamination event may not be detected.

Problems with mixture complexity, interpretation, and contamination assessment can be alleviated through the use of computer technology to automate DNA mixture interpretation and comparison to reference samples. This presentation will detail the methods that a small laboratory has used to automate DNA profile interpretation with the TrueAllele® computer system.

By using TrueAllele® to automate DNA profile interpretation, a human analyst can upload an entire 96-well plate to the TrueAllele® Investigative Database at the end of the workday, return the next morning, and examine the results. Potential matches and exclusions are identified immediately. In addition, the TrueAllele® Investigative Database can identify matches to every evidence profile and reference sample previously tested, and generate investigative leads to cases already worked.

Automated interpretation may also assist the examiner in the inference of genotypes suitable for export to CODIS. The TrueAllele® software will also automatically compare all mixture data to crime scene investigators and laboratory staff, thus giving the analyst the ability to easily identify contamination. By automating DNA mixture interpretation, the analyst's hands-on time can be reduced, more investigative leads may be identified, more CODIS-eligible profiles may be generated, and all DNA mixtures may be assessed for potential contamination.

DNA Bottlenecks, Computer Automation, Investigative Database

B31 A Comparison of Open-Source Software for Assessing the Weight of Evidence in Forensic DNA Profiles

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After attending this presentation, attendees will better understand how known complex mixture samples are treated by different probabilistic genotyping models as implemented in various software tools.

This presentation will impact the forensic science community by providing information concerning how different probabilistic genotyping models treat the same profile.

It has been a challenge, historically, to compare different software approaches and implementations for assessing the weight of evidence. The various approaches use different models, which make different assumptions and are implemented in different ways. This makes it difficult to compare the results between approaches. An additional quagmire is that no true answer exists, so it is not possible to compare results from any model to a static neutral standard. Furthermore, for some time, software that automatically modeled variables such as peak height and stutter was only available as expensive proprietary programs. Finally, training is key to using any of these programs in an optimal way, and the expertise to execute a variety of programs does not exist in one individual or even one group.

Recently, the number and type of open source free-of-charge software programs has expanded to include options for more sophisticated modeling of multiple parameters. Thus, it became possible to perform a reasonable comparison between different models. Part of this study was the recent opportunity to spend time at the special forensic statistics program sponsored by the Isaac Newton Institute for Mathematics at Cambridge University. This time was spent learning and organizing four different software programs for the purpose of comparison. The programs were: Lab Retriever, LRmix Studio, European Forensic Mixtures (EuroForMix) and likeLTD v. 6.1. Lab Retriever and LRmix Studio use discrete models in that the programs only consider nominal alleles, but do not automatically model other parameters, such as peak height and stutter. EuroForMix and likeLTD v. 6.1 model multiple parameters and also use an iterative process to find the best-fit answer.

Another advantage that has recently become available is a set of 164 complex mixture samples created from known contributors, each amplified five times, for a total of 820 samples. This sample set was created as part of a National Institute of Justice (NIJ) and will be made publicly available. The profiles from these mixture samples provide a rich source of material with which to perform informative comparisons between the different software programs. A subset of these samples will be compared to determine how profile complexity affects the Likelihood Ratio (LR) under different approaches. Results from these comparisons will be presented.

Open-Source, Probabilistic Genotyping, Model Comparison

B32 The Development of Polymorphic Combined DNA Index System (CODIS) Short Tandem Repeat (STR) Primers for Unbalanced DNA Mixture Analyses

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After attending this presentation, attendees will understand the advantages of using polymorphic STR primers for unbalanced DNA mixture analyses.

This presentation will impact the forensic science community by detailing the development of polymorphic CODIS STR primers and how these primers can be utilized to detect a minor contributor in a DNA mixture.

Currently, there are several methods available to help analysts assess mixed DNA profiles, including comparison of relative peak heights of STRs, Y-chromosomal Short Tandem Repeat (Y-STR) analysis, or the use of expert software systems; however, these methods have several limitations. For instance, it is often difficult to detect minor contributors in extremely unbalanced mixtures due to Polymerase Chain Reaction (PCR) amplification bias, Y-STR haplotypes being shared by paternal relatives, and the question of whether expert software system analysis violates defendants' rights (e.g., the Confrontation Clause).[1-3]

In the research presented, an alternative method for analyzing DNA mixtures that exploits polymorphic PCR primer sites was explored. Combining a Single Nucleotide Polymorphism (SNP) or an Insertion/Deletion (INDEL) polymorphism with an STR can create a tool suitable for analyzing mixtures when one component is in much lower quantity than the other. In this case, a conserved primer flanks one side of the STR, while the other primer is designed to anneal to a specific SNP or indel allele on the opposite side of the STR. This allows an analyst to specifically target one DNA component in a mixture depending on the polymorphism present. For example, if a major contributor is homozygous for a deletion at one locus, the primer set designed for the insertion would be used to target the minor contributor. Specifically targeting SNP or INDEL alleles absent in the major contributor overcomes the PCR amplification bias that otherwise occurs.[4]

Polymorphic primer sites near the expanded set of CODIS STR loci were identified using the University of California, Santa Cruz database genome browser and allele-specific primer pairs were designed using Primer3 web version 4.0.0.[5-7] PCR conditions were optimized for each primer set by varying cycle number, primer concentration, and annealing temperature using a thermal gradient thermocycler. DNA collected from volunteers was then isolated from buccal swabs, quantified, and amplified in singleplex reactions. When amplification was successful with just one allele-specific primer set for a given locus, the individual was classified as homozygous; successful amplification with both allele-specific primer sets for the same locus was classified as heterozygous. Results were confirmed by sequencing the SNP or INDEL locus.

After the DNAs were quantified and genotyped, mixtures at ratios ranging from 1:10 to 1:1000 were created. DNA from an individual homozygous for an SNP or INDEL associated with a marker was used as the major contributor with input quantities ranging from 1ng to 100ng, and DNA from an individual heterozygous or homozygous for the other SNP or INDEL allele was used as the minor contributor with input quantities as low as 20pg. Using this method resulted in detection of minor contributor alleles via gel electrophoresis at DNA mixture ratios of up to 1:1000. Primers were then labeled with fluorescent dyes for fragment analysis via capillary electrophoresis. Mixtures genotyped with the labeled primers were consistent with known minor contributor alleles.

In conclusion, the availability of polymorphic CODIS STR primers provides a valuable tool for forensic biologists. Successful amplification of the minor contributor will allow the STR genotype to be compared to a reference sample or database, and established allele frequencies for the STR loci can be used to calculate random match probabilities. The development of polymorphic primes will allow analysts to deconvolute and easily apply statistics to unbalanced DNA mixtures that may otherwise be uninformative.

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DNA Mixtures, Polymorphic Primer Sites, CODIS Short Tandem Repeats

B33 An Evaluation of DNA·VIEW® Mixture Solutions Beta Software with Two-, Three-, and Four-Person Mixtures for Forensic Applications

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After attending this presentation, attendees will have a basic understanding of how DNA·VIEW® Mixture Solutions can be utilized for forensic applications such as mixture analysis. Attendees will also understand the impact of this software on DNA mixture interpretation.

This presentation will impact the forensic science community by demonstrating the power, speed, and accuracy of DNA·VIEW® Mixture Solution as a possible tool for forensic mixtures interpretation.

DNA mixture interpretation is a rising issue in the field of forensic science and can be very challenging. Most current software used for forensic mixture interpretation are continuous models that use the Markov Chain Monte Carlo (MCMC) method to analyze mixtures and produce likelihood ratios. These also often require the user to input the number of assumed contributors prior to initiating the analysis of a mixture; a new analysis is required to test a different number of contributors. DNA·VIEW® Mixture Solution is new software that uses mathematical algorithms, rather than MCMC, to determine the probabilities of the evidence under various scenarios. The software calculates the probabilities for multiple prosecution hypotheses (Hp); for example, the probabilities of the evidence given that it includes the Person Of Interests (POI), perhaps a known victim, plus one, two, three, etc., unknown individuals. Similarly, it calculates the probabilities for multiple defense hypotheses (Hd). The probabilities of the most desirable Hp and Hd are then divided to obtain a fair likelihood ratio. The systematic exploration takes the place of guesswork regarding contributors and numbers of contributors.

Previously extracted DNA from four individuals from a Hispanic population was supplied by The George Washington University, Department of Forensic Science. The samples were quantified using the Quantifiler® Duo DNA Quantification Kit. Different volumes of these reference samples were then used to create two-, three-, and four-person mixtures with multiple ratios and diluted down to different DNA amounts for Polymerase Chain Reaction (PCR) reactions (2ng, 1ng, 500pg, 250pg, and 100pg). A total of 75 mixtures were created. The mixtures created ranged from simple to more complex with clear major contributors and the minor contributor remaining the same in all mixed samples. DNA samples, including single-source samples, were then amplified using AmpF*l*STR® Identifiler® PCR Amplification Kit and detected on the 3130 Genetic Analyzer. Short Tandem Repeat (STR) profile analyses were performed with the Genemapper® ID-X version 1.4; a text file was created from each electropherogram data and imported into the latest DNA·VIEW® Mixture Solutions beta software version and interpreted. The software was analyzed on its ability to produce consistent likelihood ratios over several runs, its run time in the analysis of different mixtures, and its accuracy in determining the likelihood of a minor contributor being present in a mixture.

From the data, DNA·VIEW® Mixture Solutions provides consistent and repeatable results for all mixtures. DNA·VIEW® Mixture Solutions was also able to rapidly calculate and (if desired) deconvolve mixtures with times as low as a few seconds for simple two-person mixtures and ranging up to about one and one-half hours for more complex mixtures at low DNA concentrations, though such time may be affected by computational power of the instrument. Finally, the software seemed to accurately represent the compositions of the mixed samples for the most part as complex four-person mixtures with low DNA and would result in very little of the minor contributor being detected.

In conclusion, DNA·VIEW® Mixture Solution is an efficient, fast, consistent, repeatable, and intuitive program that has many potential applications in the field of forensic science and can assist in the efficiency of processing DNA mixtures.

DNA·VIEW®, Mixtures, DNA Contributors

B34 An Evaluation of DNA Results With Propositions at the Activity Level: How to Identify the Features That Influence the Bayes Factor — An Example With a Stabbing Scenario

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The goal of this presentation is to demonstrate how to identify the features that influence the Bayes factor using a Bayesian Network, allowing the evaluation of DNA cases at the activity level. This allows the identification of the type of experimental studies that are needed in a specific case and will help the scientist focus on a limited number of variables of interest. This presentation illustrates the variable of interest for a specific stabbing scenario.

This presentation will impact the forensic science community by assisting attendees in evaluating DNA cases at the activity level.

Traces with low levels of DNA can be the result of a secondary transfer or even a tertiary transfer. Consequently, factors such as transfer, persistence, recovery, and background need to be considered. Scientists should consider their biological results given activity-level propositions. The issue here is that many experts do not feel they can do so. One of the reasons is that each case has its own features. Can numerical values from experimental studies performed under controlled conditions be used for evaluation in real-life cases? Experiments allowing a wide variety of options for any unknown specific feature should be conducted, but it is difficult to design an experiment that takes into account all possible variations of all factors; however, the most significant fact is not that the feature varies but whether the variation of the feature has an impact on the value of the results (i.e., Bayes factor).

The goal of this review is to illustrate how to identify the features that influence the Bayes factor using a Bayesian Network. This permits identification of the type of experimental studies needed in the case at hand. It helps scientists focus on a limited number of variables of interest, helping them to evaluate DNA results at the activity level. Cases presented as demonstrations are cases in which the main proposition is that the person of interest stabbed the victim and the alternative propositions are either that the person of interest shook the hand of the real offender or that the person of interest has nothing to do with the stabbing or the offender (i.e., an unknown person stabbed the victim).

Results demonstrate that in both situations the greatest impact on the value of the results were extraction efficiency, sampling efficiency, proportion of contact between hand and target surface, transfer proportion, type of surface, and type of contact. The background, the environmental conditions, the quantity of DNA on the person of interest's hand and on the alternative offender's hand have the least impact.

Considering the alternative proposition, "The person of interest has nothing to do with the stabbing, an unknown person stabbed the victim," the most impactful feature is the conditional match probability. This feature has no impact on the value of the results under the other alternative proposition (i.e., the person of interest shook hands with the actual offender).

For a case, once extraction efficiency and sampling efficiency have been established and the type of surface and of contact are known and instantiated, the features of interest remain proportion to contact and transfer proportion.

Activity, Bayesian Network, DNA

B35 A Cost-Benefit Analysis of Kinship Testing Involving Siblings and Half Siblings

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After attending this presentation, attendees will understand the current status of kinship testing in forensic science, the costs and benefits associated with testing an increasing number of siblings in order to more accurately establish a biological relationship, and the effect of substituting half siblings for full siblings for the identification of a biological relationship in question.

This presentation will impact the forensic science community by improving knowledge regarding kinship testing involving siblings and by informing a variety of agencies of the most cost-effective approach for kinship testing involving full siblings and half siblings. The results from this study will be utilized to inform the Department of Homeland Security of the costs and benefits of testing multiple members of a family and will indicate if this practice can offer greater success in the identification of a biological relationship. Depending on the results of this research project, some agencies and organizations may alter their current practices to attain a higher success rate for the identification of biological relationships in question.

A DNA kinship test assesses the relatedness between two or more individuals. A kinship test is used as a method for confirming the presence of a biological relationship between two individuals for immigration purposes, parentage testing, forensic casework, and the identification of victims of mass disasters; however, the testing of siblings in kinship testing can become complicated, since siblings can share between 0% and 100% of their DNA. Since there is a high level of variation among the amount of DNA shared between two siblings, this research investigates whether kinship testing should be performed on more than two siblings when possible. Additionally, the effect of substituting half siblings for full siblings is examined in order to assess if less information is obtained using half siblings in a kinship test. With this information, the cost-benefit relationship of utilizing an increased number of siblings in kinship testing was examined. Many factors, such as the cost of the DNA testing, the access to more than two family members for kinship testing, and the variation in the amount of DNA shared between siblings, are discussed in the examination of this cost-benefit relationship.

In order to study this cost-benefit relationship, 415 known DNA samples sourced from the Applied Genetics Technology Corporation (AGTC) in Denver, CO, were provided by the Department of Forensic Science at The George Washington University. The samples were collected from 96 total families from Caucasian, Hispanic, African American, and Asian populations, with 24 families belonging to each ethnic group. The previously extracted DNA samples were quantified using the Quantifiler® Duo DNA Quantification Kit and amplified using the GlobalFiler™ and VeriFiler™ Direct Polymerase Chain Reaction (PCR) amplification kits. The samples were analyzed using an Applied Biosystems® 3130 Genetic Analyzer and GeneMapper® ID-X software version 1.4. Using the likelihood ratios obtained from statistical calculations between the biological full siblings and half siblings, the strength of the biological relationship in question was determined. By comparing the likelihood ratio calculations between siblings, it was determined whether the biological relationship is strengthened when using an increasing number of siblings in the kinship test, and if there is a significant difference in the strength of a biological relationship when substituting half siblings for full siblings.

This presentation will provide a cost-benefit assessment of kinship testing using multiple siblings, will examine if using a greater number of siblings in kinship testing yields a more accurate identification of a biological relationship, and will determine the effect of substituting half siblings for full siblings. With this knowledge, more cost-effective approaches for kinship testing involving siblings can be adopted and practiced.

This research was funded by the Department of Homeland Security.

Kinship Analysis, Sibship Testing, Siblings

B36 Science Matters: Using DNA to Solve Missing Persons Cases in New York City

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After attending this presentation, attendees will better understand that many of the human remains for which a DNA profile is successfully obtained would still remain unidentified without the awareness of families and their willingness to provide reference samples for comparison. Attendees will hear success stories from a Missing Persons Day and gain insight into the ability of this event to provide resolution to missing persons cases.

This presentation will impact the forensic science community by providing vivid examples of success when the power of a DNA database is fully exploited. Many DNA profiles in the database belonging to unknown individuals would remain unidentified without families of the missing being made aware of the need to submit reference samples. Identifying a missing person not only provides resolution to the family but also creates leads in cases in which foul play or violence is suspected in the disappearance of the missing person.

There are nearly 100,000 active missing persons cases in the United States. Potential success in connecting these cases to Unidentified Human Remains (UHRs) is often dependent on the awareness of families and their willingness to provide DNA reference samples for comparison. The collection of family samples may sometimes be the only way to identify unknown remains that pass through the nation's mortuaries with nothing more than a DNA profile as a possible pathway to identification. Attendees of this presentation will gain insight into the ability of a special event, Missing Persons Day, to provide resolution to missing persons cases.

New York City maintains a massive 100-acre cemetery on Hart Island that is used for burying the indigent, unclaimed, or unidentified. The impetus for conducting DNA testing on the city's UHRs came from the success of DNA methods developed in response to the terrorist attacks of September 11, 2001. State-of-the-art techniques for testing bone were used to obtain DNA profiles from samples that would have otherwise been difficult to recover. This novel protocol has since been applied to thousands of bones from UHRs.

Many of the UHRs for which a DNA profile is successfully obtained would still remain unidentified without the willingness of families to provide samples for comparison. In 1975, a teenage male disappeared. Although a police report was filed, no DNA reference samples were collected because the case preceded DNA testing. The next year, a trash bag with human remains was recovered from a highway shoulder in a neighboring state. When bones from the trash bag were tested for DNA years later, the profile was uploaded to the national DNA database. In 2014, New York City initiated an annual event called Missing Persons Day, designed to connect families of the missing with resources and to provide an opportunity to submit DNA samples. One of the attendees at this event was a relative of the missing teenage male. When the relative's sample was collected and the profile uploaded to the database, it revealed kinship to the profile obtained from the bones in the trash bag.

There are many instances in which identifications have been made that would have otherwise been impossible without the ability of the Missing Persons Day event to raise awareness of the need for families of the missing to submit reference samples. In 2003, a man from one of New York City's outer boroughs went missing. Shortly thereafter, skeletonized remains were found in a suburban area approximately 30 miles away. There was no investigative progress and both cases remained unsolved for years. In 2014, the children of the missing man attended the Missing Persons Day event and submitted reference samples. The DNA profile from the children showed kinship to the profile developed from the skeletonized bone whose source was determined to be their father.

The Missing Persons Day event is billed as a humanitarian effort in which attendees are given the opportunity to visit a laboratory that is managed by the city's Department of Health and be connected to police detectives, chaplaincy services, mental health counselors, and forensic scientists. Conducting the event at a venue not associated with law enforcement is particularly important in urban areas, where potential attendees could have skepticism of the police or fear of repercussions regarding their own criminal history or immigration status. In 2013, a male in his 30s was crossing the United States-Mexico border illegally when he lost contact with family. The next year, his family attended the Missing Persons Day event and submitted reference samples. The DNA profiles obtained from these samples revealed kinship to a bone that was recovered in the desert near the border.

Since its inception in 2014, the New York City Missing Persons Day has aided in eight identifications. These are cases that would have remained unsolved without the willingness of families to submit DNA samples as part of the search for their loved ones. The full potential of the national DNA database is realized only when events such as Missing Persons Day make families aware of the need to provide samples for comparison. Identification of a missing person may also provide valuable leads in cases in which death of a UHR is ruled as homicide.

Missing, DNA, Bone

B37 The Evaluation of the Effects of Linked Markers on Kinship Testing

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After attending this presentation, attendees will better understand the implications of linked loci in kinship calculations and how best to approach such loci in familial relationship testing.

This presentation will impact the forensic science community by demonstrating to what degree three sets of linked loci (vWA and D12S391, CSF1PO and D5S818, and SE33 and D6S1043) are linked and by illuminating the potential impact of treating these as independent instead of linked. The results of this research may impact the processes involved in familial kinship testing for both domestic and foreign immigration cases by more accurately depicting pedigrees.

Kinship testing cases are common practice and important in many immigration cases globally. The kits used to analyze the DNA in such cases vary, but very often will contain linked markers. Linked markers are used occasionally in forensic DNA analyses, including kinship calculations, often with little regard for their potential impact on the calculations. The simple product rule should not be used with linked markers that are shown to not segregate independently. What should be considered is the diplotype frequency with its own frequency for recombination based on a diplotype database.

DNA from previously extracted samples from 96 families with multiple children (both full and half sibling children) from four ancestry populations (European, Asian, Hispanic, and African) from past paternity cases were supplied by The George Washington University, Department of Forensic Science, sourced from the Applied Genetics Technology Corporation (AGTC) in Denver, CO. These samples were quantified using Quantifiler® Duo, amplified using GlobalFiler™ and VeriFiler™ Direct PCR amplification kits, run on an Applied Biosystems® 3130 Genetic Analyzer, and typed using GeneMapper IDX® software version 1.4.

The results of the genetic typing were exported into Excel® where allele and diplotype frequencies were calculated for each population of n=24 families per population. Additionally, recombination frequencies were calculated at each linked diplotype that were further utilized to determine if they exhibited linkage disequilibrium.

To demonstrate the impact of using these loci in conjunction with the product rule for kinship cases, familial relationships likelihoods were calculated assuming independent assortment of the genes. These relationship likelihoods were then compared to relationships calculated by accounting for linkage to show the true impact of not accounting for linked loci.

This presentation will discuss the level of linkage between vWA and D12S391, CSF1PO and D5S818, and SE33 and D6S1043, demonstrate the impact of ignoring the linkage disequilibrium between the loci pairs, and discuss more efficient ways of approaching the use of linked loci in familial testing.

Research Funded by The Department of Homeland Security.

Kinship, Linked Markers, Diplotypes

B38 Short Tandem Repeat (STR) Profiles and Ethnic Affiliation — Chemometric Evaluation

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After attending this presentation, attendees will better understand an alternative approach for the interpretation of ethnic affiliation from unknown DNA trace evidence.

This presentation will impact the forensic science community by providing an alternative method for the estimation of ethnic affiliation with a proposition stemming from the chemometric evaluation of the data.

The issue of ethnic affiliation may arise in a forensic context. For instance, the determination of the race of a donor of a stain, left at the crime scene, may be thought to ease the investigation by contrasting such evidence with an eyewitness account. The DNA typing is a powerful tool in the determination of the identity since the majority of genetic variation is ascribed to inter-individual differences. Although the contribution of population differences is quite small to the overall variability, the genetic profiles have been used to infer the population of origin.[1,2] As the Short Tandem Repeat (STR) microsatellites are still intrinsic to the forensic domain, the present study focuses on the use of STR profiles as the basis for inferring ethnic affiliation. In the course of the past two decades, a few approaches have been presented. The methods implemented in the population genetics software usually follow the profile frequency approach, procedures based on the genetic distance, or the approaches that use tools derived from the multivariate data analysis methods. The latter two are said to be free of considering the Hardy-Weinberg (HW) and Linkage (L) equilibriums.

The goal of this study is to contrast those ethnic affiliation approaches with the proposition stemming from the chemometric evaluation of the data. For the purposes of the present study, the STR profiles were generated from the allele frequency tables available for different populations. As the derivation of the STR profile depends on the set of markers, this study inspects the usefulness of different, used-in-daily-practice sets (e.g., the Combined DNA Index System (CODIS), the European Standard Set (ESS), and the International Society for the Study of the Origin of Life (ISSOL)). For a fair comparison of models, the test set of profiles was held out for testing models developed on the training data. The ethnic affiliation methodology for each approach was to be set in the Bayesian framework to enable the forensic weight of the evidence assessment. Different chemometric approaches were inspected and evaluated.

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Ethnic Affiliation, Chemometrics, Unknown DNA Trace Evidence

B39 A Statistical and Allele Frequency Evaluation on the Methods of Kinship Calculations

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After attending this presentation, attendees will be better informed regarding the variations found in the statistical calculations used by commonly utilized DNA kinship analysis programs.

This presentation will impact the forensic science community by demonstrating that the variability present in statistical calculations of kinship analysis programs can have effects on kinship determination.

Kinship analysis of Short Tandem Repeat (STR) profiles is often used in cases of parentage testing. Kinship analysis programs calculate the likelihood ratios of paternity, maternity, and sibship using allele frequency databases. It is important to determine the extent of variation between numerical precision within these programs since small variances in value can yield different analyses and conclusions. The first portion of this presentation reports on the differences in numerical precision of these programs.

The choice of race-specific allele frequency databases brings additional variation in statistical calculations of kinship. Race-specific allele frequency databases provide for the genetic similarities and differences found within racial groups and become important for kinship analysis due to the expected genetic similarities of individuals within a biological family. Accuracy in kinship analysis is especially relevant in cases involving half siblings due to the increase of genetic dissimilarity. The second half of this presentation focuses on how the use of a race-specific allelic frequency database can affect accuracy in determining sibship between related individuals.

To identify the effects of using race-specific allele frequency databases for sibship determination, an analysis was conducted on the Short Tandem Repeat (STR) profiles collected from 96 families comprised of 415 individuals. The samples were sourced from the Applied Genetics Technology Corporation (AGTC) in Denver, CO, and were provided by the Department of Forensic Science at The George Washington University. The profiles were obtained from DNA samples collected from prior paternity and immigration testing and were self-defined as belonging to Asian, African American, Hispanic, or Caucasian backgrounds. Twenty-four families were chosen from each racial group to ensure equal representation within the data. The samples were quantified with Quantifiler® Duo DNA Quantification Kit, amplified with GlobalFiler™ and VeriFiler™, and analyzed using an Applied Biosystems® 3130 Genetic Analyzer and GeneMapper® ID-X software. Kinship analyses for the families were conducted on the software DNA•VIEW®. Sibship calculations yielded likelihood ratios that compared the likelihood of sibship with the likelihood of no biological relationship between the individuals. Four sibship likelihood ratios were calculated from four race-specific allelic frequency databases (Asian, African American, Caucasian, and Hispanic databases) for each sibship case, regardless of the individuals' reported backgrounds.

Preliminary analysis of the results conducted with DNA•VIEW® reported accurate determination of sibship for all cases involving full siblings and half siblings. In the cases determining full sibship, 94% demonstrated likelihood ratios as expected by race, with the true racial background of the family members showing the least discrimination. In cases determining half sibship, 50% demonstrated likelihood ratios expected by racial background. A chi-squared analysis of each case was conducted, and all cases showed no independence ($p \leq 2*10^{\wedge -18}$) between the likelihood ratios reported from the four race-specific allelic databases, thus demonstrating a significant difference in allele frequency values between the databases.

The results from the sibship analyses suggest that race-specific allelic frequencies have a diminishing effect on discriminating less related individuals versus more related individuals and supports prior research that half sibling analysis require highly discriminatory loci for accurate sibship determination.

Overall, this two-part evaluation of statistical calculation and allele frequencies seeks to inform the public of numerical variations involved within kinship programs and reaffirms the importance of race-based data for accuracy, allowing for the development of a more standard approach to kinship analysis.

Kinship Analysis, Statistics, Race

B40 The Development of a Biogeographic Ancestry and Phenotype Single Nucleotide Polymorpism (SNP) Panel Using Ion Torrent™ Chef System

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After attending this presentation, attendees will have a better understanding of the performance of the Ion Torrent™ Chef Next Generation Sequencing (NGS) system and development process of an NGS panel for SNP.

This presentation will impact the forensic science community by providing results from a novel development of an NGS panel for SNP in order to predict biogeographic ancestry and phenotype of an individual. This presentation will also increase understanding of the practical utility and benefits of the NGS technology.

It is essential to perform fast and accurate identification of all types of traces that belong to mass disaster victims or unknown biological evidence collected from crime scenes. Only gender identification can be achieved by using standard STR kits. Additional physical characteristics could provide important information regarding suspects/individuals. Recently, SNP markers have been used for identification, determination of phenotype, and prediction of ancestry. "Molecular eyewitness" is defined as the determination of the suspect's or individual's population origin by using Ancestry Informative SNPs (AISNPs) and the determination of the suspect's or individual's physical characters by using Phenotype Informative SNPs (PISNPs). Since there are a few studies related to geographic region that includes the interest region of Southwest Asia, it is essential to study novel and informative markers for accurate ancestry assignment for this region. Hence, the objective of this study was to develop an SNP panel on Next Generation Sequencing (NGS) devices for use in predicting biogeographic ancestry and phenotype (i.e., eye, hair, and skin color) of an individual.

In this developed panel, this study sought to determine a set of AISNP markers that could be used to differentiate Southwest Asia and Mediterranean regions from Europe and other continental regions; therefore, SNPs were selected from previous research.[1-3] The set of PISNP markers was integrated into the set of AISNP markers in order to develop one reaction system on the NGS instrument. PISNPs were used to determine physical characteristics such as eye, hair, and skin colors and were chosen from previously published papers.[4-6] As a result of this study, a biogeographic ancestry and phenotype panel, including 160 SNP markers, was developed by using the Ion Torrent™ Chef instrument. The optimization and validation of the system was performed by using commercially available reference samples (e.g., 9947a, 007). Sensitivity and reproducibility parameters were determined for the optimization and the validation of the Ion Torrent™ Chef panel.

In conclusion, the developed NGS SNP panel, which can be used for predicting the biogeographic ancestry and physical characteristics of an unknown, will contribute to the direction of the investigation and to clarify the incident quickly for unknown contributors, cold cases, or the identification of missing persons and disaster victims. In this presentation, results from the NGS panel will be presented in addition to additional evaluations of individual prediction results using statistical regression and Bayesian methods for the forensic use of this panel.

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Biogeographic Ancestry, Phenotype, Ion Torrent™ Chef System

B41 The Utility of the Precision ID Ancestry Panel for Predicting Ancestry From High-Quality and Forensic-Type Samples

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After attending this presentation, attendees will understand that a commercially available panel of Single Nucleotide Polymorphism (SNP) markers can be used to effectively predict the ancestry of known samples. Attendees will learn that genotype accuracy can be examined by testing congruence between the manufacturer's software and a third-party, well-accepted software. Attendees will also learn the impact of DNA degradation on ancestry predictions using this panel and the manufacturer's software.

This presentation will impact the forensic science community by providing the results of an initial assessment of the novel Precision ID Ancestry Panel for determining genotypes from samples of differing ancestry. This presentation will provide valuable information as to the applicability of the panel for forensic-type samples and the potential limitations of using this panel as tool for investigative leads.

Human individualization is typically accomplished by analyzing Short Tandem Repeats (STRs); however, in cases in which only a partial or incomplete STR profile is obtained, SNPs could provide valuable information to aid the investigation by providing information on biogeographic ancestry. Thermo Fisher Scientific, which developed the high-throughput Ion Torrent™ PGM™ sequencer, released the Precision ID Ancestry Panel, a 165-SNP panel for forensic ancestry prediction.

This study was directed at assessing the accuracy, reproducibility, and sensitivity of this novel panel and with the ability to provide accurate ancestry predictions for: (1) seven high-quality DNA samples that represent the three major ancestries of forensic interest in the United States (Hispanic, Caucasian, and African American); and, (2) forensic type samples, such as a toothbrush, bone, hair, shaving razor, cigarette butt, and nail clippings (*n*, 9). Libraries were prepared in triplicate using 0.2ng, 0.5ng, and 1.0 g DNA as input for the high-quality DNA samples (*n*, 63), and in duplicate where possible for the forensically relevant samples using 0.05ng–1.0ng of DNA (*n*, 39). Data was analyzed using the manufacturer's Human Identification (HID) SNP Genotyper plug-in (v.4.3.1) as well as CLC Genomics Workbench. Only 2% of all possible Quality Control (QC) flags were raised for the high-quality samples by the plug-in QC filter; 59% of these flags were due to the major allele frequency being outside the manufacturer's defined thresholds. A total of 9.8% of all possible flags were raised for the forensic type samples by the plug-in QC filter; 45% of the flags were due to locus drop-out.

A simulated degradation study was also conducted using the data generated from the seven high-quality samples prepared using 1.0ng of DNA. Data was divided into SNP subsets based on known amplicon lengths and commonly observed degradation lengths (i.e., SNPs with amplicon lengths <50bp, <75bp, <100bp, and <200bp), and ancestries subsequently predicted for each subset using the FROG-kb database (http://frog.med.yale.edu/FrogKB/). Incorrect ancestry predictions did occur in approximately 20% of the samples, primarily when Ancestry Informative Single Nucleotide Polymorphisms (AISNPs) with amplicon lengths <75bp were used in analyses. Even though the forensic type samples had more flagged SNPs than the high-quality DNAs, 72% of samples still had concordant ancestry predictions between replicates, demonstrating that this panel has the potential to be used in forensic casework with further testing.

SNP Typing, Ancestry, NGS

B42 Enhance the Power of Discrimination of Semen Identification by a Combination of Microfluidic Chips and Erase Kits

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The goal of this presentation is to overcome the problems of the differential extraction method by using the microfluidic chip technique combined with the Erase Sperm Isolation Kit (combination method).

This presentation will impact the forensic science community by reducing the interference of female DNA in the isolated sperm DNA and enhancing the power of discrimination in the semen identification.

A key point of forensic examination is how to effectively locate sperm in sexual assault case evidence. Practically, the differential extraction method was applied to isolate male DNA from the mixtures of sperm and epithelial cells; however, this is a time-consuming and less effective process. Currently, the Erase Sperm Isolation kit, a commercially available reagent, hydrolyzes cell-free female DNA before extraction of sperm DNA to reduce contamination of female DNA in isolated sperm DNA. Additionally, the microfluidic chip technique can separate sperm from the mixtures of sperm and epithelial cells according to the differences of density, size, and settling rate between sperms and epithelial cells.

The goal of this study was to overcome the problems of the differential extraction method by using the microfluidic chip technique combined with the Erase Sperm Isolation kit (combination method). Five semen samples and two buccal swabs were collected from adult men and women, respectively, as were two mixtures prepared with the ratios of sperm and epithelial cells of 1:1 and 1:3, respectively. These mixtures were stored at room temperature for 1, 3, 7, and 14 days, then their DNA profiles were analyzed. The combination method presented excellent results — the ratios of complete Short Tandem Repeat (STR) DNA profiles (15 loci, without female DNA interference) of sperm DNA were approximately 80% and 60% in the 3 and 14 days, respectively, whereas the results using the differential extraction method were less than 30%, accompanying more than 60% of interference of female DNA, in all time periods. These data indicated that the combination method can greatly decrease the female DNA interference in STR DNA profiles. The effectiveness of the combination method with nine forensic specimens that were positive for semen stains was also examined. The data of STR DNA profiles revealed that interference of female DNA was observed in only one case; however, only two cases were obtained in the complete STR DNA profiles. The possible reasons may that the amounts of isolated sperm DNA were too small to obtain the complete STR DNA profiles. In contrast, sperm DNA isolated by the differential extraction method presented high interference of female DNA (seven cases), and only one case of STR DNA profile was complete (15 loci, without female DNA interference). Taken together, these results suggested that the combination method can greatly reduce the interference of female DNA in the isolated sperm DNA and enhance the power of discrimination in semen identification.

Semen Identification, Microfluidic Chip, Differential Extraction

B43 Investigating the Impact of Protein and Peroxidase Blood Enhancement Reagents on DNA Recovery From Laundered Clothing

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After attending this presentation, attendees will better understand the use of protein and peroxidase reagents for blood detection and their potential effect on DNA recovered from laundered clothing. Additionally, attendees will gain insight into the potential transfer of DNA evidence from one article of clothing to another during the laundering process.

This presentation will impact the forensic science community by providing a comprehensive analysis of the effects of six blood enhancement reagents on DNA recovery from laundered clothing, allowing forensic examiners to make more informed decisions when analyzing this difficult type of evidence. This research will serve as an advantageous resource for forensic science professionals to use when deciding the type of enhancement reagent to use given various fabric compositions.

Blood is a commonly encountered biological fluid in criminal investigations concerning a violent incident, and visual traces of the fluid on a suspect's clothing can be diminished through laundering. Despite the potential to arouse suspicion and help reconstruct a crime, the mere presence of blood on laundered clothing is not often sufficient enough to make conclusive inferences about the nature and circumstances of a specific person's involvement in a crime. Because of this, it is essential that a method exists that both enhances a forensic examiner's ability to visualize a dilute bloodstain on a piece of laundered fabric while maintaining and preserving the quality of the DNA evidence that may be present. This study proposes to analyze the effects of laundering and the application of commercially available blood enhancement reagents commonly used to improve visualization of dilute bloodstains on DNA recovery.

Following Institutional Review Board (IRB) approval and informed consent from volunteers, venous blood was collected in sterile vacutainer EDTA vials. Six commonly used and commercially available enhancement reagents were chosen: Hungarian Red, Coomassie Blue, Amido Black, luminol, Bluestar® Forensic Magnum, and aqueous Leuco Crystal Violet (LCV). Then 100µL of human blood was deposited onto cotton, polyester, denim, and wool in triplicate, and these samples were laundered under standard washing conditions with blank controls. Following laundering, a selection of samples from each fabric type was enhanced with each of the six reagents. DNA was extracted from these samples using a QIAamp® DNA Investigator Mini Kit and quantified using a NanoDrop™ OneC Ultraviolet/Visible (UV/Vis) spectrophotometer.

Following laundering and enhancement, quantifiable amounts of DNA originating from bloodstains were obtained from all fabric types. Although washed blood samples often had a lower DNA recovery than unwashed blood samples, all untreated washed samples had a suitable DNA recovery, ranging from 0.9ng/mL to 38.2ng/mL. Similarly, samples treated with all enhancement reagents except Amido Black had DNA yields higher than the washed blank samples that ranged from 1.1ng/mL to 23.0 ng/mL. Despite this, measurements indicated that the application of some blood enhancement reagents, particularly Amido Black, may affect DNA recovery. Across all fabric types, samples treated with Amido Black had a low DNA recovery comparable to blank samples, returning yields as low as 0.7ng/mL on cotton samples. Across the board, washed blank fabric samples had a higher amount of recovered DNA than the unwashed blank samples; however, these samples were treated exactly the same with the exception of laundering. While unwashed blank samples had an average DNA yield of 3.57ng/mL, washed blank samples had an average DNA yield of 8.59ng/mL. Because of this, it is suggested that cross transfer of DNA between samples during the laundering process is possible.

Despite these results, the NanoDrop™ OneC UV/Vis spectrophotometer does not provide differentiation between human-specific DNA and DNA from other sources. Although unlikely that other DNA sources are largely contributing to the quantities of DNA present within the sample, it remains a possibility that not all of the quantified DNA yield is representative of human-specific DNA that would be probative during a criminal investigation.

This study highlights the importance and value in choosing enhancement reagents that enhance bloodstains while maintaining the integrity of critical DNA evidence on laundered clothing. The results provide a crucial resource for forensic investigators and future researchers to aid in the utilization of laundered fabric evidence.

DNA, Laundered, Enhancement Reagents

B44 Investigating Novel Methods for Estimating Time Since Deposition (TSD) of Bloodstains in Forensic Samples

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The goal of this presentation is to inform attendees of three techniques for estimating the TSD of blood in relation to forensic cases.

This presentation will provide the forensic science community with insight into multiple methods that exhibit potential to estimate the age of bloodstains. These include the measurement of enzymatic activity, protein concentration, and the degradation ratio of two ubiquitously expressed RNAs.

Blood is the most commonly encountered biological fluid found at violent crimes and can provide probative evidence in terms of DNA profiles and pattern analysis; however, these stains can also reveal previously untapped potential in determining the time of deposition of a bloodstain resulting from a trauma or event. The ability to estimate TSD of bloodstains has been researched in the past utilizing many different methods, yet results varied with no complete agreement on one method for implementation into real casework. The goal of this study was to investigate a variety of methods which exhibit potential for TSD estimation. These include investigating over time, enzyme activity, the quantification and spectrophotometric observation of total protein, and the degradation of two RNA species.

Following Institutional Review Board (IRB) approval, venous blood was collected from volunteers with informed consent into sterile EDTA vacutainer tubes. Then 100µL of blood was deposited on white cotton cloth, in triplicate, and allowed to age in a cool, dark environment for 24 hours, 48 hours, 1 week, 2 weeks, 1 month, 3 months, and 6 months. Enzyme activity of Alkaline Phosphatase (ALP) was determined using a colorimetric reaction that was measured using a Nanodrop® OneC Ultraviolet/Visible (UV/Vis) spectrophotometer. Total protein was extracted, quantified, and viewed spectrophotometrically using the UV/Vis spectrophotometer. Total RNA was extracted using the RNeasy Mini Kit, quantified, and expression analysis was performed using Real Time-Polymerase Chain Reaction (RT-PCR) targeting beta-actin and 18 S RNA.

After spectrophotometrically observing the enzymatic activity of ALP, the concentration was determined using a standard curve. It was found that from fresh blood to six-month blood the concentration dropped from 178.9U to 32.36U. When examining the amount of quantified total protein, it was found to have not decreased as drastically, ranging from 5.912mg/mL to 4.981mg/mL in the same six-month period. When each sample is spectrophotometrically observed, three specific peaks are seen at 412nm designated λ, 541nm designated β, and 576nm designated α. These peaks have historically shown correspondence to the derivatives of hemoglobin and decrease in parallel with the conformational changes that correspond to hemoglobin's degradation. The most specific change is found between 541nm and 576nm. These two separate peaks begin to fuse into one smaller peak, relating the eventual conformational change of hemoglobin into hemichrome. The most pronounced peak, found at 412nm, remains present over time; however, it diminishes from an absorbance of 1.52 to 0.50 over the six-month period. Quantifiable amounts of total RNA were extracted from all samples ranging from 22.159ng/µL to 7.000ng/µL, with no real trend of an increase or decrease over time observed. Both Beta-actin and 18S RNA were detected in all samples and have shown a general trend to decrease in expression over time.

This study has shown, using several different methods, the great potential present in the ability to estimate the TSD of bloodstains. Further research is yet to be conducted; however, the results obtained thus far show great promise for the future. It is believed that not one single method will provide the answer; rather, the utilization of multiple methods in concert with each other will ultimately provide the investigator with greater accuracy.

Aging, Blood, Degradation

B45 Isoalleles Revealed by Massively Parallel Sequencing (MPS) Provide Increased Resolution and Discrimination in Forensic Casework

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After attending this presentation, attendees will understand what isoalleles are and how they can be used in forensic casework.

This presentation will impact the forensic science community by demonstrating the power MPS holds for increased discrimination in forensic casework.

Short Tandem Repeat (STR) typing by Capillary Electrophoresis (CE) is the standard for DNA processing in forensic laboratories; however, this method is limited in the amount of information that it yields for analysis by only providing length-based information from STRs. In contrast, STR typing by MPS yields both size- and sequence-based information. This ability makes it possible to detect isoalleles (alleles that are homozygous by length but heterozygous by sequence). Because these are sequence-based differences, they are not observable in CE-based STR assays. Not only can sequence variation between individuals at the same loci be revealed, but this kind of diversity is often observed at one or more alleles in a DNA profile.

Battelle and the Ohio Bureau of Criminal Investigation (BCI) have collaborated to evaluate a customized MPS workflow utilizing the Promega® PowerSeq™ Auto/Y prototype amplification kit and the Illumina® TruSeq® DNA PCR-Free library preparation kits for sequencing on the Illumina® MiSeq® instrument. Sixteen buccal swabs (extracted and quantified) and 49 database samples (directly amped) were processed with this workflow and analyzed for the presence of isoalleles. In this study, 17 of 65 samples displayed at least one isoallele. The most common locus displaying isoalleles was D3S1358, a locus with a compound repeat, where it was observed in five individuals. Isoalleles were also observed in four samples for locus D21S11, which is a complex locus. The sample with the highest number of isoalleles contained three at different loci within the profile.

Due to the relatively common appearance of this characteristic during this study, isoalleles present a unique potential for resolving contributors of mixed samples. Several mixtures at different ratios were prepared and sequenced. Analysis of these mixtures revealed that isoalleles are able to yield valuable information when attempting deconvolution of MPS mixture data. In one mixed sample, there were three loci where the contributors to the mixture showed isoalleles. There was an additional locus where the sequence data allowed the allele type of the minor contributor to be differentiated from the stutter of the major contributor's allele. These preliminary studies suggest MPS will be a valuable tool for analyzing mixed samples in forensic casework.

As one part of a larger validation study, genetic transmission was studied to ensure that the markers included in the PowerSeq™ Auto/Y prototype amplification kit are consistent with the expected Mendelian inheritance pattern. The pattern of transmission from mother and father to offspring allowed an evaluation of the inheritance pattern as well as the stability of genetic transmission events. Isoalleles were observed in this study and were traceable from parents to their offspring. In one family, a male child had a heterozygous genotype at locus D1S1656 with isoalleles of two different sequence variants that were traced back to the mother and father. In another family, the father exhibited isoalleles at D2S441 (10a and 10b). Upon sequencing the amplified products in the offspring, it was observed that the daughter had inherited the 10a allele while the son inherited the 10b allele.

The studies performed as part of this evaluation highlight the prevalence of isoalleles and their high value to forensic casework. Whether in mixture cases, paternity cases, missing persons cases, or familial searches, MPS can provide valuable information that extends beyond those of traditional CE-based methods.

MPS, Isoalleles, Casework

B46 The Development of a Mitochondrial DNA (mtDNA) Assay for Forensic Human Inclusion/Exclusion Screening Using Real-Time Polymerase Chain Reaction High-Resolution Melt (PCR HRM) Analysis

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After attending this presentation, attendees will understand how PCR coupled with HRM can be used as a presumptive mtDNA screening tool for degraded DNA samples in forensic casework.

This presentation will impact the forensic science community by demonstrating a new method of screening mtDNA evidence and comparing it to reference samples to determine if further testing is required or to sort preliminary groupings. It is the intention that this process could be used as a presumptive step prior to standard DNA sequencing in forensic laboratories. In addition, equipment required for this additional screening is readily available to forensic DNA scientists in major crime laboratories.

Forensic scientists currently use mtDNA to identify missing persons and remains recovered from mass disaster cases. It is often valuable in these situations due to its higher copy number compared to nuclear DNA, which can become highly degraded and thus compromised. By comparing the recovered mtDNA to maternal lines, a potential identification can be made. While less discriminating than nuclear DNA, mtDNA is an excellent tool for screening and has been shown to differentiate human populations. It is highly advantageous for crime scene laboratories to be able to exclude or include a possible suspect(s) before focusing additional time and resources into further investigation.

Within the mitochondrial genome, there have been reported to be hypervariable regions known as one, two, and, more recently, a third has been discussed (HVI, HVII, and HVIII). Mutation rates in these areas are known to be five to ten times higher than nuclear genes due to low fidelity of mtDNA polymerase and the lack of repair mechanisms within the mtDNA overall. These regions are located within the control region of the mtDNA and code for no known medically or phenotypically significant genes. Additionally, other highly variable polymorphisms are located within and around these three hypervariable regions. Through the culmination of data from mtDNA sequencing over multiple populations throughout the world, databases such as MITOMAP have compiled known areas of variation designated as Single Nucleotide Polymorphisms (SNPs).

Many current techniques for SNP assays involve utilizing complex primer set-ups like SnaPShot® or fluorescently labeled Dideoxynucleotide triphosphates (ddNTPs), which ultimately conclude with capillary electrophoresis for visualization of the amplicons. As an alternative to costly sequencing or Short Tandem Repeat (STR) -like methods, real-time PCR HRM has been proposed as a more cost effective and faster approach to differentiate between mtDNA SNPs. In addition to forensics, research using HRM has also been applied to coding regions of mtDNA related to mutation-causing human diseases, such as MELAS and LHON, and evaluating polymorphisms within the control region of common carp.

In this study, primers have been constructed to amplify the variable regions of interest in the human mitochondrial genome (HVI, HVII, and HVIII), as well as SNPs of interest specifically modified with GC-tail primers to increase melt temperatures of specific polymorphisms and thus better differentiate identifying peak heights resulting from HRM. DNA Standards including 9947A, 2800M, and K562, as well as IRB-approved volunteer buccal samples were evaluated via real-time PCR-HRM and agarose gel electrophoresis. A known T477C transition within the HVIII of 2800M showed a distinguishable temperature shift in comparison to 9947A and K562. More specific primers were designed to highlight SNPs of interest, such as the mtDNA 16519 (A/G) variant which was probed with three sets of primers. The addition of the GC-tail to the G variant allowed for a visibly increased melt temperature in comparison to the A variant on the resulting melt curve and thus facilitated differentiation.

Mitochondrial DNA, Real-Time PCR, High-Resolution Melt

B47 Differentiation of Henna-Based Hair Dyes Using Attenuated Total Reflectance/ Fourier Transform Infrared (ATR/FTIR) Spectroscopy

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After attending this presentation, attendees will better understand how ATR/FTIR spectroscopy and a valid statistical classification method can be used to analyze spectra of henna-based hair powders of varying colors and from several manufacturers.

This presentation will impact the forensic science community by describing a new application of ATR/FTIR, a rapid, non-destructive spectroscopic technique, to discriminate henna-based hair dye powders in the analysis of forensic evidence.

Hair is frequently encountered at crime scenes, especially those in which a struggle has taken place between the victim and the perpetrator, but also from daily hair shedding. Microscopy is used to evaluate hair evidence. Henna (*Lawsonia inermis* L.) is a plant that has been used for centuries for hair dyeing and applying body decoration, such as body painting and palm coloring, primarily in Asia/the Middle East. Henna is increasing in popularity in the United States because of the desire of people to use a more natural method for cosmetics. Henna-based hair dyes are available in a variety of colors that are different due to the varying combinations of plant powders that include henna, cassia, and indigo. Henna is pulverized into a powder for purchase, which is then made into a paste for application. To make the paste, the powder is mixed with water. When the henna dye is applied, the henna only coats the outside of the strand instead of permeating it, making this a safe and natural alternative to synthetic dyes. As the interaction of the molecules on the diamond surface are probed in ATR/FTIR spectroscopy, the chemical features of the henna and other dyes in henna-dyed hair are recorded when it is applied to the hair. The data results were gathered from the spectra and Principle Component Analysis (PCA) was performed to create classification models, which statistically classified the powders into distinct groups based on color. ATR/FTIR analysis is a powerful tool for trace examiners because it is a quick and non-destructive technique and requires a minimal amount of sample with little-to-no sample preparation. Although the trace analyst could strip the hair of the chemical, the henna can serve as an additional tool to differentiate hair evidence.

This study evaluated the ATR/FTIR absorbance spectra for 49 different henna-based hair dye powders from 4,000-500cm^{-1} with a spectral resolution of 1.929cm^{-1} using a Thermo Fisher Scientific Nicolet™ iS™10 spectrometer running the Omnic™ software and equipped with the Smart iTR™ ATR attachment. Background spectra of air were recorded at ambient temperature. The samples were purchased from eleven different suppliers. Thirteen colors were chosen due to their availability from the individual suppliers. Thirty-two scans were recorded for each replicate; each henna sample was recorded multiple times and was used to build an FTIR henna spectral library. The average ATR/FTIR absorbance was averaged over all scans by saving the data as .CSV text files that were imported into Microsoft® Excel® for spectral analysis. Predominantly variations were observed in the fingerprint region. Absorbance ratios were analyzed at selected frequencies to produce a table of ATR/FTIR results that emphasized the variations and could be clustered using PCA statistical analysis. Statistics were used to differentiate the henna samples from one another. The Unscrambler Multivariate Analysis Software PCA will be employed for statistical analysis. ATR/FTIR spectroscopy combined with chemometrics increases the selectivity of the method.

To compare to the ATR/FTIR results, the powders and pastes were also analyzed using a smartphone application called ColorAssist. ColorAssist uses the iPhone® camera with or without the flash to capture RGB values of colored materials. The dry powders and wet paste could not be differentiated by color or manufacturer using an Excel® scatter plot of RGB data results. This research supports the use of ATR/FTIR spectroscopy to differentiate henna-containing evidence. The ATR/FTIR database will provide another tool to help solve forensics cases and can be applied in most laboratories as they are widely equipped with this instrument.

Henna, ATR/FTIR Spectroscopy, Hair

B48 A Dietary Supplement of Choline and Seminal Choline Crystals: A Consideration for Seminal Fluid Identification by Florence Iodine Reagent

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After attending this presentation, attendees will understand the morphological differences and similarities between crystals produced by the dietary supplement choline and seminal fluid using the Florence Iodine (FI) test. This study considers an important area of seminal fluid identification as false positive in the presence of a choline dietary supplement and other sources that can be encountered during an evidence examination for seminal fluid.

This presentation will impact the forensic science community by reviewing and comparing the crystal formation of choline from seminal fluid and from a dietary supplement using light and polarized light microscopy.

Choline is a non-protein component of semen that can be found in high concentration in seminal stains. The choline test is used as a presumptive or an initial test for the presence of seminal fluid in many laboratories around the world.[1] As a presumptive test, it can be very important forensically from a different perspective. It can reduce the time spent searching and screening an item by focusing on a certain area that provides an indication of the searched-for stain. This test will also reduce the cost of operation by using less of the expensive confirmation testing. The test can also be less destructive on suspected semen stains as it uses less sample.

Generally, the need for tests for seminal fluid is vital in forensic sexual case examinations if there is no sperm found in the microscopic examination. The possibility of confronting no-sperm samples is increasing in forensic work due to vasectomy and azoospermia conditions in men worldwide. Another condition that can cause an of absence of sperm includes penetration without ejaculation.

Worldwide, vasectomy is increasing; in the United Kingdom, more than 45,000 operations are performed each year in National Healthcare System (NHS) hospitals and an unknown number in private clinics.[2] The trend for vasectomies worldwide is 30 million, 43 million, 44 million, 28 million, and 30 million in 1982, 1991, 2001, 2009, and 2015, respectively.[3] On the other hand, azoospermia can be defined as the absence of sperm in the ejaculate. This condition can be found approximately in 1% of all men and in 10% to 15% of infertile males.[4]

The need to identify seminal stains that have no sperm has produced several tests, such as the choline test, the Acid Phosphatase (AP) test, the Prostate Specific Antigen (PSA) test, and the Rapid Stain Identification (RSI) test for Semenogelins (Sg).[1] The AP and PSA tests are considered to be presumptive tests that were found to be less specific than the FI test for choline.[5] The Sg gene is also present in primates, which can indicate that it can also be found in non-human sources.[6]

In this research, an attempt was initiated using light and polarized light microscopy to characterize the morphology of choline crystals from seminal fluid and from dietary supplements, which were studied microscopically after using FI. The results can be used in choline source evaluation. The use of choline in addition to other presumptive tests can increase clarification of the case.[7] There is a need for more research on the subject of choline crystal formation tests and the chemical differences between various sources of choline in addition to other modern testing.[8]

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Choline Dietary Suppliment, Seminal Fluid, Sexual Crime

B49 The Effect of Body Mass and Cadaveric Bloat on DNA Quantity and Downstream Short Tandem Repeat (STR) Success

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The goal of this presentation is to provide attendees with information regarding the effect of decomposition on the quantity and quality of DNA extracted from the muscle tissue of human cadavers for identification purposes. Attendees will become familiar with how the processes of DNA degradation and damage occurring throughout the early stages of the human decomposition process affect downstream STR typing success.

This presentation will impact the forensic science community by emphasizing the importance of sample collection before a body enters the bloat stage to ensure the highest chance of successful genotyping using STRs for human identification purposes.

Human remains may be recovered for identification during forensic casework, missing persons cases, or after a mass disaster. These bodies may be found in various stages of decomposition, including fresh, early decomposition, bloat, advanced decomposition, and skeletonization. In such cases, DNA profiling using STRs is routinely applied to identify the remains; however, DNA is known to degrade after death, and harsh environmental conditions such as heat, humidity, and exposure to sunlight speed up decomposition and lead to increased DNA damage and degradation. In addition, body mass is also known to affect the rate of decay. This project assessed the amount and integrity of DNA recovered from low (average 53kg) and high (average 84kg) body mass cadavers through the early stages of decomposition and determined the success of STR profiling throughout each stage.

This study investigated the quantity and quality of DNA extracted from the quadriceps muscles of six cadavers during the first 12 days of decomposition where soft tissue persisted. The cadavers were placed in an outdoor environment in either April or October in southeast Texas. DNA from the quadriceps muscle was extracted and assessed to determine DNA quantity and quality via real-time Polymerase Chain Reaction (PCR) (DNA concentration and degradation ratio), and genotyping was performed using a commercial STR amplification kit.

As expected, the quantity and quality of DNA decreased as decomposition progressed, accompanied by a decrease in the number of reportable alleles and peak heights across the STR profiles. Complete STR profiles were obtained from all bodies until day four of decomposition. Although variation between the cadavers was evident, an interesting trend was observed between low and high body mass cadavers. The DNA quantity and STR profile quality decreased rapidly at the onset of bloat (around day six) in the low body mass cadavers, whereas in the high body mass cadavers, a similar decrease occurred several days later (day ten), near the end of the bloat stage. This phenomenon resulted in complete (or near complete) STR profiles being obtained from the larger cadavers for up to four days longer than the smaller bodies. On average, the number of STR alleles recovered from all cadavers decreased during bloat, indicating the importance of collecting tissues for DNA analysis as early as possible from all decomposing bodies, with the bloat stage marking the most rapid decrease in DNA quantity and STR success during the initial stages of decomposition for both high and low body mass cadavers.

Human Decomposition, STR Typing, Human Identification

B50 The Collection of DNA From Fingerprints on Weathered Trash Bags

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After attending this presentation, attendees will recognize the importance of the cyanoacrylate fuming technique for the development of fingerprints left on garbage bags after being subjected to weathered conditions. Attendees will also understand the importance of obtaining touch DNA from the developed fingerprints.

This presentation will impact the forensic science community by demonstrating that touch DNA can be obtained from the developed fingerprints on weathered garbage bags.

At crime scenes, any evidence found can be considered probative. Research is being conducted to discover new techniques for the development of fingerprints, the impact of weather conditions on the development of the fingerprint, and/or if the substrate the fingerprint was left on affects the collection. Each of these aspects may affect how fingerprints are collected. Fingerprints are left behind on surfaces by transferring three main products from the secretory glands — sebaceous, eccrine, and apocrine glands — on the human skin. These products include a mixture of organic, inorganic, and environmental contaminants. Every latent fingerprint left behind has different ratios of each component, which can affect the durability, resistivity, and quality of the fingerprint.

The Cyanoacrylate (superglue) Fuming Method (CFM) has been found to be effective for the development of latent fingerprints on non-porous surfaces.[1] This analytical tool involves subjecting a latent fingerprint to cyanoacrylate vapors in an enclosed chamber that polymerizes with the fingerprint residues to form a polycyanoacrylate polymer along the ridges.[2] Stated in the *Portland Press Herald*, approximately 30% of latent fingerprints collected from crime scenes are usable for fingerprint comparison; this illustrates the importance of being able to collect DNA from the developed fingerprints to analyze and obtain a DNA profile.[3] Cyanoacrylate fuming is a non-destructive process, which means DNA transferred from the individual in the latent fingerprint can be extracted and analyzed, which allows for the source of the fingerprint(s) to be determined.[4]

The goal of this research was to collect and develop fingerprints from weathered trash bags through the process of cyanoacrylate fuming, subject each sample to an automated extraction, quantify each sample through quantitative Polymerase Chain Reaction (qPCR), Short Tandem Repeat (STR) profiling, then complete a comparison between the known and unknown samples.

During the research, a series of experiments were conducted to determine if the weather (hot, cold, and light versus dark) affects the quality of latent fingerprints when developed with cyanoacrylate. Each fingerprint was analyzed to determine if a fingerprint comparison could be completed after fuming, then a DNA sample was taken. A control was placed in a room with sunlight and ambient temperature. The effect of sunlight on developed fingerprints was also tested to determine if the quality of the fingerprint changes. This was completed by placing a garbage bag in a dark cabinet that was not opened. Each variable had multiple bags present that contained a total of 20 fingerprints per bag to test if fingerprint quality and DNA analysis is affected by time. A second variable of time was looked at that included leaving the garbage bags outside for a period of 24 hours, 2 weeks, 1 month, and 3 months. After the development of each latent fingerprint, a second method was developed to look at the collection and extraction of DNA from the visible fingerprint. Chosen individuals were given multiple garbage bags and were asked to place their thumbprints on the side of each garbage bag. Each bag was then subjected to a different variable and the fingerprint quality and DNA analysis was tracked and analyzed. For consistency between the amounts of touch DNA, a protocol was determined, which included the time since washing of their hands and the objects each person touched before placement. The focus of future research is the development of a method to test whether the cyanoacrylate fuming technique can develop latent fingerprints on garbage bags, more specifically on the side of garbage bags.

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Cyanoacrylate Fuming, DNA, Fingerprints

B51 Blast Suppression Foam Does Not Inhibit DNA Recovery and Analysis

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After attending this presentation, attendees will better understand the effects Aqueous Foam Concentrate-380 (AFC-380) blast suppression foam has on DNA recovery and analysis.

This presentation will impact the forensic science community by verifying that the use of AFC-380 does not obstruct DNA evidence collection or deter its subsequent analysis by Polymerase Chain Reaction (PCR).

To develop investigative leads in response to an Improvised Explosive Device (IED) explosion, biological evidence is collected from the exploding apparatus or surrounding material post-blast and analyzed. DNA collected from IED-blasted material can provide decisive evidence for ensuing criminal investigations or intelligence operations; however, recovery of high-quality DNA from exploded IEDs is infrequent and is met with considerable obstacles. The explosive force and heat produced by a blast can destroy or degrade DNA. PCR inhibitors may also be co-extracted, impeding amplification of target fragments and preventing identification of possible suspects. Aqueous blast suppression foam is commonly used in military and anti-terrorism applications to contain blasts associated with IEDs by suppressing the shock wave associated with detonation. The use of blast suppression foam in response to IED threats requires an understanding of any potential detrimental effect on DNA recovery, quality, and succeeding forensic analysis.

In this study, the effects of blast suppression foam on DNA recovery and analysis by two different quantitative Polymerase Chain Reaction (qPCR) methods were investigated. Human blood was spotted onto various sample materials, (e.g., PVC pipes, metal pipes, paper, and cloth). Samples were exposed to AFC-380, both with and without detonation, and compared to unexposed/undetonated controls. All samples were collected and swabbed, and DNA was extracted using the EZ1™ DNA Investigator Kit. DNA extracts were evaluated for nuclear DNA quantity and quality using the Quantifiler® Human Plus DNA Quantification Kit and for mitochondrial DNA quantity and quality using a custom qPCR method.

Overall, following analysis of nuclear and mitochondrial DNA by PCR, 86.2% of the samples were within the standards range of the assays (>0.005ng/µl nuclear DNA or >10 copies mitochondrial DNA). Out of the 13.8% that were not within standards range, 10.4% were blasted, indicating detonation-damaged biological evidence isolated from those particular samples. In addition, internal PCR controls of recovered samples that were treated with AFC-380 amplified similarly as untreated samples. Importantly, treatment with AFC-380 did not cause DNA degradation.

The results of this investigation provided no evidence that AFC-380 reduces DNA quantity (aside from potential DNA dilution effects), degrades DNA, or results in PCR inhibition. This study validates that use of AFC-380 should not obstruct the recovery and subsequent analysis of DNA collected from critical explosive device evidence.

DNA Analysis, Blast Suppression Foam, PCR

B52 Preliminary Experiments on Human Bloodstain Age Estimation by ^{1}H,^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy

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After attending this presentation, attendees will understand the advantages and the possibilities offered by high-resolution ^{1}H and ^{13}C NMR in studying the aging of bloodstains found at a crime scene. Indeed, the possibility of dating blood still represents a challenge of paramount importance in the forensic sciences research field and, although numerous techniques have been proposed in recent years, an accurate estimation of the time elapsed since the crime was committed by using bloodstains is still not possible.[1]

This presentation will impact the forensic science community by presenting the preliminary results on the use of NMR spectroscopy in monitoring the changes of bloodstain spectra as a function of time.

Despite the typical lower sensitivity and higher cost of this technique with respect to other techniques which are more commonly found in forensic laboratories, mainly Mass Spectrometry (MS) and Raman spectroscopy, this techniques' main advantage relies on the possibility of acquiring spectra that directly represents the molecular composition of the whole sample, thus realizing an "-omics" approach, similar to what has recently been proposed for food matrices.[2-4]

Since blood constitutes a complex biological matrix, the possibility of monitoring the modifications of the different classes of molecules present in the whole blood over time is extremely appealing in a forensic framework.

Fresh peripheral blood was collected from different healthy adult volunteers. The obtained simple bloodstains were aged without the addition of any anticoagulants.

NMR spectra were recorded on a Bruker FT-NMR AVANCE III HD 600 MHz spectrometer with a CryoProbe™ BBO H&F 5mm probe and ^{1}H NMR data were acquired using standard Bruker pulse sequences: zg (1D sequence), zgcppr (1D sequence with pre-saturation, using composite pulse for selection), ledbpgp2s1d (Bipolar Longitudinal Eddy Current Delay (BPPLED) pulse sequence), and cpmgpr1d (Carr-Purcell-Meiboom-Gill (CPMG)).

Multivariate data analysis was applied to the collected NMR spectra in order to condense redundant information, to examine overall differences, trends in variation, and relationships between samples and variables, therefore identifying the pools of compounds able to discriminate the age of a particular bloodstain found at the crime scene.

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Bloodstain Aging, Nuclear Magnetic Resonance, Multivariate Analysis

B53 The Influence of Ceramic Tiles' Surface Characteristics on the Analysis of Bloodstain Patterns

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After attending this presentation, attendees will be more aware of the many differences existing in the surface characteristics of traditional as well as modern ceramic tiles, currently employed worldwide both for floor and wall covering applications, and the consequent implications on the analysis of bloodstain pattern characteristics associated with a bloody criminal event. Although recent research on Bloodstain Pattern Analysis (BPA) was also necessarily directed toward the study of the effect of surface characteristics of different types of substrates, similar studies on ceramic tiles are completely missing in the scientific literature.[1-3] Indeed, a wide range of ceramic tiles is available on the market with different technical characteristics, such as porosity and surface roughness, which will inevitably affect the morphology of the patterns arising from blood impact on that specific tile. Moreover, in the last decade, digital decoration of ceramic tiles by inkjet printing technologies began replacing conventional decoration techniques, smoothing the way toward many new possibilities, including the decoration of structured surfaces with ever-more-complex textural characteristics, for example, those reproducing natural wood and stone.[4,5]

This presentation will impact the forensic science community by, for the first time, presenting and discussing results of BPA experiments performed on traditional as well as innovative and digitally decorated ceramic tiles. The importance of this study also relies on its global character, as Italy and particularly the ceramic district of Sassuolo (Province of Modena, Emilia Romagna region) covers approximately 38% of the international trade of ceramic tiles.[6]

Therefore, in this study, ceramic tiles with different surface finishing and textures (from the ceramic district of Sassuolo) were considered as the substrates for simple BPA experiments; for example, drop patterns reproduction. In particular, ceramic tiles with structured, smooth, matte, and glossy surfaces were selected and accurately characterized in terms of surface roughness, mineralogical (X-ray diffraction), and microstructural analysis (Scanning Electron Microscopy (SEM)) in order to correlate the surface properties with the morphological characteristics of the obtained bloodstains.

Blood from healthy swine was utilized according to the studies by Christman, and the experiments were performed according to Laber and Epstein.[7,8] The resulting bloodstain patterns were recorded by means of a Nikon® D5100 reflex digital camera.

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BPA, Ceramic Tiles, Digital Decoration

B54 Method Validation for DNA Recovery From Cartridge Casings

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The goal of this presentation is to inform attendees regarding the efficacy of two DNA extraction methods for cartridge casings, soaking and swabbing, with comparisons also made between cartridges and casings, ammunition brands, and treatment (loading) methods.

This presentation will impact the forensic science community by enhancing the small group of recent studies that have involved DNA recovery from casings and may influence attendees to take these findings back to their labs and begin the process for changing their extraction methods.

Touch DNA is gaining popularity in the forensic science community. Increased kit sensitivities and better methodologies have contributed to obtaining DNA evidence from steering wheels, firearms, and, as with the focus of this study, cartridge casings.[1,2] Skin cells have the potential to be left behind on anything a person encounters.[3] Most ammunition is loaded into a magazine by hand, so there is a chance that DNA could be left behind on the ejected casings often left at crime scenes. The main goal of this study was to validate the best method for case use by the San Francisco Police Department (SFPD) Crime Lab. Swabbing involved using a swab moistened with sterile water, followed by a dry swab. The soaking method required placing the casing itself in a tube and surrounding it with the extraction components as well as swabbing the cartridge when removed from the liquid portion.

Overall, 96 swabbed casings and 96 soaked casings were tested. The profile of the loading analyst was the only one observed, with the exception of three samples. These were treated as blank samples when calculating results. The swabbed casings returned an average of 0.788pg/µL of DNA, while soaked casings averaged 1.46pg/µL, a significant difference. The majority of profiles produced by both methods had less than five alleles with 26.04% of swabbed and 43.75% of soaked casings generating profiles. The average percentage of alleles recovered by swabbed casings was 8.93% with an increase to 14.12% for soaked casings. With the help of STRmix™ software, soaked casings also produced a greater quantity of likelihood ratios exceeding one sextillion.

Additionally, 48 cartridges were tested and proved less successful than casings with an average percent allele recovery of 3.1% in comparison to 11.5%, which was a significant difference. There was also varied success across brands and treatments. The brands tested were .40-caliber Winchester®, Remington®, Blazer®, and Speer® ammunition with normal, handled, and saliva-transferred treatment methods. The treatment methods involved grabbing cartridges directly from the storage envelope and loading (normal), licking the thumb before grabbing each cartridge and then loading (saliva transferred), and carrying the ammunition in pockets for three hours and loading (handled). The Blazer® casings and saliva-transferred treatment had the highest percent allele recoveries with 30% and 29%, respectively. Blazer® was significantly higher than all other brands. The saliva-transferred method was significantly higher than the normal and handled treatments. The lowest recovery was the Remington® brand with 1.3% and the normal treatment with 2.6%. The ejection port was also swabbed after each emptying of the magazine with the target profile observed in 41.7% of samples.

SFPD will be adopting the soaking method in their lab due to the general trend of higher success within each variable. Even though every comparison did not return a significant difference, it would be their best attempt to recover DNA. In the future, this study could be conducted with multiple loaders to see if results improve, and with more samples to increase data normality.

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Cartridge Casings, Soaking Method, Touch DNA

B55 New Strategies and Recommendations for Front-End Separation of Compromised Biological Mixtures Using Cellular Fluorescence Profiling and Flow Cytometry

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After attending this presentation, attendees will better understand how cellular properties such as fluorescence, optical scattering, and diversity of surface antigens can be used to analyze and ultimately separate contributor cell populations in aged or degraded biological mixture samples.

This presentation will impact the forensic science community by introducing new methods and technical guidelines for separating biological mixture contributors prior to DNA extraction using flow cytometry. This can improve the efficacy of mixture interpretation in DNA caseworking units.

Previous studies have demonstrated that hybridizing cell mixtures with fluorescently labeled antibody probes, and then isolating fluorescent cells using flow cytometry, can be an effective technique for separating certain types of cell mixtures; however, mixture samples that have been compromised and/or degraded demonstrate a significant decrease in the efficiency of antibody probe binding as well as an overall loss of intact cell targets. This presents an ongoing obstacle for adopting a cell separation workflow for forensic applications. Therefore, the goal of this study was to investigate the molecular dynamics of cellular decomposition in simulated forensic samples and develop new strategies for mitigating biochemical processes that can lead to non-specific probe interactions and subsequent inefficiencies in cell sorting.

To accomplish this, a series of two-person blood mixtures that varied in drying time (between 24 hours and 128 hours), contributor ratio (10:1, 5:1, 3:1, 1:1), and total starting volume of blood (between 20µl and 500µl) was analyzed. This study found that non-specific interactions with the antibody probe were a significant factor in blood mixtures that had been dried for more than 24 hours, such that target cell populations were difficult to identify against non-target populations. This could be mitigated by reducing the antibody-to-cell ratio in the hybridization to <0.1µg per 20,000 cells. Subsequent sorting experiments performed on two-person mixtures with varying contributor ratios demonstrated that the Short Tandem Repeat (STR) profile of the minor contributor was successfully enriched in each sorted cell fraction when compared to the STR profile of the unsorted mixture. For example, in the 10:1, 5:1, and 3:1 mixtures, the ratio of minor contributor increased to ~1:2, ~1:1, and ~1:1 (major:minor), respectively. Although there was no systematic relationship between the degree of profile enrichment and the original contributor ratio, it was observed that the position of the sorting gate had a significant effect on the quality of the resulting STR profile, such that gates designed to capture cells at the tail ends of the fluorescence distribution of the mixture demonstrated the highest enrichment for the target cell population. To test the robustness of this sorting criteria, three different dried blood mixtures were created with 100µl total volume each with a 1:1 contributor ratio and the tails of the fluorescence distribution were sorted in a blind fashion (i.e., without measuring the fluorescence histograms of single source cell populations). The resulting sorted cell fractions showed enrichments of 2:1 and 3:1 for the A02 positive and A02 negative contributor respectively. This was consistent across multiple mixture replicates.

Last, this study conducted a limit of detection test using the above sorting workflow by drying blood mixture samples with 100µl, 50µl, and 20µl total starting volumes and 1:1 contributor ratios for each. Results demonstrated enrichments of the target population of 2:1 to 23:1, suggesting that clear separation can be obtained for blood mixtures that originally contained as little as 20µl of total volume.

Overall, these results suggest that antibody probes coupled to front-end separation with flow cytometry can be used to enrich target cell populations in whole blood mixtures that have been dried and/or compromised. Protocol changes to a traditional cell separation workflow, such as reducing the concentration of antibody probe, targeting cells with different optical properties, and sorting cells representing the "tail ends" of the distribution of fluorescence values for the mixture samples, can be effective strategies for minimizing the effects of cellular decomposition during front-end probe labeling and separation.

Biological Mixtures, Mixture Interpretation, Flow Cytometry

B56 Probing Potential Interferences in DNA Extraction of Semen Collected on Surface-Enhanced Raman Spectroscopy (SERS) -Active Forensic Evidence Swabs

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After attending this presentation, attendees will better understand the effects of synthesis reaction temperatures on silver characterization of SERS-active forensic evidence swabs. Attendees will also know how the fabrication of the swabs relates to the silver recovered in the extraction solution.

This presentation will impact the forensic science community by introducing a method using silver-coated forensic evidence swabs to successfully identify semen and subsequently extract and genotype DNA, following the insertion of a centrifuge step in the extraction protocol. The following technique has advantages over current approaches because it is sensitive, time efficient, and non-destructive. This could result in the potential identification of a variety of human bodily fluids to be used for DNA analysis.

SERS-active forensic evidence swabs are coated in silver to achieve a technique known as SERS. SERS was developed because Raman spectroscopy is an unfavorable occurrence that can be amplified by placing a metal extremely close to the sample. It was chosen for this research because of its distinctive ability to outline specific biological molecules and do so with minute amounts of sample. This technique would allow for a more convenient method to identify human bodily fluids in the forensic field.

Plain nylon swabs were placed in a synthesis reaction with silver oxide and hydrogen gas via the hydrogen reduction method.[1] This reaction produces silver nanoparticles that grow on the individual fibers of the swabs, thus making them silver-coated. A $[Ru(bpy)_3]^{+2}$ solution was swabbed and used to measure the Raman signal because of its easily identifiable Raman spectrum.

A trial was performed to see how the variation of reaction temperatures affected the efficiency of the swabs. $[Ru(bpy)_3]^{+2}$ solution was swabbed using five swabs of each temperature, which were dried to be tested with a Horiba LabRAM HR Raman microscope. All five data sets were averaged for each swab temperature and compared to observe the best signal. This procedure was similarly used for swabs containing seminal fluid samples. Atomic absorption spectroscopy was also used to obtain the quantity of silver on the swabs before the extraction process. Dissolving the swabs in nitric acid and filtering the solution extracted the silver present on the coated swabs. The solutions were then measured with a Perkin Elmer® Atomic Absorption spectrometer.

To ensure that measureable DNA could be recovered from the silver swabs, an extraction protocol was performed. The DNA recovery results showed no interference with silver nanoparticles, although there was a sizeable amount left in solution during extraction. To resolve a potential issue with silver leaching into the buffer solution, a centrifuge step was added to remove the nanoparticles from suspension, hence allowing for the extraction of the silver-free supernatant. An Applied Biosystems™ PrepFiler™ Forensic DNA Extraction Kit was used on plain silver-coated swabs to obtain solutions just before and after the centrifuge step. To show that the silver was not interfering with results, Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES) was performed on both buffer solutions. Electron microscopy imaging was also conducted on the post-extraction swabs to better characterize how the silver left on the swabs was changing. Data analysis was conducted to determine if a correlation was present between the reaction temperatures and the efficacy of the swabs.

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SERS, Semen, Silver Nanoparticles

B57 The Optimization of Human Hair Proteomic Processing for Single Hair and Ancestral Analysis

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After attending this presentation, attendees will understand the impact of using genetic information from hair peptides to aid in human identification. Attendees will also realize why proteomic processing is important in generating meaningful proteomic datasets.

This presentation will impact the forensic science community by shifting human identification into a dual DNA-proteomics perspective. Further implications will also be made in making identifications and ancestral classifications based on genetic information found in protein from a single human hair.

Forensic hair evidence obtained by microscopic morphological comparison has been criticized as being subjective, unreliable, and not reproducible. Mitochondrial DNA analysis has been able to remedy the reliability of hair analysis, but DNA can degrade substantially under environmental insults, particularly with greater distance from the scalp. Unlike DNA, protein has peptide bonds that are more resistant to cleavage. This research explores a proteomic approach to hair analysis. Variations in protein expression level reflect variations in transcription factor levels, their affinities for response elements, and possibly epigenetic and genetic variation. Another form of variation is the presence of Genetically Variant Peptides (GVPs) that offer the prospect of even more discriminating analysis.[1]

GVPs are the result of non-synonymous Single Nucleotide Polymorphisms (SNPs); therefore, genetic information such as SNPs can be predicted via the protein sequence and confirmed with parallel DNA sequencing. With high-resolution mass spectrometric instrumentation, single amino acid polymorphisms can be detected in the amino acid sequences of keratin, keratin-associated, and other proteins. As a result, GVPs can be used to help identify an individual or even classify ancestral origin.

This research focuses on increasing peptide yield from hair protein by optimizing its chemical processing. Further goals include comparing European and African hair GVPs and assigning the differences in population frequency found therein, as well as decreasing the working hair length to 20mm (~100µg). Data have been obtained to optimize conditions for disulfide reduction, alkylation, and peptide digestion. Temperature, time, agitation types, and concentrations of the reagents have been tested. The four metrics used to determine the best parameters for processing are yield of the insoluble fraction, yield of the soluble fraction, unique peptide number, and the number of GVPs. Results indicate that lower temperatures were better than higher temperatures. Agitation by stirring resulted in higher solubilization than swirling or remaining static. A time course quantifying the insoluble fraction has shown that trypsinization for six hours solubilizes most of the hair by mass and results in detection of the most unique and total peptides. The optimized hair processing procedure, with shorter times for both reduction and digestion, has yielded improvements in detectable GVPs, and yields similar numbers of GVPs compared to other approaches that rely on urea and a mass spectrometry compatible detergent; however, the increase in peptide and GVP generation is more pronounced in European hair as opposed to African hair. This is mainly due to the lower quantity of keratin-associated proteins detected in African hair. Overall, the data show optimized proteomic processing of human hair results from shorter reduction and digestion at room temperature with gentle stirring.

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Proteomics, Hair, Genetically Variant Peptides

B58 Quantifiler® Trio or PowerQuant® System? Is One Kit Better at Predicting the Success of Short Tandem Repeat (STR) Typing of the Male Component of Sexual Assault Evidence?

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After attending this presentation, attendees will better understand the relationship between the quantitation data for the male component of a complex mixture and the success of developing a meaningful male autosomal STR or Y-chromosomal Short Tandem Repeat (Y-STR) profile.

This presentation will impact the forensic science community by providing information about quantitation and prediction of STR genotyping success that can help streamline the DNA analysis process and decrease the sexual assault kit backlog.

The sexual assault kit backlog in forensic casework remains a prominent issue across the nation, despite additional funding from government programs such as the DNA Backlog Reduction Program.[1] The nationwide backlog problem has been attributed to factors including the timely submission of the sexual assault kits to the laboratories, the time required to analyze the complex samples in conjunction with the shortage of resources, shortage of qualified forensic scientists, and overall increased demand for forensic services.[2] A recent study, conducted in 2009, disclosed approximately 406,000 forensic biology requests estimated to be backlogged for DNA casework by the year's end nationwide.[3] DNA quantitation data using multi-target quantitation kits can help streamline the analysis process by identifying the total autosomal and male DNA concentrations, while also assessing the quality. Analysts can use this data to predict STR genotyping success, triage the samples for autosomal or Y-STR profiling or both, and determine if it is worthwhile to continue with DNA testing.

In this study, male and female buccal swabs were extracted organically and quantitated. A lower limit sensitivity study was conducted for both the Applied Biosystem® QuantifilerTrio® kit and the Promega® PowerQuant® System to determine the lower limit of DNA detection and percent Coefficient of Variation (CV). A dilution series was created ranging from 1ng to 0.0015ng for the male and female samples and run in triplicate with each quantitation kit. Next, a dilution male and female mixture series was prepared by increasing the female-to-male ratio of DNA template in a dilution series ranging from 1:1 to 163,840-fold while holding the male DNA template quantity constant at 0.010ng. The dilution mixtures were run in duplicate using both the QuantifilerTrio® kit and the PowerQuant® System and the performance differences were assessed.

When the sensitivity was assessed, the PowerQuant® and QuantifilerTrio® kits showed similar results with reproducible data. A low percent CV was observed down to 10pg of template DNA; thus, 10pg was the quantity of male DNA chosen to hold constant for the male:female ratio study. Dilution male:female mixtures were prepared in duplicate with each quantification kit. Using the quantitation data obtained for both kits, an average quantity value for each dilution was calculated for PowerQuant® and QuantifilerTrio® for both the autosomal data and the Y-chromosome data. There was little difference observed between the quantity averages for the autosomal data for each kit. Further statistical analysis will determine whether the difference is significant or not. Both kits were able to detect the male contributor when the female contributor was many thousand times greater in concentration. The data obtained thus far is promising; however, more work is necessary to establish which quantification kit is superior at detecting male DNA in an excess of female at extreme ratios.

Overall, this study will provide information to the forensic science community about using DNA quantitation to predict STR genotyping success to further streamline DNA analysis of sexual assault kits. Further work on this project will include analysis of degraded tissues from a variety of sources utilized to construct complex male:female mixtures. The ability to utilize the quantitation and degradation data in order to predict STR outcomes will be provided since all mixtures will be analyzed using the Promega® PowerPlex® Fusion and Applied Biosystem® Y Filer® STR kits.

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DNA Quantitation, Sexual Assault Kits, STR Genotyping

B59 Ion Mobility Mass Spectrometer (IMMS) — A Useful Confirmatory Tool for Analyzing Drugs and Explosives

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After attending this presentation, attendees will have developed an understanding for the practical analytical capability the interface between an Ion Mobility Spectrometry (IMS) and Mass Spectrometry (MS), often referred to as IMMS, can offer the forensic chemist. In forensic instrumental analysis, separation techniques such as Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography/Mass Spectrometry (LC/MS), and Capillary Electrophoresis/Mass Spectrometry (CE/MS) are considered confirmatory test instruments. On the other hand, the IMMS technique, not yet fully implemented as a routine instrumental analytical technique in crime laboratories, can be classified as a confirmatory test instrument with advantages not well understood within the forensic community. IMMS offers value-added data not possible from these other interfaces. Separation of isomers, isobars, and conformers, reduction of chemical noise, and measurement of ion size are possible with the addition of ion mobility cells to mass spectrometers. In addition, structurally similar ions and ions of the same charge state can be separated into families of ions, which appear along a unique mass-mobility correlation line.

This presentation will impact the forensic science community by broadening knowledge about IMMS. Such knowledge may prove useful and be applied to confirmatory test analysis in forensic investigations. This presentation will add to research already conducted in forensic instrumental analysis by broadening understanding of yet-to-be fully implemented instrumental techniques in the crime laboratory. Such knowledge may well speed the implementation of IMMS as a routine analysis in the crime laboratory.

Any instrumental method used for confirmatory tests must be capable of producing reproducible, reliable, and accurate data. The IMMS technique can play a major role in achieving this goal. Data generated from IMMS can be used to evaluate the quality, reliability, and consistency of analytical results, which is an important part of any good analytical laboratory practice.

In conclusion, IMMS has the following advantages: simultaneous separation and identification of isomeric, isobaric, and conformer type drugs and explosives; charge state and trend line separations; isotope separation of complexed mixtures; short separation time; reduction of false positive rates; and random MS noise can be separated from real signal in IM space.[1-5] With all these advantages, why not employ the technology as a routine analytical tool for generating confirmatory data with forensic samples? The technology holds great promise for routine analysis in crime laboratories.

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Ion Mobility Mass Spectrometry, Drugs and Explosives, Confirmatory Tests

B60 A Preliminary Characterization of Sexual Assault Lubricants: A Comparison Between Direct Analysis in Real-Time Time-Of-Flight/Mass Spectrometry (DART®-TOF/MS), Gas Chromatography/Mass Spectrometry (GC/MS), and Fourier Transform Infrared (FTIR) Spectrometry

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After attending this presentation, attendees will understand the utility of the classification of lubricants as a novel technique for the analysis of sexual assault trace evidence using DART®-TOF/MS, in comparison to GC/MS and FTIR spectrometry.

This presentation will impact the forensic science community by aiding in the classification of unknown sexual lubricant samples based on components that are indicative of a type or class, thus providing investigative leads and innovative techniques in the analysis of trace evidence.

Unfortunately, sexual assaults are a reality in today's society. Due to the increasing use of condoms, there may be a reduced potential of finding DNA evidence in sexual assault cases. This requires a novel approach to the analysis of other trace evidence. The classification and characterization of lubricants is a relatively new approach in providing a method to analyze unknown trace evidence in such instances. In this study, approximately 20 samples from different sexual lubricant manufacturing types were tested, including water-based, silicone-based, oil-based, organic/edible, and others, which may include Personal Hygiene Products (PHPs) and lotions. The tested lubricant samples may be sub-classified as regular, sensation, flavored, spermicidal, and anesthetic based on additives in the formulation designed to impart a specific functionality (i.e., lidocaine or capsaicin).

This research sought to analyze lubricants and PHPs using DART®-TOF/MS, GC/MS, and FTIR methods that could aid in the identification of components in sexual lubricants. The goal of this study was to create a classification and characterization scheme to provide investigative leads from unknown lubricant samples. Neat lubricants as well as extracts were analyzed in both positive and negative ionization modes using DART®-TOF/MS in replicates of five. Neat lubricants and extracts (i.e., chloroform and hexane) were also analyzed via FTIR in triplicate. The neat lubricant samples were not analyzed directly by GC/MS; in turn, only the extracts were analyzed in triplicate. This information provided the necessary data to identify unique markers that define each individual lubricant class. Multivariate statistical techniques were used to create a classification scheme for the lubricants from the DART®-TOF/MS, GC/MS, and FTIR results. The outcomes of the classification schemes are expected to separate the different manufacturing types into different groups, as well as sub-classes within each manufacturing type. Unknowns were classified based on each Linear Discriminant Analysis (LDA) model from each instrument and were used to determine which instrument provides the best classification.

To provide a real-world example, cotton swabs from sexual assault kits were utilized as the collection media. Small amounts of lubricant and PHP samples were deposited onto the cotton swab and allowed to sit for one hour. Subsequently, a portion of the cotton swab was extracted in a 1:1 dichloromethane and methanol mixture for DART®-TOF/MS and GC/MS analysis. The remaining portion was extracted with either chloroform or hexane for FTIR. The extract results were compared to the classification scheme developed from the neat samples to determine how accurate the classification of real-world samples would be. The classification scheme developed from this preliminary study may ultimately enable future identification of questioned lubricant samples collected from crime scenes.

Sexual Lubricants, DART®-TOF/MS, Characterization

B61 An Analysis of Standard Glass Reference Materials Via Advanced Chemical Techniques for Forensic Applications

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After attending this presentation, attendees will understand how various techniques, including Glass Refractive Index Measurement (GRIM), Neutron Activation Analysis (NAA), Time-Of-Flight/Secondary Ionization Mass Spectrometry (TOF/SIMS), and Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) have been used to characterize several internationally available glass reference materials. The goal of this research was to measure elemental concentrations and stable isotope ratios in several glass reference materials, some of which have not been previously characterized, and thus enhance their value as reference materials.

This presentation will impact the forensic science community by illustrating that the main research outcomes presented in this study are stable isotope ratios and trace element concentrations in a range of soda lime and borosilicate glass reference materials. These values can be used for matrix-matched calibration of instruments used for the elemental analysis of glass, and thus have impact in regard to improving the quality of trace evidence examination. Furthermore, this presentation describes the scope and limitation of TOF/SIMS for the semi-quantitative analysis of trace elements in glass for forensic purposes.

All the reference materials (soda-lime glasses National Institute of Standards and Technology (NIST) 612, Float Glass Standards (FGS) 1, FGS 2, and borosilicate glasses NCS DC61104 and NIST 93a) were analyzed to determine if they had refractive indices relevant to forensic casework. This was accomplished via the oil-immersion temperature variation method using a Foster + Freeman GRIM® 3 instrument.

The soda lime glass reference materials were quantitatively analyzed by NAA at the Australian Open Pool Australian Light water (OPAL) nuclear reactor. Compositional data were generated by NAA for more than 20 elements in FGS1 and FGS2, including at least nine that were not measured in the previous research by Latkoczy et al., or the American Society for Testing and Materials (ASTM) inter-laboratory study.[1,2] NAA analysis of NIST 612 gave concentrations for 29 elements, including 10 for which no data are given on the certificate of analysis, and these values agree with the average values from other literature sources.[3,4] Trace element concentrations were also measured using LA-ICP/MS and semi-quantitatively determined using TOF/SIMS. The precision of the measurements using each of the techniques was used to determine the relative suitability for each technique for forensic microanalysis of glass.

The borosilicate glass reference materials NCS DC61104 and NIST 93a were analyzed using multi-collector ICP/MS to measure boron and lithium concentrations and stable isotope ratios and TOF/SIMS was used for the semi-quantitative analysis of trace element concentrations. Currently, these standards only have published concentration values for major elements and not for trace element analysis or any isotopic ratios.[5,6] Sensitive High-Resolution Ion Microprobe (SHRIMP) is a multi-collector mass spectrometric technique that can be used for the direct analysis of very small particles with high mass and spatial resolution. This technique was used to conduct replicate measurements of boron and lithium isotope ratios of several chips taken from different regions of the glass standards to check for compositional homogeneity. NAA and LA-ICP/MS analyses of these materials were not possible due to the high boron content of the samples.

The main research outcomes given in this presentation are stable isotope ratios and trace element concentrations in a range of soda lime and borosilicate glass reference materials. These values can be used for matrix-matched calibration of instruments used for the elemental analysis of glass, and thus have impact in regard to improving the quality of trace evidence examination. Furthermore, this presentation describes the scope and limitation of TOF/SIMS for the semi-quantitative analysis of trace elements in glass for forensic purposes.

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Glass Reference Materials, Micro-Chemical Analysis, Neutron Activation Analysis

B62 Quantifying the Uncertainty of Measurement for Gas Chromatography/Mass Spectrometry (GC/MS) Acceptance Criteria

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After attending this presentation, attendees will better understand the current acceptance criteria used for the identification of chemical substances with GC/MS. Attendees will also understand how the variability measured over more than six months in three different laboratories on five different instruments compares with recommended acceptance criteria provided by different governing bodies.

This presentation will impact the forensic science community by providing a numerical basis for the acceptance criteria generated with respect to GC/MS instrumentation. Additionally, the demonstration of a simple technique to determine the analytical uncertainty of measurement with the use of a 2 sigma (2s) criterion will assist practitioners with determining the uncertainty of measurement for their own analytical instrumentation. Finally, a demonstration of the analytical uncertainty of measurement in comparison to the published values will assist with the development of standardized language for the assessment and presentation of analytical uncertainty of measurement in court.

This research is not hypothesis driven; however, the experimental results are important and novel in the sense that the reproducibility of GC/MS results are rarely evaluated in such a detailed manner. The results provide practitioners with an appreciation for how realistic uncertainty compares with the acceptance windows recommended by different organizations.

The selectivity and confidence of compound identification by GC/MS is affected by the uncertainty in measuring the retention times and fragment ion abundances. Forensic chemists need to understand the factors that influence retention time and mass spectral measurements so that they can better present and defend the results in court.

Data analysis was conducted on GS/MS measurements collected using 13 different drug standards from three different laboratories using five different instrumental setups. An expanded uncertainty of two times the standard deviation from the mean (2σ) was used to identify the uncertainty of measurement for the retention time and relative ion abundance measurements made within these laboratories. The rationale for the use of a 2σ criterion is that this corresponds with an estimate of the 95% confidence interval, assuming normally distributed data.

Based on the numerical results, the retention time acceptance criteria currently recommended by different agencies are considerably wider than the average 2σ per week or per month determined through this study. Similarly, differences exist between recommended acceptance criteria for the uncertainty of measurement of relative ion abundances and this data set; however, when the uncertainty of measured relative ion abundances was averaged across all substances and all laboratories, the measured uncertainties agree quite well with the recommended acceptance criteria. The numerical analysis based on this data set indicates that the acceptance criteria within an instrument within a laboratory should be considerably tighter for the retention time. Also, depending on the tune frequency of the mass spectrometer, the relative ion abundance measurements should have narrower acceptance criteria than typically recommenced. The application of tighter acceptance criteria would provide more selective identification than the currently used broad-applicable recommendations.

Variability, Acceptance Criteria, GC/MS

B63 The Characterization of Nylon Fiber Color by Ultra High-Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS)

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After attending this presentation, attendees will recognize the merits of using UHPLC coupled with Time-Of-Flight/Mass Spectrometry (TOF/MS) in the analysis of nylon fiber dyes.

This presentation will impact the forensic science community by demonstrating that UHPLC-TOF/MS is suitable for the analysis of dyes in nylon fibers.

Nylon is a synthetic polyamide with repeating units made of hydrocarbons linked by highly polar amide groups. Acidic dyes are commonly used to color nylon fibers, where the dye molecules interact with the amide groups via hydrogen bonding. Current techniques for analyzing dyes in nylon fibers include microspectrophotometry, thin-layer chromatography, high-performance liquid chromatography, and capillary electrophoresis.

There are advantages to using UHPLC-MS to analyze nylon fiber dyes. The results provide chemical-level information about the dyes that would not be available via analysis by microspectrophotometry or thin-layer chromatography. Besides molecular mass information, partial structural information may be accessible if a fragmentation technique is available. On the downside, the method is destructive and other analyses, such as microspectrophotometry, would need to precede it.

Depending on the type of dye used to color the fiber, extraction can be difficult. A solvent for extracting the dye from the nylon fiber must be capable of solvating the dye molecules by reducing their affinity for the nylon fiber. Equal parts deionized water, pyridine, and ammonium hydroxide were mixed and used as extraction solvent. The fiber and solvent were heated at 100°C for one hour to achieve optimum dye extraction. Then various nylon fibers of different colors from several manufacturers were subjected to this dye extraction protocol followed by analysis of the extracted dyes via UHPLC-MS. Each sample was run under both positive and negative ion modes. The UHPLC column used was a Brownlee SPP by Perkin Elmer® (2.7µm:C18 2.1x30mm) with a method run time of four minutes.

The method yielded signals in both positive and negative ion modes for the dyes extracted from samples of different colors. Manufacturers typically use a proprietary blend to obtain different dye colors by mixing dyes in different ratios. Consequently, different colors from the same manufacturer display signals corresponding to the same dye components, in different ratios. Some dyes were present in all colors, while others were only present in certain colors. The data generated from UHPLC-MS analysis yielded a fingerprint for each sample that could be used to improve the confidence in the forensic examination of nylon fibers.

Nylon Fiber, Dye, UHPLC-MS

B64 A Sticky Situation: How Adhesive Collection of Fibers Affects Analysis

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After attending this presentation, attendees will understand how the use of tapes as a collection method could negatively impact the comparison of fibers.

This presentation will impact the forensic science community by explaining the effect of adhesive tape collection on common fiber analytical techniques. The outcome of this study will be the creation of a standardized methodology for forensic fiber collection using tape.

This study focuses on three techniques commonly employed in the analysis of fibers: fluorescence microscopy, Microspectrophotometry (MSP), and Fourier Transform Infrared (FTIR) spectroscopy, along with stereomicroscopy and polarized light microscopy. These techniques were utilized to examine contamination issues by the adhesive on fibers exposed to a variety of collection tapes.

In this study, four adhesive types were chosen: water-soluble trace evidence tape, Post-it® adhesive, fingerprint tape, and duct tape. These are commonly available products to crime scene investigators or tapes commonly found in crime scenes. Both natural and man-made fibers were selected for testing to provide a wide diversity of different morphological and chemical matrices for exposures to the adhesives. The fibers tested against each adhesive included cotton, wool, polyester, and nylon.

For fluorescence microscopy, four filter cubes were used with an Olympus® BX51. A scale of brightness was created to compare the fluorescence of each sample. The MSP data was gathered by a CRAIC™ MSP AX10 and analyzed by comparing the spectra in the Ultraviolet (UV) and visible region. A Nicolet™ FTIR with a Continuum Microscope was used to collect the FTIR spectra through the infrared to near visible wavelengths. A baseline analysis was made for each of the fibers and each of the adhesives (the controls). Then, the fiber was placed on the adhesive and removed again and the combination of fiber and adhesive was analyzed (combination fibers).

The fibers taken from the adhesives revealed contamination for most of the combinations. Results disclose changes in fluorescence, both enhancing and quenching, especially in the fingerprint adhesive and the trace evidence tape. For MSP, there were distorted peaks on the combination fibers compared to the control fiber. These peaks matched with the adhesive control, demonstrating that the contamination on the combination fibers comes from the adhesive from which the fiber is collected. The FTIR spectra for some contamination fibers revealed a mixture of peaks from the fiber control and the adhesive control.

These results establish the need for caution when using tape to collect fibers and when retrieving fibers from adhesives found in a crime scene. When comparing two fibers, the possibility of adhesive contamination must be recognized and, if possible, an analysis of the adhesive conducted to compare the spectra. Using the least-contaminating adhesive for collection is the most effective way to compare two fibers in these analyses.

Fibers, Tape Collection, Trace Evidence

B65 The Identification of Fine Plastic Materials by Thermal Desorption and Pyrolysis Combined With Direct Analysis in Real Time-Mass Spectrometry (TDP/DART®-MS)

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After attending this presentation, attendees will understand the value of TDP/DART®-MS for the rapid identification of minute plastic materials. This analysis method does not require any sample pre-treatment, such as solvent extraction.

This presentation will impact the forensic science community by explaining how TDP/DART®-MS can be effectively applied as an identification of minute plastic materials, such as synthetic fibers.

Drugs present in biological and autopsy specimens cannot be detected without first selecting the pretreatment and analytical conditions appropriate for the drugs. Thus, it is extremely important to investigate the analytical conditions suitable for specific compounds and samples; however, in recent years, due to the situation in which new substances appear one after another, including New Psychoactive Substances (NPS) that threaten society, it is very difficult to examine individually the analytical conditions that are appropriate for each new substance. Thus, a comprehensive analysis system for drugs that requires minimal investigation of pretreatment and analytical conditions is greatly desired. This study is investigating an analytical method for directly analyzing drugs in blood that does not require any pretreatment. In a previous study, by using TDP/DART®-MS for drugs in urine, each drug was separated and detected through thermal gradient heating for all drugs.[1] The detected ions were correctly identified according to their measured accurate mass and product ion spectra. Moreover, for the quantitative analysis, the calibration curves were prepared with urine-added drugs at concentrations ranging from 0.01μg/ml to 1μg/ml, and the curves were linear in that range; however, the detection sensitivity was not satisfactory, and this study sought higher sensitivity. In this presentation, the results of the investigation to improve the detection intensity of drugs will be described.

The samples were standard drug-mixture solutions and drug-mixtures loaded blood and urine (i.e., blank blood and urine samples with several types drug mixtures added). Mass spectra were obtained by using a quadrupole Time-Of-Flight (qTOF) MS equipped with a DART® ion source and a TDP unit. The TDP unit was mounted between the DART® ion source and the MS. Mass spectra were measured in positive-ion mode as the samples were heated from ambient temperature to 300ºC. Additionally, in order to improve the detection intensity, the solvent extraction for deproteinization treat and the analysis systems were investigated. For the investigation of solvent extraction for deproteinization treat, Ethanol (EtOH), Methanol (MeOH), and Acetonitrile (ACN) were used. For the investigation of the analysis system, the glass tee-tube (the HOOD) between an ion source and a qTOF was attached. This glass tee-tube can work to prevent the diffusion of volatilized drugs from the blood samples.

As a result of the investigation of solvent extraction for deproteinization treat, the highest sensitivity was attained using ACN. In addition, when using the HOOD, the peak areas of the extracted ion current gram of each drug were increased. It is concluded that the volatilized drugs had been ionized more efficiently by attaching the HOOD. Also, it is concluded that the sensitivity is further increased by increasing the heating rate of the samples.

Reference(s):

1. Hiroko A. et al. Forensic Drug Analysis by Thermal Desorption and Pyrolysis Combined With Direct Analysis in Real Time-Mass Spectrometry (TDP/DART®-MS). *Proceedings of the American Academy of Forensic Sciences*, 69th Annual Scientific Meeting, New Orleans, LA. 2017.

Identification of Plastics, TDP/DART®-MS, Adhesive Tape

B66 The Evidentiary Significance of Automotive Paints From the Northeast: A Study of Red Paint

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After attending this presentation, attendees will understand the significance of population studies specifically pertaining to automotive paints.

This presentation will impact the forensic science community by providing physical and chemical population data on automotive paints in the Northeast, thus informing criminalists about the significance of automotive paint characteristics.

This research was completed to provide data relating to the significance of automotive paint chips found in a specific population. Research has previously been conducted regarding Midwestern automotive paint populations as well as populations regarding the layer chemistry of the paints.[1,2] But to date, no research has been conducted on automotive paints from the Northeast. This research looked at paint samples from the Northeastern portion of the United States and uses common techniques in addition to emerging techniques for automotive paint analysis.

The populations of automotive paints are constantly changing and, thus, need to be thoroughly monitored. By investigating these populations, forensic scientists can begin to understand what significance each individual automotive paint may hold. In order to do this, the physical appearance, layer structure, and layer chemistry can be analyzed to provide a forensic examiner with more detail that can be used to give strength to a conclusion made during an automotive paint examination.

This population study involved the discrimination of red automotive paints using a comparative analysis approach and data analysis. The red samples were chosen as a target group from a larger automotive paint population based on popularity among consumers and manufacturers. The first portion of the analysis used stereomicroscopy, brightfield, and polarized light microscopy to analyze all samples collected in the population. This study analyzed the paint samples from approximately 200 automobiles ranging from 1989 to 2017. The macroscopic and microscopic characteristics of each sample analyzed included: relative surface color, presence of effect pigments, relative size of effect pigments, number of different pigments, number of layers, layer color, layer texture, and relative thickness of the layers. The population data obtained varied from the previously released reports from a Midwestern and North American automotive paint populations.[1,3] The Midwestern study analyzed 300 samples and the North American study was conducted on a much larger scale, but each demonstrates the importance of doing this type of study. For example, the present research had a 20% gray-colored frequency which differed from the less then 10% obtained in the Midwestern study and 16% in the North American study. The target color of red had a 13% frequency in the current study, as compared to the 15% in the Midwestern and 10% in North American studies.

Next, only the red automotive paints were further analyzed using a comprehensive sequence. This helped to determine the differentiating power of the analytical sequence as well as analyze the chemical properties of similarly colored paints. Current laboratory methods were used to analyze the red automotive paints, and included Ultraviolet/Visible Microspectrophotometry (UV/Vis MSP), Scanning Electron Microscopy with Electron Dispersive X-ray Spectroscopy (SEM/EDX), and Fourier Transform Infrared (FTIR) microspectroscopy. In addition, this research used Raman microspectroscopy, an emerging technique for automotive paint analysis that has been demonstrated to provide valuable pigment information.[1]

This study was conducted to highlight the significance of automotive paint comparisons and the characteristics each sample possesses. The frequency data and the degree of differentiation is important information as it can provide a foundation for determining the significance of indistinguishable samples.

Reference(s):

1. Palenik, Christopher S., Skip Palenik, Ethan Groves, and Jennifer Herb. Raman Spectroscopy of Automotive and Architectural Paints: Insitu Pigment Identification and Evidentiary Significance. *Microtrace LLC.* (n.d.): n. pag. 2016.
2. Zięba-Palus, J., and Borusiewicz, R. (2006). Examination of multilayer paint coats by the use of infrared, Raman and XRF spectroscopy for forensic purposes. *Journal of Molecular Structure.* 792-793, 286-292. doi:10.1016/j.molstruc.2006.03.072.
3. *Global Automotive 2016 Color Popularity Report.* Axalta. (n.d.). Retrieved from http://www.axaltacs.com/content/dam/New%20Axalta%20Corporate%20Website/Documents/Brochures/Axalta%202016%20Color%20Popularity%20Report.pdf.

Paint, Population Study, Analytical Sequence

B67 Assessing the Capability of Combining Elemental and Phase Mapping in Automotive Paint Systems Analysis Using Scanning Electron Microscope/Energy Dispersive Spectroscopy (SEM/EDS)

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The goal of this presentation is to familiarize attendees with new methods that are potentially useful in the analysis of automotive paint systems.

This presentation will impact the forensic science community by demonstrating how the combination of elemental analysis and phase mapping using SEM/EDS can provide useful information during forensic analysis of automotive paint systems. The use of this new method may also help confirm elemental compositions among the layers of paint systems, where overlaying of the principal X-ray lines of some elements occurs.

The analysis of automotive paint systems is of utter importance in forensic investigations of hit-and-run accidents to help to identify possible involved vehicle(s) and/or accident dynamics. During the manufacturing process of automobiles, a number of layers of coatings are applied sequentially to the car body to fulfill visual and functional demands. Each layer can be composed of different ratios of organic and inorganic binders, pigments, and additives, which in combination create characteristic automotive paint systems that could be highly distinguishable. When coupled with Fourier Transform Infrared Spectroscopy (FTIR) in forensic paint analysis, SEM/EDS provides comprehensive information regarding the inorganic and organic components of each coating layer. Standard elemental mapping using SEM/EDS provides detailed elemental information, but the indication of possible chemical compounds is limited. In this work, the capability of phase mapping to identify possible constituents and provide supplementary information in addition to elemental mapping is investigated.

The analytical method was developed and tested on 13 automotive paint systems. The paint systems were embedded in epoxy and the surface was thoroughly polished to expose the cross sections of the coating layers. Elemental data were collected in replicates on the Backscatter Electron (BSE) images of the cross sections. Based on the X-ray elemental maps and the quantitative information, such as weight percentage of each element, areas of distinctive composition of elements were identified as phase maps using commercially available software. The distribution, spectra, and major contributing elements of the phases were analyzed in each sample and their corresponding chemical compounds were identified based on mole fractions calculated using weight percentages.

Results demonstrate that phase analysis can be a useful tool in identifying possible inorganic compounds present in automotive paint systems. Elemental and phase maps collected in replicates are repeatable. A slight difference was observed between the experimental and empirical mole fractions, which will lead to further investigation in the range of experimental values for accurate and precise identification of the compounds. In addition, phase mapping may also be used to determine the presence of elements in cases where overlaying of the principal X-ray lines was found. More specifically, Barium (Ba) and Titanium (Ti) were co-detected across two coating layers in three samples, and elemental maps were not sufficient in confirming whether the elements were only present in one coating or in both layers due to the overlaying of K_α line of Ti, and the L_α line of Ba. Phase maps obtained from two of the samples allowed the exclusion of Ba from the E-coats of both samples and produced more resolved spectra for analysis.

The results from this work may provide a tool to identify inorganic compounds in complex automotive paint layers that cannot be accomplished by FTIR and elemental SEM/EDS analyses alone. The application also reveals a promising potential in assisting the interpretation of X-ray lines overlay in standard SEM/EDS analysis.

Automotive Paint Analysis, Phase Mapping, SEM/EDS

B68 The Enhancement of Human Scent Profiles as Forensic Evidence

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After attending this presentation, attendees will understand how human odor profiles can be collected and evaluated, why human scent can be utilized as an individual or class characteristic, and why there is a need for profile enhancement of odor profiles for all ethnicities.

This presentation will impact the forensic science community by portraying the reproducibility of findings in previous Caucasian and Hispanic human scent analyses as well as the novel incorporation of African American odor profiles.

Human scent has been previously defined as a complex mixture of Volatile Organic Compounds (VOCs) detected in the headspace above a scent sample.[1] Humans generate odor from several areas of the body, including scalp, hair, mouth, hand, axillae, and feet. The analysis of the chemical composition of human scent has enabled scientists to portray variations within these factors (age, gender, and ethnicity) to distinguish between individuals. Due to the novelty of human scent research, human scent evidence has been undervalued in the court of law; however, this type of evidence has significant value when physical evidence is not available at crime scenes. In order to increase the individualization and differentiation power of human scent evidence, this study sought to further investigate the identification of chemical signatures within the hands and axilla of specific ethnicities and genders.

During the study, the axilla odor of 60 participants was sampled. Upon collection, samples were extracted using both Headspace/Solid Phase Micro extraction (HS/SPME) and Liquid Extraction (LE) and analyzed using Gas Chromatography/Mass Spectrometry (GC/MS). The utilization of SPME immediately followed by LE complements the extraction of semi-volatile and non-volatile compounds, hence filling in the gaps of the compounds that could not be recovered using HS/SPME alone. This ensured that a full VOC profile was obtained, allowing for improved statistical analysis without requiring any additional sample collection. The samples were evaluated statistically to extrapolate data unique to specific individuals and groups.

Scientific advances have enabled the forensic science community to use scent as a feature for individual or class characteristic determination. The several analyses of body odors using the VOCs emitted have proven that, if enhanced, human scent can be just as useful as fingerprints and DNA in the attempt to identify individuals. In future work, the VOCS emitted from the underarm of human subjects can potentially correlate to specific Human Leukocyte Antigen (HLA) alleles. Additionally, once the unique odor profiles of each ethnicity can be identified and reproduced efficiently, the development of live human scent training aids for canines can commence.

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Human Scent, Volatile Organic Compounds, Solid Phase Microextraction

B69 Phase Equilibria of Complex Fluid Mixtures: Modeling and Measurements With the Advanced Distillation Curve With Reflux (ADC-R)

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After attending this presentation, attendees will better understand how the vapor-liquid equilibrium of complex fluids (such as ignitable liquids) can be measured using a modified distillation apparatus and how such measurements may be used to interpret results of forensic headspace analysis.

This presentation will impact the forensic science community by providing a metrology to improve understanding of the complex, real-world fluids that those in this community encounter daily. The experimental approach presented may support forensic analysis conducted by headspace methods, including arson fire debris analysis, by providing quantitative, low uncertainty data describing the relationships between the vapor and condensed phases of a sample of interest.

Building on the successful development and widespread implementation of the ADC approach to measuring the volatility of complex fluids like fuels, the ADC-R metrology was developed to address the gap in our ability to experimentally determine the vapor-liquid equilibrium of fluids containing more than two components.[1] High-quality measurements of the volatility of pure components and binary mixtures are readily available, but the fluids encountered by a criminalist, like fuels, contain many more components.[2] For such complex mixtures, the ADC-R collects data about the chemical composition of both liquid and vapor phases across a variety of temperatures, elucidating the entire two-phase region at atmospheric pressure.

Two simple mixtures were used to demonstrate the ADC-R method: a decane + tetradecane binary mixture and the Huber-Bruno surrogate, a ternary mixture developed to represent the volatility of an aviation turbine kerosene.[1,3] These simple mixtures used to develop and test the approach were chosen because they are well understood, having been extensively measured in previous work. The experimental T-P-x-y data were compared to existing mixture models. For both test fluids, the measurements of vapor-liquid equilibrium were in very good agreement with model predictions. The data were also used to improve the binary interaction parameters used in modeling.

This study concludes that the ADC-R is an appropriate method for measuring the T-P-x-y behavior of the complex and polydisperse fluids used in the real world. Gasoline and other ignitable liquids relevant to the forensic community may be measured using ADC-R. This approach and the data it may make available in the future are relevant to the interpretation of forensic headspace analyses, for example, the carbon strip method conventionally used to analyze arson fire debris.[4]

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4. Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration With Activated Charcoal. 2016, ASTM International.

Chemical Analysis, Trace Evidence, Ignitable Liquids

B70 The Development of the Precision ID GlobalFiler™ Next Generation Sequencing (NGS) Short Tandem Repeat (STR) Panel

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After attending this presentation, attendees will be aware of the development of sequencing STRs on an NGS platform and better understand visualizing and analyzing data with a new software platform.

This presentation will impact the forensic science community by presenting an NGS system developed and optimized for STR sequencing and data analysis. The resulting STR NGS profiles can be used in adjunct with partial Capillary Electrophoresis (CE) STR profiles from difficult casework samples.

Not unlike CE STR kit development, extensive studies were performed for new primer and master mix designs. Since these designs are made for NGS, they were developed to be compatible with library and template preparation techniques. Additionally, novel bioinformatics algorithms and software were invented to visualize and analyze data and allow analysts to transition from CE and NGS data and vice versa.

The Precision™ ID GlobalFiler™ NGS STR Panel targets 31 autosomal STRs and 4 sex-determining markers. The Precision™ ID DL8 Kit for Library Preparation on Ion Chef™, the Ion S5™ Precision™ ID Chef & Sequencing Kit for Template Preparation, and Ion S5™ Sequencing Systems for sequencing were developed to sequence STRs. Additionally, there are 53 Single Nucleotide Polymorphisms (SNPs) In Flanking (SIFs) regions surrounding the repeat. These SIFs allow for further discrimination of mixtures between major and minor contributors, as well as what may look like a stutter with CE versus a minor contributor allele.

In concert, the new Converge 2.0 Software provides an optimized pipeline for STR panel sequence analysis and reporting. Developed with a GMID-X-like interface, with browser-based navigation, Converge can be directly or indirectly connected to the Ion Torrent™ server. This allows secondary analysis data to be automatically transferred to Converge after sequencing runs are complete.

A study utilizing 34 known samples were sequenced with the Precision™ ID system workflow and concordance with previously generated CE genotyping assessed. In addition, 32 of the samples were used to create 1:10 and 1:20 mock mixture sample sets of both genders (male:female, male:male, and female:female). The mixture samples were a stress test for both the STR bioinformatic algorithms and detection of a low-level male contributor(s). High levels of concordance between the CE genotype and Massively Parallel Sequencing (MPS) genotype were observed and resulted in accurate results displayed in simplified STR and International Society of Forensic Genetics (ISFG) long-sequence nomenclature. Stutter ratios in the MPS system are elevated in comparison to those in a CE fragment length-based system due to the multiple rounds of PCR performed; however, even with higher stutter, the more complex and compound STRs allow for determining between stutter and minor contributor when the repeat motif of the minor contributor differs significantly from the stutter allele of the major contributor.

Next Generation Sequencing, Massively Parallel Sequencing, Mixture Analysis

B71 A Novel Workflow for Identifying Phenotypic Polymorphisms in Detoxification Enzymes Associated With Drug Metabolism

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After attending this presentation, attendees will have an alternative workflow to proprietary enrichment and library preparation pipelines provided by the major manufacturers of Massively Parallel Sequencing (MPS) platforms for the identification of Single Nucleotide Polymorphisms (SNPs), small Insertions and Deletions (INDELs), and Copy Number Variants (CNVs) in detoxification enzymes related to drug metabolism.

This presentation will impact the forensic science community by presenting an alternative workflow for identifying genetic polymorphisms associated with drug metabolism that forgo the proprietary workflows developed by manufacturers of next generation sequencing platforms. Alternative approaches have potential cost-saving implications as phenotypic polymorphisms are considered in molecular autopsies.

The capabilities and affordability of MPS are bringing personalized medicine into the realm of possibility for many patients. As costs continue to fall for MPS analysis, pharmacogenomic approaches offer an opportunity to provide insight into personalized drug metabolism rates. Most current efforts in this area have focused on opiate metabolism by members of the cytochrome P450 family of enzymes, typically in regard to accidental overdoses.

Polymorphisms associated with metabolism function are largely classified into the type of genetic lesion and the phenotypic effect associated with each particular mutation. The types of lesions are broken down into SNPs, small INDELs of nucleotide sequence, and additional CNVs from gene duplication events. (Due to being the most prevalent and similar to INDELs, both SNPs and INDELs will be referred to as SNPs for the remainder of the discussion.) As mentioned previously, the mutations are then further classified based on the phenotypic effect they have on enzyme function. These are broken down into silent mutations (in which no effect on metabolism is observed), gain of function mutations (in which an increased rate of metabolism is observed), and reduction/loss of function (in which an ablated or reduced rate of metabolism is observed).

As mentioned above, the expanding capabilities and reduced costs of MPS platforms allow an expansion of this technique to not only provide information for molecular autopsies associated with cardiac arrest or opiate overdoses but could provide insight into the levels of intoxication an individual has based on personalized drug metabolism rates. This information could be pertinent in establishing levels of impairment for culpability associated with drug-induced accidents.

One limitation to wider implementation of such technologies is that the costs are still slightly more than would be feasible for routine testing. To remedy this, a series of iterative price reductions are likely necessary to bring the costs down enough to make this type of testing routine. One area in which large cost savings are possible is the area of target enrichment and library preparation. Currently, manufacturers of MPS platforms have proprietary custom target enrichment/library preparation workflows that ease the design of the assay by taking the work load from the practitioner and exporting it to their production facilities. Without budgetary restraints, this scenario is favorable and desirable.

In situations in which budgetary constraints don't allow the higher price associated with proprietary custom target enrichment/library design, an alternative method that is only slightly more labor intensive is proposed.

Utilizing Primer-BLAST, primers were designed for long Polymerase Chain Reaction (lPCR) around the gene for the human carboxylase enzyme (CES1), which is known from literature to affect methylphenidate metabolism. The gene is encoded over an approximately 31kb region on the q-arm of chromosome 16 in humans. This study designed lPCR primers that broke the gene into two overlapping amplicons of approximately 17kb each. These amplicons were then pooled at equimolar concentrations and served as the enriched target for MPS library preparation. A third party enzymatic library fragmentation kit was used to cleave the lPCR amplicons into fragments of 200bp for MPS suitability. Another enzyme from the same kit was used to repair the cleaved ends and a third enzyme was utilized to ligate adaptors for barcoding/indexing of fragments prior to clonal amplification on an automated library preparation platform. Once the samples were placed onto the automated library preparation platforms, the recommended workflow of the manufacturer was followed through sequencing. SNPs were identified via both the manufacturer's bioinformatics software package as well as independently via generated fastq files and freely available software (IGV).

Analytical thresholds for identified SNPs were established based on depth of coverage in a region and percentage of reads observed for individual SNPs. This was based on an initial training set of 32 individuals sequenced twice for concordance between runs. Additionally, the nature of the mutations was determined based on 172 individual samples that were classified based on their sensitivity to methylphenidate and clustered via principle component analysis.

The method described could be adapted to other targets and purposes, such as molecular autopsy and other detoxification enzymes. Additionally, sonic fragmentation of amplicons could be used in a similar fashion.

Phenotypic SNPs, Detoxification Enzymes, Pharmacogenomics

B72 From the Ashes: Genetic Identification of Burned or Cremated Human Skeletal Remains

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After attending this presentation, attendees will better understand the potential and limitations of genetic investigations of burned and cremated human remains using traditional forensic DNA analysis techniques and next generation sequencing.

This presentation will impact the forensic science community by providing an avenue to gain maximum information from fire-damaged and cremated human remains that have historically been very difficult to identify.

Identification of human skeletal remains is a routine task for forensic DNA analysts; however, this task can be more challenging when the recovered bone fragments have been badly burned or charred, as occurs in cases such as mass disasters, house fires, or car accidents. Additionally, after commercial cremation, there may be a need to confirm the identity of the remains, for reasons including civil or criminal cases, paternity or kinship analysis, or identification of missing or deceased individuals.

Examination of skeletal remains typically begins with anthropologists who can employ various metric analyses to assess whether the remains are human or animal and assist in determining a biological profile including sex, stature, and weight; however, diagnostic bone fragments may not be available in remains damaged by fire or cremation. Furthermore, the accuracy of metric analyses of cremated samples is typically dependent on having the entire cremated remains, which is not always the case.[1] As such, DNA analysis may be the only option for identification.

Genetic identification of severely burned or cremated remains using traditional short tandem repeat analysis or mitochondrial DNA (mtDNA) sequencing has historically been limited due to low quantity and extreme degradation of remaining DNA and, in the case of cremated remains, concerns regarding contamination; however, advances in DNA analysis chemistries and technologies present an opportunity to re-assess these processes.[2]

Presented here are the results from five case studies involving aged, severely burned, or cremated human remains. DNA was extracted using an in-house-developed method modified from a commercial silica-based extraction kit. All extracts were quantified using real-time Polymerase Chain Reaction (PCR) to determine nuclear or mtDNA recovery. Nuclear DNA was assessed using the InnoTyper® 21 Kit. This kit targets small amplicons (60bp-125bp) and is intended for use with samples containing low quantity and highly degraded DNA. Mitochondrial DNA was assessed using an in-house-developed, whole-mtDNA genome probe-capture assay for sequencing on the Illumina® MiSeq®. Multiple samples from individual sets of remains were examined to assess consistency across results.

Results indicate that low levels of nuclear and mtDNA can be recovered from burned and cremated bone. Degradation indices obtained via quantitative PCR (qPCR) showed the recovered DNA was highly degraded. Partial InnoTyper® 21 profiles were obtained from concentrated extracts; however, exaggerated stochastic effects, such as allele drop out and peak-height imbalance, were observed in some profiles due to the low amount of starting template, complicating the profile interpretation. Results also revealed that sufficient mtDNA could be recovered from some remains for whole mtDNA genome sequencing using the probe capture approach and massively parallel sequencing. Overall, results determined that genetic information can be obtained from burned or cremated skeletal remains using emerging chemistries and technologies, providing an option for identification when traditional methods fail.

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Cremated Remains, DNA Identification, Next Generation Sequencing

B73 An Analysis of Challenging Forensic Samples Using Probe Capture Next Generation Sequencing (NGS)

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After attending this presentation, attendees will understand the advantages and application of a customized probe capture NGS system designed for forensically challenging samples.

This presentation will impact the forensic science community by illustrating how the custom probe capture NGS method is useful for the recovery and sequencing of the whole mitochondrial genome of forensically challenging samples (e.g., telogen hairs and touch DNA).

Forensic biological samples that are highly degraded, limited, and mixed (such as telogen hair and touch DNA) can be challenging for conventional Short Tandem Repeat (STR) genotyping. An alternative strategy for analyzing forensically challenging samples is analysis of mitochondrial DNA (mtDNA); however, conventional mtDNA sequencing methods such as Sanger Sequencing are limiting in discrimination power because they often fail to detect low level heteroplasmy or mixtures that are common in forensic samples. NGS methods have the potential to overcome many of the limitations of conventional methods used for analyzing mtDNA markers due to the high-throughput massively parallel clonal sequencing nature. In conjunction with using NGS, this study developed a custom probe capture enrichment system targeting the entire mitochondrial genome and 451 nuclear Single Nucleotide Polymorphism (SNP) markers for highly degraded and mixed samples. This approach uses DNA probes to enrich targeted regions from randomly fragmented DNA libraries for clonal, massively parallel sequencing, thereby maximizing recovery of short DNA fragments characteristic of forensic samples.

This custom probe capture NGS assay was successful in recovery and sequencing of the entire mitochondrial genome from forensically challenging samples, including 33 telogen hair roots, 22 telogen hair shafts, and 19 touch DNA samples recovered from spent cartridges using the double-swab method with cotton and flocked swabs. Thirty of 33 telogen hair roots yielded 100% coverage of the mitochondrial genome while 3 telogen hair roots had >80% coverage of the mitochondrial genome at >100x read depth. Seventeen of the 22 telogen hair shafts yielded 100% coverage of the mitochondrial genome while 5 showed >80% coverage of the mitochondrial genome at >100x read depth. Of these 5 telogen hair shafts, 4 failed conventional PCR-based amplification while 1 showed weak results. Furthermore, a subset of the DNA libraries of these telogen hair roots were captured and sequenced using a customized SNP probe capture assay, yielding 65.1% to near 100% coverage of the 451 nuclear SNPs. In addition to telogen hairs, touch DNA collected using cotton swabs exhibited an average of 93.7% coverage of the mitochondrial genome while touch DNA collected using flocked swabs exhibited 100% coverage of the mitochondrial genome at >100x read depth. A subset of these touch DNA samples collected using flocked swabs exhibited 100% coverage of the mitochondrial genome at >500x read depth. The major mtDNA variant sequences were consistent for all touch DNA samples and with the subject's profile. The mtDNA haplogroup was determined to be H7 for all four of the touch DNA samples and the reference.

In conclusion, this probe capture NGS system is shown to be useful for recovery and sequencing of the entire mitochondrial genome for forensically challenging samples, including telogen hair roots, telogen hair shafts, and touch DNA recovered from spent cartridge casings. Furthermore, both nuclear SNP and mtDNA markers can be analyzed from a single DNA library prepared from challenging samples that are limited in quantities and compromised in qualities. Therefore, when a sample with degraded or a low amount of DNA is encountered, this probe capture NGS method will allow for both SNP and mtDNA analyses without consuming more DNA extract.

Probe Capture, Next Generation Sequencing, Touch DNA

B74 An Innovative Massively Parallel Sequencing (MPS) 74-Microhaplotypeplex Forensic Assay for Improved Deconvolution of Mixed DNA Samples

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After attending this presentation, attendees will appreciate the potential of using MPS for short DNA regions containing multiple Single Nucleotide Polymorphisms (SNPs), called Microhaplotypes (MHs), to deconvolute DNA mixtures.

This presentation will impact the forensic science community by proposing a novel analytical approach on an MPS platform to improve the forensic analysis of mixed DNA samples.

Microhaplotypes are novel genetic markers with two or more SNPs within a short distance of each other (≺300 nucleotides), which jointly define a multi-allelic locus. MHs are useful for identification purposes and prediction of biogeographic ancestry, but also demonstrate great potential for mixture deconvolution as they are characterized by having important differences from conventional Short Tandem Repeat (STR) markers, such as same size alleles, lower mutation rate, and the absence of stutter peaks.[1] As the mainstay Sanger sequencing does not allow determining the cis/trans relationship between individual SNP alleles (i.e., the haplotype), the MPS technology overcomes such limitations by clonal sequencing of individual strands and the detection of the haplotype of interest within a specific locus.[2]

In this study, an innovative MPS multiplex panel of 74 MH loci, with optimum characteristics for improved deconvolution of DNA mixtures, was developed and tested on the Ion Chef/Ion S5™ MPS platform. To simulate a range of scenarios typically encountered in casework samples, a series of unbalanced two- to four-person DNA mixtures characterized by different ratios between contributors was prepared. The performance of the assay in targeting one or more minor contributors in the presence of up to 160-fold excess of major contributor(s) was tested using different amounts (1ng-10 ng) of input of genomic DNA (gDNA).

For data analysis of the Ion AmpliSeq™ sequencing results, the latest released beta version of Microhaplotyper Plugin was used for genotyping the DNA contributors. The MPS 74-MHplex forensic assay was shown to detect the minor contributor at higher mixture ratio than conventional autosomal STR markers, regardless of the sex of the individual. For two-person mixtures, a full MH profile of the minor contributor was reported at 1:10 and 1:20 ratios, whereas an increased number of allele/locus drop-out events was observed proportionally at 1:40, 1:80, 1:100, and 1:160 ratios; fewer were also reported at lower mixture ratios for three- and four-person DNA mixtures. Albeit allele drop-outs reduced the total number of exploitable MH loci, the Random Match Probability (RMP) calculated for the minor contributor(s) was higher or within the range of values typically obtained from full/partial autosomal STR profiles at low and high mixture ratios, respectively. In addition, MH mixture profiles of two to six persons were statistically simulated to determine the global distribution of alleles detected for 74 MH loci. Interestingly, promising results suggest the possibility of estimating the potential number of contributors based on the total allele count observed for each specific two-, three-, four-, five-, and six-person mixture-group.

These findings indicate that the MPS 74-MHplex assay is an insightful forensic DNA tool for improving the deconvolution of mixed samples and simultaneously extrapolating useful biogeographic ancestry information of the detected contributors.

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Microhaplotypes, Mixture Deconvolution, Massively Parallel Sequencing

B75 Massively Parallel DNA Sequencing Applications for Forensic Mixture Analysis

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The goal of this presentation is to characterize massively parallel DNA sequencing data for the analysis of mixture samples.

This presentation will impact the forensic science community by making a powerful case for the promise of Massively Parallel Sequencing (MPS) technology to enhance current capabilities for mixture deconvolution.

The introduction of MPS technology for forensic DNA analysis offers many advantages over legacy Capillary Electrophoresis (CE) typing due to the elucidation of sequence information in addition to fragment size, as well as the ability to multiplex many forensically relevant markers. The information gleaned from DNA sequence data relative to CE fragment size may provide increased discriminatory power among individuals, which could prove valuable for the deconvolution of mixture samples. Despite the clear theoretical advantages of MPS data for mixture analysis, mixture deconvolution software tools have not been developed to utilize the added information obtained from sequence data.

To characterize MPS data for mixtures samples, more than 100 artificial mixture samples were established, ranging from two to five contributors (male and female) in varying ratios to include minor contributor input at 3% of the total mixture quantity. Samples were generated using eight single-source contributors (four males and four females) previously characterized using CE and MPS technologies and selected for size- and sequence-based allele diversity. A dilution series of all single-source samples was created to establish profiles at various input levels from 500pg to 15pg. These samples were processed using the Promega® PowerSeq™ Auto/Y System (targeting 22 autosomal Short Tandem Repeats (STRs), 23 Y-chromosomal Short Tandem Repeats (Y-STRs), and amelogenin) across five sequencing runs on the Illumina® MiSeq®. Data were analyzed with Battelle ExactID® software. Results were further characterized using custom software applications to identify allele and stutter sequences and to recognize contributor profiles among the mixture data.

Data from the single-source samples were evaluated for genotype accuracy and patterns in stutter and drop out across the dilution series. Single-source samples generated with full DNA input (500pg) were accurate and complete relative to data generated with CE and other MPS platforms. Across the 8 contributors, 8 out of 46 loci exhibited at least one isoallele, which are identical by length but differ in sequence. Loci D2S1338 and D12S391 displayed the largest gains in resolution due to sequence differences among same-size alleles. Patterns in sample- and locus-specific drop out were characterized across the dilution series. Stutter patterns were largely consistent across dilution series and reflected similar patterns to those observed in CE data.

Mixture samples were queried for each contributor profile and were compared with expectations based on single-source sample performance. Minor contributors were detected even when providing as little 3% (approximately 15pg) of the template DNA in the mixture. The relative representation of each contributor in the MPS data was evaluated by comparing read counts for alleles present only in a single contributor, as well as by comparing read counts attributed to Y-chromosome versus autosomal STRs to compare male versus female contributions. By all measures, the relative read counts attributed to each contributor were highly consistent with the true proportions of each sample contributing to the mixtures.

The current data set provides novel insight into the value of MPS data for mixture analysis. The results support a growing body of evidence suggesting that MPS technology provides accurate and reliable data for forensic STR markers, with an improved power of discrimination for loci exhibiting isoalleles. Moreover, the current demonstration of the sensitivity of MPS data to capture minor contributors, together with the strong reflection of the true contributor ratios in the relative read counts, makes a powerful case for the promise of MPS technology to enhance current capabilities for mixture deconvolution.

Massively Parallel Sequencing, Mixture Deconvolution, Forensic DNA Analysis

B76 Enzymatic Cascades for Biochemical Identification From Sweat

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After attending this presentation, attendees will better understand that sweat is a complex, non-invasive, biological fluid capable of discriminating between multiple individuals based on the respective concentrations of biochemical content in the sweat of each individual.

This presentation will impact the forensic science community by providing a new method and purpose for sweat analysis that is capable of differentiating metabolic compounds inherent in each individual's sweat. This presentation will demonstrate the potential of sweat analysis for biochemical identification purposes to increase security measures by serving as a locking mechanism for electronic devices. Future research into sweat analysis will also create an alternative to the time-expensive DNA identification processes via the bio-affinity detection of small molecules in sweat.

Sweat is a non-invasive, biological fluid that is continuing to attract attention by the scientific community as it contains various amino acids and other low molecular-weight compounds. The concentrations of the biochemical content within an individual's sweat are controlled by hormone metabolism processes that are variable and fluctuate daily based on factors such as age, gender, diet, and activity levels. Therefore, no two individuals will have the same hormone levels at a given time and the concentrations of these sweat components should be specific to each individual; however, the limitation of instrumental detection limits prevents the determination of an individual based on a single analyte. By monitoring multiple analytes, the probability of correctly identifying a person based on these metabolic analyte concentrations increases. The combination of these levels has the potential to increase security measures by serving as a locking mechanism for electronic devices containing personal information, such as smartphones and tablets, and are much more difficult to duplicate than current security measures.

Three compounds commonly found in sweat and fingerprints were studied using three separate and respective single-analyte enzymatic assays for the analysis of sweat by ultraviolet-visible spectrophotometry. The separate use of all three methods has the ability to determine the concentrations of each compound within the sweat of the sample originator. Analysis was first performed on 50 mimicked sweat samples that were created and tested based on the physiological concentrations of amino acids and small molecules known to be present in sweat. Additionally, a collection and extraction method was successfully developed in order to collect and test authentic sweat samples from volunteers. The use of similar enzymatic assays has been previously demonstrated when analyzing fingerprints. Since the amino acid content was sufficient for authentic fingerprint detection, the amino acid content in authentic sweat should be the same, if not greater. The application of sweat collection was designed to decrease the sampling error, as compared to fingerprint collection, and increase the amount of sweat collected to aid in the analysis of small molecules at low concentrations.

Biomarker analysis is a well-established discipline in forensic science that involves the analysis of biological samples for the presence of various substances indicative of personal attributes, such as the identification of an individual through DNA in blood. Although the forensic science field has developed rapidly over the years, the investigation processes are lengthy and the majority of the routinely used forensic science techniques require proper sample collection at the crime scene, followed by transportation to a laboratory facility before any informative analyses. This has led to a backlog in the analysis of serology samples. The research presented here addresses this situation by introducing the use of bio-affinity-based assays for quick and straightforward analyses of sweat. Because only miniscule amounts of enzymes and substrates are necessary, this method only requires very small amounts of samples and can be developed into a field kit for on-site testing. Furthermore, the bio-affinity-based cascades are remarkably versatile and can be adjusted for the analysis of a wide range of substrates. By utilizing re-programmable bio-affinity-based cascades, this approach has the potential to create a new method for identification, which can decrease crimes through increased security measures and move the strictly laboratory-based analyses to rapid on-site analyses that do not require specialized laboratory training. This could lead to the revolution of the field of forensic science and result in the acceleration of many criminal investigations.

Identification, Sweat, Cascades

B77 A Comparison Study of a Mass Spectrometry (MS) -Based Serological Assay With Existing Casework Models

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After attending this presentation, attendees will better understand the advancements achieved with protein MS for the identification of five human body fluids and their impact on existing casework models.

This presentation will impact the forensic science community by assessing the performance of a validated MS-based serological assay in an operational environment. The results of this study demonstrate an increase in sensitivity and specificity over existing workflows.

Protein MS has emerged as a technique to supplant traditional enzyme- and antibody-based tests for the identification of body fluids. As part of a multi-year initiative, a robotic, fully automated sample preparation workflow coupled with protein MS has been developed and validated for the confirmatory identification of five biological fluids (saliva, blood, seminal fluids, vaginal fluids, and menstrual fluids). Sample preparation is conducted in 96-well plate format using the Agilent® AssayMAP Bravo automation platform with protein identification on an Agilent® 6495 triple quadrupole mass spectrometer coupled to an Agilent® 1290 series Ultra High-Performance Liquid Chromatography (UHPLC). Combining these two pieces of hardware, up to 100 samples can be prepared and analyzed in one day. A fit-for-purpose developmental validation has been completed by integrating appropriate studies according to guidelines set by the Scientific Working Group for Forensic Toxicology (SWGTOX) and the Scientific Working Group on DNA Analysis Methods (SWGDAM). Developmental validation studies assessed reproducibility/repeatability, sensitivity, stability, mixture analysis, specificity, carryover, ion suppression, and limit of detection.

In addition to the developmental validation studies, the workflow was assessed to demonstrate that the technology is "fit-for-implementation" in relation to existing casework models (e.g., immunochromatographic/antibody screening, and DNA/Short Tandem Repeat (STR) typing protocols) and the overall operational workflow of a forensic laboratory. A series of appropriate mock casework samples that assessed recovery of substrates, overall assay sensitivity, impact of potential contaminants, multi-fluid mixtures, sample degradation, and mock sexual assault kits were prepared and processed in tandem by MS, currently employed serological tests, and by standard DNA profiling methods.

Commercially available serological tests included antibody- and enzyme-based platforms targeting blood (RSID™ Blood, ABAcard® HemaTrace), seminal fluid (RSID™ Semen, ABAcard® p30), and saliva (RSID™ Saliva, SALIgAE®). DNA extracts were processed via organic/organic differential extraction, Quantifiler™ Trio DNA Quantification Kit, GlobalFiler™ Polymerase Chain Reaction (PCR) Amplification Kit/Y Filer® Plus PCR Amplification Kit, and analyzed using Applied Biosystems® 3500 Genetic Analyzer. Two hundred fifty samples were assayed across the three approaches. For serological analysis, the MS approach offered superior detection limits (e.g., human blood detection from less than 10nL of whole blood recovered from a swab) while also providing true confirmatory results. Furthermore, the MS method can reliably detect vaginal and menstrual fluids, for which commercial assays do not exist. While genetic testing of STRs has historically proven to be much more sensitive than traditional serological methodologies, MS and DNA analysis are more comparable in terms of sensitivity limits. For low-level samples (e.g., picoliter quantities of body fluid), a relationship between the number of protein targets identified and corresponding peak height intensities with the number of alleles detected from the known donor was observed. For example, a low-level seminal fluid sample produced two out of five target seminal fluid markers and generated 67% of an STR profile. Overall, the MS method allowed for clear, unambiguous, serological identification of body fluids to the point where the technology can be called "comparable" to STR testing.

In conclusion, the implementation of the MS approach offers comparable sensitivity to current genetic testing methodologies, providing an advantageous relationship between a positive body fluid identification and the likely success of downstream DNA analysis. This provides a greater tool to forensic examiners seeking to perform sample prioritization and deliver confirmatory contextual information in a criminal investigation.

Serology, Proteomics, DNA Testing

B78 Confirmatory Identification and Genotyping of Human Seminal Fluid Collected on Surface-Enhanced Raman Scattering (SERS) -Active Forensic Evidence Swabs

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After attending this presentation, attendees will better understand a novel method for serological screening of forensic evidence for confirmatory identification of human seminal fluid.

This presentation will impact the forensic science community by providing results for a method that enables rapid, highly sensitive, non-destructive identification of semen. These data will ultimately be used to expand the utility of the method to other human biological fluids.

Forensic evidence is often screened to determine whether biological fluids are present prior to attempting DNA analysis. Fluid-specific serological tests employing immunological and biochemical indicators are typically used to make these determinations. Initially, a presumptive test establishes the possibility that a particular fluid is present. Next, a confirmatory test is used to identify the material and species of origin; however, these methodologies lack sensitivity and specificity. Additionally, because these tests are performed sequentially, biological fluid identification can be expensive, labor intensive, and require consumption of precious samples.

Raman spectroscopy is an optical technique that characterizes the inelastic scattering of light that is indicative of the composition of a particular molecular species. Due to the low probability of the Raman scattering event, Raman analysis of small amounts or concentrations of analyte can be problematic. SERS is an extension of the Raman spectroscopic technique in which analyte signals can be enhanced by several orders of magnitude when on or near a nanostructured metallic surface. Interaction of the electronic structures of the analyte and SERS substrate increases the analyte's Raman cross-section (i.e., the likelihood of inelastic scattering) through a chemical and/or electromagnetic enhancement mechanism. Some studies have shown that Raman spectroscopy is well suited for the analysis of biological fluids because it is rapid, highly selective, and non-destructive.[1] In the forensic laboratory, Raman spectroscopic analysis could enable a reduction in the number of serological tests performed on an evidentiary item since it can be used for simultaneous identification of all relevant biological fluids; however, Raman spectroscopy alone may not provide the level of sensitivity required for forensic samples, especially in cases in which low laser excitation powers are preferred and/or fast analysis times are required. As a result, SERS may be a more appropriate approach. This presentation describes a novel method to identify human seminal fluid on nylon-flocked swabs coated with silver nanoparticles.

For this study, sample collection swabs were prepared by synthesizing silver nanoparticles on the surface of COPAN® 4N6FLOQSwabs™ using the hydrogen reduction method.[2] Distribution and size of nanoparticles were characterized using a scanning electron microscope. Efficacy of SERS enhancement was tested by swabbing a dried sample of $[Ru(bpy)_3]^{+2}$ with a prepared collection swab, followed by detection with a Horiba LabRam HR Raman microscope. A 3X serial dilution of sole-source human seminal fluid was performed to create a total of seven samples with concentrations ranging from 15ng/µL to 21pg/µL of genomic DNA. Diluted semen (10µL) was pipetted onto the surface of swabs with and without SERS nanoparticles. Raman data of analyte adhered to the SERS-active swabs were collected using 632.8nm laser excitation and various collection geometries and integration parameters and analyzed to determine a limit of detection of the method. Following spectroscopic analysis, DNA was extracted from each swab using the Applied Biosystems® PrepFiler® Forensic DNA Extraction Kit. Several controls were also integrated during extraction. Extracts were quantified using the Applied Biosystems® Quantifiler® Trio kit. Quantifiable DNA was detected in all extracts except those obtained from swabs with low initial concentrations. Recovery was not affected by SERS particles or exposure to the Raman laser, as no difference was observed in DNA recovered from semen on naked swabs, SERS swabs, and positive controls. Additionally, quantification values were compared to Raman results to determine whether a correlation exists between SERS signal intensity and DNA recovery. DNA from a subset of samples was amplified using the Applied Biosystems® Quantifiler® kit. Full STR profiles were obtained for all samples with high starting concentrations. As expected, stochastic effects were observed in data obtained from samples with the lowest starting concentrations; however, no differences were observed in DNA profiles from SERS extracts versus non-SERS extracts. These results demonstrate that confirmatory identification of human seminal fluid using SERS is robust, sensitive, and does not affect downstream analyses.

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Serology, Semen, SERS

B79 Identification of Body Fluid Using Multiplex Polymerase Chain Reaction (PCR)

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After attending this presentation, attendees will better understand the development of a novel trace method for the determination of body fluids based on epigenetic markers and sequencing techniques. The procedure involves a multiplex amplification of tissue-specific methylation sites followed by pyrosequencing and/or massively parallel sequencing to determine the identity of the tissue.

This presentation will impact the forensic science community by providing results from an optimization study and evaluation of a multiplex reaction that combines previously developed singleplex reactions determined in prior studies. This presentation will contribute to efforts being made worldwide to expand the information that can be gleaned from an individual's DNA for use in a forensic investigation.

When investigating a crime, it can sometimes be more important to determine the origin of a DNA extract in order to prove criminal intent. For example, in situations such as child abuse, it is the type of body fluid present that is important and not the presence of the suspect. In recent years, multiple DNA methylation markers have been developed for use in the prediction of whether a DNA sample originated from blood, saliva, vaginal epithelia, or semen.[1,2] These loci, known as tissue-specific Differentially Methylated Regions (tDMRs) can exhibit hypomethylation in one body fluid type while exhibiting relatively high methylation levels in other body fluids. Additionally, methylation markers have been found for the prediction of biological age, but demonstrate that the body fluid can influence the results.[3,4] Therefore, it is necessary to first determine from which body fluid the DNA originated. The goal of this project is the development of a multplexed amplification that would permit the analysis of a single sample of genomic DNA for all body fluid types. Such a procedure can save valuable evidence and time, as well as permitting a quicker and more comprehensive result.

In this experiment, a multiplex of previously published tDMRs, found in BCAS4, CG06379435, PFN3A, and ZC3H12D, was created and the amplification process was optimized for further analysis. This optimization was achieved by careful design of the primers to minimize mispriming events, as well as altering the concentrations of primers and Magnesium Chloride ($MgCl_2$) in order to attain roughly equal representation of each amplicon in the final PCR product. Next, a set of 16 samples of extracted DNA — consisting of saliva, blood, vaginal epithelia, and semen — were treated with bisulfite in order to convert unmethylated cytosines to uracil.[5] Methylated cytosines are not converted, permitting determination of relative levels of methylation based on sequence analysis. A multiplex amplification of the above tissue's specific methylation sites was then performed and the resultant DNA sequenced using a pyrosequencer. The results of pyrosequencing were performed using four separate pyrosequencing runs of the amplified samples. An additional subset of samples was also analyzed simultaneously using massively parallel sequencing. The results of the pyrosequencing and massively parallel sequencing were compared to ensure that the same results were being attained in either method.

The 16 DNA samples were correctly identified as belonging to one specific body fluid type. In either sequencing method, the percent methylation at each of the amplified regions was found to be consistent with the results of these methylated regions when amplified individually.[6] Going forward, it would be advantageous to build upon this multiplex by adding in age-specific methylation markers. These markers would rely on the identification of the body fluid in order to increase the accuracy of the age prediction model.

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Body Fluid Identification, tDMR, Massively Parallel Sequencing

B80 The Effect of Organic Acid Influenced by Sample pH on False Positive Test Results Using Immunochromatographic Assays

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After attending this presentation, attendees will better understand how the presence of organic acids at various pH levels can affect the accuracy and reliability of results obtained using immunochromatographic assays for multiple biological fluids from a number of manufacturers.

This presentation will impact the forensic science community by emphasizing the importance of using immunochromatographic tests as a presumptive indication of biological fluids and will illustrate how results from these assays should not be overstated. Instances of non-specific binding events utilizing various immunochromatographic assays as influenced by sample pH and the presence of organic acids will be evaluated.

A majority of forensic biological evidence requires detection of bodily fluids as a means to identify and prioritize items that should be processed for genetic analysis. The screening and confirmatory identification of these bodily fluids can greatly aid an investigation; however, it is important to understand the limits of the test employed. The current methodology most routinely applied to forensic casework for the detection of biological fluids is immunochromatographic assays. Manufacturers of these tests, including Seratec®, Abacus Diagnostics®, and Independent Forensics, market such assays for blood, semen, saliva, and urine detection. Regardless of manufacturer or target analyte, these tests function in a similar manner and therefore suffer from the same limitations. Target biomarkers present in lower concentrations in other biological fluids have demonstrated the potential to produce positive reactions.[1-3] Additionally, false positive reactions due to cross-reactivity with non-target molecules with similar conformational epitopes are possible as are non-specific binding events.[4] This latter category of false positives was further investigated in this study.

Common immunoassay tests utilized in forensic casework, including ABAcard® p30 and ABAcard® HemTrace® by Abacus Diagnostics®; RSID™-Urine, RSID™-Semen, RSID™-Blood, and RSID™-Saliva by Independent Forensics; and PSA Semiquant, HemDirect, and Amylase Test by Seratec®, were evaluated. A 300mM solution of citric acid was prepared across a pH range from 1.78 to 12. Citric acid solution was added according to the manufacturers' recommendations for each test with regard to sample incubation times, volumes, and run times for analysis. Invalid test results, negative results, and false positive results were recorded over the indicated pH range. Repeatability was evaluated by testing the lowest and highest pH citric acid solution to produce a false positive result in triplicate for each test. Deionized water solutions over the pH ranges that produced false positive results for citric acid solutions were also analyzed.

False positive results were observed for each assay evaluated and were seen between a pH of 2 to 12 on various tests. Repeatability was observed for all tests evaluated. All deionized water solutions produced negative test results as expected, indicating the role of organic acids in generating non-specific binding events. It should be emphasized that based on the findings exhibited in this study, immunochromatographic tests display presumptive findings due to lack of specificity. Additional organic acids were also evaluated at multiple concentrations as were possible mechanisms leading to false positive test results.

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Immunochromatographic Assays, False Positives, Organic Acid

B81 The Growing Phenomenon of the Epidemic of Synthetic Opioids and Forensic Science: Impact and Response

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After attending this presentation, attendees will better know and understand the unprecedented spread of synthetic opioids, the trends that most law enforcement agencies face as a direct result of widespread opioid use and illicit sales, and what is currently being accomplished to better understand and combat this unceasing epidemic.

This presentation will impact the forensic science community by raising awareness of the spread of synthetic opioids and by examining perspectives from a variety of forensic science areas, including field investigations, criminalistics, forensic pathology, toxicology, and pharmacology.

In the past year, there have been more known fentanyl-related overdose deaths in the United States than in the past 60 years. The current crisis is multi-faceted and involves a global supply of the substances being smuggled into the United States. The increase of drug fatalities over the past few years demonstrates that more potent and more sophisticated designer drugs are not only available on the streets but are also finding their way into the population following internet purchases and receipt through the mail.

This session intends to address this epidemic from multiple perspectives. In March of 2017, the American Academy of Forensic Sciences, seeing a need to gather its diverse resources to evaluate the current synthetic opioid crisis, established an *ad hoc* committee. Four task groups (Analytical Chemistry, Education and Outreach, Medicolegal Issues, and Health and Safety) were created to approach this challenge from a targeted perspective. A summary of these efforts will be provided through a discussion of lessons learned and opportunities gained. Experts of various disciplines will intervene and share their experience and expertise on this topic from various perspectives.

Discussion will include how the Prohibited Mail Narcotics program with the United States Postal Inspection Service is committed to protecting its employees, customers, and the public from the dangers of handling mail pieces containing illegal narcotics and the violence associated with drug trafficking.

The facets of a medicolegal death investigation paradigm shift in Cuyahoga County, OH, will be described. A new paradigm was adopted involving consideration of the use of internet and electronic media communication, purchase and distribution of product, and new safety concerns in the investigation of drug overdose deaths. The approach to telephone interviews on death reports, scene investigation and safety considerations, and interactions with law enforcement personnel, family members, witnesses, Emergency Medical Teams (EMTs), and medical personnel has evolved.

Recently developed methodologies for signature profiling of illicit fentanyl and fentanyl-related seizures at the Drug Enforcement Administration (DEA) Special Testing and Research Laboratory will be discussed. These methodologies include the quantitative determination of fentanyl, adulterants, and diluents, the quantitative determination of occluded processing solvents, and the identification of trace synthetic impurities from clandestine synthesis. The use of profiles of fentanyl isotopes, trace components, occluded solvents, and cutting agents to chemically link fentanyl seizures in which relationships were previously unknown or only suspected will also be discussed.

Detection, identification, and reporting of these novel and emerging illicit opioids is technically challenging, mainly due to their structure similarity and their mixture with other drugs. A Real-Time Communication Network has been created by the DEA Southeast Laboratory to provide technical assistance to forensic scientists and to facilitate the rapid dissemination of information regarding the analysis of synthetic opioids in real time. The use of this communication tool to share information of emerging synthetic opioids as they are being identified will be reviewed.

From a medicolegal perspective, the development and implementation of an online website that provides public access to a wide array of drug-related death surveillance resources and tools will be described. This database is populated by medical examiners and coroners' agencies and can be accessed by the public with no intermediary. This resource gives users access to detailed information regarding specific drugs, demographic information pertaining to the decedent, and to investigational findings related to the circumstances of the death.

An overview of the role of pharmacology and toxicology of opioids will be provided and the resources and analytical methodologies used to identify and quantify new drugs in driving under the influence cases and postmortem toxicology will be examined. The challenges of interpreting the role of these drugs in human performance and fatalities will be presented. In addition, data collected by a large reference laboratory that performs testing for municipalities and counties across the United States will be presented.

Synthetic Opioids, Fentanyl Analogs, Pharmacology

B82 The Data Needed to Realize the Value of Forensic Science

Sheila Willis, PhD, Forensic Science Ireland, Garda HQ, Phoenix Park, Dublin, IRELAND*

After attending this presentation, attendees will better understand the dangers associated with reporting test results in isolation and the effect of addressing activity-level propositions rather than source-level propositions.

The presentation will impact the forensic science community by highlighting the broader role that forensic scientists must play to ensure their information and findings do not contribute to miscarriages of justice.

The concept of forensic scientists addressing propositions at different levels of the hierarchy is well documented in the scientific literature.[1] The higher up the hierarchy, the greater is the value provided by the forensic scientist.

This presentation will outline the impact of addressing activity propositions rather than source propositions and highlight the type of data forensic scientists need to ensure their information is not misleading or used by others in a misleading manner.[2,3] Source propositions are those in which the scientists seek to establish whether two materials share the same origin or not, while activity propositions address what materials are expected to be found given a specific activity. Activity propositions need data on the probability of materials transferring in given scenarios and provide much more useful information to forensic questions. Following this logic, it is seen that the scientist may address whether two groups of glass fragments share the same source. This demands information on the discriminating power of the techniques used and the frequency of occurrence of specific parameters. This may be useful in court but will not be as helpful as considering whether the findings support the suspect being the man who broke the window rather than some other man. To address the latter, the scientist needs information for the court on the likelihood of glass transferring given the details of the breaking glass and the time since the incident to address the prosecution proposition and will need to know the probability of specific populations having glass on their clothing to address the defense proposition. The weight of evidence or likelihood ratio is the ratio of these two probabilities. This type of information is less well documented and sometimes less explicit than the data on techniques and frequency of occurrence.

Confining reports to source propositions can be misleading. Consider a situation in which a rare fiber is recovered from a garment and found to be indistinguishable from a reference sample. In isolation, the court is likely to consider this information to be helpful, but consider again if, given the contextual information, the scientist expects that multiple fibers should be transferred and recovered. It is advanced that the court needs this information and that the scientist has a duty to supply it.

The logic applies to all trace evidence and is, according to this presentation, also the case for low levels of DNA in which the discriminating power of the technique may overwhelm the other relevant factors.

The basis of this presentation will be the three principles learned from Evett: (1) that forensic science findings need to be interpreted in a context; (2) that at least two propositions should be addressed; and (3) that the scientist is well placed to address the probability of the findings rather than the probability of the propositions.[1]

This presentation will include examples from personal experience in which source rather than activity propositions were misleading and examples to illustrate why the context is important.

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Data, Principles, Activity

B83 Criminalists' Ethics in the Era of Social Media

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After attending this presentation, attendees will better understand the impact and additional pitfalls the expanding everyday use of social media has on the ethical behavior of criminalists.

This presentation will impact the forensic science community by providing a forum for the discussion on how social media can inadvertently draw an ethical criminalist to participate in actions that are counter to professional ethical codes of conduct.

Social media such as Facebook®, Twitter®, LinkedIn®, YouTube®, and Instagram™ have revolutionized the distribution and "life span" of information. The ability to instantly share our lives and activities with potentially millions of people by providing truncated bits of data without taking the time for thoughtful editing has changed the way society interacts. Social media is a valuable tool but can also be dangerous.

All professions have some form of code of conduct to provide guidance for ethical behavior. Forensic science is no different; there are codes such as the American Academy of Forensic Sciences (AAFS) Code of Ethics and Conduct and the American Board of Criminalistics (ABC) Rules of Professional Conduct. Forensic science codes of ethical or professional conduct can be found in many professional associations. As forensic science professionals, we strive to adhere to these codes and perform our work ethically, with honor and integrity. Unbeknownst to us, many of us have used social media without first evaluating the link between our ethics and social media outlets. Without recognizing and understanding the pitfalls, social media has made it more difficult to adhere to the codes of conduct. Actions that used to be a personal communication between two individuals have become public knowledge and available on line, forever.

Other professions, such as attorneys and health care professionals, are actively studying the impact of social media and providing their practitioners with additional ethical guidance. The federal government has prepared a guidance document for federal employees. Forensic scientists need to enter the conversation soon as too much time has already passed and damage may have already happened. Examples of social media damage in the criminal justice system include cases resulting in mistrials due to witnesses, jurors, and judges inappropriately commenting on cases through their social media accounts.

Ethics Code sections will be examined and discussed to help identify pitfalls. In addition, other concerns such as *Brady*, reputation management, and employment impacts will be considered. Can postings on Facebook®, YouTube®, or Instagram™ keep you from being hired by a government agency? Can defense attorneys or prosecutors use postings to show *Brady* violations? What impact can your on-line life have on your professional life?

Social Media, Ethics, Conduct

B84 A Report on the Forensic Science Research and Evaluation Workshop: A Discussion on the Fundamentals of Research Design and an Evaluation of Available Literature

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The goal of this presentation is to make attendees aware of the information contained in the published report.

This presentation will impact the forensic science community by serving as a guide to improve research design and literature evaluation.

This report is based on a "Forensic Science Research Evaluation Workshop" sponsored by the National Science Foundation and the National Institute of Justice and was held at the American Association for the Advancement of Science headquarters in Washington, DC. The impetus for the workshop was recent criticisms of the forensic sciences from public, legal, and scientific sources. One of the more important critical reports was the 2009 National Research Council Report, *Strengthening Forensic Science in the United States: A Path Forward.*[1] It was highly critical of the scientific foundations for several of the forensic disciplines, declaring that "Little rigorous systematic research has been done to validate the basic premises and techniques in a number of forensic science disciplines." and "… a statistical framework that allows quantitation … is greatly needed."[1]

Since the early 1990s, DNA analysis testimony began stating the probability of identified matches. Researchers have only recently begun to look at a mathematical and statistical basis for pattern comparison analysis, such as latent fingerprints, fired bullets, and toolmarks.[2-4] Materials such as paints, fibers, and tapes that are often found as physical evidence at crime scenes could be of greater value should we establish a statistical significance of an association with a suspect or victim. Knowing the abundance and variation in composition of these materials could be used to establish probability estimates of these materials randomly being found at the scene. Historically, there has been resistance to this approach because information on manufactured materials has been considered to be too difficult to maintain due to production changes; however, the establishment of well-maintained centralized databases are possible and should be developed.[5]

The workshop was formed to discuss the fundamentals of research design and the evaluation of the literature in order to conform to higher scientific standards in critical thinking and laboratory performance. The publication from the workshop is intended to provide some grist for evaluating and elevating the research efforts in the forensic sciences and it may be of value for the Organization of Scientific Area Committees (OSAC) members advanced practitioners, peer reviewers, and students of forensic science.[6,7]

The workshop was organized by a planning committee. Three subject areas were chosen, each consisting of a half-day session as follows: (1) experimental design and statistics; (2) interpretation and assessment; and, (3) policy implications. The goal was to bring together a range of 17 experts in the experimental and behavioral sciences, law, policy, and government funding to address the need for a higher standard of forensic science research. Each session consisted of one plenary speaker and four to five additional speakers. Each speaker had one-half hour to present their topic.

Each participant submitted a short essay of the topic they presented at the workshop, and they are included in the publication.[6] Additional observations and conclusions were made during panel discussions and these are included after the write-ups of each section.

In the summary of the report publication, there is an outline of topics to design research and evaluate forensic science literature.[6] The outline provides important considerations when planning a research project, reviewing submitted papers for publication, or simply determining the scientific quality of forensic literature. The report is intended to be a guide to assess forensic science research and its literature. The topics are not all-inclusive and are meant as a starting point. Each write-up has significant references to assist in a greater depth of background on the subject. For each specific discipline within the forensic sciences, evaluators will need a thorough knowledge base of the specific discipline to properly evaluate writings. If the evaluator is not strong in statistics, it is recommended that they confer with a statistician. A close look will be required to determine if the statistics used are appropriate. With this, it is hoped that a greater level of forensic science research is attained in the future.

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Forensic, Research, Evaluation

B85 FORESIGHT 2020 Project: Connecting Labs, Laboratory Information Management Systems (LIMS), and FORESIGHT for Benchmarking Performance

Max M. Houck, PhD, 140 7th Avenue, S, Davis Hall, St Petersburg, FL 33701*

After attending this presentation, attendees will understand a project connecting forensic LIMS to a forensic industry benchmarking process named FORESIGHT that provides labs with data for process improvement, efficiency, and quality control.

This presentation will impact the forensic science community by providing information regarding how the FORESIGHT 2020 project will make data submission to FORESIGHT and the National Institute of Justice (NIJ) grant compliance reporting easier, how analytics can improve laboratory performance and efficiency, and how using metrics increases accountability and cost effectiveness in both governmental and for-profit labs.

FORESIGHT 2020 is an information technology project that has developed software connecting forensic science LIMS to a forensic industry benchmarking process named FORESIGHT. Benchmarking is the comparison of an organization's processes and performance metrics to industry best practices from other organizations. Metrics can be descriptive (cases submitted or items tested, for example) or ratio based (reports per employee or tests per item, for example). Ratios allow for robust comparisons between laboratories providing a foundation for communication about better practices. FORESIGHT is the result of an NIJ award to improve the quality and quantity of industry information to forensic labs for process and quality improvement. FORESIGHT has been active for a decade in the forensic and public health industries, with hundreds of participating agencies worldwide. Forensic laboratories have used FORESIGHT data to improve efficiency, cut costs, reduce backlogs, and increase wages and compensation for employees.

The FORESIGHT 2020 software allows labs to submit data to the FORESIGHT process with a few clicks of the mouse, thereby easing data submission, improving the quality of the data, and increasing participation in FORESIGHT, resulting in broader representation of the nation's forensic laboratories' performances. The laboratories will have data to help with process improvement, efficiency, and quality control. In addition, the software helps achieve compliance with NIJ granting requirements and reports, and an analytical dashboard will assist the lab's self-awareness of real-time statistics, caseloads, and productivity.

The FORESIGHT 2020 software is currently in beta testing at laboratories around the country. Once beta testing is completed, the software will be made available for laboratories to download and install for use with JusticeTrax, STaCS, Porter Lee, or StarLIMS systems. The software is free under a grant to the American Society of Crime Laboratory Directors (ASCLD) from the Laura and John Arnold Foundation.

FORESIGHT, Benchmarking, Quality Assurance

B86 Rates of Loss and Replacement of Very Small Particles (VSP) on the Contact Surfaces of Footwear During Successive Exposures

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After attending this presentation, attendees will better understand the rate of loss and replacement of VSP from the contact surfaces of footwear, methods to measure this rate, and the potential contribution to the evaluation of trace evidence on footwear.

This presentation will impact the forensic scientific community by providing information necessary to properly appreciate and interpret VSP on the contact surfaces of footwear.

The separation of particle signals arising from different sources is one of the enabling operations for Particle Combination Analysis (PCA).[1] Although it is well-recognized that criminals track dust to and from every crime scene, dust particles on a suspect's shoes are very seldom used as evidence linking the accused to the crime. The major obstacle preventing the use of this type of evidence is that the shoes have mixtures of particles arising from activity before, during, and after the crime itself.[2] Methods separating the evidentiary particle "signal" from background noise would enable a powerful new and widely applicable forensic capability. This capability would augment traditional footwear pattern evidence with objective quantitative associations, addressing one of the specific issues raised in the 2009 National Academy of Sciences (NAS) Report. To help pursue this possibility, methods are being developed and tested that will lead to better understanding of the loss and replacement of VSP on the contact surfaces of footwear.

Prior work established that a 250m walk (approximately 175 steps per shoe) removes and replaces particles on the outermost contact surfaces of footwear.[3] It is important to achieve a better understanding about how quickly this replacement occurs. This understanding will: (1) help interpret the significance of the trace evidence found on the contact surfaces (representing the most recent environment(s) to which the footwear was exposed — how recent?); and, (2) provide a foundation for the differential analysis of these traces and those found on other areas of the footwear.

Two distinctly different and commonly encountered types of shoe soles were used in this study: athletic shoes (with flexible rubber soles) and work boots (with hard rubber soles). Three well-characterized environmental sites with characteristic VSP profiles (distinguishable by defined qualitative and quantitative particle characteristics) were used for footwear exposures under dry, dusty conditions.

Thirty-six pairs of shoes (18 pairs of each type) were exposed to a "loading site" by walking distances of 175 steps/shoe: six pairs (12 shoes) of each type in each of the test site environments. For each set of 12, two shoes (one pair) was set aside as a control (0 steps in the second environment). Each of the remaining 5 pairs of shoes were exposed for a different number of steps to a second of the three environmental sites: 5, 10, 25, 50, and 100 steps/shoe.

VSP were recovered from the contact surfaces of all shoes by swabbing, analyzed by polarized light microscopy, and interpreted using: (1) a chi-square measure of distance; and, (2) a Latent Dirichlet Allocation model developed at South Dakota State University.

Substantial loss and replacement of VSP occurs on contact surfaces of footwear in as little as five steps/shoe. By 25 steps/shoe, the replacement is substantially complete. Knowledge of the rapid loss and replacement on contact surfaces provides a basis to explore differential analysis of: (1) VSP signals from the contact areas of footwear; and, (2) those from more recessed areas of the footwear sole.

This project was supported in part by awards from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.

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Trace Evidence, Footwear, Particle Signals

B87 The Determination of Key Factors in Particle Combination Analysis to Enable Systematic Improvement, Optimization, and Transition to Practice

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After attending this presentation, attendees will better understand the current state of development of a particle combination analysis capability, results from recent and ongoing research, important next steps for development, opportunities for collaboration, and expected impact — both near and long term.

This presentation will impact the forensic science community by providing the information and perspective necessary to systematically improve particle combination analysis.

Particle combination analysis using Very Small Particles (VSP) is a new approach, highly significant for its potential to expand the number of cases to which trace evidence can meaningfully contribute and for its ability to include a quantitative statistical approach to data interpretation.[1,2] Research has demonstrated this approach has exceptional promise to expand the number of cases in which trace evidence can be used and to provide quantitative measures of evidential value. The laboratory analyses are highly efficient, utilizing existing crime laboratory personnel and equipment.

The current state of development of particle combination analysis will be briefly reviewed: what has been demonstrated, what has been suggested, and what remains to be accomplished. Prior research, employing *reasonable choices* of analytical and statistical parameters, has: (1) demonstrated the presence of highly discriminating VSP profiles on the surfaces of common items of physical evidence; (2) characterized VSP combinations using analytical instrumentation and expertise commonly available in forensic laboratories; (3) developed statistically rigorous measurements of correspondence between VSP profiles; and, (4) produced objective measures for the resulting probative value.[2-4]

The reasonable choices of analytical and statistical parameters employed in prior research were sufficient to demonstrate feasibility and potential. Systematic development and validation of these methods require that the analytical and statistical parameters be more critically examined, and that the key factors influencing the performance of the methods be identified.

The optimization of a VSP analysis protocol requires that factors influencing the reliability, costs, and selectivity be identified. Separating factors (a quantity or quality that does have an influence upon the system) from variables (a quantity or quality that might have an influence upon the system), requires a screening stage of experimental design. The result will be identification of a few important, controlling factors that must be addressed in order to meaningfully optimize the protocol. It will also provide information, such as the variability and magnitude of effects that will be needed for the next stage of process improvement.

Determination of the key factors and the magnitude of their effects will result in a significantly improved capability. Analytical and computational parameters, previously selected as *reasonable choices*, can be revised and replaced, with a combined effect that will have a material impact. Second, these results will provide necessary input to experimental designs that will permit systematic improvement and optimization. Identification of key factors will enable these critical steps and further the transition of particle combination analysis to practice. Third, and most importantly, the results will contribute directly to the fundamental advancement of a new quantitative and broadly applicable approach to trace evidence. Well-documented factors and effects for one VSP analysis protocol will allow parallel, collaborative assessments of alternative options for high efficiency analysis of VSP (such as micro Raman methods, micro X-Ray Fluorescence (microXRF), genetic analysis, or alternative Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) protocols).

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Trace Evidence, Particle Combination Analysis, Very Small Particles

B88 A Forensic Comparison of Sandy Soils Using Raman Spectroscopy, X-Ray Diffraction, and Synchrotron Powder Diffraction (PD)

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After attending this presentation, attendees will better understand an additional technique for the analysis of sandy soils, the ability to replicate that technique, and an understanding of the place of the technique in a forensic soil analysis approach.

This presentation will impact the forensic science community by raising the ability of practitioners to analyze sometimes-problematic sandy soils. This in turn may increase the profile of forensic soil analysis as a whole, leading to further developments and optimal use of a valuable evidence type. In addition, this presentation is a demonstration of opportunities for synchrotron research, an emerging field with funding available.

The examination and comparison of soil and related materials transferred in situations of contact is a powerful method for linking persons, vehicles, equipment, and locations. The evidential value of soil comparison derives from its widespread distribution, highly variable composition, relative ease of transfer, persistence, and resistance to degradation.[1] Ideally, sufficient soil is recovered to perform a range of tests allowing meaningful comparisons with samples from known locations. These tests include the comparison of the inorganic and/or organic fractions, with multiple techniques utilized.[2-4] Possible associations can be established in which the difference in characteristics lie within the expected limits of natural variation and measurement uncertainty.

Nonetheless, forensic soil comparisons employing bulk methods of analysis provide only limited discrimination where the soils have similar geological origin, or where insufficient sample is available to measure bulk properties reproducibly. Sandy soils present an additional challenge, with minimal organic material and heavy minerals resulting in limited material for comparisons, regardless of the bulk amounts present. One such area of forensic importance is the Swan Coastal Plain in Western Australia, covering much of the Perth metropolitan region and dominated by strongly leached sandy soils. While urbanization has resulted in an enrichment in the soil by organic and clay materials, areas of predominantly quartz sands remain.[5]

Primary and secondary minerals recovered from quartz grains within sandy soils allow additional scope for the differentiation of soils dominated by siliceous grains. A method for their recovery and analysis has been developed at ChemCentre and has been successful in analyzing trace mineral coatings on quartz sands from the Swan Coastal Plain in Perth, Western Australia. It is proposed that the approach is applicable to a broader range of sandy soils in general.

This study outlines efforts undertaken to characterize the primary and secondary minerals using Raman Spectroscopy, X-Ray Powder Diffraction (XRPD), and Synchrotron Powder Diffraction (PD). More than 40 samples of sites from within the Perth Metropolitan region have been examined, with the goal of further validating and expanding the technique previously undertaken using laboratory-based X-Ray Diffraction (XRD) on more than 350 samples. The chemometric analysis of PD beamline data obtained at the Australian Synchrotron from these samples exhibits differentiation and associations even within areas of minimal geological differences. Preliminary experiments attempting to analyze single sand grains using PD at the Australian Synchrotron were also undertaken.

This presentation will detail the approach, interpretation, and its place within a standard soil analysis framework. Examples of the use of the approach in casework will be provided. It is envisaged that the approach and associated database can be expanded and allow for the increased differentiation of highly leached, sandy soils from locations outside of Western Australia.

Portions of this research were undertaken on the powder diffraction beamline of the Australian Synchrotron, Victoria, Australia.

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Soils, Inorganic, Synchrotron

B89 The Use of Scanning Electron Microscopy With Energy-Dispersive X-Ray Spectroscopy (SEM/EDS) for Quantitative Forensic Comparisons of Blue Glass

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After attending this presentation, attendees will understand the problems encountered during the quantitative analysis of SEM/EDS spectra of blue glass samples and how to overcome them.

This presentation will impact the forensic science community by instructing attendees on how to overcome problems of black-box software while using SEM/EDS for the quantitative analysis of blue glass samples.

EDS, especially when employing an SEM as an excitation source, is a core elemental characterization technique for micro and ultra-micro samples. This technique has long been used to add to the evidential value of micro-transfer trace evidence by microscopical forensic examinations. Glass chips are one specific class of transfer evidence for which this technique has been employed. As a strategy for comparing glass samples by EDS, criminalists often normalize the data and compare ratios of peak heights or peak areas to one common major element, such as silicon. Courts today often require data regarding the frequency of occurrence of similar spectra to assist the trier of fact concerning the weight of this comparative evidence. This research hopes to add insight into this matter.

In this presentation, X-ray spectra obtained from 46 blue glass samples will be compared. Previous work has been completed that looked at the Ultraviolet/Visible (UV/VIS) spectra of these blue glass samples using a fiber optic micro-spectrophotometer in which few spectral differences led to possible differentiation among certain samples in this set. As color is the first logical step in comparing glass samples for analysis, it could sometimes be subjective to the observer. This research allowed for the comparisons to be instrumentally supported. The UV/VIS spectra will be compared with the SEM/EDS spectra to determine if differentiation among these glass samples is possible using these two techniques alone or in conjunction with one another. Sample preparation will be discussed as often how the sample is prepared for analysis will define any further analysis.

Being mindful of the caveat given by Dr. Peter Zoon of the Netherlands Forensic Institute (to be careful about how we generate and use data obtained with computerized black box software), the quantitative data generated from the blue glass samples and a pair of standard reference materials (620 and 621) from the National Bureau of Standards (NBS) were evaluated by a commercial software package utilizing both standardless and with-standard algorithms. In addition, the raw data was processed using the National Institute of Standards and Technology's (NIST's) Desk Top Spectrum Analyzer II (DTSA II). By use of the NBS standard reference materials, the advantages of both DTSA II and the commercial software package can be assessed. Statistical treatment of the data in the form of principal component analysis allows determination of the discrete characteristics of elemental composition to classify and discriminate between these glass samples. The results of these investigations, as well as some topics to be mindful of, will be presented.

SEM/EDS, Glass, Quantitative

B90 Chemical Pattern Recognition in Glasses: What Can Be Extracted From Spectroscopic Data Sets?

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After attending this presentation, attendees will understand X-Ray Fluorescence (XRF) microscopy in applications to glass analysis, which is an important element in forensic identification of these materials.

This presentation will impact the forensic science community by providing key aspects of glass analysis and an example of a practical application of XRF spectroscopy to materials identification.

XRF spectroscopy is a useful tool in the identification of substances and in confirming their identity with little or no sample preparation. New capabilities of the energy-dispersive XRF analytical microscope (micro-XRF) enable recording not only spectra of small glass particles (as small as 50-100 microns) but also hyper-spectral images of any object with high spatial resolution (<10 micrometers). Hyper-spectral image is a set of the data which contain information about position of the point along with a full XRF spectrum at this point. This means the data can be mined for unsuspected elements after the measurements have been made, and that statistical methods can produce chemical distributions of the elements and/or material classification based on Principal Component Analysis (PCA), in particular, with association between elements that can aid in the identification of bonded phases. For example, statistical analysis of micro-XRF data for glass can be used to locate the make, model, and year of cars by analyzing a glass chip. This presentation will provide practical insights into the application of the micro-XRF to the analysis of glass and soil.

The XRF analytical microscope was used in this study. XRF spectra of the glass were collected using 30keV acceleration voltage and with an X-ray spot size of 50 microns. XRF spectra of the glass strongly depend on X-ray optics, sensitivity of the detector, and accelerating voltage. In addition, background from the substrate will contribute to the spectrum of the small glass pieces because excitation X-ray penetrates through the glass and interacts with the substrate. This effect becomes very importance for the particle size of 300 microns (or less) or powder. The change in the spectrum due to the shape or size will lead to the different quantification of the sample (different composition). A method that allows one to minimize this effect or take it into consideration was developed. In this presentation, examples of the spectra from bulk material, small glass pieces, and powder will be shown.

The spectra of glass from several car manufactures and commercial glass (microscope slides, window glass, fuse glass) in the range of 1.00keV–40.96keV (<400 spectra) were collected. The spectra were truncated and analyzed in the spectral range of 1.00keV–15keV because major X-ray lines are in this range. A standard Fixed Point Multiplication (FPM) algorithm without any correction and/or calibration was used to calculate concentration of Na_2O, MgO, Al_2O_3, SiO_2, K_2O, CaO, TiO_2, MnO_2, Fe_2O_3, As_2O_5, and CeO_2 in all samples. All spectra and concentration data sets were scaled before PCA was applied. The correlation between classification based on spectral analysis and concentration analysis was found.

Glasses, XRF, PCA

B91 A Forensic Analysis of Automotive Paint Evidence Using Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

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After attending this presentation, attendees will have a fundamental understanding of the significance of automotive paint evidence as it pertains to automobile crashes, hit-and-run accidents, and vehicular homicides. Furthermore, attendees will be familiar with a novel rapid analytical protocol involving DART®-MS for the chemical interrogation of automotive paint evidence.

This presentation will impact the forensic science community by demonstrating a unique analytical methodology for the forensic analysis of automotive paint evidence. The following research seeks to highlight the potential of this technique for the analysis of paint and polymer evidence in an effort to add another instrumental method to the current suite of analytical techniques available to the forensic examiner.

While no current universal methodology exists for forensic paint examination, general frameworks for the analysis of paint evidence can be obtained through guidelines developed by the Scientific Working Group on Materials Analysis (SWGMAT) and the American Society for Testing and Materials (ASTM).[1,2] These guidelines advise that forensic examiners use a combination of microscopic and instrumental techniques to characterize both the organic and inorganic components of the paint samples. Most forensic paint examinations begin with optical microscopy, followed by Infrared (IR) spectroscopy and, if necessary, pyrolysis-Gas Chromatography (py-GC), often interfaced to a Mass Spectrometer (MS). Py-GC/MS is the most discriminating technique available to the forensic paint examiner for differentiating between samples with similar binder compositions. Although the discriminating capability of this technique is high, it is a destructive technique and sample analysis is extremely time intensive.

As a result, DART®-MS was employed to investigate if this technique can pyrolyze and characterize automotive paint coatings. DART®-MS is an ambient ionization technique capable of rapidly analyzing samples in any physical state with high resolution and accurate mass detection, while requiring minimal sample preparation. An optimized protocol has been developed and used to pyrolyze a small subset of automotive clear coats obtained from black vehicles. The mass spectral data were compared to results obtained from a standard py-GC/MS protocol developed by the Florida Department of Law Enforcement. Preliminary data indicate that DART®-MS with a gas heater temperature of 550°C is able to pyrolyze and analyze automotive coatings within five minutes, a significant improvement on current py-GC/MS methodologies. Interpretation of the data obtained from both instrumental techniques revealed that the information was complementary in nature. Additional analysis of the paint samples was conducted using the ionRocket DART®-MS system, which enables more precise temperature control and a greater temperature gradient. Approximately 10 to 20 automotive clear coats were then characterized using the DART®-MS protocol and multivariate statistics (cluster analysis, principal component analysis, and linear discriminant analysis) were utilized to assess the chemical diversity of the clear-coat population (intra- vs. inter-sample variability).

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Automotive Paint, DART®-MS, Py-GC/MS

B92 Comparing X-Ray Diffractometry (XRD) and Fourier Transform Infrared (FTIR) Spectrometry for the Analysis of Forensic Evidence

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After attending this presentation, attendees will understand the value of XRD as a method of analysis applicable to a wide variety of forensic evidence, including soil, drugs, plastics, and papers. Attendees will become aware of instances in which XRD is superior to FTIR, particularly in providing compositional information about samples. Attendees will also become aware of the replacement of large XRD facilities with benchtop XRD units and the availability of new solid-state detectors that obviate the need for liquid nitrogen cooling.

This presentation will impact the forensic science community by increasing awareness of the value of XRD as a replacement for or supplement to FTIR in the analysis of certain types of forensic evidence.

Most forensic science laboratories tend to use FTIR as their go-to analytical method for the identification of unknown samples. The FTIR spectra of unknowns may be searched for in a variety of spectral libraries (e.g., drugs and polymers); even if no spectral match for the unknown is found, structural information about the unknown can be obtained. FTIR instruments are relatively inexpensive and well within the budgets of most forensic science laboratories. XRD has long been recognized as being similar to FTIR in the richness of the data it provides. For example, the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) places XRD in the same category of analytical techniques as FTIR, Raman scattering, and mass spectrometry. In the past, XRD instrumentation was large (often requiring its own laboratory with attendant cooling units) and the detectors required liquid nitrogen cooling to function. New benchtop XRD instruments have reached the market; these have a small footprint and their new solid-state detectors eliminate the need for liquid nitrogen cooling. Forensic science laboratories should consider adding XRD to their repertory of analytical techniques. XRD can be particularly useful for the identification of minor crystalline constituents of samples where the infrared absorptions of these constituents are weak due to their low concentrations or where their absorptions are obscured by those of more abundant components of the samples.

To compare XRD and FTIR, two types of forensic samples were analyzed by both techniques. Sixteen different brands of trash bags and 28 different brands of black electrical tape were analyzed by both methods. These are two frequently encountered types of forensic evidence. The FTIR spectra of both the trash bags and the electrical tapes were dominated by absorptions of the polymers comprising the samples, with minor contributions from inorganic additives. In some cases, the infrared absorptions of the minor constituents were masked by the infrared absorptions of the polymer. The XRD patterns provided more information and better discrimination among samples than FTIR. For trash bags (composed primarily of polyethylene), the XRD patterns exhibited a clear distinction between Low-Density Polyethylene (LDPE) and Linear Low-Density Polyethylene (LLDPE). The trash bags were found to contain crystalline additives in varying concentrations (talc, calcite, or a combination of talc and calcite), while the electrical tapes contained a variety of additives whose XRD patterns were readily distinguished from one another. The differentiation of forensic samples is particularly important when questioned and known samples are compared in order to identify possible sources of the questioned samples.

This research demonstrates the value of XRD in analyzing forensic evidence, particularly when it is important to identify minor constituents whose infrared absorptions are very weak.

X-Ray Diffraction, FTIR, Analysis

B93 The Persistence and Environmental Degradation Patterns of Sexual Lubricants and Personal Hygiene Products (PHPs) Using Direct Analysis in Real-Time Time-Of-Flight/Mass Spectrometry (DART®-TOF/MS) and Gas Chromatography/Mass Spectrometry (GC/MS)

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After attending this presentation, attendees will be able to visualize the degradation profiles of lubricants and PHPs under various environmental conditions and time periods. The discriminating capabilities of DART®-TOF/MS and GC/MS will also be demonstrated. Each technique will be evaluated through statistical treatments such as Hierarchal Cluster Analysis (HCA), Principal Component Analysis (PCA), and Linear Discriminant Analysis (LDA).

This presentation will impact the forensic scientific community by introducing a framework in which sexual assault evidence can be analyzed given trace amounts and degraded samples on fabric matrices.

Recently, condoms have been used to conceal DNA evidence gathered from seminal fluid, thereby bypassing conviction of the perpetrator. As a result, sexual lubricants have been considered as trace evidence in sexual assault crimes; however, the residuals from lubricants tend to resemble that of personal hygiene products, which may give rise to false positives in analysis. In a recent study in which the neat form of lubricants and PHPs were analyzed using DART®-TOF/MS, a statistical distinction between lubricants and PHPs was demonstrated.[1] With this research, a protocol in which both sample groups are distinguished can be utilized in sexual assault cases. Furthermore, a way to be able to trace a degraded profile back to the neat lubricant profile is required as the delayed reports of the assault are likely, thus resulting in degraded or trace amounts of residuals collected from the victim versus the neat, unaltered profile. The purpose of this study was to analyze the degradation profiles of lubricants and personal hygiene products over time.

The samples were analyzed using two techniques, DART-®TOF/MS and GC/MS. GC/MS will be utilized in the analysis of samples to illustrate how degraded samples change over time; however, co-elution issues and the inability to analyze silicone-based samples without prior sample extraction or pyrolysis makes it difficult to develop a single simplistic method for the analysis of sexual lubricants. Conversely, DART®-TOF/MS is an ionization technique that employs a heated, excited gas stream to ionize the sample in an ambient atmosphere in either positive or negative modes. The lack of chromatographic separation allows for the analysis of silicone-based lubricants in addition to the other marketing types. Additionally, no sample preparation is generally required for analysis and real-time, high-resolution mass spectra are produced.

Thirty-two samples consisting of 12 bottled lubricants, 10 PHPs, and 10 condoms were analyzed and subsequently degraded. For this study, both persistence over specified time intervals, (0, 3, 6, 9, 12, 16, 24, 48, and 72 hours) and induced environmental conditions were evaluated. To determine the environmental and persistence effects on the degradation of the lubricants, .25g of each lubricant was deposited and rubbed into common fabrics (e.g., underwear and bedding). Samples were exposed to different environmental conditions, then analyzed to determine how the chemical composition of major and minor components changed over a three-day period, thereby identifying the persistence of the specific components after use. Each lubricant sample was exposed to the following situations in an indoor environment: cold temperature (5°C), room temperature (22°C), and hot temperature (39°C) under the specified exposure times with and without direct Ultraviolet (UV) exposure.

Following sample analysis, chemometric methods were applied to the dataset and compared against the neat dataset of both lubricants and PHPs. Using HCA and PCA, a clear distinction between lubricants and PHP samples was observed, with correlation decreasing with prolonged exposure to degradation conditions. The degraded profiles of lubricants and PHPs were found to be statistically different using significant peak classifiers as discriminating factors. LDA was used to compare the degraded samples against the neat dataset to evaluate if the classifications from degraded profiles would provide similar groupings, displaying the relationship of neat and degraded samples alike.

The goal of this study was to evaluate the degraded profiles of lubricants and personal hygiene products over time under induced environmental conditions. Additionally, a method in differentiating the neat and degraded profiles of both lubricants and PHPs was developed. The ultimate goal is to create a protocol in which lubricants and PHPs (in their neat and degraded forms) can be examined as common pieces of evidence in sexual assault cases.

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DART®-TOF/MS, Lubricant Degradation, Extraction Process

B94 Using Eye Tracking to Understand Decisions Made by Forensic Latent Print Examiners

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The goal of this presentation is to assist attendees in understanding how the eye-tracking behavior of professional latent print examiners is associated with their decisions.

This presentation will impact the forensic science community by describing the underlying bases of forensic latent print examiners' conclusions.

A variety of studies have been conducted evaluating the latent print examination process, but there has been relatively little research regarding the fundamental basis of this visual task: how examiners use their eyes to accomplish these tasks, and what visual information guides the examination process.[1-13] This presentation will discuss the results of a study conducted to gain a greater understanding of how latent print examiners perform analysis and comparison tasks and to gain a greater understanding of why examiners make different determinations. By asking examiners to annotate or mark up their work, some understanding of the information those examiners relied upon in making their determinations has been gained, but this is limited to the information they felt was worthy of annotating. Eye gaze can assist to better understand the factors that lead to differences in examiners' interpretations and, ultimately, their conclusions. The eye behavior of forensic examiners provides insight into how examiners make their decisions.

Eye-tracking technology was used to monitor examiners as they performed their tasks to learn what visual information they used and how they worked with that information. Eye tracking allows the determination of not only how examiners differ in the features they look at in the images, but also how they aggregate information from those images; that is, using spatial as well as temporal aspects of eye tracking. Analyses of why some examiners make inconclusive conclusions when others exclude or individualize will provide information that can be used to improve the reliability of the latent print comparison process, particularly for the more challenging comparisons.

In this study, more than 130 hours of eye-gaze information was collected from 121 practicing latent print examiners as they performed more than 2,000 fingerprint comparisons and more than 1,200 other tasks that were designed to isolate specific behaviors that arise during comparisons. The latter tasks included ridge counting, ridge following, and searching for a designated feature group in a comparison print. Eye gaze was sampled at a rate of 1KHz using a camera and infrared illumination. The raw data was calibrated, partitioned into saccades (rapid movements) and fixations (median duration of 0.27 seconds), and mapped onto image coordinates for subsequent analysis. Examiner annotations from previous studies were available for the majority of the comparisons.

This presentation will discuss how eye behavior is associated with examiners' determinations, including the extent to which eye behavior can explain erroneous determinations (false positive and false negative conclusions) and non-consensus determinations (conclusions that differ from the majority of examiners). This presentation will also discuss how eye behavior is affected by the difficulty of comparisons, and how the presence or absence of visual context affects eye behavior. The results of this study reveal explanations for how some errors occur. This presentation will describe how examiners use information in the latent print and how that relates to comparison efficiency and risks of misinterpretations. Variability in the eye-tracking data and issues in the interpretation of this data that will be important to future research will be discussed.

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Latent Prints, Eye Tracking, Examiner Behavior

B95 A Case Impact and Operational Cost Analysis of Blind Verifications in Latent Print Examinations

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After attending this presentation, attendees will better understand the case impact (as defined by conflict or non-consensus conclusions) as well as impact on operational costs (as defined by time spent verifying) of blind verification procedures.

This presentation will impact the forensic science community by presenting data and general observations accumulated during blind verifications of all latent print comparison conclusions at the United States Army Criminal Investigation Laboratory's (USACIL) Latent Print Branch.

Blind verifications have been advocated as a panacea for many of the concerns related to latent print examinations. Error rate studies demonstrate the potential for blind verifications to both uncover non-consensus conclusions as well as limit examiner errors. Other stakeholders believe blind verifications would mitigate contextual bias that standard verifications (non-blind) would not. Although both ideas hold merit, there has been a surprising lack of research data to understand the impact of blind verification procedures on actual casework operations and in which scenarios the procedures would yield the most impact.

For six months, USACIL's Latent Print Branch completed blind verifications of all comparison conclusions in live casework. Typical latent print examinations comprise three main phases: Analysis, Comparison, and Evaluation (or ACE). The fourth phase, referred to as Verification, is typically a repeat of the previous phases of ACE. In most laboratories, the verifying examiner will have access to all the work previously completed by the original examiner, including resulting conclusions from a comparison between an unknown impression (a latent print) and a known impression (or a set of known standards). In blinded situations, the verifying examiner will only be supplied with the compared latent print(s) and the known standard(s) these were compared to. Task irrelevant information, such as the original examiner's annotations or conclusions, the source of the known standards (e.g., an Automated Fingerprint Identification System (AFIS) "hit"), and the relationship between the known standards and the case (e.g., "victim") are not available to the blind verifier; however, task relevant information, such as substrate or matrix, may be requested by the blind verifier. At the completion of the blind verification, the two examiners compared conclusions. Differences in opinion were noted and resolved in accordance with laboratory conflict-resolution procedures and documented for purposes of this evaluation.

This presentation will discuss the data and general observations accumulated when comparing blind verification procedures to non-blind verification procedures in terms of case impact (rates of conflicting conclusions) and operational costs (time required to conduct verifications) as well as incidental observations related to interpersonal dynamics that were displayed during the evaluation period.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the United States Department of the Army or United States Department of Defense.

Blind, Verification, Impact

B96 Statistical Interpretation and Reporting of Fingerprint Evidence at the United States Army Criminal Investigation Laboratory (USACIL)

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After attending this presentation, attendees will have a greater understanding of how fingerprint evidence may be quantified, policies and procedures guiding the use of such methods in practice, and next steps to ensure the method is accessible by the broader forensic community.

This presentation will impact the forensic science community by discussing the progress and challenges of implementing a method for statistical interpretation and reporting of fingerprint evidence at the USACIL.

The results of forensic fingerprint examinations are traditionally based on the visual comparison and subjective opinions of forensic examiners and reported as categorical statements of inclusion or exclusion of a particular individual as the source of a latent print. In 2009, the National Research Council (NRC) encouraged the forensic science community to develop tools to evaluate and report the strength of forensic evidence using validated statistical methods rather than relying solely on the subjective opinion of forensic examiners. The recommendations of the NRC are consistent with those of the President's Council of Advisors on Science and Technology Report (PCAST) in 2016. The primary concern of the NRC and PCAST is the legal field's inability to assess the reliability of fingerprint comparison results for a given case at hand without validated statistical data concerning the strength of the findings, thus bringing into question the scientific validity of fingerprint evidence and its admissibility in criminal courts.

Over the past couple of years, the USACIL has been taking incremental steps forward to facilitate the transition from solely subjective, experience-based practices to integrating more robust, scientifically demonstrable, and data-driven practices for latent print examinations. As a part of this effort, the USACIL has developed, validated, and implemented a method that facilitates the evaluation and reporting of the statistical strength of fingerprint evidence. In March 2017, the USACIL began reporting the statistical strength of fingerprint evidence within the military criminal justice system and is navigating a way forward for the broader forensic fingerprint discipline toward stronger scientific foundations and improved practices. This presentation will provide a general explanation of the statistical methods employed, discuss policies and procedures governing its use in casework, observations related to its operational and technical impact and challenges following implementation, and discuss how other federal, state, and local forensic service providers can implement similar reforms within their laboratories.

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Fingerprints, Statistics, Likelihood Ratio

B97 An Update on the Academy Standards Board (ASB) Firearms and Tool Marks Consensus Body

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The goals of this presentation are to describe the purpose of the ASB Firearms and Tool Marks (FATM) Consensus Body and to present updates as to the Body's work on developing and approving standards in the disciplines of firearms and tool marks analysis.

This presentation will impact the forensic science community by providing attendees with information on the progress the FATM Consensus Body has made and is making in the areas of firearms and tool mark examinations.

The American Academy of Forensic Sciences created the ASB as a response to the need for standardization from the forensic community. This was in line with recommendations in the 2009 National Academy of Sciences (NAS) Report. It is now working in cooperation with the Forensic Science Board of the Organization of Scientific Areas (OSACS). The purpose of the FATM Consensus Body is to focus on standards and guidelines related to the examination of firearm and tool mark evidence. This includes the comparison of microscopic tool marks on bullets, cartridge cases, and other ammunition components and may also include firearm function testing, serial number restoration, muzzle-to-object distance determination, tools, and tool marks.

This presentation will discuss the inception of the ASB, its legal status as an entity, its membership, its mission, and a discussion on its consensus bodies with particular emphasis on the discipline of firearms and tool marks. Created in 2016, and under ASB Secretariat Brad Wing, the FATM Consensus Body is comprised of 33 members representing a number of disciplines that include subject matter experts, general interest, consumer groups, user/government (federal, state, and local), academia, producers, and user/industry. In addition to members, there are observers as well as managers from the ASB. Observers and other non-members are encouraged to participate by reviewing documents and offering comments. Standards can be developed by the Consensus Body or offered by other bodies such as the OSACs. Once documents containing recommended standards and guidelines are submitted to the Consensus Body, the documents will be reviewed by members of that body, and changes to those documents may be made to meet the American National Standards Institute (ANSI) publishing guidelines. Once the documents have been reviewed, they will be offered up for public comment. All public comments must be reviewed and addressed by the Consensus Body. After final review, the documents will be published as guidelines or standards.

It is imperative that all members participate, and that those individuals and bodies that are affected by proposed standards and guidelines participate by subscribing to the FATM Consensus Body and listen to meetings which are broadcast on Join Me™, read the documents, and offer constructive comments.

ASB, Consensus Body, FATM

B98 An Objective Comparison of Striated Tool Marks Produced From Ten Consecutively Manufactured Cold Chisels Measured by Contact and Optical Surface Profilometry and Comparison Microscopy

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The goals of this presentation are: (1) to inform attendees of the developments in quantitative measurement comparison of striated toolmarks; (2) to introduce recent developments in tool mark profile measurement using 2D and 3D surface profile instruments; and, (3) to demonstrate the robustness of proposed mathematical-based comparison scoring methods on a challenging tool mark test set.

This presentation will impact the forensic science community by informing and maintaining currency in the development, testing, and validation of the emerging technology of tool mark comparisons using 3D profilometry and objective mathematical comparison methods.

In the forensic science specialty of tool mark identification (which includes firearm identification), "tool marks" are the result of a harder surface acting upon a softer surface, usually but not exclusively metal alloys. The method for tool mark comparison relies on an objective side-by-side evaluation of the agreement by comparison microscopy; however, the opinion of "sufficient agreement" for conclusion to identify a tool source is subjective in nature, relying on the education, training, and experience of the examiner.

The first extensive study focusing on quantifying matching patterns of tool mark stria was performed by Biasotti in 1959.[1] His work demonstrated that consecutive matching stria groups were diagnostic for a quantitative measurement of similarity when two striated tool marks are microscopically compared, now termed Quantitative Consecutive Matching Stria (QCMS). Known Matching (KM) and Known Non-Matching (KNM) rifling land impressions on fired bullets were compared and the consecutive matching stria were counted. In non-matching comparisons, small QCMS numbers were observed, demonstrating the quantity of agreement observed by chance, understanding that agreement sufficient for identification must exceed that quantity. The minimum number of QCMS that was necessary for an accurate conclusion of the firearm source was empirically determined. The QCMS method is currently used by many examiners to quantify the agreement in striated tool mark comparisons.[2-7]

Optical 3D systems are emerging as objective methods in forensic tool mark measurement and comparison. These adapted instruments are useful due to the increasing computational power of modern computers and control systems tasked with handling high-definition 3D data.[8-17]

This is the first scientific investigation to employ consecutively manufactured tools (chisels) to produce striated tool marks whose contour profiles were measured by: (1) contact stylus profilometry; (2) non-contact 3D optical profilometry employing focus variation instrumentation; and, (3) the QCMS method. Striated tool marks were created pairwise in a controlled manner from ten consecutively manufactured cold chisel blades. Comparison tests between tool marks created in this manner have the best potential for producing microscopic agreement between two or more different tool sources, resulting in false positive identification. The striated tool mark profiles were measured using contact stylus 2D profilometry and non-contact optical 3D profilometry. Profile similarity and differences of KM and KNM tool marks were compared using two mathematical methods: Cross-Correlation Function Maximum (CCF_{MAX}) and the recently developed Congruent Matching Profile Segments (CMPS).

Both 2D- and 3D-acquired profile comparisons exhibit a wide separation between KM and KNM CCF score distributions when the full-length profile comparisons were made. Similarly, large separations of KM and KNM score distributions were also the result of segmented profile comparisons performed by CMPS. Replicas of the KM and KNM chisel tool marks were also compared using comparison microscopy, and the similarity and differences were measured by the QCMS method. Results of these comparisons also demonstrate a wide separation of distributions between the QCMS "runs" in KM and KNM tool mark comparisons, and no KNM comparisons exceeded three consecutive matching stria. The score distributions were also examined in a subjective statistical manner for their theoretical estimations of matching probability and demonstrate an exceedingly rare probability of sufficient agreement that would result in false positive identifications.

Conclusions: The results support the use of quantitative methods of profile comparisons for the discrimination of matching and non-matching striated tool marks. Both contact and non-contact methods of profilometry are useful in later mathematical comparison and score methods; however, in actual casework, the non-contact methods would be more acceptable because the contact stylus method may introduce stylus marks onto the evidence tool marks and, therefore, change the original evidence features. Additionally, the full profile comparison method in the CCF_{MAX} method is not as applicable to casework because the vast majority of striated tool marks on evidence items or collected at crime scenes exhibit partial tool marks from the full tool-working surface. The CMPS method exhibits the best promise for an objective comparison method and is better designed to compare much smaller profile segments of the striated tool mark profile to typically larger reference profiles. The evaluation of the tool mark peak frequency could also be employed to select optimum profile segment widths for the tool marks to be compared.

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Tool Mark, Objective Comparison, Profilometry

B99 The Critical Angle of Bullet Impacts in Common Materials Seen in Forensic Casework

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The goal of this presentation is to demonstrate the angles of bullet impacts into materials that will penetrate, ricochet, or fragment.

This presentation will impact the forensic science community by informing attendees that the approximate angles to which bullets will penetrate or ricochet a material can be crucial to the reconstruction of a shooting event.

A case was investigated that involved the death of a young woman who was killed from a rifle bullet at long range.[1] There was debate as to whether the bullet that struck the victim was a direct shot or had ricocheted off a river between the shooter and the victim. Data from previous research related to bullet ricochet characteristics served as a basis to perform independent testing.[2] An AK-style rifle similar to the one used in the incident was fired into water at various angles and the departure angles from ricocheted bullets were documented through witness panels. During this experiment, the impact angle at which the bullet ricocheted off the water was evaluated. The impact angle that yielded penetration into the water was approximately seven degrees and greater. Although consistent with previous literature, the question was raised as to whether this angle would vary across other substrates encountered in forensic casework.

It is known that a bullet will ricochet off the surface of a substrate, such as wood, at one angle and penetrate the surface at steeper angle.[3] The angle at which a bullet will penetrate/perforate versus ricochet off a substrate is referred to as the critical angle.[4] An experiment was devised to measure critical angles from 25 shots using two common law enforcement pistol and rifle calibers with standard bullet types. Substrates utilized for this experiment included wallboard, automotive steel, wood, and two different types of glass.

Critical angle results from this experiment varied from five to nine degrees, depending on the surface composition. Attendees will be presented with the specific critical angle determinations from these tests as well as the detailed interaction of the bullet and surface that was captured with high-speed videography.

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Critical Angle, Bullet Impact, Ricochet

B100 Battelle's Final Report on the National Institute of Justice (NIJ) -Sponsored Initiative: A Feasibility and Guidance Study of Massively Parallel Sequencing (MPS) for Forensic DNA Applications

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After attending this presentation, attendees will better understand the MPS technology, its potential viability for forensic applications, and the identified gaps and considerations for implementation perspectives.

This presentation will impact the forensic science community by providing an awareness of MPS technology advancements in the field of forensic DNA analysis, the potential strengths and limitations of the specific technology application, and critical information relevant to the laboratory-specific decision process for potential investment in such a technology.

Forensic DNA analysis through Capillary Electrophoresis (CE) -based typing of Short Tandem Repeat (STR) is a well-established and successful technology with widespread legal and technical acceptance and is integral for the generation of more than 16 million DNA profiles registered within the Federal Bureau of Investigation's (FBI's) National DNA Index System (NDIS). The emergence of MPS presents opportunities for potential success beyond conventional CE-based techniques, specifically with respect to degraded specimens (missing persons) and possibly complex samples (mixtures). More significantly, MPS provides a broader scope of informative and discriminating data through Single Nucleotide Polymorphisms (SNPs) for identity, physical appearance, ancestry, and kindred relationships. Consistent with newer technologies, MPS also introduces levels of change, some of which are disruptive to the present approaches routinely applied by forensic DNA laboratories, ranging from nomenclature, workflow, and instrumentation, to data interpretation and reporting.

In 2014, Battelle first reported the receipt of a National Institute of Justice (NIJ) grant for initiating a two-year study (2015-17) to assess the technical readiness and feasibility of the MPS technology for forensic applications. The study consisted of both Performance Testing (Phase 1) to assess system capabilities and optimization of commercially available MPS products, and Inter-Laboratory Testing (Phase 2) for conducting a series of carefully designed studies to address key validation criteria. The Phase 2 effort specifically included the integral testing participation of technical leads from the Armed Forces DNA Identification Laboratory (AFDIL); the Bureau of Alcohol, Tobacco, Firearms and Explosives (ATFE); the California Department of Justice (CAL DOJ); the FBI; Harris County Institute of Forensic Sciences (HCIFS); the National Institute of Standards and Technology (NIST), the New York City Office of the Chief Medical Examiner (NYC OCME), and the North Carolina State University. The validation study included assessment of the technology with respect to reproducibility, precision, concordance, sensitivity, mixtures, and non-probative casework. For the latter, each participating laboratory selected up to 14 samples, varying in types and often reflective of the respective missions for each laboratory. This presentation will convey the final results of this comprehensive study, including strengths, gaps, and considerations of strategic roadmaps for the MPS technology acceptance and transition. In total, a contemporary assessment of the technical readiness of the MPS technology will be provided, including examples of actual casework processed across two of the commercially available analysis workflows.

Massively Parallel Sequencing, Abundance, Iso-Allele

B101 Massively Parallel Sequencing (MPS) of Short Tandem Repeats (STRs) and Microhaplotypes for Mixtures

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After attending this presentation, attendees will understand how to utilize microhaplotypes and STR data generated by MPS.

This presentation will impact the forensic science community by presenting a novel method using microhaplotypes to determine the number of contributors in a mixture sample as well as infer biogeographical ancestral information.

Primers, library and template preparation chemistries, and algorithms were designed to analyze these markers. The AmpliSeq™ library preparation chemistry was developed to handle up to 100ng of input DNA, along with using a high-multiplex panel, which is important to increase the probability of recovering the minor contributor.

Microhaplotypes are multi-allelic genomic markers that contain more than one SNP residing in genetic proximity, whereby each haplotype is represented as statistically phased SNP genotypes.[1] These markers provide an ideal tool for mixture analysis, with a lower baseline and better intralocus balance over STRs in an MPS context. Furthermore, microhaplotypes are surrounded by conserved sequences, which can be sequenced with high accuracy, so therefore lack the complexity and interpretation issues of stutter. On the other hand, MPS exposes the additional diversity in compound and complex STR loci when repeat and flanking sequence is compared.[2-4] To investigate the sensitivity of the two marker types for mixture detection, a range of major to minor donor ratios and number of individuals was sequenced.

Seventy-eight microhaplotypes that have been previously typed on 83 populations showing high numbers of alleles and ancestry informativeness were targeted for design in multiplex with 31 autosomal STRs and 4 Y-markers.[5] DNA was extracted from samples taken from individuals of different biogeographic ancestries. Mixtures were created at ratios of 1:1, 1:3, 1:10, 1:20, and 1:50, along with mixtures containing up to four donors. Libraries for MPS were barcoded with both manual and automated Ion Chef™ library preparation methods. The libraries were then quantified and subsequently run through template preparation on the Ion Chef™, then sequenced on the Ion S5™. Using GlobalFiler™, fragment analyses were also performed on the same mock mixture samples to gather STR concordance and performance data. Reads were aligned to target regions of the reference human genome and haplotypes, biogeographic ancestry, number of contributors, mixture ratios, and minor and major contributors were determined. Converge™ 2.0 software was used to visualize sequence, STR profiles, strand bias, and number of contributors.

With the capacity to sequence many markers in parallel, MPS underscores the power of microhaplotypes and STR sequence as powerful forensic markers for handling mixture samples when Capillary Electrophoresis (CE) systems are not capable of generating conclusive results.

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Microhaplotypes, Massively Parallel Sequencing, Mixtures

B102 Investigating Rates of Mitochondrial DNA (mtDNA) Heteroplasmy in Different Haplogroups Using Massively Parallel Sequencing (MPS)

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After attending this presentation, attendees will understand MPS analysis of mtDNA haplotype and heteroplasmy and whether rates of heteroplasmy are linked to populations of different haplogroups.

This presentation will impact the forensic science community by introducing a robust MPS approach to sequencing mtDNA and enhancing the discrimination potential of mtDNA typing by evaluating rates of heteroplasmy across haplogroups.

MPS, a high-throughput form of next generation sequencing, allows increased resolution of mtDNA heteroplasmy and is at the forefront of efforts to expand the utility of forensic mtDNA typing. Maternally inherited mtDNA is present in hundreds to thousands of copies within one cell, has a high mutation rate, and passes through multiple bottlenecks, which allows for a range of variant percentages in the mtDNA sequence. Heteroplasmy is a heterogeneous collection of sequence variants in the cytoplasm of the cell. Heteroplamic rates from a population of European haplogroups (for example, H, J, K, T, and U) have provided evidence that heteroplasmy is common in the Control Region (CR) of the mitochondrial genome (mtgenome). A maternal lineage of mtDNA will share the same collection of major variant Single Nucleotide Polymorphisms (SNPs) and insertions/deletions (indels) called the haplotype. Certain haplotypes are common in population groups and may be shared among unrelated individuals.[1] In line with this, a haplogroup consists of similar haplotypes that have risen from related ancestral lineages. It is hypothesized that there is potential for differences in rates of heteroplasmy linked to population haplogroups, based on assumption and empirical observation that the position and rate of heteroplasmy may be linked to the haplotype sequence.

This current project has used a robust MPS approach to measure, analyze, and report rates of heteroplasmy on a per sample and per nucleotide basis for 750 samples in population groups reporting to be non-European (NIJ-2016-DN-BX-0171). Buccal cells were collected from 750 unrelated non-European individuals and MPS analysis conducted on the CR using Nextera® XT library preparation and 300X300 paired-end reads on an Illumina® MiSeq®. Secondary analysis was performed using GeneMarker® High-Throughput Sequencing (HTS) software to evaluate haplotype and heteroplasmy, and HaploGrep to determine haplogroups.

The forensic science community requires further validation of MPS analysis of mtDNA to encourage adoption of this technology into working laboratories and investigations. This project utilized a reliable MPS workflow developed in a laboratory, along with proper software for evaluation of MPS mtDNA data. Implementation of this optimized procedure combined with establishment of rates of heteroplasmy across the CR in different haplogroups will significantly enhance the accessibility to, and discrimination potential of, mtDNA typing. This highly resolved reporting will encourage the forensic science community to increase implementation of mtDNA analysis as a whole.

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Mitochondrial DNA, Massively Parallel Sequencing, Heteroplasmy

B103 Is That Peak Real or Is It a Mis-Type? Only the Profile Donor Knows for Sure

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After attending this presentation, attendees will gain an appreciation for the possibility of frank mis-types in forensic DNA profiles, specifically with regard to peaks in stutter positions of a contributing profile.

This presentation will impact the forensic science community by raising awareness of the possibility for frank mis-types in forensic DNA profiles, especially in low-template samples and in particular, in stutter positions of alleles from a true contributor.

Samples containing low levels of DNA and/or mixtures of DNA from multiple individuals are routinely encountered in forensic DNA casework. These samples can be considered inclusively as "complex" samples. Such samples are challenging to interpret because of the inherent uncertainty in determining the genotypes of the contributors to the evidence profile. To further investigate the challenges involved in interpreting complex samples, a specific sample set was created. This sample set comprised two-, three-, and four-person mixtures. The template amounts were 30pg, 50pg, 100pg, and 500pg. The mixture ratios were 1:1, 2:1, 4:1, and 9:1. The various mixtures resulted in a total of 164 complex samples, each of which was amplified five times for a total of 820 profiles. The sample set provides a rich source of data than can be queried in numerous ways. Because these samples derive from known sources, ground truth is known, and the alleles expected in each of the profiles can be determined.

The Polymerase Chain Reaction (PCR) amplification typing artifact known as stutter introduces an element of ambiguity into the interpretation of forensic DNA profiles. Most analysts are aware of issues that arise from a stutter peak deriving from a relatively high-quantity contributor. These peaks can sometime be higher than expected, introducing the possibility of calling a false allele; they can also potentially mask a real allele in a low-quantity minor component of a mixture; however, there has been little discussion of the behavior of stutter in low-template mixtures and of the potential for the generation of a false profile that does not represent the true profile of the input sample.

In reviewing the aforementioned dataset, it became apparent that a non-trivial proportion of profiles would result in frank mis-types if read blind. Most, but not all, of these instances occurred in low-template samples, and the vast majority of these mis-types were attributed to peaks in either the N-1 or N+1 stutter positions of known input alleles. This phenomenon will be discussed and examples will be shown.

DNA, Mis-Type, Low Template

B104 Genotyping Challenging DNA by the Isolation of Polymerase Chain Reaction Products (IPCRp)

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The goal of this presentation is to demonstrate the application of the IPCRp method for genotyping challenging DNA samples, in particular, touch and low copy DNA samples.

This presentation will impact the forensic science community by increasing competence when genotyping challenging DNA samples, such as touch and low copy DNA.

Genotyping field-collected samples of low quantity amplifiable copies or Low Copy Number (LCN) DNA has been a considerable challenge primarily due to the constraints of the PCR reaction itself in which the amount of the amplified targeted DNA at the end of the PCR cycle process is directly proportional to the amount of the template DNA at the start of the reaction. Increasing the number of cycles or going through another cycling process, as in the case of the nested PCR method, would increase the amount of a PCR product. Optimizing the PCR reaction for increased cycle number requires advanced technical skills and specialized reagents; however, even when done properly, high PCR cycle reaction creates additional problems due to increased stutter and background noise, the most important factors that have an adverse effect on the genotyping of LCN DNA. The IPCRp genotyping method reportedly can increase the detection levels more than 10-fold compared to classical PCR, does not require increased cycle number, and eliminates background noise in the process of genotyping.

The IPCRp method was used for genotyping touch DNA samples. Fingerprints from a stainless steel bar were swabbed and DNA was extracted by QIAGEN® Micro Kit, followed by IPCRp amplification and genotyping on a Capillary Electrophoresis (CE) genotyper platform. The 6-Plex PCR kit was designed according to the IPCRp method protocol with both forward and reverse primers labeled with fluorescent dye and biotin, respectively. The kit simultaneously amplified THO1, FGA, CSF, D21, TPOX, and D7 loci. Following the PCR, the double-stranded PCR product was captured on a streptavidine plate, washed, and only dye-labeled targeted DNA in single-stranded configuration was released and loaded to the genotyping platform. The loading quantities of the PCR product into streptavidin plates and the CE instrument were optimized. With the IPCRp amplification/genotyping method, a full genotype was obtained out of 10pg of template DNA when 12µl of captured targeted DNA was loaded to a 310 Genotyper®.

The full profile was obtained from all touched DNA samples, the stutter levels were not increased, and there was considerable reduction in background noise. Results from genotyping touch DNA samples and low copy number DNA will be presented. It is recommended that the IPCRp method be used in every case with genotyping challenging samples.

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Touch DNA, Low Copy DNA, IPCRp

B105 Forensic Biology Under the Microscope

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The goal of this presentation is to help forensic analysts identify and recover minute quantities of biological material from evidence with the aid of a dissecting microscope when necessary.

This presentation will impact the forensic science community by demonstrating the successful production of screening results and DNA profiles from biological evidence collected with the aid of a dissecting microscope that may otherwise have gone untested.

Attendees will learn about techniques that have been developed for recovering DNA from tiny amounts of evidence. The recovery of biological material, with samples merely millimeters in size, presents unique challenges in DNA processing. Without the proper techniques, many laboratories may overlook the value of testing minute amounts of biological evidence. Biological material from physical evidence may be difficult to access, potentially wedged in the cracks of the piece of evidence or trapped in the weave of a fabric. Somewhere between the analysis of DNA from a single cell and large quantities of blood, bone, or tissue is the realm of biological evidence that can be visualized and recovered using a dissecting microscope.

Over the past 17 years, DNA Solutions® has developed tools and techniques that allow enough biological material to be recovered from these types of difficult samples for screening and DNA analysis. Manual DNA isolation techniques have been coupled with sensitive Short Tandem Repeat (STR) kits to produce unique DNA profiles from some evidence that might have gone untested. Casework examples will be presented, ranging from the processing and recovery of residual biological evidence from medical trocars that were used in surgery to the successful production of a DNA profile from a tiny speck of blood identified on a pill from a large chain pharmacy. The processes used to successfully perform DNA analysis from traces of dried mucus on a handkerchief that had been recovered from within a commercial food product will be outlined, as well as the determination of the wearer of a set of clothes covered in the victim's blood following a brutal murder. Finally, the development of a DNA profile from hollow point bullets after the bullets had been cleaned for ballistics matching and stored for more than four years will be described. The DNA profiles were able to be matched to the victim, confirming their origin. Each of these cases highlights the tools (micromanipulators) and techniques (vacuum systems) developed at DNA Solutions® and used to successfully recover minute amounts of biological evidence and produce data critical to the investigations surrounding each case.

Forensic Biology, Dissecting Microscope, Minute Evidence

B106 High Resolution Melt (HRM) Curve Analysis for Preliminary Screening of Short Tandem Repeat (STR) Loci at Quantification — Obtaining More Information Earlier in the DNA Workflow

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After attending this presentation, attendees will better understand how HRM curve analysis may be used with various statistical tools to screen forensic DNA samples for determination of specific genotypes or geno-groupings.

This presentation will impact the forensic science community by providing a method to detect exclusionary data as well as potentially detect the presence of a mixture prior to multiplex STR amplification and analysis.

Currently, the DNA laboratory workflow does not allow the possibility of quickly identifying exclusionary contributors or determining whether a sample is a mixture until end-point DNA profile interpretation and identification. This presents a problem for low template DNA samples, particularly those from touch DNA samples where numerous small areas of a touched item are individually swabbed to avoid inadvertently creating mixtures. Thus, those samples with low DNA yields may not produce full or even partial STR profiles and since this is determined at the end-stage of the DNA workflow, the possibility of combining multiple single-source extracts may be lost. A screening assay at an earlier step could be a beneficial tool, allowing an analyst to determine if consumption of the sample is warranted or if swabs from different areas of the evidence item should be combined prior to STR amplification. The quantification step is the most logical place to add this screening assay, due to the multifaceted capabilities of quantitative Polymerase Chain Reaction (qPCR) instruments and human DNA quantification kits. Previous work demonstrated that commercially available quantification assays did not themselves produce melt curve products nor was their quantitative accuracy altered by the presence of an additional HRM dye. These data also indicated that genotyping or geno-grouping single-source samples and identification of mixture samples is possible using HRM curve analysis of the D5S818 and D18S51 STR locus using EvaGreen® intercalating dye and a QIAGEN® Rotor-Gene® Q. The current data focuses on a statistical approach for examining duplexed HRM data from two STR amplicons (D5S818 & D18S51) using two different qPCR platforms.

Buccal swab DNA was obtained from individuals who expressed one of seven desired D5S818 and D18S51 genotypes (n=20 for each genotype at each locus). An optimized amplification reaction was used to amplify both STR targets followed by HRM analysis on the QIAGEN® Rotor-Gene® Q and the ABI® 7500 qPCR platforms. Quantitative examination of all peaks identified in the melt curve (including shoulder peaks) revealed that while temperature shifts alone were not significant enough to determine genotypes, the additional differences in the number of peaks present and the primary peak:shoulder peak height ratios were significant enough to allow statistical assessment. Statistical modeling of the resulting data was accomplished using the Principle Component Analysis (PCA) -based Rotor-Gene® Q ScreenClust HRM® software, ABI® 7500 instrument Sequence Detection Systems (SDS) software, and Linear Discriminate Analysis (LDA) approach using R statistical software. Each statistical model evaluates a set of known samples to create training groups for each genotype, which are then used for comparison against each unknown for identification of the most closely related and/or least dissimilar genotype or genotype grouping. From those groupings, the accuracy of this prediction was calculated for each approach. At the D5S818 locus, the ScreenClust HRM® software provided an accuracy of 23.9% when classifying the data between seven genotypes. To improve the predictive power using the PCA-based model, geno-groupings were created from the seven genotypes by combining genotypes with similar melt patterns together into groups. The accuracy was re-evaluated and demonstrated improvement to 46.6% with three geno-groups. The same set of samples was re-evaluated using LDA in R. This resulted in an accuracy of 58.9%. Geno-groups were created again to improve predictive value and the resulting three groups yielded an accuracy of 81%.

These data support the use of an LDA-based statistical model of HRM data from highly polymorphic STR amplicons for identification of a geno-group. Incorporation of this amplification and melt analysis into a commercially available quantitation kit could provide early exclusionary information and potentially provide detail about the number of contributors to a DNA sample *prior* to STR multiplex amplification and CE analysis. Future experiments will include integration of this HRM duplex into commonly used human DNA quantification chemistries and evaluation of both single-source and mixture samples.

High Resolution Melt Curve, qPCR, STRs

B107 Maximizing the Amount of DNA Recovered: A Study of Mawi DNA Technologies' iSWAB™-ID Collection Device for Forensic Science Application

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After attending this presentation, attendees will understand the utility of a new device for the collection and stabilization of cells and Deoxyribonucleic Acid (DNA). Attendees will also learn about: (1) problems that may exist with the current collection and recovery of cells and DNA; and, (2) the benefits and limitations of implementing the new collection technology into the field of forensic sciences.

This presentation will impact the forensic science community by introducing a device that enhances the collection and recovery of cellular material for subsequent DNA testing. The mechanism of the device allows for an increase in specimen release into proprietary direct lysis buffer that is compatible with commonly used forensic amplification kits and produces robust and reliable Short Tandem Repeat (STR) profiling.

DNA evidence often contains low amounts of cells; therefore, the importance of proper collection and storage to protect the DNA and ensure that maximum recovery of cells is achieved cannot be overemphasized. New techniques and inventions have made the collection of DNA evidence more efficient and consistent through the development of different types of swabs, lysing buffers, and various other improvements; however, DNA specimen release can be impeded by the structural properties of the swabs and the chemistries of buffers used for collection. Relatively substantial portions of the biological material, ranging from 20%-76%, can be lost through retention on the swab or loss during the extraction process.

The purpose of this study was to define conditions and limitations of the use of Mawi DNA Technologies' iSWAB™-ID collection device for the collection of forensic samples and subsequent DNA testing. Originally, the iSWAB™-ID collection device was designed to improve bio-sampling in the medical field in underprivileged areas where refrigeration is unavailable. Its unique room-temperature-stable chemistry and device design for optimal bio-sampling can potentially be adapted for forensic purposes. Experiments were designed to answer questions about the efficiency and effectiveness of the iSWAB™-ID collection device for forensic purposes. The following parameters of the iSWAB™ buffer and collection device were tested: (1) ability to collect dried stains; (2) ability to recover cellular material from different types and conditions of swabs; (3) ability to lyse different cell types; (4) ability to stabilize DNA over an extended period of time; and, (5) ability to perform in downstream Polymerase Chain Reaction (PCR) testing and produce quality STR profiles.

The Quantifiler® Duo DNA Quantification Kit on an Applied Biosystems® 7500 Real-Time PCR instrument was used to estimate the quantity of human DNA present in each sample. For comparison with samples extracted with currently used methodologies, STR typing was performed, following DNA quantification, on all samples of DNA extracted with the iSWAB™-ID collection device using the GlobalFiler™ PCR Amplification Kit and Identifiler® Plus PCR Amplification Kit and analyzed using the GeneMapper™ ID-X software.

Cumulatively, the data indicates that the iSWAB™-ID collection device is efficient and convenient while providing enhanced DNA recovery and full, high-quality STR profiles when targeting as little as 0.5ng of DNA; however, some limits exist, which include the potential of making low-input samples too dilute for subsequent analysis. The results of this study illustrate the potential benefits and limitations of implementing the iSWAB™-ID collection device in the forensic field.

This study was partially supported by Mawi DNA Technologies. The opinions, findings, and conclusions or recommendations expressed are those of the author(s) and do not reflect those of Mawi DNA Technologies.

Biological Specimen Collection, DNA Recovery, Direct Lysis

B108 An Evaluation of QIAGEN® Investigator® 24plex GO! Using Crime Scene Substrates and Direct Amplification

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The goal of this presentation is to educate attendees in direct amplification of body fluids without the time-consuming and labor-intensive extraction and quantification steps. After attending this presentation, DNA analysts will be able to generate DNA profiles from body fluids when commonly found crime scene substrates containing inhibitors remain in the amplification reaction during the thermal cycling procedure.

This presentation will impact the forensic science community by generating DNA profiles quickly. The results can be obtained within a very short time period. This in turn can help to quickly locate a perpetrator or exonerate the innocent. No new instrument is needed and the method can be easily implemented in the forensic laboratory.

Short Tandem Repeat (STR) analysis is a technique performed routinely in forensic laboratories. Markers in these regions are amplified by Polymerase Chain Reaction (PCR) technology using specific primers, which have made them valuable in the forensic community. In violent personal crime cases, blood, saliva, semen, and nasal secretions are routinely encountered on various substrates. Direct amplification of these body fluid stains without time-consuming, labor-intensive extraction and quantitation steps has proven useful in forensic science laboratories.

Direct amplification entails amplifying the DNA present in a body fluid stain without extraction or quantitation steps and injecting the amplified product in a capillary electrophoresis system. The QIAGEN® 6-dye amplification system, Investigator® 24plex GO! allows the identification of 22 polymorphic STR loci and includes two innovative internal PCR controls (quality sensors). The quality sensors, QS1 and QS2, provide information regarding whether there is degradation and/or inhibition. These also indicate the absence of DNA or a failed PCR reaction. The current research utilizes this amplification kit to directly amplify minute amounts of blood, saliva, semen, and nasal secretions placed on various simulated crime scene substrates while the substrates remained in the reaction during amplification. Substrates chosen for deposition of these body fluids included various types of fabric ranging from white cotton to blue denim jeans and leather as well as cigarette butts, chewing gum, woodchips, straw, grass, and other objects.

Blood from four deceased donors was obtained from a forensic pathologist. Four semen samples were purchased from commercial vendors. Saliva and nasal secretions were collected from four individuals following the guidelines of the Office of Research Protection (ORP). Each body fluid was diluted in a 1:1 ratio. For blood, semen, and nasal secretions, 0.2µL of each diluted sample was deposited on a 1.2mm punch or cutting of each substrate. For saliva samples, 0.5µL of the body fluid was deposited on each substrate. After drying overnight, the stains were transferred to individual tubes. Next, 5µL of Investigator® GO! Lysis Buffer was added to each punched substrate containing blood, saliva, and nasal secretions and left at room temperature for 20 minutes with occasional mixing.

For semen samples, after drying overnight, 0.2µL of 1M DTT solution was added to each substrate containing semen samples. Each semen-stained substrate was then incubated at 56°C for 30 minutes. In the next step, each sample was shaken at 600rpm for another 30 minutes using a thermal mixer.

Each substrate containing only one type of body fluid was then subjected to amplification. For this step, 20µL of the reaction mixture was added to all tubes and amplification was performed following recommended protocol. During the amplification step, each substrate containing one of the four body fluids remained in the reaction mixture. Amplified products were injected into the 3130xl Capillary Electrophoresis (CE) system. GeneMarker® HID analysis software v 2.9 from SoftGenetics® was used for fragment analysis.

All stains created from the four body fluids using simulated crime scene substrates were amplified successfully, even when the substrates remained in the reaction mixture during amplification steps. All stains were created as single-source samples to generate DNA profile from one single donor. Thus, no mixture analysis was necessary in this project.

Consistent and concordant profiles were obtained from all body fluid-stained substrates. The S peak on QS2 locus occasionally dropped out, indicating inhibition in the sample, even when a complete profile was obtained. Known inhibitors such as soil or dye were present in some of the substrates. Therefore, this observation was not unexpected. Despite the substrates being present during the thermal cycling steps, the reagents were able to overcome inhibition and amplify DNA from challenging samples.

This study suggests that the Investigator® 24plex GO! is a valuable tool that can be easily incorporated in the analysis of body fluids such as blood, saliva, semen, and nasal secretions in forensic laboratories. Since there is no extraction and quantitation involved in the described procedure, the results can be obtained within a very short period of time. This can quickly help find a perpetrator or exonerate the innocent.

Direct Amplification, Investigator® 24plex GO!, Inhibitors

B109 An Evaluation of the Quality of Short Tandem Repeat (STR) Profiles Generated by Rapid DNA Instruments

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After attending this presentation, attendees will understand the performance of the Rapid DNA instruments and whether the Rapid DNA instruments can be applied for casework samples.

This presentation will impact the forensic science community by reporting on the quality and characteristics of STR profiles produced by two different Rapid DNA instruments.

The Rapid DNA instruments are optimized to produce STR profiles from reference buccal swabs without human review. The applicability of the Rapid DNA instruments to disaster victim identification and casework samples is also of great interest to the forensic community.

In this study, the quality and the characteristics of STR profiles produced by the DNAscan™ 6C Rapid DNA Analysis System using the High DNA Content (HDC) Flexplex™ chemistry and the Low DNA Content (LDC) Flexplex™ chemistry, and the RapidHIT™ System using the GlobalFiler® Express chemistry were evaluated. High DNA content samples, such as reference buccal swabs, mock casework, bloodstain, and saliva stain samples were analyzed. Buccal swab samples were collected from volunteers. Blood dried on the glass slide and saliva on water bottles were collected by wiping using the swabs moistened with sterile distilled water. Cotton swabs manufactured for the DNAscan™ 6C Rapid DNA Analysis System were used. A full DNA profile without an incorrect allele calling and low-quality flag issued by the Rapid DNA expert systems was counted as a success. A profile with an allelic dropout at any loci caused by consumables or instrument defective was counted as a failure. A profile with a labeled non-allelic peak, such as a dye blob and spectral pull-up as an allele, was also counted as failure. The .fsa files were exported from both systems. Human review data analysis using the GeneMapper® ID-X Software was performed after the peak detection threshold for each system had been established using an electropherogram obtained from clean blank swabs.

The success rates for buccal samples for both Rapid DNA systems were approximately 80%, as reported by other publications. It was found that the success rate for mock casework samples was less than 50% due to low-quality flags that indicate the presence of non-allelic peak and/or the substantial variability in peak height and/or inter-loci balance, was more frequently issued by the Rapid DNA expert systems than buccal samples. No indications of carryover and cross contamination were observed in any of the blank samples by the Rapid DNA expert system and the GeneMapper® ID-X Software analysis. It was shown that the success rate can be improved significantly when .fsa files were analyzed by GeneMapper® ID-X Software.

An allelic ladder is run concomitantly with samples in each cassette. When the allelic ladder fails to meet the quality parameters of the Rapid DNA expert systems, the pre-loaded virtual allelic ladder implemented in each of the Rapid DNA instruments is employed to designate the sample alleles; however, it was found that some incorrect alleles were called by the Rapid DNA instruments in such cases. Therefore, it was considered that the success of the allelic ladder run was important for the correct allele calling.

Rapid DNA, Data Analysis, Allelic Ladder

B110 The Recovery of the Mitochondrial Genome From Cremated Human Remains

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After attending this presentation, attendees will better understand how mitochondrial DNA (mtDNA) may be recovered from highly degraded samples, such as cremated remains, and analyzed using Massively Parallel Sequencing (MPS).

This presentation will impact the forensic science community by introducing a protocol that provides the means for DNA identification from severely burned human remains when other methods for identification have been exhausted or are not feasible.

The combination of a DNA extraction method designed for calcified tissues combined with a whole mitochondrial Genome (mtGenome) multiplex Polymerase Chain Reaction (PCR) assay and MPS will yield sufficient sequence information necessary for DNA identification of cremated human remains.

In cases of fires or explosions, forensic identification by traditional methods such as fingerprinting or anthropological analysis is often not possible. DNA identification of charred or incinerated remains may be the last resort for family members wishing to identify their loved ones. The recovery of DNA from burned human remains has been notoriously difficult. Some studies have been successful in typing DNA from charred remains and dental pulp; however, concerns exist regarding the quality and purity of DNA that is recovered.[1,2] Nuclear DNA in bone is often limited and PCR inhibitors can impede amplification of Short Tandem Repeats (STRs). The circular structure and subcellular sequestration of mtDNA may provide some protection from degradation, and high copy number of mtDNA per cell increases sensitivity of DNA typing assays, making it better suited for DNA identification of severely degraded samples such as burned remains.[3]

In this study, mtDNA was sequenced from an individual whose remains had been incinerated in a commercial crematorium. Ashes and small bone fragments were recovered from the cremated remains of the deceased. Bone fragments were externally cleaned with bleach and pulverized. DNA was extracted from ash and pulverized bone using a kit optimized for calcified tissues.[4] MtGenome copies from each extract were quantified, and Hypervariable region 1 (HV1) was amplified and sequenced via Sanger sequencing. Whole mtGenome was amplified using a multiplex PCR assay, and libraries were prepared and sequenced via MPS. HV1 and whole mtGenome sequences were compared to one another for concordance. All sequences were also compared to a maternally related reference to establish identity.

Results indicate that mtDNA can be successfully extracted from cremated remains. The combination of a calcified tissue-specific DNA extraction method with multiplex PCR and MPS is a useful technique to maximize recovery of genetic information from such severely compromised sample types.

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Mitochondrial DNA, Massively Parallel Sequencing, Cremated Remains

B111 The Development of an X-Chromosome Insertion-Deletion (InDel) Multiplex for Forensic Applications

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After attending this presentation, attendees will have a better understanding of the development and application of the X-chromosome InDel marker system for use with forensic DNA samples.

This presentation will impact the forensic science community by providing results from a developed novel X-chromosome InDel as a key aspect for the analysis of special forensic investigations as it can contribute to traditional DNA testing systems in cases in which those systems do not produce results due to the presence of degraded DNA.

Short Tandem Repeats (STR) loci are commonly used genetic markers for the purpose of forensic identification; however, there are many situations in which a full DNA profile cannot be achieved from a crime scene due to degradation of the biological samples over time. Recently, there is a tendency toward Single Nucleotide Polymorphism (SNP) and InDel among forensic scientists since these markers have smaller DNA sequences; therefore, a successful DNA profile can be achieved from even a small amount or degraded biological samples. InDel polymorphism is a type of genetic variation that is formed by the addition or loss of one or several bases in the human genome. InDel loci have small (60bp to 200bp) amplicon length (Polymerase Chain Reaction (PCR) products) and have begun to be used in forensic identification. X-chromosome InDel polymorphism can be used in specific kinship investigations (incest cases, etc.).[1-5] The goal of this study is to develop a multiplex InDel panel, consisting of 18 X-chromosome loci with a short amplicon size, for forensic purposes.

Eighteen X-chromosome InDel markers were chosen based on the following criteria: (1) mean heterozygosity of more than 30% in Europeans; (2) a minimum of 250Kb differences between each InDel loci; and, (3) the amplicon length was less than 300bp. InDel primers were designed using the PRIMER3 software and tested for hairpin and primer-dimer secondary structures with the AutoDimer software. Singleplex PCR was applied to the InDel loci, then multiplex PCR was performed containing PCR master mix, optimized primer concentrations, and 1ng-10ng of genomic DNA. InDel loci fragments were separated using an ABI® PRISM® 310 Genetic Analyzer and analyzed with GeneMapper® v3 software. The validation study of these 18 InDel loci was performed with the following parameters and aspects: analytical threshold, sensitivity and stochastic threshold, heterozygous balance, precision and accuracy, repeatability and reproducibility, genotype concordance (9947a), DNA mixtures, and case samples.

Eighteen InDel loci were successfully amplified using a single multiplex reaction. Optimization and validation parameters of the 18 InDel Multiplex was successfully applied. The high performance of this 18 InDel multiplex fluorescent PCR system makes it a valuable additional system to the current STR systems and can be used for forensic purposes.

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X-Chromosome, InDel, Panel Development

B112 The Impact of Antioxidant Beverages on the Chemiluminescent Detection of Bloodstains at Crime Scenes

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The goal of this presentation is to inform attendees of the recent findings related to the antioxidant inhibition of the chemiluminescent detection of bloodstains.

This presentation will impact the forensic science community by improving insight into the inhibition of the reaction between chemiluminescent reagents, specifically luminol and Bluestar Forensic®, and blood found at crime scenes. The possibility of this inhibition has been suggested in several published articles but has yet to be studied.

The identification of blood deposited at a crime scene is crucial to the reconstruction of events leading to and following a crime, as well as corroborating or rejecting statements and alibis. Many biochemical reagents are at the crime scene investigators disposal to both screen for, and confirm, the presence of visible and non-visible bloodstains. One such reagent for the detection of non-visible, or latent, bloodstains is a chemiluminescent reagent known as luminol, which comes in multiple formulations, the most widely utilized being Bluestar Forensic®. It was recently reported, but not further studied, that the presence of antioxidants in contact with a blood stain may hinder the reaction of such chemiluminescent reagents, giving way to a false negative reaction.[1,2] Conceivably, blood is the most commonly encountered bodily fluid at crime scenes. Therefore, further investigation of the possibility of antioxidants within the environment potentially masking bloodstains is necessary to address the opportunity for hindered investigations.

This study investigated the potentially negative effects of seven different antioxidant sources on the reaction between chemiluminescent reagents and blood. Methods involved staining both absorbent and non-absorbent surfaces, carpet and tile, respectively, with 2mL of four dilutions of blood: neat, 1:10, 1:100, and 1:1000. Each bloodstain, after a 24-hour drying period, was then treated with 5mL of one of seven antioxidant sources: orange juice (100%, not from concentrate); green tea (one processed Pure Leaf® cold green tea beverage and one unprocessed organic hot green tea leaf beverage); a supplement drink (Bai® Antioxidant Infusion); red wine (Pinot Noir); coffee (premium roast); or black English breakfast tea. Bloodstains were also treated with Coca-Cola®, which contains no antioxidants, acting as a control. Following a 24-hour drying period, each sample was then treated with one of two chemiluminescent reagents, luminol or Bluestar Forensic®, and documented for chemiluminescent intensity. A Canon® EOS Rebel T3i digital SLR camera was used to document each reaction to later compare to control samples and better approximate the chemiluminescent intensity.

The results of this study revealed red wine and coffee to negatively impact the chemiluminescent reaction of both luminol and Bluestar®, creating false negatives on both surfaces, with all dilutions. Samples tested with Coca-Cola® all produced moderate-strong positive chemiluminescence, showing the application of beverages to not act as a barrier for the reaction, rather potentially the antioxidants present in red wine and coffee inhibiting the reaction. The other five antioxidant drinks produced positive chemiluminescent reactions; however, these reactions were impacted and could lead to misinterpretation. Orange juice, both green teas, the supplemental drink, and black tea produced weak-moderate reactions on both surfaces, with Bluestar® fading very quickly compared to typical reactions, which could be misinterpreted as a false positive.

This research has highlighted the importance of the choice of method and considerations to be taken when screening evidence items/crime scenes for blood and of the possibility of antioxidants in the environment having a negative impact. The results of this study provide a valuable and novel contribution to the forensic science field as the impact of antioxidants has largely been unexplored, yet warrants investigation.

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Blood, Antioxidants, Chemiluminescence

B113 The Development and Evaluation of Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) Assay for Forensic Saliva Identification

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After attending this presentation, attendees will better understand a novel combined approach for the detection of saliva as a model for body fluid identification using real-time RT-LAMP.

This presentation will impact the forensic science community by demonstrating that real-time RT-LAMP is an ideal tool to identify saliva. It shows great potential in forensic casework by presenting the possibility of identifying body fluids via messenger RNA (mRNA) and is capable of identifying body fluid stains at crime scenes by combining with Direct LAMP and multiplex LAMP technology.

LAMP employs a DNA polymerase which contains the 5' to 3' polymerase activity, but lacks 5' to 3' exonuclease activity. It replaces and releases the complementary strands instead of hydrolyzing them. The previously released strands can be used as a template and amplified with proper primers. Via auto-cycling mediated by the DNA polymerase, it keeps displacing target strand DNA and creates new targets as it amplifies. Thus, LAMP reaction can take place at a constant temperature.[1] LAMP has been proven to be a rapid, sensitive, accurate, and economical technique for DNA amplification widely used in detection of various kinds of pathogens, microorganisms, fungi, and parasites.[2,3]

RT-LAMP with the saliva-specific marker, Statherin (STATH), was used to identify the presence of saliva in this study. The 18S rRNA gene was used as the internal control. Seven body fluids, including venous blood, saliva, semen, menstrual blood, sweat, urine, and vaginal secretions, were collected from volunteers using procedures approved by the Institutional Review Board (IRB) of the Central Police University in Taiwan. Total RNA was extracted from each body fluid with the RNeasy® Mini Kit and then quantified using NanoDrop® ND-1000 UV spectrophotometer. One-step RT-LAMP was performed using Loopamp RNA Amplification Kit. For Direct LAMP, body fluid samples were simply boiled before RT-LAMP without RNA extraction.[4] The LAMP product was confirmed by electrophoresis and restriction enzyme digestion. The real-time turbidimeter was used to monitor the change of turbidity, which indicated the real-time accumulation of LAMP products. Calcein was used for fluorescence detection. Additionally, Polyethylenimine (PEI) was also used for multiplex LAMP.[5]

Saliva samples were successfully identified using LAMP with STATH primer sets without false positive and negative (at least 12 samples for each body fluid and negative controls were tested). The Threshold time (Tt) for saliva was 39.4±2.9min. LAMP products with 18S rRNA primer sets were detected from all body fluids samples (Tt: 26.8±2.2mins for venous blood, 44.2±2.3mins for saliva, 33.2±2.2mins for semen, 44.7±5.6mins for menstrual blood, 44.7±2.5mins for sweat, 32.9±0.4mins for urine, and 38.2±2.8mins for vagina secretions). Evaluation for LAMP products of all samples by fluorescence and electrophoresis is consistent with the results. It also revealed that less than 1µl of saliva could still be detected, which is equal to or slightly better than identifying body fluids by mRNA profiling (1µl of saliva).[6] STATH and 18S rRNA could be successfully identified simultaneously using multiplex RT-LAMP with PEI. Saliva samples without RNA extraction were also successfully identified by Direct LAMP.

Results demonstrate that LAMP exhibited high potential for providing an ideal tool in saliva identification for its simplicity, efficiency, specificity, and sensitivity. One-step RT-LAMP takes less than one hour to complete the entire reaction. Traditional two-step procedures including reverse transcription and PCR comparatively cost more money, time, and labor and raise a higher risk of contamination. Also, traditional PCR-based methods require a heavy and expensive thermal cycler for reaction, making it difficult to perform at the crime scene. RT-LAMP could be performed using one piece of laboratory equipment instead of a dedicated machine. It was also shown that Direct LAMP without RNA extraction and fluorescence detection with the presence of calcein could be performed simply and is applicable to test remotely from the laboratory, such as at a crime scene. The assay was also applied successfully to the putative identification of saliva from non-probative forensic samples, demonstrating that LAMP is capable of identifying body fluid stains at the crime scene. This is the first application of RT-LAMP for the identification of saliva.

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Saliva Identification, Reverse Transcription LAMP, Statherin (STATH)

B114 Identification of Kratom (*Mitragyna Speciosa*) DNA Using a Real-Time Polymerase Chain Reaction High-Resolution Melt (PCR HRM) Assay

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After attending this presentation, attendees will be able to evaluate how a newly developed real-time PCR assay coupled with post-PCR HRM can be used for kratom identification when dealing with degraded or mixture plant samples in forensic casework.

This presentation will impact the forensic science community by demonstrating a new method to identify kratom from other illegal and "legal high" plants that may be in forms indistinguishable by eye and through microscopic techniques. This process could be used as a cost-saving alternative to chemical analysis currently in use, such as Fourier Transform Infrared (FTIR) or Gas Chromatography/Mass Spectrometry (GC/MS). In addition, equipment required for this screening is readily available to forensic DNA scientists in major crime labs.

Kratom (*Mitragyna speciosa)* is a plant that is a member of the family Rubiaceae, or coffee family, and is commonly used for the analgesic, opiate-like, and stimulant properties from the alkaloids it produces. The most famous and widely cited of these alkaloids is mitragynine as it is the most abundant metabolite, although there are more than 40 alkaloids produced by the plant, including 7-hydroxymitragynine. While it is widely referred to as kratom worldwide and in its native Southeast Asia, it is also referred to by other names in various countries, including biak-biak or ketum in Malaysia, krathom, kakuam, ithang, or thom in Thailand, and mambog in the Philippines. Its popularity among users primarily relies on its stimulant and pain-relieving properties, which have been reported to be 17 times more potent than morphine.

The first country to formally control the use of kratom was Thailand in 1943. In recent years, its use as a recreational drug has increased worldwide and numerous case studies have been reported in the literature documenting various effects on users and in the extreme instances, including its presence in cases of death in which the drug was taken in combination with other legal and illegal substances. Western nations have responded to this recent increase of prevalence by all-out banning, controlling, and/or imposing restrictions to only be used with a medical prescription. The United States Drug Enforcement Administration (DEA) currently includes kratom on the list of Drugs and Chemicals of Concern. As of September 2016, the DEA reopened the comment period to consider labeling kratom as a Schedule I substance; thus, its legality currently defers to a state-by-state basis.

Current methods of identification seen in the literature include visualization techniques such as colorimetric tests and microscopy and quantitation through alkaloid chemical analysis such as Hydrogen-1 Nuclear Magnetic Resonance (^{1}H NMR), GC/MS, FTIR, High-Performance Liquid Chromatography (HPLC), and Direct Analysis in Real-Time Mass Spectrometry (DART®-MS), to name a few. Recent DNA techniques have relied on Restriction Digest Length Polymorphism (RFLP) assays.

In this study, primers designed for the PCR-HRM assay targeted the Secologanin Synthase 2 (SLS2) and Strictosidine Beta-D-Glucosidase (SGD) genes within the *M. speciosa* genome. Amplification of SLS2 and SGD genes produced amplicons of 168bp and 66bp, respectively. The SLS2 primer set was specific for *M. speciosa* against other "legal high" plant species such as *Cannabis sativa*, *Datura wrightii,* and *Papaver orientale* with a melt curve of 77.09°C. Further sensitivity testing indicated continued detection of SLS2 amplicon at concentrations as low as 0.05µg/mL.

Kratom, Real-Time PCR, High-Resolution Melt

B115 Internal Validation of a Quantifiler® Trio Setup Method on the Hamilton® Microlab® Sequential Transfer and Aliquotting Robot (STAR)

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After attending this presentation, attendees will have a better understanding of the importance of increased automation in the forensic DNA laboratory workflow, specifically as it relates to Quantifiler® Trio set up on the Hamilton® STAR.

This presentation will impact the forensic science community by demonstrating that the Hamilton® STAR has the capability to set up a 96-well plate for quantitation and achieve reliable results that are comparable to those obtained manually and perhaps even more precisely than a manual setup. This presentation will also highlight the benefits of combining different automated methods on the STAR in order to create a more seamless process with less analyst intervention, leading to fewer opportunities for contamination or human error.

The Hamilton® Microlab® STAR is an automated liquid-handling robot that can be programmed to perform a variety of tasks, such as extraction, quantitation set up, normalization, amplification set up, and capillary electrophoresis set up, in the forensic DNA workflow. Robots such as the Hamilton® STAR are useful in forensic DNA laboratories because they reduce the risk of contamination between samples and increase the precision of pipetting. The use of air-displacement pipetting reduces the risk of contamination presented by liquid-displacement pipetting used in some other robots. The Hamilton® STAR also reduces the amount of bench work time for analysts, allowing more time for analyzing data and technical review, which are the major bottlenecks in most forensic DNA laboratories. The use of a worksheet that establishes each sample's destination allows flexibility of sample processing, and a barcode reader for samples adds a second check that prevents sample mix-up. The Hamilton® STAR is able to process up to 96 samples at once, which allows for more samples to be processed in less time than with manual preparation. With increased automation in their workflow, forensic DNA laboratories can increase their efficiency, thereby reducing case turnaround time.

In this presentation, the quantitation set up method developed by Sorenson Forensics® was validated for use on the Hamilton® STARs currently used by the New York City Office of Chief Medical Examiner's Department of Forensic Biology. The internal validation study was conducted in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines, including cross contamination, volume verification, and precision studies as well as manual and robot-to-robot comparison studies. In addition, automation workflow strategies for large metropolitan forensic DNA laboratories will be discussed.

Hamilton® STAR, Automation, Quantitation

B116 The Identification of Forensically Relevant Body Fluids Using Methylation-Specific Polymerase Chain Reaction (PCR) and High-Resolution Melt (HRM) Analysis

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After attending this presentation, attendees will be informed of the benefits of using Methylation-Specific PCR (MSP) and HRM analysis to differentiate between different body fluids.

This presentation will impact the forensic science community by informing attendees of the ability to quickly and easily determine the presence of a specific body fluid prior to completing DNA analysis.

The combination of MSP with HRM analysis is a method that can demonstrate the difference between various forensically relevant body fluids. This type of body fluid identification involves targeting expressed genes and comparing their methylation status to other cell types.[1] DNA extracted from different body fluids undergoes a bisulfite conversion process that converts any unmethylated cytosines to uracil, which ultimately becomes thymines during PCR amplification. Forward and reverse primers are designed to amplify a target region of DNA that contains multiple CpG sites. The methylation status of the cytosines results in an alteration to the Melting Temperature (T_M); methylated cytosines produce a higher T_M than unmethylated cytosines. The primers can then be designed to bind specifically to methylated or unmethylated template strands.

Several studies have previously identified gene sequences that are differentially methylated in several relevant body fluids. The majority of these studies used pyrosequencing to identify changes in the methylation status of semen, blood, and saliva. The goal of this study is to use MSP and HRM analyses to visualize these differences and identify each body fluid. Using those results, two or more primers will be multiplexed to ensure a greater specificity in determining the presence of a forensically relevant body fluid.

Throughout this study, ten known primer sets and several primer sets of new design were studied to enhance body fluid identification. The forward and reverse primers were analyzed during their methylated and unmethylated forms to display the various T_Ms. ZC3H12D and DACT1_F have already been identified and analyzed using HRM analysis.[1,2] The eight remaining primer sets were obtained from studies involving sequencing to determine the differentiation likely to occur among body fluids. These include FGF7, cg06379435_W, BCAS4, cg06379435_S, DACT1_B, USP49, DDX4_B, and B_SPTB_03.[3-6] This study has proven the ability to differentiate semen from other body fluids. Further analysis is being performed to differentiate between blood and saliva, as these body fluids tend to display a similar T_M for many primer sets. The next step for this study is to combine two or more of the primer sets into a multiplex assay which will increase the specificity of the distinct body fluid identification.

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Body Fluid Identification, Methylation-Specific PCR, High-Resolution Melt Analysis

B117 A Comparison of Extraction Methods for Amplification of Nuclear DNA From Hair Shafts Using the InnoTyper 21® DNA Typing Kit

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The goal of this presentation is to share data from studies comparing multiple methods for nuclear DNA extractions from hair shafts, demonstrating that full nuclear DNA profiles can be obtained from hair shafts with as little as 30 picograms of DNA using the InnoTyper 21® DNA amplification kit.

This presentation will impact the forensic science community by assessing and demonstrating methods that can obtain full nuclear DNA profiles from highly degraded DNA from evidence samples, such as hair shafts.

Forensic crime laboratories receive hair shafts as evidentiary samples and process them for DNA evidence as a means of identification of individuals. Nuclear DNA is often too scarce and degraded to enable sufficient profile recovery from hair shafts using standard methods. There are many more copies of mitochondrial DNA in a cell than nuclear DNA. Therefore, it is common practice to target mitochondrial DNA instead of nuclear DNA when processing a hair shaft for DNA evidence.

Researchers at InnoGenomics® Technologies have developed a nuclear DNA quantification system, InnoQuant®, which targets 80-basepair and 207-basepair fragments and a novel amplification kit, InnoTyper 21®, which targets ~60bp-125bp fragments. Targeting short DNA fragments creates the potential to obtain sufficient nuclear DNA data from samples containing highly degraded DNA or low template DNA. Rather than targeting loci that contain Short Tandem Repeats (STRs), these kits target retrotransposable nuclear elements, which are non-coding genomic DNA repeat sequences, or "mobile insertion elements," comprising approximately 40% of the human genome.

This study compares nuclear DNA extraction methods from hair shafts such as the QIAamp® Fast DNA Tissue kit, direct lysis procedures using ZyGEM® prepGEM™ enzyme, and direct lysis procedures using pronase. Pronase is a mixture of proteases isolated from the bacteria *Streptomyces griseus.* It is reported that pronase will cleave peptide bonds into single amino acids. A common challenge with extracting DNA from hair shafts is the breakdown of keratin proteins in the hair shaft to release the DNA. Therefore, it was hypothesized that direct lysis extraction methods utilizing pronase will result in sufficient keratin degradation and therefore release enough nuclear DNA from hair shafts to be quantified with the InnoQuant® quantification kit and typed using InnoTyper 21® DNA typing kit.

This study exhibited mixed results with direct lysis using pronase at concentrations of 0.05mg/mL and 0.01mg/mL, generating a range of full, partial, and null InnoTyper 21® profiles. Higher pronase concentrations of 0.25mg/mL produced significant PCR inhibition, causing amplification failure. The QIAamp® Fast DNA Tissue kit extraction method resulted in the highest average concentration of DNA (0.0495ng/µl) extracted from hair shaft samples and the most complete profiles using the InnoTyper 21® kit. Full profiles of nuclear DNA from hair shafts were obtained using the InnoTyper 21® amplification kit from samples with as low as 0.0021ng/µl of DNA; however, not all samples at this concentration resulted in successful amplification. There was a large discrepancy between partial profiles which ranged from 2 to 18 loci. The success of DNA typing appeared to be dependent on the donor source as well as the extraction method.

Hair Shafts, DNA Extraction, Degradation

B118 The Limits of Detection (LODs) of Surface-Enhanced Raman Spectroscopy (SERS) -Active Swabs Used to Screen for Human Bodily Fluids (HBFs)

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After attending this presentation, attendees will better understand the sensitivity associated with a novel technique for the screening of HBFs for confirmatory identification of semen, saliva, and their mixtures.

This presentation will impact the forensic science community by providing the LODs of a technique that enables rapid, highly sensitive, non-destructive identification of semen, saliva, and their mixtures. The sufficient concentration of each HBF and mixture analyzed that are needed to produce an analytical signal that can be reliably discriminated from the signal produced without the pure HBFs or mixtures will be determined. The methodology will ultimately be extended to acquire the LODs of other pure HBFs (blood and vaginal fluid) and other mixtures.

The screening of HBFs is routinely conducted in crime laboratories prior to DNA analysis. First, a presumptive test is conducted to determine whether a specific bodily fluid can be present, followed by a confirmatory test, which establishes the identity of the material; however, the existing methods are prone to false positives and false negatives and therefore lack sensitivity and specificity. Furthermore, these tests require extensive sample pre-treatment, expensive chemicals, and are time consuming and destructive for the sample.

Raman spectroscopy is an analytical technique that provides information about molecular vibrations that can be used for sample identification and quantitation. The suitability of Raman spectroscopy for the analysis of bodily fluids has been reported as this technique allows for the rapid, highly selective, and non-destructive analysis of the samples.[1] Raman may also offer the possibility of performing one type of measurement for all HBFs, while also preserving the DNA evidence contained in such samples; however, the high sensitivity required for forensic samples may not be obtainable with Raman spectroscopy alone. Therefore, SERS may be more suitable for the analysis of HBFs as it enables the enhancement of analyte signals by several orders of magnitude. This increases the sensitivity of the Raman and therefore its ability to detect small amounts, dilute samples, and mixtures, which can be found at a crime scene.

Silver nanoparticles were attached to nylon Copan 4N6FLOQSwabs™ using the hydrogen reduction method.[2] Seminal fluid from was obtained from Lee Biosolutions™, Inc. Mixtures were prepared with different semen/saliva ratios. A range of concentrations of the semen and saliva, as well as their mixtures, were produced to measure their LODs. Different swabbing techniques were explored and Raman data of analyte adhered to the SERS-active swabs was measured using a 632.8nm laser excitation. Distinctive SERS bands of semen and saliva were used to identify their contributions in the mixtures. Based on the signal-to-noise approach, the LODs were then determined. The measured signals from the bodily fluids of known low concentrations were compared with those from blank samples using a signal-to-noise ratio of three. The minimum concentration at which the bodily fluids and mixtures could be reliably detected was then established.

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Serology, SERS, Limits of Detection

B119 The Extraction of Touch DNA From Chemically Developed, Aged Fingerprints

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After attending this presentation, attendees will better understand how latent fingerprints deposited onto a paper substrate are developed with various chemical reagents, what touch DNA is and how it can be extracted from paper, and how touch DNA can still be recovered several days to weeks after a fingerprint has been chemically developed.

This presentation will impact the forensic science community by demonstrating that fingerprint evidence can be tested for touch DNA even after being chemically developed. This is important in cases such as cold case files or exoneration cases in which fingerprint evidence has been in storage over time after being chemically developed, but DNA testing was either not available or not requested.

Fingerprints recovered from crime scenes are rarely perfect and often require enhancement. For latent fingerprints deposited onto porous surfaces such as paper, developing reagents like ninhydrin can be applied to make the print visible by staining it dark purple.[1] If after development a latent print cannot be used for comparison, the next step may be to test the print for touch DNA. Touch DNA is left behind by a person upon touching a surface and leaving behind DNA-containing skin cells.[2] The development of touch DNA has provided forensic scientists with a tool for extracting DNA from fingerprints when traditional developing methods cannot produce a usable print.

If a fingerprint is not usable for comparison purposes, it can then be tested for touch DNA; however, laboratory backlogs can sometimes leave developed fingerprint evidence sitting in storage for several days, weeks, months, and even years before it can be tested. Several studies have been conducted to examine the effect that developing reagents, such as 1,2-indanedione, have on the recovery of touch DNA from developed fingerprints deposited onto porous surfaces over time.[3,4] It has been shown that DNA can be recovered up to 21 days post-development after treatment with 1,2-indanedione, but research is lacking as to how other reagents for porous surfaces, such as ninhydrin, 5-methoxy ninhydrin, 5-methylthio ninhydrin, genipin, and lawsone, affect future DNA analysis.[3]

To examine the effect of chemical reagents, fingerprints from four participants (two males, two females) were deposited onto personal checks, developed with the reagents listed above, then allowed to age for various time intervals (1-180 days). Reference buccal swabs from the participants were collected for subsequent DNA analysis. A set of control fingerprints were also collected from each participant and were not treated with a reagent. After aging, the samples were swabbed, quantified, amplified, and then analyzed using capillary electrophoresis to determine if a DNA profile could be generated. Results determined that even after treatment with chemical reagents, a genetic profile can be generated for the aged samples. This demonstrates that even in cases in which treated fingerprint evidence is left to sit in evidence for extended periods of time, DNA can still be extracted and used to help identify a suspect.

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Touch DNA, Aged Fingerprints, Ninhydrin

B120 The Impact of the Length of Time of Personal Contact on Secondary DNA Transfer

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After attending this presentation, attendees will appreciate how much contact may be necessary for secondary DNA transfer to occur and the potential impact on forensic investigations. The learning objectives are to evaluate the detection of secondary DNA transfer using next generation amplification kits, to investigate the length of contact time that might result in secondary DNA transfer, and to assess the impact of secondary DNA transfer on determining how evidence relates to a crime scene. The hypotheses are that secondary DNA transfer: (1) can occur during brief periods of contact; and, (2) can complicate interpretation of DNA evidence.

This presentation will impact the forensic science community by demonstrating that secondary DNA transfer can occur during brief encounters and that it can have a significant impact on understanding evidence in relation to a crime scene.

The analysis of trace amounts of DNA from items possibly handled by a suspect during the commission of a crime often plays a crucial role in criminal investigations. In some cases, DNA left on a touched object can be the only link to the perpetrator. The increased capability to detect minute traces of DNA from a perpetrator at a crime scene has been a continuous goal of the forensic community. While enhancing the sensitivity of Short Tandem Repeat (STR) kits to increase the likelihood of obtaining results from trace DNA samples, there also appears to be a simultaneous increase in the detection of extraneous DNA, the presence of which can complicate the identification of a suspect.

Empirical research has not only demonstrated the primary transfer of DNA via direct contact with an object, but also the secondary transfer of DNA whereby an individual's DNA is transferred to an object or another individual via an intermediary. The indirect transfer of DNA as an explanation for the presence of trace DNA samples at a crime scene appears to be becoming more prevalent in forensic investigations and during the subsequent court proceedings. In Europe, the focus of forensic investigation appears to be moving toward not only identifying the source of a DNA profile, but identifying how the DNA was deposited on an evidentiary item — directly or indirectly.[1,2]

Expanding upon the Cale et al. study by introducing additional variables, this project used handshaking to simulate contact that could lead to secondary DNA transfer.[3] Participants shook hands for varying lengths of time: 10, 30, and 60 seconds. Plastic knives were handled immediately following contact. The knife handles were subsequently sampled for DNA using a wet swabbing technique. The samples were amplified with the GlobalFiler® Polymerase Chain Reaction (PCR) Amplification Kit and analyzed on an ABI® 3130xl genetic analyzer. The Mixture Analysis Tool within GeneMapper® ID-X version 1.5 was utilized to facilitate data interpretation.

Data was obtained from all samples (n=72). Interpretable DNA profiles were obtained from 38 samples. The DNA yields for samples that resulted in interpretable profiles ranged from 50pg to 3ng. In 26 of the 38 interpretable profiles, single-source profiles or mixed DNA profiles with the major component matching the primary contributor were obtained. Five contributor inversions were observed, where the secondary contributor matched the major component of a mixed DNA profile. Indistinguishable mixtures of both contributors were obtained from seven samples, making it difficult to identify the primary handler of the knife. In the remaining 34 samples, the presence of alleles from the secondary contributor and/or extraneous DNA rendered the profiles inconclusive. Consequently, secondarily transferred DNA complicated the interpretation of DNA typing results in those samples.

Mixed DNA profiles are often obtained from trace DNA samples in which multiple handlers of an object can be detected. Typically, the greatest proportion of the DNA comes from the most recent handlers, but not necessarily the last handler.[4] In some instances, an individual who did not directly handle the object can be detected. The results of this study demonstrate that under certain conditions, secondary DNA transfer can occur during brief interactions. The presence of DNA transferred through an intermediary to an object can make the identification of the primary handler of an object difficult. Forensic DNA analysts need to be cautious when assessing the likelihood of one mode of transfer over another as an explanation for the presence of an individual's DNA at a crime scene.

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Transfer, Trace DNA, GlobalFiler®

B121 Statistical Modeling of the Case Information From Ohio's Sexual Assault Kit (SAK) Testing Initiative

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After attending this presentation, attendees will be informed concerning: (1) the application of logistic regression model to forensic science; and, (2) lessons learned from the Ohio SAK Initiative.

This presentation will impact the forensic science community by demonstrating that the application of logic regression modeling to SAK processing can be used to provide insight into whether a sample might yield a Combined DNA Index System (CODIS) -eligible DNA profile.

There is limited comprehensive data documenting the progression and results of statewide SAK forensic testing. The state of Ohio has now analyzed SAKs that did not initially undergo DNA testing, and a wealth of information now exists regarding the DNA test results from nearly 14,000 kits processed because of Ohio's SAK Initiative. Case information from a sample of 2,500 completed SAKs was collected in order to investigate a number of relationships regarding SAK data. Data collected from the SAK case files included: (1) pertinent information from the nurse notes taken at the time of the kit collection; (2) whether or not a particular forensic sample was collected; (3) whether or not a sample yielded foreign DNA; (4) whether the sample produced a CODIS-eligible DNA profile; (5) whether the sample resulted in a CODIS hit; and, (6) the total number of samples run in the first round of testing.

The majority of the focus of the study involved analyzing the results of DNA testing by swab or sample location (vaginal, anal, oral, hair, clothing, etc.). A logistic regression model was fit to the data to predict whether or not a kit contained CODIS-eligible DNA profiles. The individual effects of variables identified early in the study on the response were observed. The number of days a victim waited between the date of the sexual assault and SAK collection significantly impacted the probability of obtaining a CODIS-eligible DNA profile. As the wait time increased, the probability of obtaining a CODIS-eligible DNA profile decreased. The probability of obtaining at least one CODIS-eligible sample from a kit varies as a function of victim age and days to kit collection. The impact of years to kit submission to the laboratory for testing is being further investigated. Days to submission were more significant than years until testing. This study demonstrates that application of logic regression modeling to SAK processing can be used to provide some insight into whether a sample might yield a CODIS-eligible DNA profile.

CODIS, SAK, Statistics

B122 The Development of Epigenetic DNA Methylation Markers to Predict Tobacco Smoking of Unknown Suspects

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The goal of this presentation is to demonstrate to attendees a new method to predict tobacco smoking behavior based on DNA samples recovered from crime scenes.

This presentation will impact the forensic science community by proposing a novel and effective pyrosequencing-based technique able to determine smoking habits of a suspect based on the blood or saliva samples left behind at a crime scene. This presentation will illustrate the model used to distinguish tobacco smokers from non-smokers.

Recent developments in the analysis of epigenetic DNA methylation patterns have demonstrated that certain genetic loci show correlation with the tobacco smoking.[1,2] It is the goal of this study to identify a set of epigenetic methylation markers that exhibit variations in DNA methylation with tobacco smoking and can be used in forensic laboratories. In forensics, finding a suitable biomarker for tobacco smoking could be a very useful tool for predicting the individual's lifestyle. These results may prove beneficial in determining the potential identity of a suspect by narrowing the range of those who could have been the source of the recovered samples.

Different epigenome association studies have reported various genetic loci in which methylation levels were associated with tobacco smoking. From those previous studies, five genetic loci (AHRR, ALPP, IER3, GFI, and F2RL3) that contained smoking-related CpG sites have been identified; however, those results were based on chip arrays that only provide information on single CpG sites.[1,2] Initially, a total of 52 novel CpG sites located on the five genetic loci were examined to check their correlation with tobacco smoking. Among those tested, CpG sites at the AHRR gene were found to have the strongest correlation with tobacco smoking. Biological samples of blood and saliva were collected from volunteers based on smoking status: current smoker and non-smoker. DNA samples were extracted and bisulfite was modified in order to convert the unmethylated cytosines to uracil while maintaining the methylated ones as cytosine. Next, the DNA was Polymerase Chain Reaction (PCR) amplified, and the methylation level at each CpG site was quantified by pyrosequencing.

A prediction model for tobacco smoking was constructed using a set of CpG sites at the AHRR gene. This DNA methylation-based model was very effective in predicting a history of smoking habits using blood samples and was less informative when tested using saliva. Methylation patterns for all CpG sites used in the model exhibited a statistically significant decrease in average methylation levels for smokers when compared to non-smokers. The results indicate that specific CpG sites in AHRR could be used as potential epigenetic markers to predict specific individual lifestyle (tobacco smoking) using blood and saliva specimens. As a result, these epigenetic markers could be used to provide investigative leads in cases with unknown perpetrators.

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Tobacco Smoking, DNA Methylation, Pyrosequencing

B123 The Development of a Paper Microfluidic Device for the Detection of Organic Smokeless Powder Residue

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After attending this presentation, attendees will better understand current research relating to the development of microfluidic Paper-based Analytical Devices (μPADs) as an easy and cheap alternative to current presumptive field-testing of low explosives, particularly smokeless powders. Minimal training is required to operate these devices and they are ideal for use in the field by the military and law enforcement. Attendees will also gain a basic understanding of the organic compounds contained in smokeless powders.

This presentation will impact the forensic science community by providing insight into the possibility of cheap, user-friendly, presumptive testing devices for smokeless powder residues.

In this project, colorimetric tests are implemented on paper microfluidic devices, permitting organic residues from smokeless powders to be detected in the field. Paper microfluidic devices are usually prepared from chromatographic paper. For these particular devices, wax printing was followed by lamination at elevated temperatures to create hydrophobic wax barriers and hydrophilic channels. Capillary action is then used to mobilize liquids containing dissolved analytes through the channels of the device. Colorimetric reagents are placed at the end of each channel for detection of the individual compounds, which are, in this case, organic additives. Paper-based microfluidic devices were initially designed for application in medicinal and disease testing in remote areas where the lack of refrigeration limited the ability to store expensive reagents. Because reagents are dried on the device prior to use, shelf lives are prolonged when compared to these liquid reagents. μPADs now have a wide variety of applications. This research been exploring forensic applications of this technology. In this project, a paper microfluidic chip has been developed that involves presumptive, colorimetric tests for multiple different organic compounds contained in smokeless powder residues.

Residue from smokeless powder-based explosive devices mainly consists of nitrocellulose and other organic compounds. Contained within the powder are additive packages that consist of energetic materials such as nitroglycerine, deterrents such as dibutyl phthalate, plasticizers such as ethyl centralite, and stabilizers such as diphenylamine. For this project, a paper microfluidic device for the detection of various organic smokeless powder additives is currently being developed. For example, potassium hydroxide has been used for the colorimetric detection of dinitrotoluenes producing a green color and the Greiss reagent can be used for the detection of nitroglycerine producing an orange/brown color. These tests, among others, were first prepared in solution and then optimized for use on paper.

These devices are currently undergoing developmental validation to measure the reproducibility, stability, and sensitivity of the analysis. These paper-based devices should prove useful to the analysis of smokeless powder residue, as the chips are compact and minimal time is needed to produce results. The ultimate goal of this project is to design and test a series of these devices for the presumptive detection of a variety of explosives residues in the field.

Smokeless Powder, Paper Microfluidics, Low Explosives

B124 Determining the Threshold of Identification Via Gas Chromatography/Mass Spectrometry (GC/MS) of Weathered Gasoline Extracted From Nylon Carpet

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After attending this presentation, attendees will understand how the amount of weathering a gasoline sample undergoes can impact the threshold of identification for GC/MS for ignitable liquid analysis.

This presentation will impact the forensic science community by establishing a method for determining the threshold of identification for ignitable liquids. This method may be used to optimize GC/MS instrument analysis, allow for comparison of data between instruments, and eventually let a minimum threshold of identification be set and become part of quality assurance programs.

The Organization of Scientific Area Committees (OSAC) defines the threshold of identification as the minimum concentration of ignitable liquid that can be identified from GC/MS spectra using accepted pattern identification criteria. A method for determining this threshold for ignitable liquids has not been established. Variation in the ion ratios of weathered gasoline samples has also not been investigated.

Due to its frequent use as an accelerant, gasoline was used as the ignitable liquid in this study. Weathered gasoline samples were used because they are more representative of what is recovered from arson scenes.

Neat gasoline samples were weathered to varying percents (50%, 75%, 90%, and 99%) by volume. All samples were prepared in duplicate. A system blank and a positive control were also prepared alongside the samples. The nylon carpet matrix was prepared by burning the substrate for ten seconds with a blow torch. The burned carpet samples were spiked with gasoline and an internal standard. The spiked carpet was then extracted using passive headspace according to the American Society for Testing and Materials (ASTM) 1412. The carbon strips were desorbed using carbon disulfide.

GC/MS analysis, using an Agilent® 7890a GC with a 5975C MS, was performed in this study. A temperature program consistent with ASTM 1618 was used to analyze the samples.

Data was interpreted by examining the ratio of the base peak area counts to the qualifier ion area counts for ten target compounds. The area counts for the internal standard were used to correct for instrument variation. Target compounds were chosen based on ASTM 1618 guidelines and compounds that would still be present in a largely weathered sample. The chosen compounds were dodecane, 1,2,3,-trimethylbenzene, 2-ethyl-1, 4-dimethylbenzene, 1-ethyl-2,4-dimethylbenzene, 1,2,3,4-tetramethylbenzene, 1-methyl-indane, 3-phenylbut-1-ene, naphthalene, and 2-methyl-napthalene. Neat gasoline peak ratios were used to establish an acceptable range for the peak ratios of the weathered samples. The acceptable range was considered to be within ±20% of the neat gasoline peak ratio.

GC/MS, Arson, Threshold of Identification

B125 The Characterization of Propellants in Shotgun Cartridges by Isotope Ratio Mass Spectrometry (IRMS) and X-Ray Fluorescence (XRF) Spectroscopy

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After attending this presentation, attendees will better understand the application of Gas Chromatography (GC) IRMS and XRF to the characterization of propellants in shotgun cartridges.

This presentation will impact the forensic science community by demonstrating how isotope ratio analysis by GC/IRMS and elemental analysis by XRF can differentiate propellants in shotgun cartridges from various brands and countries.

Although firearm-related deaths are not as common as in the United States and other countries, a shotgun is occasionally encountered in violent cases in South Korea. In this research, nine shotgun cartridges (shotgun shells) of seven different brands that were produced in four different countries were purchased to characterize the propellants and differentiate between various brands and countries. First, organic components of propellants in the pre-shot cartridges were analyzed by Thin Layer Chromatography (TLC), color test, and Gas Chromatography/Mass Spectrometry (GC/MS). In order to be able to differentiate the shotgun cartridges, the stable isotope ratios of carbon and nitrogen ($\delta^{13}C$, $\delta^{15}N$) were obtained by GC/IRMS, targeting Nitroglycerin (NG) in the propellant from each cartridge. For shotgun shells in which NG was not detected, bulk samples were analyzed by Elemental Analysis/IRMS (EA/IRMS) to obtain stable isotope ratios of carbon and nitrogen ($\delta^{13}C$, $\delta^{15}N$) for nitrocellulose and additives of the propellants. This is because most explosives (including propellants in shotgun shells) are mixtures with additives at low levels.

The results of the stable isotope analysis revealed that NG is a major component of the propellant in the five shotgun shells from four different brands out of nine cartridges. Although it was not possible to detect NG in four shotgun shells of three different brands, nitrocellulose and additives were identified in four cartridges. In addition, it was possible to differentiate shotgun cartridges based on their brands and countries by means of their characteristic stable isotope ratios of carbon and nitrogen. Elemental analysis of propellants from nine cartridges was also performed by X-Ray Fluorescence (XRF)spectroscopy. It was found that each shotgun shell displayed characteristic compositions of multiple elements. As a result, they could be differentiated based on their relative amounts of potassium (K), iron (Fe), cupper (Cu), and lead (Pb), categorizing them into five groups.

This study demonstrated that XRF and IRMS are useful techniques to differentiate propellants in shotgun shells as screening and confirmatory methods, respectively. With the combination of two methodologies, it is expected that the differentiation of propellants in shotgun shells is readily possible in forensic firearm analysis.

Shotgun Cartridges, IRMS, XRF

B126 An Explosives Analysis With Portable Ion-Trap Gas Chromatography/Mass Spectrometry (GC/MS) for Battlefield Forensics

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After attending this presentation, attendees will better understand how portable ion-trap GC/MS with Solid-Phase Microextraction (SPME) sampling is used to detect explosive residue for applications in the area of battlefield forensics.

This presentation will impact the forensic science community by evaluating the method parameters used by the portable GC/MS to determine the most efficient mode of explosives testing in the field.

Recent advancements in portable GC/MS instrumentation have enabled the field analyses of explosives and explosive residues by emergency responders, the military and law-enforcement organizations. For battlefield forensics, portable GC/MS instruments are used for the detection and confirmatory identification of threats pre- and post-explosion to provide intelligence, investigation, and adjudication information. Traditional analysis for explosives involves sample selection, collection, packaging, transport, laboratory analysis, and data interpretation; however, there are many challenges to this type of analysis, and if there are any errors in the process, such as incorrect sampling or packaging, the results may be meaningless. Time is also important. The ability of a commander to make decisions in near real-time with data collected and interpreted at the scene can be critical. Additionally, because many explosives readily decompose or evaporate, it is imperative that analysis be completed in a short time frame, which is not always possible due to remote military locations or laboratory backlogs. Although the on-scene detection and analysis of explosives by portable Ion Mobility Spectrometry (IMS) has been used for years, this is a presumptive test that suffers from both false positive and false negative results. Using modern, portable GC/MS instruments in the area of battlefield forensics is crucial as it provides an easy user interface that provides clear confirmatory results in a short time frame, which is important when the chemical being searched for may endanger lives.

The portable GC/MS system used in this research uses a Low Thermal Mass (LTM) resistively heated capillary column directly linked with a miniaturized toroidal ion trap mass spectrometer. The LTM capillary GC column enables fast separation and thermal recovery, resulting in rapid consecutive runs of approximately five minutes. Sample collection was performed using Solid-Phase Microextraction (SPME) with direct injection via thermal desorption from the SPME into the GC/MS inlet. SPME is especially useful in field-portable settings because it provides an easy, small, lightweight, and solvent-free method for sample collection. Using SPME for explosives analysis removes the need to perform extensive extraction procedures that significantly complicate the analysis and is thus difficult to perform in the field.

In this research, 12 different explosives covering both military (e.g., PETN, RDX) and homemade (e.g., TATP, HMTD) explosives were deposited onto the SPME fiber and separately injected into the GC/MS. Various parameters (e.g., injection port temperature, column temperature, and ramp rate) were adjusted to determine an optimal method for detection and identification of the explosive. This method development is important because there is a need for a cooler-temperature method than the standard test method employed to test for dangerous toxic industrial chemicals and chemical warfare agents. Many explosives degrade at higher temperatures, which can make their analysis challenging. The current standard method for the portable GC/MS employs an inlet temperature of 270°C, a column start temperature of 50°C with a hold time of ten seconds, and a ramp rate of 2°C/second, resulting in a total run time of 180 seconds. Eleven of the 12 explosives were detected and identified at amounts of 200ng. Explosive compounds that have traditionally been difficult to identify, such as TATP, were readily detected using all methods tested. RDX was the only non-identified explosive, which was not surprising given that it is notoriously difficult to detect by GC/MS due to its rapid thermal decomposition. It was concluded that a lower temperature method than what is currently implemented is a superior alternative for the detection of explosives in the field by portable GC/MS because it yields less degraded results.

Portable GC/MS, Explosives, Battlefield Forensics

B127 An Examination of Striations on Bullets Discharged From 3D-Printed Metallic Gun Barrels

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After attending this presentation, attendees will better understand the 3D printing process to design and manufacture firearms. Direct Metal Laser Sintering (DMLS) will be used as an example to discuss the new manufacturing process and its potential influences in firearms examination.

This presentation will impact the forensic science community by providing new data in firearms examination of 3D-printed metallic gun barrels and tool marks left on bullets associated with them. Two identical metallic gun barrels 3D printed from the same digital file were used to test the impact of 3D manufacturing in firearms examinations.

3D printing is a manufacturing process that potentially can transform a virtual digital model into a real-world 3D solid object. This process has been a popular technique to produce a phototype of any design. Because this manufacturing process is becoming affordable and available, 3D printing is becoming a method of choice to manufacture functional product in many industry, including firearms.

In this work, two identical metallic gun barrels were 3D-printed from the same digital file from the same printing chamber. The model was built based on the physical dimensions of a 1911 pistol using a computer software without the use of 3D scanners. Because the roughness of the 3D-printed gun barrel surface, some exterior surface of the barrels required some hand-fitting in order to fit into a 1911 pistol. The surface of the interior part of the gun barrel was left untreated in this study. After fitting the barrels, 100 cartridges were discharged from each of these two identical barrels to examine and compare striations left on the bullets.

The first 50 rounds, from both barrels, discharged without incident. The only issue noted was that a substantial number of test fires did not properly extract and resulted in a stove-piped cartridge case. At approximately the 65th test fire, from each barrel, the slide started to lock up and would not cycle back to allow the cartridge cases to extract. Due to the locked slide, it became necessary to tap the slide back with a ball-peen hammer to remove the fired cartridge case. Multiple attempts were made to clean, dremel, and oil the chambers in hopes of improving extraction, none of which proved successful, and the locking up continued through the end of the 100 test fire cycles. Post-printing surface treatment of the barrel may be needed to eliminate this issue. Examination of the 100 fired bullets from either barrel revealed that all 100 bullets could easily be identified to each other. The striations maintained their consistency in individual characteristics over the course of the test firing. When comparing the test fires from two identical 3D printed barrels, the bullets were easily eliminated as having been fired from the same barrel.

Firearms Examination, 3D Printing, Direct Metal Laser Sintering

B128 Bridging the Gap Between Categorical and Probabilistic Statements in Fire Debris Analysis

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After attending this presentation, attendees will better understand Receiver Operating Characteristic (ROC) curves and how they depict the relationships between categorical decisions and evidentiary value.

This presentation will impact the forensic science community by exploring these and other relationships between categorical decision thresholds and likelihood ratios as applied to fire debris evidence.

The paradigm shift from categorical statements to statements of evidentiary value are facilitated by ROC curves and the visual representation they provide of the relationships between the strength of the evidence, decision risk and cost, and other related parameters.

A categorical statement implies that a decision has been made regarding some aspects of a forensic examination. For example, it may be categorically stated that a fire debris sample contains residue of an ignitable liquid from one of the American Society for Testing and Materials (ASTM) E1618 classes.[1] When a categorical statement is made, it is also implicit that the analyst has set some threshold upon which the decision is based. Unfortunately, the current system of fire debris analysis does not define and implement a numerical scale for setting a decision threshold, and consequently analyst bias is possible. In addition, a categorical decision coupled with a variable decision threshold provides no information regarding the strength of the evidence. It is possible to understand the relationship between decision thresholds and evidentiary value with the assistance of the ROC curve.[2]

Generating a ROC curve requires assigning a score to each sample in a data set. The ground truth (positive or negative for ignitable liquid) must be known for each sample. Samples of known ground truth can be generated in the laboratory; however, the samples must be realistic and representative of casework samples. Assigning a score to each sample is the more complicated task. The score should be a single value and should reflect the probability that a sample belongs to the positive class (i.e., those samples containing ignitable liquid residue). Samples containing a more prominent ignitable liquid profile should receive a higher score. The scores can come from numerical calculations, the application of rules lists, decision trees, or other methods.[3] Once a scoring method has been determined, it is applied to all the samples in the known ground truth data set and each sample is assigned a score. Since the ground truth is known for each sample, along with an assigned score, it is easy to generate a ROC curve by using each score as a threshold and assigning each sample with a score greater than or equal to the threshold as belonging to the positive class. Once the class assignment is made, calculate the true positive and false positive rates and plot these values on an x, y coordinate system. After stepping through all of the possible thresholds, the points are connected to generate the ROC curve. The tangent to the ROC curve at any score gives the likelihood ratio for samples with that score. The slope of a line from the origin (0, 0) through a point on the curve that has been defined as a decision threshold corresponds to the likelihood ratio for all decisions based on that thresholding score.[4]

These and other relationships between categorical decision thresholds and likelihood ratios will be explored for fire debris evidence.

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2. T. Fawcett. An introduction to ROC analysis. *Pattern Recogn. Lett.* 27(8) (2006) 861-874.
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Fire Debris, Likelihood Ratios, ROC Analysis

B129 Useful Characteristics to Assist With Identifying Hornady® Bullets and Their Potential Caliber

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After attending this presentation, attendees will be aware of the various features that can be used to identify Hornady® polymer-tipped bullets. Furthermore, attendees will understand the potential use of cannelure height as a means to determine the specific caliber of .38 caliber-class Hornady® Critical Defense® bullets.

This presentation will impact the forensic science community by increasing awareness of the various features of Hornady® polymer-tipped bullets and the many ways they can be identified. In addition, this presentation will provide insight regarding the potential for cannelure height to be used to determine the specific caliber of .38 caliber-class Hornady® Critical Defense® bullets.

Bullet examinations are a pivotal aspect of firearm/tool mark examinations, especially for cases in which a suspect firearm is not recovered. Information, such as caliber, ammunition manufacturer, and the potential type of firearm, are valuable details that help guide detectives' investigations. For this reason, it is important for examiners to be knowledgeable about the various characteristics that can be used to identify specific ammunition brands and bullet calibers.

Hornady® is an ammunition manufacturer whose signature polymer-tipped bullets (specifically, Critical Defense®, Zombie Max™, and Critical Duty®) have begun to appear more often in casework. There are a few important characteristics that examiners should be aware of to assist in identifying these bullets. Initially, the bullets can be recognized by their polymer tips. The majority of the polymer tips are red; however, pink and green have also been used. Additional features, such as the number of cannelures and the characteristics of the expanded hollow point, can be useful for identifying Hornady® polymer-tipped bullets.

Furthermore, the potential to differentiate 38 caliber-class Hornady® Critical Defense® bullets based on cannelure height, in combination with bullet weight, was explored. In casework, firearm examiners often receive damaged bullets, which can make caliber determination challenging. The ability to utilize cannelure height, along with bullet weight, as a means to determine caliber could further assist in providing specific, useful, investigational information to detectives.

An assortment of 38 caliber-class Hornady® Critical Defense® ammunition was obtained. A bullet was pulled from each specific caliber. The cannelure height was measured from the base of the bullet to the base of the cannelure using calipers and a stage micrometer. From the measurements, it was determined that cannelure height does differ with respect to specific caliber; however, some of the cannelures were fairly close in height. Therefore, it would be difficult to narrow down to one specific caliber. In general, the cannelure height increased as bullet weight increased.

Hornady®, Cannelure, Caliber

B130 The Effects of Varying Decomposition Settings on Powder Stippling Patterns on Skin

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After attending this presentation, attendees will better understand the persistence of Gunshot Residue (GSR) patterns over time under varying decomposition variables and be aware of methods to best identify GSR on decomposed skin.

This presentation will impact the forensic science community by illustrating the effect of decomposition on GSR patterns and indicating optimal methods to identify such patterns when visual identification is no longer possible. The chemical methods discussed in this presentation, adapted from traditional form, may assist investigators in the field in identifying firearm evidence on decomposed bodies.

The purpose of this study was to examine the effect of varying decomposition exposure settings on GSR powder patterns, particularly powder stippling patterns, on skin using animals as a model for human skin. The GSR patterns were examined using photography, stereo-light microscopy, and chemical methods.

GSR is of particular forensic importance in firearm-related incidents as it can be used in forensic reconstruction in regard to distinguishing range-of-fire since GSR patterns are defining features of an intermediate range-of-fire.[1] Powder stippling is a specific type of GSR pattern that results from the impact of unconsumed and partially consumed gunpowder kernels with live skin that leads to small, hemorrhagic injuries in live tissue; however, the preservation and condition of these patterns throughout decomposition are dependent on a variety of factors, as even human activity can impact exposure.[2,3] Some case reports have even indicated attempts to conceal the smell of putrefaction via the application of insect repellant.[4]

In this study, 11 anesthetized bull calves were shot with a handgun at a distance of approximately two inches and immediately euthanized. They were then subjected to decomposition in an open field fully exposed and under three altered decomposition settings: buried, covered in vegetation, and covered in insect repellant. Bull calves were chosen since studies have shown that bovine tissue is similar in structure to human skin and exhibits similar decomposition activities to human cadavers.[5] For comparison, the experiment with bull calves was coupled with a series of trials in which fresh pig skin was shot to create GSR patterns and subjected to decomposition fully exposed. The pig skin GSR patterns and bull calves' powder stippling patterns were photographed throughout all stages of decomposition and examined with a stereo-light microscope. When GSR patterns became difficult to identify visually, the patterns were swabbed and subjected to modified Griess and Sodium Rhodizonate tests to test for chemical components of GSR, including nitrites and lead, respectively. Tissue specimens were collected from select bull calves for histology as an additional method of visualization for the presence of GSR.

It is hypothesized that decomposition will progressively alter the physical appearance of the gunshot lesion and GSR pattern, but that visual identification of the patterns will persist up until the stage of advanced decay. Furthermore, purposeful efforts to limit exposure (burial, vegetative covering, or chemical masking) will increase the persistence of patterns that permit visual identification. Finally, it is hypothesized that chemical analyses and histology may be able to identify the GSR pattern in an advanced state of decomposition. Preliminary results of this study have shown that Sodium Rhodizonate tests are the more sensitive chemical method for identifying the presence of GSR in advanced states of decay. The pig skin trials have illustrated that the visual persistence of GSR is impacted by the firearm and ammunition used. Stereo-light microscopy has also identified the presence of unburnt GSR kernels on skin approximately two months decomposed.

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Gunshot Residue, Decomposition, Shooting Reconstruction

B131 "Free Range" Gunshot Primer Residue: A Study on Multiple Transfers of Gunshot Primer Residue

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After attending this presentation, attendees will better understand the number of times gunshot primer residue can transfer from one surface to the next.

This presentation will impact the forensic science community by providing attendees with insight into the dynamics of gunshot primer residue particle transfers from one surface to a second surface to a third surface. This presentation describes the methods used to test this theory of transfer of gunshot primer residue.

Gunshot primer residue is produced by a firearm when it is discharged. The primer for centerfire cartridges is mainly composed of lead styphnate, barium nitrate, and antimony sulfide. The residue from the primer explosion escapes from openings in the gun and can be deposited on a person's hands and clothing. These particles can be collected and analyzed using automated scanning electron microscopy energy dispersive X-ray. Characteristic gunshot residue primer particles have a molten appearance and are composed of barium, antimony, and lead. There have been several studies that examine the transfer of gunshot primer residue to the interior of police cars from a person who has gunshot residue on their person. There have not been studies conducted that try to determine the number of times gunshot primer residue particles could transfer from one surface to another.

This presentation will detail the results of a study on the transfer of gunshot primer residue from a gunshot primer residue-contaminated area to a clean subject who enters the area and subsequently transfers the gunshot residue particles to other surfaces outside the contaminated area.

The firearms section of the laboratory was conducting firearm familiarization training. One of the analysts of the Trace Evidence section of the laboratory attended the training. This analyst does not handle firearms at all. At the end of the training, the analyst was asked to stub her clothing, her cubicle chair, the driver seat of her car, and any chairs that she may have sat in at her home in the same clothing she had worn to the training. These stubs were analyzed using scanning electron microscopy energy dispersing X-ray spectroscopy instrumentation using standard laboratory procedures for the analysis of gunshot primer residue.

This research takes a novel approach by investigating the likelihood of multiple transfers of gunshot primer residue particles between surfaces.

Gunshot Residue, Scanning Electron Microscopy, Contamination

B132 The Sub-Particle Composition and Morphology of Gunshot Residues (GSR)

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After attending this presentation, attendees will have a more developed understanding of the variation in sub-particle composition and morphology of different types of GSR and its significance to GSR evidence evaluation.

This presentation will impact the forensic science community by reporting on work that has been conducted in the sectioning and sub-particle analysis of GSR from different types of ammunition and firearms, allowing for a more thorough understanding of the variation in and differentiation of GSR particles that can be demonstrated at this level.

This presentation explores the key hypothesis that GSR particles are not homogeneous and their sub-structure carries valuable information that can be exploited in GSR examination.

Complex interactions in the moments after firing result in the formation of GSR particles.[1] Incorporation of key elements is thought to come principally from the ammunition's primer; however, inclusions from the projectile, casing, and barrel of the firearm are not uncommon.[2] When it comes to the assessment of GSR evidence, the usual standard technique, Scanning Electron Microscopy with Energy-Dispersive X-Ray Microanalysis (SEM/EDS), operates on a whole particle analysis level. While this has been useful in providing a qualitative assessment of the presence of particles characteristic of GSR, detailed characterization and differentiation of particles originating from different ammunition types has been elusive.

Recently, there has been research to suggest that the complexity of GSR formation results in distinct differences in both the sub-particle composition and morphology. Prior studies have indicated that beyond the external morphology and composition, sub-surface morphological differences can be used as a means of identifying particles from different ammunition types.[3] More recent work has suggested the use of a Focused Ion Beam (FIB) to examine the internal composition and morphology of GSR particles as a means of obtaining more in-depth information about a particle's composition and origin.[4,5] Practically, the casework experience has shown that in some situations three component particles are observed in situations where one component – antimony – is absent from the primer mix but is present in the projectile.[6,7] This is most often the case with .22 caliber ammunition, which is common in Australia. The prospect of being able to assess the origin of different particles, or specific components within them, would provide useful information in forensic investigations and GSR research.

This study applies FIB techniques supported by SEM/EDS and X-ray mapping, as well as Auger Electron Spectroscopy (AES) and Time-of-Flight/Secondary Ion Mass Spectroscopy (TOF/SIMS), to GSR particles originating from different ammunition calibers and types, with a view to demonstrating their sub-surface features and heterogeneity. Specific morphological and compositional features were then evaluated as to their relationship to the origin of the particles, with a view to determining if they originate purely from elements in the primer or of a mixed primer and projectile origin. GSR samples were collected from the hands of shooters, following the discharge of a number of different ammunitions. Characteristic particles were then selected and elemental X-ray maps collected using an SEM/EDS system. These particles were then cross-sectioned or had slices collected from them using a gallium ion FIB. The cross-sectioned face of these particles was re-mapped with SEM/EDS to assess internal morphology, composition, and discrete phases. Slices collected from these particles were analyzed using AES and TOF/SIMS in order to provide further compositional information.

Ultimately, this study has demonstrated that in many cases there are distinct sub-particle compositional and morphological features that clearly demonstrate the non-homogeneous nature of GSR particles. GSR examiners could use these features to improve the probative value of GSR examinations.

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Gunshot Residue, Criminalistics, Focused Ion Beam

B133 The Refinement of a Mathematical Model to Predict Evaporation of Gasoline

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After attending this presentation, attendees will be familiar with the application of a mathematical model that can be used to generate chromatograms of evaporated ignitable liquids. More specifically, this presentation will discuss the refinement of the model to generate chromatograms corresponding to evaporated gasoline in a more accurate manner.

This presentation will impact the forensic science community by providing a method by which chromatograms corresponding to evaporated gasoline can be generated mathematically, thus eliminating the need for experimental evaporation. The modeled chromatograms can be used as a reference collection that can aid in the identification of gasoline present at any evaporation level in fire debris samples.

During an intentional fire, ignitable liquids used as accelerants undergo evaporation, among other processes. To aid in identification, chromatograms of the ignitable liquid residue are compared to a database containing chromatograms of ignitable liquid reference standards. Databases typically contain chromatograms corresponding to the unevaporated liquid, as well as the liquid evaporated to different levels to account for chemical changes as a result of evaporation; however, experimentally evaporating a liquid to different levels can be a time-intensive process with many variables to take into account, for example, agitating or heating the reference liquid.

A mathematical model was developed that predicts the evaporation rate constant of compounds as a function of retention index (I^T). The model was developed using diesel, a heavy petroleum distillate that contains compounds that elute over the I^T range 800–2,200. The predicted evaporation rate constants are used to determine the fraction remaining of each compound which can then be used to generate the modeled chromatogram.

In the initial assessment of model performance, Pearson Product-Moment Correlation (PPMC) coefficients were used to compare modeled chromatograms to those obtained from the corresponding, experimentally evaporated liquid. For the petroleum distillates tested, PPMC coefficients greater than 0.9 indicated strong correlation between the modeled and experimentally derived chromatograms; however, performance was diminished when the model was applied to gasoline, with a mean PPMC coefficient of 0.806±0.004 indicating only a moderate correlation for comparison of the modeled chromatogram and the corresponding chromatogram for a 50% experimentally evaporated gasoline.

This presentation will focus on refinement of the model to improve performance in generating chromatograms corresponding to evaporated gasoline. As a more volatile liquid, gasoline contains compounds that elute in the I^T range 400–1,500; a majority of these compounds were not included in the original model. Thus, the model was refined to broaden the I^T range and, hence, include these more volatile compounds.

To accomplish this, gasoline was experimentally evaporated from 10%–90% (by mass) in 10% increments. Each evaporated gasoline was analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) initially using a 100% polydimethylsiloxane column (100m x 0.25mm i.d. x 0.50µm d.f.). The longer column and thicker stationary phase film were necessary to identify the highly volatile compounds present in gasoline. Using the more conventional 30-m column, these compounds eluted with the solvent front and were not observed.

The performance of the model improved with the more representative chemical profile for gasoline in the experimentally evaporated samples. For example, for comparison of predicted and gasoline experimentally evaporated to 50% by mass, the PPMC coefficient increased from 0.8 to greater than 0.97, which indicated strong, rather than moderate, correlation. To investigate the potential for further improvement, the model was refined to span the wider I^T range 400–2,200. To refine the model, the abundance of the early eluting compounds (e.g., butane, pentane, hexane, and branched isomers of these alkanes) was plotted as a function of evaporation time. The resulting decay curves were fit to the first-order kinetic rate equation to determine evaporation rate constants, which were then plotted as a function of I^T. These data were combined with corresponding data from the original model and linear regression was applied to redefine the model.

This presentation will describe refinement of the model in more detail and will further discuss the improvements in model performance to predict chromatograms corresponding to gasoline evaporated to any level.

Mathematical Model, Gasoline, Evaporation

B134 Assessing Microheterogeneity of Surrogate Post-Detonation Urban Debris (SPUD) Standard Reference Material (SRM) 4600 Using Microbeam X-Ray Fluorescence (µ-XRF)

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After attending this presentation, attendees will understand the importance of developing a well-characterized, homogeneous SRM to aid in the development of methods needed to characterize the debris expected from the aftermath of a nuclear attack.

This presentation will impact the forensic science community by discussing how the development of this SRM will facilitate the ability to analyze debris from an improvised nuclear device detonation in a city, aiding intelligence efforts in identifying the responsible parties.

Concerns over the use of nuclear or radiological materials in terrorist attacks have led to the emergence of the nuclear forensics field. This discipline focuses on best practices to evaluate such material both before and after the detonation of a nuclear device. A thorough characterization of pre- and post-detonation materials may aid intelligence efforts to attribute responsibility for an attack by identifying the fuel type, weapon design, production process, production date, and other features of the device.[1] Well-characterized SRMs and Certified Reference Materials (CRMs) are needed to ensure that nuclear forensic measurements will be sufficiently accurate and precise to be legally defensible.

Currently, there is a shortage of SRMs/CRMs needed to develop methods for analyzing pre- and post-detonation nuclear material.[1] In some cases, cost, scarcity, or quality of existing materials is problematic, whereas in other cases, a suitable material simply does not exist. Given the likelihood that a large city would be the target of an attack, efforts have been undertaken to prepare to analyze the debris expected from the detonation of an improvised nuclear device in an urban environment. The National Institute of Science and Technology has led a collaboration to develop two vitrified SPUD SRMs: SRM 4600 and SRM 4601.[2] A glass-like material was selected for its durability, control over what can be added to the mixture, straight-forward mass balance, and relative ease of homogenization. The matrix of the SRMs is a composite of materials and metals present in concrete (cement, crushed stone, and sand) and steel, two of the most common urban building materials. SRM 4600 was doped with natural depleted uranium to serve as a blank; SRM 4601 was doped with Uranium-235 (approximately 22% highly enriched uranium) to serve as a test sample.

As part of the process to certify the major and minor components of the SRMs, non-destructive µXRF measurement with Principal Component Analysis (PCA) on the resulting data was used.[3,4] Sixteen 1.5-gram samples of loose solid SRM 4600, each representing a replicate of the production lot, were prepared in XRF sample cups sandwiched between two sheets of X-ray-transparent film. To assess the homogeneity of the sample, 10,000 spectra were collected at both a single, fixed location and random locations from each of the sixteen "sandwiches." Collecting 5,000 spectra from the same location provided measurements of variability due to the instrument and systemic sources of error. The 5,000 spectra collected from random locations across the "sandwich" represent variability due to both the instrument and sample heterogeneity.

Quantifying the homogeneity of a sample is necessary to identify the effects of "nuggets," small subsamples of the bulk material enriched in certain elements.[3,4] These nuggets, which can vary in size and composition, cause the XRF data to stray from a normal distribution, precluding the use of common statistical measures of variability. PCA is able to take multiple statistical factors from the X-ray map and single location data in order to establish a detection limit for nuggets. The nominal beam diameter of the instrument used is 400µm, and a larger spot size can be simulated by averaging adjacent data points. This process will average out any imperfections present, and the averaging can be repeated iteratively until the PCA treatment indicates no detectable nuggets. From these analyses, a Minimum Sample Mass (MSM) can be calculated, which defines the smallest mass of sample needed for all elements to be homogenous. Preliminary calculations for SRM 4600 suggest an MSM of 3mg is required for reproducible measurements of element concentrations to not be susceptible to sample heterogeneity. This presentation will discuss the analysis of the 16 replicates of SRM 4600, which were analyzed by the above methodology to verify the accuracy of the preliminary MSM calculations and determine if the MSM can be decreased.

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Nuclear Forensics, Post-Detonation, Standard Reference Material

B135 Statistical Characterization of Aluminum (Al) Powders in Explosives Using Automated Particle Micromorphometry

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After attending this presentation, attendees will better understand the forensic potential of automated particle micromorphometry and statistical analysis to aid in making comparisons between questioned and known Al powders.

This presentation will impact the forensic science community by demonstrating the application of Al particle micromorphometry as a quantitative method for the characterization and comparison of explosive evidence, which may also provide valuable lead identification for forensic investigations.

Starting materials for an Improvised Explosive Device (IED) are readily obtainable from local commercial sources. Al powder, a common metallic fuel, has a wide variety of legitimate uses and is widely available without significant regulatory constraints.[1] Al powders can be obtained from industrial manufacturers or can be produced inexpensively using basic instructional manuals and videos. Due to the online sharing of instructional manuals and published books on how to construct IEDs, bomb makers are now informed on the easily accessible household materials that can be used to make explosive chemical mixtures.[2]

Previous results using S Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) showed morphology and surface characteristics can differentiate some methods of Al powder manufacturing (i.e., industrial vs. homemade). Particle micromorphometry may be used as a complementary method to gain additional information to differentiate Al powder sources. This presentation addresses fundamental factors of Al particle metrology, including sample slide preparation, imaging parameters, and potential methods to minimize sampling biases; the statistical methods used to analyze these large multidimensional datasets will be discussed.

Al powder samples were obtained from legitimate industrial manufacturers, various "in-house" production methods, and seized IEDs. The amateur methods were replicated to produce Al powder from easily available sources, including 25 brands of Al foil, 7 brands of metallic spray paints (40 cans), 24 Al ingots melted from Al cans and filed or lathed, 23 pyrotechnics, and 40 catalyst packets from two brands of binary exploding targets. To prepare microscope slides for imaging, a subsample containing ~1,000µg from bulk Al powder was placed into a microtube containing Permount® mounting medium. The solution was mixed until evenly dispersed, then an aliquot of the subsample was placed dropwise onto a microscope slide and a coverslip added. Preliminary statistical analysis on these microscope slide preparations that contained only four subsamples and one aliquot from each subsample determined there were large within-sample variations; therefore, more subsamples are needed for a representative sample of the bulk Al powder. A subset of 17 Al powder samples were prepared using ten subsamples and three aliquots for each subsample. Leave-One-Out Cross-Validation (LOOCV) was performed on this subset of samples and it was statistically determined that seven subsamples of the bulk Al powder and three aliquots for each subsample were sufficient to obtain a representative sample of the bulk Al powder.

Transmitted light microscope images ($n \approx 4{,}200$ fields of view/sample) of the Al samples were acquired using an automated stage and automated Z-focus. Dimensional analysis was calibrated using a National Institute of Standards and Technology (NIST) -traceable stage micrometer; polystyrene spheres of 100µm, 50µm, and 10µm were used as secondary standards to assess linear calibration. Images were batch processed using commercial image analysis software and customized code. Each image was converted to a binary image to enhance edge detection and the particles were counted and measured. Seventeen parameters were measured for each particle within the image field of view, including area, aspect ratio, perimeter, roundness, mean diameter, mean feret, radii (maximum and minimum distance from particle centroid to edge), radius ratio, box height, box width, and fractal dimension. The large multidimensional datasets ($n \approx 90{,}000$-$500{,}000$ particles/sample) were analyzed using an open source statistical package.

The datasets are too large and complex to analyze at this point without some dimensionality reduction. Preliminary work focused on the use of a weighted summary of 17 morphometric measurements on the subsamples. This was achieved by first taking weighted averages of the particles per Fields Of View (FOV) yielding a 17-dimensional vector for each FOV (i.e., average particle for each FOV). Then, the average of the average vectors for each FOV per aliquot was calculated, giving a 17-dimensional vector per aliquot. Likewise, the average vector across the aliquots corresponding to a given subsample was calculated, resulting in one 17-dimensional vector for the entire subsample for each sample. The classification accuracy between sources of Al powders using this weighted approach and results from the various multivariate statistical methods tested will be presented.

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Improvised Explosive Devices, Metal Fuel, Image Analysis

B136 A Case Study: Rubber Buckshot Tissue Penetration Capability

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After attending this presentation, attendees will better understand the penetration capabilities of rubber buckshot pellets fired at various distances and at different media.

This presentation will impact the forensic science community by demonstrating the inability of rubber buckshot to penetrate ballistic gelatin, a tissue simulant, from approximately eight yards. The experiments in this presentation were conducted due to a question posed in a case by the submitting agency; therefore, the methodology and results/conclusions can impact the forensic science community by exposing fellow colleagues to an unusual case and the approach that was taken to answer the posed question.

A determination was requested as to whether 12-gauge RIO™ brand rubber buckshot was able to penetrate ballistic gelatin, a tissue simulant, at a muzzle-to-target distance of approximately 40 yards (the approximate distance between the suspect and victim at the time of the shooting). According to the detective and multiple police statements, on November 14, 2015, the suspect indicated that the victim and his friends were trespassing on his property on their All-Terrain Vehicles (ATVs). The suspect proceeded to retrieve his 12-gauge Mossberg® model 500A shotgun and fired two warning shots using RIO™ brand rubber buckshot (marketed as less lethal ammunition). One pellet penetrated the victim's thigh; however, the pellet could not be removed for medical reasons. The victim was wearing four layers of clothing — none of the clothing was collected. The fired shotgun shells could not be located at the crime scene to verify what type of ammunition was fired. The detective had reason to presume that the suspect was not firing less lethal ammunition (i.e., rubber buck shot) but was instead firing lethal ammunition, such as lead buckshot.

Testing was conducted at the Seattle Police Department shooting range and high-speed videos were taken. The Mossberg® 500A shotgun was used to fire 12-gauge RIO™ brand rubber buckshot at cardboard and denim-covered ballistic gelatin at various distances to determine the penetration/perforation ability of the rubber buckshot. The 10% ballistic ordinance gelatin was mixed using standard practices and calibrated using a BB. During testing, the gelatin was not temperature controlled. Ballistic gelatin is intended to simulate soft tissue and does not account for skin, bone, additional layers of clothing, or other intervening materials. At approximately eight yards, all 15 rubber pellets perforated cardboard with an approximate pattern diameter of 24 inches. At approximately 40 yards, four rubber pellets impacted the cardboard, none of the four perforated the cardboard (only 4 of the 30 rubber pellets impacted the cardboard target). At approximately eight yards, two rubber pellets perforated the layer of denim, but there was no penetration of the gelatin (only 6 of the 15 rubber pellets impacted the denim-covered gelatin). Obtaining data for rubber buckshot into gelatin from 40 yards away was not feasible due to the wider pattern distribution of the rubber pellets. That being said, as a projectile travels downrange, it will gradually decelerate; therefore, if the rubber pellets could not penetrate the gelatin at approximately eight yards, it was concluded that they would not penetrate gelatin at approximately 40 yards. In conclusion, the 12-gauge RIO™ brand rubber buckshot pellets did not penetrate/perforate the cardboard at approximately 40 yards or the denim-covered ballistic gelatin at approximately eight yards.

Rubber Buckshot, Ballistic Gelatin, Case Study

B137 Multiple Factors Influencing Probabilistic DNA Mixture Interpretation of Highly Challenging Samples: The Relevance of Deep Validation Studies to Ensure Quality Assurance Requirements in Actual Casework

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After attending this presentation, attendees will better understand how important deep validation studies and Quality Assurance (QA) are in order to establish the limits and the capabilities of laboratories and experts, and how protocols, kits, instruments, and probabilistic software choices may influence the statistical results.

This presentation will impact the forensic science community by providing the ability to establish robust and reliable lab protocols useful when approaching actual casework samples.

DNA mixture probabilistic interpretation is undoubtedly one of the most challenging tasks in forensic genetics.[1,2] In the past ten years, new statistical models and software have been developed in this field to perform more sophisticated calculations to include or exclude a suspect from a complex DNA profile; but can we ever determine that we have enough information, and the right information, to accomplish this?

Multiple factors may affect DNA mixture interpretation and this is obviously due to one-by-one contributor template concentration and integrity, but also to systematic protocols chosen during sample processing. For these reasons, DNA extraction and quantitation methods, typing kits, and Capillary Electrophoresis (CE) instruments play a fundamental role in information used for probabilistic interpretation; results will only be as close to reality as the data used for calculation is reliable.[3,4] Semi-continuous and fully continuous methods play a key role as they take into account different aspects of DNA mixture profiles in qualitative terms, such as quantitative data used for statistics.[5] Therefore, QA processes, for both analysis and probabilistic calculations, can guarantee results' robustness and permit understanding laboratory performances when approaching complex DNA profile interpretation such as Low Template DNA (LT DNA) mixtures derived from trace evidence collected at the crime scene.

The detailed knowledge of each step of the entire validated method from trace evidence to interpreting a DNA mixture profile is certainly the crux to solving the most complicated problems in forensic genetics and, even more importantly, to understanding if laboratories and experts have all the puzzle pieces to accomplish this.

Multiple quantitation kits, typing kits, instruments, and probabilistic software results will be presented in order to underline their characteristics and differences, revealing what expectations experts may have for each of them during the interpretation of actual casework.

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DNA Mixture Interpretation, Validation, QA

B138 Probabilistic Prediction of the Number of Contributors in DNA Mixtures Using a Machine Learning-Based Approach

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The goal of this presentation is to present the state-of-the-art approach to the prediction of the number of contributors in DNA mixtures, an area of significant need in the forensic science community.

The prediction of the number of contributors remains a significant gap in the available suite of forensic software tools. This is an approach that is believed could be seamlessly implemented into the standard workflow. This presentation will impact the forensic science community by informing attendees of possible methods to combat this issue. Attendees will be equipped with the information necessary to bring this information back to their labs to discuss the positives and negatives of this approach.

DNA mixture interpretation remains one of the most significant areas of need in the forensic community. The success of many current mixture interpretation approaches relies on the assumption that the number of contributors is correctly predicted. When this presumption is incorrect, it may lead to decreased likelihood ratios or incorrect exclusions or inclusions, particularly when faced with increasingly complex mixtures of three or more contributors. The use of machine learning to probabilistically estimate the number of contributors in a DNA mixture is proposed in this presentation. A machine learning algorithm is a statistical tool that can, after exposure to an initial set of data, be used to classify previously unseen data. Machine learning approaches excel when faced with complex problems involving implicit patterns (such as the problem of successfully classifying three- and four-person DNA mixtures). These algorithms are specifically capable of learning probabilistic models that can be subsequently used for prediction. Such a predictive model was created using a support vector machine, and the model's performance was compared against currently used methods for predicting the number of contributors.

The model had an accuracy of greater than 98% in identifying the number of contributors in a DNA mixture of up to four contributors. A comparison to approaches utilizing Maximum Allele Count (MAC) and Markov Chain Monte Carlo (MCMC) methods exhibited a greater than 6% improvement in classifying three-contributor samples and an improvement of more than 20% when assessing four-contributor samples. The Probabilistic Assessment for Contributor Estimation (PACE) also accomplishes classification of the number of contributors for mixtures of up to four contributors in less than one second using a standard laptop or desktop computer. The initial assessment of classification via machine learning relied on samples amplified using the AmpFℓSTR® Identifiler® Polymerase Chain Reaction (PCR) Amplification Kit, primarily due to the availability of large data sets. The functionality of the system has been broadened to address the new expanded-locus Combined DNA Index System (CODIS) kits, such as the PowerPlex® Fusion PCR amplification kit, with data analysis currently nearing completion.

Considering the high classification accuracy rates of PACE, the inherent complexity of standard methods to classify three or more contributors, and the lack of rapid alternatives, this approach provides a promising means of estimating the number of contributors and, subsequently, will lead to improved DNA mixture interpretation.

Number of Contributors, Mixture, Machine-Learning

B139 Validating TrueAllele® Interpretation of DNA Mixtures Containing up to Ten Unknown Contributors

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After attending this presentation, attendees will understand the reliable interpretation of low-level DNA mixtures containing many people. Fully Bayesian computer methods can accurately extract identification information from complex DNA evidence.

This presentation will impact the forensic science community by providing empirical support for sophisticated scientific interpretation of DNA evidence often found in criminal justice applications.

Mixed samples of known genotype composition were prepared following a randomized experimental design that included very low contributor amounts. The mixture samples contained from two to ten contributors. The Cuyahoga County Regional Forensic Science Laboratory (Cuyahoga) amplified the samples using a PowerPlex® Fusion Short Tandem Repeat (STR) kit. Cybergenetics and Cuyahoga independently conducted TrueAllele® casework testing to interpret the DNA mixture data.

TrueAllele® provides fully Bayesian analysis. The system examines all STR data without human intervention, without knowing the comparison genotype; data objectivity eliminates contextual bias. The computer extracts all information from the evidence data, inferring genotypes and other parameters; the model completely eliminates unneeded calibration. Inferred evidence genotypes are compared with references only after genotypes have been separated from DNA mixture data; the two-phase Likelihood Ratio (LR) approach enforces objectivity.

The study examined sensitivity, specificity, and reproducibility of TrueAllele® match information. Fewer contributors in a mixture generally improved these metrics. With more contributors, longer Markov Chain Monte Carlo (MCMC) statistical sampling increased accuracy. Assuming excess contributors did not affect match information for true contributors, but added superfluous genotypes containing little information.

Sensitivity: As contributor number increased from two to six, average match strength decreased from 24 to 5 LR log units ("ban"). With up to four contributors, only positive log (LR) values were observed.

Specificity: As contributor number increased from two to six, average specificity decreased from -33 to -13 ban. When comparing with non-contributors, a match statistic of more than a thousand had a false positive probability of under 0.0001. With LR more than a hundred thousand, the probability decreased to less than 0.000001.

Reproducibility: Fewer contributors conferred greater reproducibility. The within-group standard deviation for two contributors was 0.17 ban, and for six contributors was 1.00 ban.

DNA Amount: Match information depended on a contributor's DNA amount, regardless of how many contributors were in the mixture. (More contributors mean less DNA per contributor.) Contributors comprising less than 10% of the mixture exhibited an average log (LR) of 3 ban; however, with fewer contributors, the match statistics were higher. Major contributors (more than 50%) averaged 28 ban. Contributors over 20% gave positive log (LR) values, regardless of how many contributors were present.

Contributor Number: When given the number of contributors a user observed in the data, the computer's mixture solutions were better than when given the expected number known from the study design. This result demonstrated application robustness, as practiced by forensic analysts on DNA mixtures.

Assumed Genotypes: With low-level minor contributors, providing the computer with known (i.e., previously matched) references reduced uncertainty in genotype inference. For a 15% minor in a ten-person mixture, the log (LR) value increased from 3 to 8 ban when assuming previously matched references as known genotypes. The log (LR) value for a 2% minor in a ten-person mixture increased from 1 to 4 ban. When assuming known genotypes, specificity improved — the non-contributor log (LR) average shifted from -3 to -6 ban.

MCMC Sampling: More MCMC sampling increased sensitivity. With six contributors, MCMC sampling at five thousand cycles gave an average log (LR) of 2.4 ban for true contributors; this increased at one hundred thousand cycles to 5.2 ban. Specificity was essentially unchanged by further MCMC sampling, so faster run times did not increase false positives. Regardless of contributor number, reproducibility at one hundred thousand cycles revealed a between-run variability of less than 1 ban.

Independent Testing: Cybergenetics average match statistic for true contributors was 8.52 ban, while Cuyahoga's average was 8.24 ban. On average, the log (LR) values for independent comparisons conducted at the two sites were within 1.15 ban.

A validation study showing reliable interpretation of complex DNA evidence will be presented.

DNA Evidence, Mixture Validation, Bayesian Computing

B140 Autosomal, Chloroplast, and Mitochondrial Data of a United States Cannabis DNA Database

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After attending this presentation, attendees will understand the basic principles behind employing both autosomal and lineage (mitochondrial and chloroplast) markers for individualizing and sourcing marijuana samples.

This presentation will impact the forensic science community by demonstrating the applicability of an autosomal and lineage panel that could not only assist law enforcement agencies in verifying legal marijuana products but also aid in the linkage of illegal cases. These methods could also serve as additional tools to previously established marijuana profiling programs used in federal agencies such as the United States Customs and Border Protection (CBP) and the Drug Enforcement Administration (DEA).

Since *Cannabis sativa* (marijuana) is a controlled substance in many parts of the world, the ability to track biogeographical origin of cannabis could provide law enforcement with investigative leads regarding its trade and distribution. Population substructure and inbreeding cause individual marijuana plants to become more genetically related. This genetic relatedness can be helpful for intelligence purposes. Using autosomal, chloroplast, and mitochondrial DNA allows not only for prediction of biogeographical origin of a plant, but also allows its genetic identification.

A previously validated 13-autosomal Short Tandem Repeat (STR) multiplex was used to genotype 496 samples. Samples were analyzed from four different sites: 21 seizures at the United States-Mexico border, Northeastern Brazil, hemp seeds purchased in the United States, and the Araucarian area of Chile. In addition, a previously reported multi-loci system was modified and optimized to genotype five chloroplast and two mitochondrial markers. For this purpose, two methods were designed: a homopolymer STR pentaplex and a Single Nucleotide Polymorphism (SNP) triplex with one chloroplast (cscp001) marker shared by both methods for quality control. For successful mitochondrial and chloroplast typing, a novel real-time Polymerase Chain Reaction (PCR) quantitation method was developed and validated to accurately estimate the quantity of the chloroplast DNA (cpDNA) using a synthetic DNA standard. In addition, a sequenced allelic ladder was designed for the homopolymer STR pentaplex.

For autosomal typing, distinguishable profiles generated from 381 samples that yielded full STR profiles and 44 duplicate genotypes within seizures were observed. Phylogenetic analysis and case-to-case pairwise comparisons of 21 seizures at the United States-Mexico border, using F_{ST} as genetic distance, revealed the genetic association of nine seizures that formed a reference population.

For mitochondrial and chloroplast typing, subsampling was performed and 141 samples were genotyped. Complete haplotypes (STRs and SNPs) were observed for 134 samples. As expected, extensive haplotype sharing was observed; five distinguishable haplotypes were detected. In the reference population, one haplotype was observed 39 times in addition to two other unique haplotypes. Haplotype sharing was observed between the United States border seizures, Brazil, and Chile, while the hemp samples generated a distinct haplotype.

Results revealed that both autosomal and lineage markers could discern population sub-structure. Phylogenetic analysis of the four populations using the neighbor joining method and F_{ST} as genetic distance were estimated with the GDA software. Parsimony analysis was then performed with the PAUP* software. The STRUCTURE software was employed to investigate the population structure among groups. And finally, the R package, adegenet, was used to visualize the genetic distance of the populations using Principal Component Analysis (PCA).

In conclusion, the results of this research demonstrate the utility of both autosomal and lineage genotyping methods for characterizing marijuana samples.

Forensic Botany, *Cannabis sativa*, DNA Database

B141 The RoarPlex – A Novel Tetranucleotide Microsatellite and Sex Identification Panel

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After attending this presentation, attendees will recognize the natural research barriers wildlife genetic studies pose and how to successfully overcome them to learn more about the species of focus. A better understanding of the benefits tetranucleotide microsatellite markers provide in comparison to their dinucleotide counterparts will also be accomplished through the analysis of the designed RoarPlex.

Due to the constantly evolving and largely emerging nature of wildlife forensics, the RoarPlex could have a tremendous influence on future studies involving large felid species. This presentation will impact the forensic science community by providing exposure to the wildlife forensics field and illustrating what can be accomplished using a forensic skill set to aid in wildlife conservation efforts.

The snow leopard (*Panthera uncia*) is an elusive species native to the mountainous regions of Central and South Asia. Targeted for its fur and bones or to protect the livestock upon which they prey, the snow leopard is an endangered species that requires immediate conservation action. Due to the snow leopard's cryptic nature, research based on direct observations required for effective conservation is problematic and can be supplemented by non-invasive surveys and genetic analysis.

A multiplex containing eight tetranucleotide microsatellite markers and a sex-determining region Y marker was developed to aid in the investigation of illegal killings of snow leopards and to assist with population abundance and distribution studies on a larger geographic scale. Previously, studies relied on dinucleotide repeats that were complicated to score and difficult to combine across data sets. Dinucleotide microsatellite markers are vulnerable to errors associated with high stutter percentages and misinterpretation of adenylylation stutter. The previous panel also included only four microsatellite markers in one reaction. Tetranucleotide repeats drastically reduce the errors caused by slippage and decrease the visual impact of adenylylation stutter, making for unambiguous allele interpretation. The addition of more microsatellite markers in one reaction exponentially increases the individual identification information available, a component comparable to the Combined DNA Index System (CODIS) increasing the number of core loci accepted. An allelic ladder was developed to ensure accurate allele designation across laboratories, with validation according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) and the International Society of Forensic Genetics (ISFG) recommendations. To accomplish the multiplex design, 16 tetranucleotide microsatellite markers originating in the domestic cat (*Felis catus*) were screened. Results indicated that 15 markers could be successfully amplified using the available primer sets due to common ancestry and similar genome structure. Using M13 labeling, the 15 amplified microsatellites were fluorescently tagged and genotyped. Resulting peaks of the successful microsatellites were more distinct and allele calling was simplified. An informative multiplex containing eight of the most optimal microsatellite markers was constructed through selective data analysis, primer redesign, and compatibility determination. A sex-determining region Y marker was inserted into the reaction mix to simultaneously accomplish sex identification. The tetranucleotide panel provided more information, including sex, in one reaction, reducing the cost, error, and time required to perform the assay.

The enhanced panel and allelic ladder simultaneously improved research methods, assisted with transboundary initiatives, and enabled data sharing, thereby increasing the impact of population studies. Despite multiplex design being focused on the snow leopard, initial studies indicate that the RoarPlex could be successful in obtaining the individual identification of other large felids, including the bobcat (*Lynx rufus*), lion (*Panthera leo*), and cheetah (*Acinonyx jubatus*), expanding its utility. Due to the constantly evolving and largely emerging nature of wildlife forensics, the RoarPlex could have a tremendous influence on future studies involving large felid species.

STR, Tetranucleotide, Multiplex

B142 The Development of the Field Isolation and Amplification of DNA Assay (FIA-DNA) Kit: A Revolutionary Method for Species Identification of Unknown Samples

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After attending this presentation, attendees will better understand the impact of wildlife forensics and genetic analysis on conservation and wildlife management plans for threatened and endangered species.

This presentation will impact the forensic science community by presenting a novel technique for genetic species identification of biological samples in the field for both international conservation and law enforcement efforts.

A major challenge in biodiversity conservation is the identification of unknown samples collected in the field. Most endangered species are elusive; therefore, many monitoring programs rely on non-invasive sampling using scat or hair. One difficult hurdle is identifying unknown biological samples in the field due to the reliance on genetic analysis in a laboratory. The use of specialized software and equipment is generally tied to labs located in developed countries such as North America, Europe, India, and Japan; however, sample collection and management are often conducted in rural and developing countries in Africa, South America, Asia, and remote areas of North America where most large animal populations remain.

This can cause delays in obtaining important information and is often infeasible for many on-the-ground conservation efforts with limited resources. Because there is no method of on-site species identification for these samples, money and time are wasted sending non-target samples to labs for expensive and time-consuming analyses. The goal was therefore to develop an FIA-DNA Kit for genetic field identification of species for both international conservation efforts and management of Pennsylvania wildlife without the need for Polymerase Chain Reaction (PCR) and agarose gel electrophoreses.

Once successfully implemented, the FIA-DNA Kit will allow field researchers and wildlife managers to perform a simple genetic analysis for species identification without requiring samples to be sent to a laboratory. The FIA-DNA Kit makes use of Loop-Mediated Isothermal Amplification (LAMP) and visual product detection using calcein, a novel technique which eliminates the need for a thermal cycler for DNA amplification and detection. This technique was coupled with a modified extraction protocol using Whatman® Non-Indicating Fast Technology for Analysis (FTA) Elute Micro Cards, allowing for portability and ease of use outside of the laboratory setting.

Preliminary results tested efficacy on snow leopard *(Panthera uncia*), bobcat (*Lynx rufus*), and coyote (*Canis latrans*) samples for species-specific identification. This method using LAMP amplification of DNA has exhibited 100% specificity for more than 30 previously extracted snow leopard scat samples. Coupling LAMP amplification with the modified FTA extraction protocol has yielded successful, repeatable detection in more than 50% of tested scat samples to date. This method can be completed in less than one hour with minimal equipment, using Ultraviolet (UV) fluorescence within the reaction tube to confirm species identification.

Results from initial field deployment tests of the FIA-DNA Kit at the Powdermill Nature Reserve in Rector, PA, will be discussed. The FIA-DNA Kit was tested for precision in identifying a wide range of scat samples, eliminating the time and cost of lengthy lab-based analyses. Due to LAMP amplification's ease of use, rapid reaction time, and isothermal conditions, which do not require a thermal cycler, this kit will be able to be used in the field to effectively identify samples to a species level.

The ability to pre-screen samples will also increase the efficiency and turnaround time for the genetics laboratory. Because the FIA-DNA Kit is easily modified to test for other species by developing primers, it will prove invaluable for other time-sensitive applications, including population surveys of game species, poaching cases, and outdoor crime scenes involving unidentified biological samples. Due to the expanding field of wildlife forensics, the FIA-DNA Kit holds endless potential for wide applicability across genetic and forensic disciplines.

LAMP, Species Identifiction, Wildlife Forensics

B143 The Development and Validation of a Dual-Genus, Multiplex Polymerase Chain Reaction (PCR) Assay for African and Asian Elephants for Forensic Purposes

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After attending this presentation, attendees will understand how a multiplex PCR genotyping assay has been developed that will detect African and Asian elephant DNA simultaneously. Attendees will also understand how to efficiently create PCR assays for wildlife identification.

This presentation will impact the forensic science community by providing a standardized PCR assay that detects elephant DNA for use in wildlife investigations. The development of this assay fills a research gap in wildlife forensic science.

African (*Loxodonta africana)* and Asian (*Elephas maximus*) elephant populations are categorized under Appendix I or II of the Convention on the International Trade in Endangered Species (CITES), respectively.[1] CITES is an agreement that regulates plant and animal species throughout the world to ensure that international trade of their products does not impact their survival.[1] An Appendix I listing includes species that are threatened with extinction, thus trade of these plants and animals is highly restricted.[1] Species not facing extinction that require extra attention and regulations so they don't become exploited and over-utilized are listed in Appendix II.[1] The primary reason for the decline of these two animals is the illegal trade of their ivory. Other reasons for the decline in the elephant population are deforestation and human conflict.

Working toward conserving endangered and threatened species takes many forms throughout the world. Conservation efforts can include educating people of all ages, *in vitro* fertilization of healthy females, or surveying populations in the field. Wildlife forensic science can be defined as a field that promotes conservation efforts and the investigation of wildlife crimes through the use of scientific techniques that can be applied to the law.[2] Crimes against animals can be deterred and/or further prosecution sought through testing with forensic genetic techniques. The creation of novel genetic assays can greatly impact wildlife forensic science, not only in identifying the species, but also the individual from which the evidence originated. This information can also be used to track illegal trade routes throughout the world. Few publications describe the development and subsequent validation of tools and assays that can generate data of evidentiary quality.[2] Molecular genetics techniques can help enforce conservation efforts; however, they must be properly developed and validated in order to be of evidentiary quality for court systems.

In wildlife crime laboratories, species of origin can often be determined by morphology. This method is limited by the expertise of the taxonomist and the condition of the animal product. Ivory is commonly carved into small figurines and trinkets. Elephant meat, hair, and hide are traded, which can make it difficult to identify the species. These limitations have led to the development of genetic tests to identify species of origin in wildlife investigations. The targeting of Short Tandem Repeats (STRs) is used in this novel assay. African and Asian elephants do have highly similar genomes; however, in portions of these highly polymorphic regions, variation exists.

In this study a dual-genus, multiplex PCR genotyping assay to identify elephant DNA for forensic purposes was developed. By eliciting information from the variable areas of the elephant genomes, both genera of animals can be identified. Following the assay development, a rigorous developmental validation was conducted according to current community recommendations set forth by the Scientific Working Group for DNA Analysis and Methods (SWGDAM). The completion of this work provides an assay that can generate data of evidentiary quality for wildlife crime laboratories.

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Multiplex Development, Elephant, STRs

B144 Public Sequence Databases: An Assessment of Their Reliability for Identifying Non-Human Biological Material

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After attending this presentation, attendees will understand: (1) the differences between the two main public databases of DNA barcode data (GenBank and Barcode Of Life Data Systems (BOLD)) with regard to the number of sequences, curation, and quality checks; (2) the success of both databases for obtaining the correct taxonomic assignment for insects, plants; and fungal taxa; and, (3) the precautions necessary when using public sequence databases in a forensic setting.

This presentation will impact the forensic science community by identifying varied challenges of using public sequence databases for the identification of unknown biological material encountered in casework.

Crime laboratories routinely receive evidence which contains non-human biological material. If identified, such material could help reduce the search area in provenance cases or identify the best method for isolating a plant toxin. DNA barcoding permits species-level identification of biological materials through the comparison of the unknown barcode sequence to a reference database.

There are two main public sequence databases containing barcode data, BOLD and GenBank, the latter for which the data is not curated. This study performed an initial assessment of both the quality and reliability of the DNA barcode data contained in these databases, a prerequisite for their use in a forensic setting. To achieve this, curated reference material was sourced from national collections, with taxa chosen based on their inclusion in BOLD but also to represent the main lineages of plants, macro-fungi, and insects (total *n*, ~150). The relevant barcode sequences from these reference samples (rbcL, matK, trnH-psbA, ITS, and COI) were generated and used for searching against both databases. The ability of each database was assessed to obtain the correct taxonomic assignment (genus and species), when using the default search parameters; GenBank outperformed BOLD for insect taxa (86% and 50%, respectively) whereas for plant and fungal taxa, both databases performed comparably (~78% and ~64%, respectively). Considering that the correct match was often not discernible among the top matches, modified searches against each database were performed to assess whether resolution improvements were possible. Given that the underlying algorithm and associated parameters for searching BOLD are fixed, modified searches were limited to changing the subset of barcode sequences against which an unknown is compared (i.e., all records, only records with species level identifications, only full-length sequences). For a blast search against GenBank, the impact of altering parameters, including word-size and the penalties/rewards for mismatches and gaps, was systematically assessed.

This presentation will outline the optimal search parameters needed to consistently obtain the correct identification of an unknown when using either the BOLD or GenBank database. Additionally, some precautions needed when using public sequence databases in a forensic setting will be identified.

Public Sequence Databases, GenBank, BOLD

B145 Optimized Recovery of DNA and Protein Components From Contact Traces on Fired and Unfired Cartridge Casings

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After attending this presentation, attendees will be aware of potential proteomic applications in forensic biology and realize that it is possible to extract DNA and protein from touch evidence without compromising yields for either type of biological material.

This presentation will impact the forensic science community by alerting crime laboratory scientists to a new tool for touch evidence analysis. This approach may ultimately improve source identification for contact traces on cartridge casings.

Current DNA typing success rates for spent cartridge casings have been reported as less than 10%, and recent studies have shown that metal surfaces, specifically brass, can be detrimental to DNA.[1-3] These types of probative but low-level samples would benefit from supplemental testing of protein polymorphisms, also known as Genetically Variable Proteins (GVPs), as has been described for keratin in hair samples.[4] Proteins are expected to be more abundant than DNA and may be less affected by heat and mechanical pressure. This data reveal that it is possible to extract DNA using a mass spectrometry-compatible lysis buffer and sequencing grade trypsin as the protease. The incubation buffer consists of 0.01% ProteaseMAX™ and 5mM DTT in 50mM NH_4CO_3. Extraction proceeds with 20 minutes of denaturation at 56ºC, three hours at 37ºC for trypsin digestion, and EMD Millipore™ MW100 membrane filtration. The concentrated DNA fraction will be above the membrane and mass spectrometry-ready peptides are in the flow through. Protein sequencing was performed by reversed-phase liquid chromatography using Easy-nLC™ 1000 High-Performance Liquid Chromatography (HPLC) and Q Exactive™ Orbitrap™ mass spectrometer. Polymerase Chain Reaction Short Tandem Repeat (PCR STR) testing utilized the Identifiler® Plus multiplex and a 3500 Genetic Analyzer. The method was first established using sebaceous-rich fingerprints on glass slides, then tested for fired and unfired 9mm nickel, aluminum, steel, and brass cartridges.

Trypsin digest DNA yields were similar to a standard proteinase K method and DNA was suitable for STR typing. Peptide fractions could be injected on the HPLC without further modification. Data revealed significant differences for protein and DNA recovery before and after firing and between the larger steel cartridges and the other three metals. Even samples with no detectable DNA still yielded peptide peaks, with the average of identified proteins ranging from 27-74 for unfired and 12-30 for fired casings. Extraction negatives were generally negative, but sampling uncleaned cartridges straight out of the box, without deliberate touching, showed high levels of protein background. This needs further investigation. Parallel sets of ten unfired touched brass cartridges were tested using dry collection with tape versus wet swabbing. While tape lifts on average did not recover more DNA, STR typing rates were slightly improved with six full or good partial profiles for tape-lifted samples compared to five for the swabbed samples. Supplemental peptide testing has the potential to increase the power of discrimination for low-level samples. The same proteomic data can also be used to infer body fluid and/or species attributions.

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Cartridge Casings, Touch DNA, Protein Sequencing

B146 Alternate Proteases and Direct Cell Lysis Methods for the Recovery of Exogenous DNA From Fingernails

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The goal of this presentation is to present data from an evaluation of the efficiency of alternative proteases and direct cell lysis methods in extracting foreign DNA from fingernails.

This presentation will impact the forensic science community by suggesting alternative extraction methods for fingernail samples beyond the use of Proteinase K (PK).

When extracting a fingernail sample, it is possible to recover endogenous Deoxyribonucleic Acid (DNA) of the nail donor from within the nail and from the surface of the nail; similarly, foreign DNA may also be present on and recovered from the nail surface.[1,2] When attempting to recover the latter, fingernail samples present particular problems. Often, the foreign component is masked by the greater mass of nail donor DNA present in and on the nail sample.[3] This masking effect is exacerbated by the use of PK in the DNA extractions, as PK, with an average of 200 cut sites per keratin molecule, is capable of breaking open the keratin matrix of the nail and exposing the nail DNA intercalated in the matrix. Directly extracting nail clippings, in contrast to swabbing or scraping, would further introduce nail DNA when using PK.[4] This present study attempts to compare alternative proteases (ZyGEM® and Acrosolv®) with fewer cut sites than PK and two direct cell lysis methods (IGEPAL® CA-630 and Mawi iSWAB™-ID) with the intent of minimizing recovery of nail DNA from within the nail and thus mitigate the masking effect often seen with fingernail samples.

The endogenous DNA extraction efficiency of each suggested method was compared with QIAGEN® QIAamp® DNA Investigator extraction of hand-washed and/or cleaned nails. In contrast to previously published literature, a comparison of the results between hand-washed and cleaned nails suggests that much of the endogenous DNA recovered from fingernail samples is derived from DNA on the surface rather than from within the nail. QIAamp® extraction with the inclusion of Dithiothreitol (DTT) recovered significantly more DNA (p=0.0088) than the sample protocol without DTT. The IGEPAL® method recovered the least DNA from the nail, whereas the Acrosolv® method recovered more DNA than the QIAamp® protocol without DTT. Recovery was observed with the Mawi iSWAB™-ID buffer, but additional experiments are needed.

Fingernails were also spiked individually with blood, saliva, and semen to assess the recovery of foreign DNA. The extractions of the spiked nail samples demonstrate variability across all samples, owing, to some degree, to inconsistencies of sample preparation. IGEPAL®'s inability to recover complete foreign profiles suggests that the method is not viable for extraction of fingernail samples. Conversely, the ZyGEM®, Acrosolv®, and MAWI extraction methods demonstrate potential as alternative extraction methods for fingernail samples and would benefit from additional experimentation.

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Fingernails, DNA Extraction, Foreign DNA

B147 Increasing DNA Typing Success With Improved Front-End Processing and Alternate Workflow Strategies

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After attending this presentation, attendees will understand that there are a number of alternatives from swab selection to designed workflow to consider in order to improve sample recovery and extracted DNA yield, as well as to minimize sample consumption. The outcome can be increased success, improved processes, and maintaining as much sample as possible for additional testing.

This presentation will impact the forensic science community by stressing the importance of collection and extraction and how these processes, if considered in a systems approach, can benefit developing investigative leads in a cost-beneficial manner. Indeed, it is these processes that contribute substantially to the success of DNA typing, especially for low-quantity samples. This presentation will describe the important features to consider to affect an efficient process of DNA recovery and reduced consumption of biological evidence.

Consumption of evidence is a critical concern in forensic DNA analyses, especially for challenged samples that contain low quantity and/or degraded DNA. Success of DNA typing is related to the amount of target material recovered from an evidentiary item. Collecting as much sample as possible, maintaining the integrity of the sample after collection, and recovering as much DNA as possible should be sought. In addition, there have been arguments in legal proceedings that samples should be split, regardless of whether there is potentially ample material or if there are only minute quantities. Sample splitting for low-quantity samples can translate into no or inconclusive results in some cases in which consuming the entire sample may have generated an interpretable result.

A better way to address increasing typing success and concomitantly minimize sample consumption is to consider novel methodologies or tools and alternate workflows. These approaches should improve sample collection by using effective collection devices (such as nylon flocked devices), by utilizing tools that release DNA well during extraction to obtain the highest yield possible (such as specialized extraction baskets), and can subsample evidence to minimize sample consumption (such as micro-sized collection systems). The issues surrounding legal arguments on sample consumption and workflows using newly developed swabs and extraction baskets will be described. The subsampling method consumes such a small portion of the stain that essentially the entire sample is preserved for additional testing or re-analysis. After collection, the sample is amplified directly. Under the amplification conditions, there appears to be an enhanced sensitivity of detection likely due to a localized Polymerase Chain Reaction (PCR) effect even at 1:99 dilutions of blood and saliva based on higher Short Tandem Repeat (STR) peak heights than standard procedures with 1ng input DNA from the same samples. Touch samples from common items and textiles yielded results consistent with the types of the donors and items. The results of this study may potentially have important implications for analysis of low quantity and/or degraded samples that plague forensic casework.

Swab, Extraction, Sampling Strategy

B148 The Evaluation and Optimization of DNA Recovery and Amplification From Bullet Cartridge Cases

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After attending this presentation, attendees will better understand the complexities and challenges associated with the collection, extraction, amplification, and analysis of touch-type DNA samples commonly encountered with ballistics evidence. Attendees will also learn techniques for improving the probability of recovering sufficient DNA to generate interpretable Short Tandem Repeat (STR) profiles.

This presentation will impact the forensic science community by providing information on the optimization of commonly employed DNA collection methods and extraction techniques in an effort to improve the ability to successfully obtain interpretable DNA profiles from both fired and unfired cartridge cases without resorting to Low-Copy Number (LCN) DNA profiling methodologies.

Cartridge cases, both fired and unfired, are commonly encountered at crime scenes. According to the Federal Bureau of Investigation (FBI) crime statistics for 2015, 71.5% of homicides, 40.8% of robberies, and 24.2% of aggravated assaults involved the use of a firearm. Despite the high frequency and latent value of cartridge case evidence, these samples are not routinely submitted for DNA analysis, as it is a commonly held belief that it is difficult or impossible to recover DNA from this type of evidence. Underlying this belief is a preponderance of published studies on generating STR profiles from cartridge cases that indicate low success rates and/or minimal profiles that are unsuitable for comparative purposes.

To optimize DNA recovery from cartridge cases, numerous collection and extraction techniques were paired and evaluated. Five collection techniques (a tape-lift method, a double-swabbing wet:dry method, a double-swabbing wet:wet method, a soaking method accompanied by sonication and lyophilization, and a soaking method accompanied by vortexing and lyophilization) were paired with each of three extraction techniques, including phenol:chloroform organic extraction, PrepFiler™ Forensic DNA Extraction Kit, and the QIAamp® DNA Investigator Kit. First, 1,800 unfired 9mm cartridge cases of various metal compositions (450 each of brass, nickel-plated, aluminum, and steel) were tested with all pairings of techniques to assess possible impact of cartridge metal-type. Next, an additional 450 fired brass 9mm rounds were tested with all pairings of methodologies to assess the effects gunshot residue can have on profile quality. Finally, 230 unfired and 230 fired brass 45 Automatic Colt® Pistol (ACP) rounds were tested to assess the impact larger surface areas would have on optimum collection methods. All samples were quantified using the Life Technologies™ Quantifiler® Trio quantification kit and subjected to post-amplification concentration using an Eppendorf™ Vacufuge® plus vacuum concentrator. Samples were then amplified with the Life Technologies™ GlobalFiler® amplification kit and analyzed using Capillary Electrophoresis (CE) or analyzed using Massively Parallel Sequencing (MPS) methods.

For unfired samples of all metal types, extraction technique had the greatest effect on DNA recovery ($\bar{x}$ *Fs*=5.775; *df*=2,8; *p*=0.0103) with organic (phenol:chloroform) extraction producing optimum results. Recovery technique optimization was dependent on caliber size/surface area of the sample. For 9mm rounds, the tape-lift collection method consistently provided higher yields of DNA than other collection methods; however, for larger caliber ammunition the tape-lift method was inferior when compared to the two soaking methods. Analysis of fired samples indicates even greater differences in the success of profile generation when using optimized methodologies resulting in full and partial genetic profiles as compared to alternative combinations of recovery and extraction methods, which generated a total loss of genetic information. Comparison of these CE data to MPS data is underway.

This study demonstrates that by optimizing the methods employed for DNA collection and extraction, it is possible to increase the likelihood of obtaining good-quality STR profiles from cartridge cases without resorting to LCN DNA profiling methodologies. The presence of Gun Shot Residue (GSR) as well as the caliber of the cartridge case may impact the choice of optimal methodology and workflow. Further studies are targeted at identifying and mitigating the impact of possible co-eluting components of GSR or reactive metallic species from the casings themselves that compromise DNA profiling success through inhibition and/or augmented DNA degradation.

Touch DNA, Cartridge Casings, DNA Extraction

B149 Crime Scene Culture: How Inadvertent Collection of Bacteria Affects DNA Profiling Success

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After attending this presentation, attendees will better understand the external factors impacting DNA evidence stability and its impact on DNA profiling success. Attendees will also learn how microbiological organisms, collected with the sample, at crime scenes can significantly impact the results.

This presentation will impact the forensic science community by increasing knowledge of the need to preserve or process DNA evidence, especially from touch or compromised samples, in a timely manner. Increasing casework analysts' knowledge of the biological factors impacting their results will allow them to take necessary precautions to ensure that the sample collected at the crime scene has not degraded prior to analysis.

In recent years, a combination of new technologies being introduced into the market and the increasing impact of the use of DNA to solve crimes has led to both increased reliance on DNA and more collections. One often-overlooked area is the impact that biological materials, collected with the samples at the crime scene, can have on the resulting DNA profile. Bacteria, fungi, and enzymes such as DNases can have a dramatic impact on the stability of a DNA sample, possibly breaking it down before it ever reaches an analyst's bench for extraction and amplification.

This presentation will describe the studies performed on mock crime scene samples to study the specific effects of microbes and enzymes on the collected sample. Blood, saliva, and touch samples were deposited on various surfaces from picnic tables and bricks to plastic knives and shoe soles. After deposition, the samples were placed outside in the environment for a few days to simulate and stimulate normal bacterial, fungal, and enzymatic activity that can occur at a crime scene. After a few days in the environment, the samples were collected using the wet/dry swab method with a cotton swab and placed in a swab box. Accelerated aging experiments were performed on the samples by placing them in various temperature (room temperature, 37°C, and 56°C) and humidity (ambient ~20%-40% RH and >60% RH) conditions.

At selected time intervals, samples were removed and analyzed for DNA profiling success and for enzymatic activity and microbial growth. Bacterial activity was evaluated by incubating the mock evidence swabs in nutrient broth to observe turbidity in addition to inoculating nutrient agar plates to obtain single colony isolates. To evaluate DNase activity from the collected mock crime scene samples, DNase Test agar plates with methyl green were streaked with the collected swabs. DNase Test agar contains embedded DNA polymers that form a complex with the methyl green. In the presence of DNase activity, the embedded DNA depolymerizes and the methyl green/DNA complex fades into clear zones of agar surrounding the DNase positive bacteria. Some of the single colony isolates demonstrated DNase activity that may have a significant impact on obtaining a DNA profile from low-level or touch samples.

By combining the data obtained from the bacterial and enzymatic assays with the data from the DNA profiling, interesting observations and correlations were determined. Not only was microbial and enzymatic activity dependent on the surface of deposition, but it also affected the stability of the DNA collected. The results presented from this study will highlight bacterial and enzymatic activity and how it correlates to the resulting DNA profile.

Collecting a sample from an individual or from a crime scene is only one aspect of obtaining a DNA profile. Depending on the surface and suspected biological stain, analysts may need to take additional steps to ensure sample integrity. True success is when that profile can be used to identify a missing person, solve a crime, or exonerate a wrongfully convicted individual.

DNase, Bacteria, Evidence Collection

B150 An Improved Method for the Analysis of Fiber Evidence Using Polarized Light Microscopy (PLM)

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After attending this presentation, attendees will better understand a unique, useful, and rapid method using a traditional PLM to classify and identify the composition of fiber evidence.

This presentation will impact the forensic science community by introducing a new approach based on mounting fibers in a fluid medium that has a Refractive Index (RI) value that is intermediate between the fiber's principal RIs. When fibers are mounted in such a fluid medium and observed with a microscope equipped with a single linear polarizer, the fiber's RI will match the mounting fluid's RI at some specific angle of rotation. This angle of match is easily measured and provides a quantitative value for classification.

Multiple fiber types may be present in a single mount and be classified individually without the need to prepare multiple mounts. Implementing this method requires a polarized light microscope with a graduated rotating stage and is most efficient when either a graduated rotating polarizer or analyzer is available.

The classic PLM method of fiber identification is to measure the principal refractive indices using the Becke line method to determine the relative RI between the fiber and its surrounding mounting fluid.[1] The classic method requires mounting of fibers in a series of fluids of differing RIs until both principal RIs are matched. Synthetic fibers and some natural fibers behave optically as uniaxial crystals. As such, fibers have a continuous gradient of refractive index. This gradient ranges from the high to low values of the principal RI. Fibers have their principal RI values aligned parallel and perpendicular to the fiber axis. In this method, a fiber is mounted in a fluid with an RI value that is between the two principal RI values. It is observed using only linear polarized light. As the orientation of the polarized light vector is changed relative to the fiber axis, the Becke line will vanish. The angle between the fiber axis and the position at which the Becke line is indistinct is readily measured using a PLM. The PLM must have a rotating stage and either a graduated rotational analyzer or substage polarizer. A rotating stage is used to align the fiber so that the initial polarization direction is parallel to the fiber axis, then the analyzer (or polarizer) is rotated until a match point is reached. This angle of matching RI is only a function of the fiber composition and refractive index of the fluid.

The angle of rotation for matching refractive index (θ) can be calculated when the refractive index of the mounting media and the principal values of the fiber are known. Using the equation of an ellipse in spherical coordinates, a spread sheet was developed to generate tables of data that aid in textile fiber analysis. For example, fibers mounted in refractive index fluid RI=1.570 have angles of matching refractive index (θ) as follows: nylon (vivrelle), 0.0°; olefin (PE), Q=22.4°; nylon 6, Q=19.4°; nylon 6, 6 Q=24.5°; polyester (PCDT) Q=55.8°; polyester (PET) Q=65.7°; polyester (PTT) Q=75.2°. These data demonstrate the ability of this method to identify several common fibers in a single preparation. The efficiency of fiber identification is greatly improved over the classical PLM method.

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Fiber, Evidence, PLM

B151 Microscopical Discrimination of Human Head Hairs Sharing a Mitochondrial Haplogroup

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After attending this presentation, attendees will better understand the current state of forensic hair analysis and the benefits a combined microscopic and genetic approach can offer for discrimination of human hair samples. Attendees will appreciate the different forms of information available from microscopy and mitochondrial DNA (mtDNA) analysis and how these can complement each other in a forensic analysis.

This presentation will impact the forensic science community by introducing data from a current inter-laboratory study on the level of discrimination possible by microscopic hair analysis, adding to understanding of the strengths and limitations of microscopic comparisons of hairs and demonstrating the importance of combining microscopic and genetic analyses when evaluating evidential hairs. Previous studies have looked at the degree to which mtDNA can be used to differentiate microscopically similar hairs. This study focuses on hair samples that may not otherwise be discriminated by mtDNA to assess how well microscopy can differentiate hairs that share a mitochondrial haplogroup. This analysis illustrates that microscopy can provide distinguishing information not contained in forensic mtDNA DNA examinations.

Interpreting microscopic assessments of hair necessitates an understanding of the human factors that are involved in these examinations, such as visual perception, experience, and training. The accuracy and reliability of microscopic hair analysis was tested using 20 sets of hair samples from participants who share mtDNA haplogroups. The test sets were created using participants who had been genotyped and the hair samples for each test were then selected based on shared mtDNA haplogroup, ancestry, macroscopic similarity, and length. The tests were assigned random alphanumeric designators and mailed to hair examiners in forensic laboratories across the United States. Additional data was collected regarding the amount of training a forensic hair examiner had, how often hair proficiency tests were taken, years of experience, the types of casework typically received, degree of specialization in trace evidence subfields, and how the examiner characterized ancestry from the hair samples. The level of consensus was assessed between examiners for each test and how "rare" or "common" it was to not be able to differentiate hairs of mtDNA-defined groups by microscopic comparison. This study addresses some of the criticisms by the President's Council of Advisors on Science and Technology (PCAST) of forensic hair analysis and provides data regarding the strengths and limitations of microscopic hair analysis.

It is generally accepted that microscopic analysis of hairs will not provide a level of individualization that allows for identification of a hair to a single person to the exclusion of all others. By choosing sets of hairs from people who share the same mtDNA haplogroup, this study sought to determine how microscopically similar hair samples from these individuals may be and the degree to which microscopy can provide reliable differentiation of these hairs. While nuclear DNA analysis can lead to an individual identification, most evidential hairs are shed without sufficient follicular tissue for such testing, therefore limiting genetic testing of the majority of the hairs found in casework to mtDNA. Furthermore, identification through a destructive DNA analysis without first assessing a hair microscopically can hinder an investigation through the loss of valuable information. This study summarizes the current understanding of the utility of microscopic hair analysis in forensic casework, demonstrates the strengths and limitations of microscopic hair comparisons, and highlights the importance of combining non-destructive and destructive tests, such as microscopy and genetic analyses, to obtain the most information from a piece of evidence.

This study rigorously tested the accuracy and reliability of microscopic hair analysis with the conclusion that microscopy can discriminate greater than 85% of the hairs that mtDNA cannot. Accordingly, microscopic analysis of hairs should be maintained in forensic laboratories because it offers a cost-effective and non-destructive test that serves as the first level of sample discrimination and provides complementary information not available from mtDNA analysis.

Hair, Microscopy, Mitochondrial DNA

B152 Determining the Effects of Storage Conditions on the Preservation of Ignitable Liquid Residues

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After attending this presentation, attendees will understand to what extent different factors influence the preservation of stored ignitable liquid residue samples.

This presentation will impact the forensic science community by providing fire debris analysts with data to support which storage factors are important to consider when the preservation of samples is desired.

Preservation of ignitable liquid residue extracts is necessary for possible re-examination of suspected fire debris samples. Preliminary research investigating the effects of storage has been completed previously, although many aspects of storage have not yet been addressed.[1-3] The work completed by Sandercock provided foundational preservation research; however, this research will examine a wider range of factors.[2] In this study, Gas Chromatography/Flame Ionization Detection (GC/FID) was used to evaluate the extent to which select storage factors influence the preservation of fire debris samples over a 30-day period. A test mixture was created containing approximately 20 compounds commonly found in ignitable liquid residues. An aliquot of the test mixture was deposited into a metal can and adsorbed onto charcoal strips through active headspace. Chromatograms between the initial time-point and 30 days after analysis were compared to understand the relative effects of the storage conditions.

A partial factorial Design of Experiments (DOE) was used to evaluate the effects of different storage factors on the ability to preserve the sample. The six factors considered were: vial type (screw cap vs. crimp cap), storage temperature (23°C vs. -4°C), method of sample concentration (ambient evaporation vs. dry nitrogen stream), extraction solvent (carbon disulfide vs. pentane), whether the sample vial was stored in a vapor-tight fire debris bag, and whether the vial was covered with Parafilm®. This partial factorial design was replicated across the different methods of preservation suggested in the American Society for Testing and Materials (ASTM) E2451 *Standard Practice for Preserving Ignitable Liquids and Ignitable Liquid Residue Extracts from Fire Debris Samples* (i.e., reconstitution of the sample onto a charcoal strip vs. cutting the strip in half).[4]

The results of this study will be used to understand the relative effects storage factors have on the preservation of ignitable liquid residue extracts and will provide a platform to both continue future work on preservation and allow analysts to make informed decisions on archiving and preserving casework. Sample preservation can be an important step in a suspected fire debris case and this research will provide analysts with insight into critical preservation factors and the effect the factors have on the data.

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Fire Debris, Ignitable Liquid Residues, Preservation

B153 The Identification of Representative Compounds in Ignitable Liquids and Substrates: Classification of Fire Debris Using a Naïve Bayes Approach

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After attending this presentation, attendees will better understand the classification of fire debris as positive or negative for the presence of ignitable liquid residue using a Naïve Bayes approach.

This presentation will impact the forensic science community by utilizing a method for the identification of representative compounds in fire debris and the determination of the presence or absence of ignitable liquid residue in fire debris using likelihood ratios. The purpose of the presentation is to propose a classification system for fire debris.

In this study, 700 burned and 100 unburned substrates and 646 neat ignitable liquids were used. The burned substrate samples were prepared using Modified Destructive Distillation Method (MDDM), Direct Heat (DH), and Indirect Heat (IH) methods. Identification of the compounds in the chromatograms of the substrate and ignitable liquid samples were analyzed by Automated Mass Spectrometry Deconvolution and Identification System (AMDIS) software, using a library containing 282 compounds, including the retention times, retention indices, and mass spectra of each compound.[1]

Logistic regression and Receiver Operating Characteristic (ROC) analysis were applied to evaluate the probability of the presence of the library compounds in substrates and ignitable liquids. For these calculations, the delRT ($1/[1+|\Delta r.t.|]$) parameter was introduced. This parameter uses the absolute value of the retention time difference ($|\Delta r.t.|$) between the compound in the library and the sample. The data used to generate the logistic regression model was comprised of multiple compounds from each of 42 ignitable liquids taken from the Ignitable Liquid Reference Collection (ILRC) database.[2] Liquids were selected from all American Society for Testing and Materials (ASTM) E1618-14 classes.[3] ROC analysis was used to determine the best logistic regression model. Compounds having a probability ≥ 0.75 were designated as being present in the sample.

Based on their estimated presence or absence, the frequencies of occurrence of the compounds in substrates and ignitable liquids were calculated as the number of occurrences divided by the total number of ignitable liquid or substrate samples. For example, methanol was identified 13 times in 624 substrate samples. Therefore, the frequency of occurrence of methanol in substrates is 2.1×10^{-2}. Some compounds were found to have a zero frequency of occurrence in substrates or ignitable liquids. For instance, acrolein was found to have a zero frequency of occurrence in ignitable liquids. Failure to observe a compound in ignitable liquids or substrates is the result of the statistical sample and not a true estimate of the frequency of occurrence in the population. The Good-Turing smoothing technique was used to estimate the probabilities of occurrence for the compounds that were not seen in the substrates and ignitable liquids.

The frequency of occurrences obtained for the 282 library compounds in substrates and ignitable liquids were used to calculate likelihood ratios for the presence of ignitable liquid in a fire debris sample using a Naïve Bayes approach. The likelihood ratio is calculated by Naïve Bayes as the ratio of the product of the probabilities of occurrence in ignitable liquids (numerator) and substrate (denominator) for each of the 282 compounds found in a fire debris sample. Results will be presented for the cross validation of the method and testing on fire debris data.

This research was supported by the National Institute of Justice, Office of Justice Programs. The findings and opinions expressed in this work are those of the authors and do not reflect those of the United States Department of Justice.

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Fire Debris, Logistic Regression, Naive Bayes

B154 The Calculation of Likelihood Ratios in Fire Debris Analysis: Model Effects

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After attending this presentation, attendees will better understand modern methods of fire debris analysis, the calculation of Likelihood Ratios (LLRs) and how model effects on the LLR can be taken into account. Model effects on the LLRs calculated for fire debris samples can arise through distributional changes, which may have an impact on the class means, variances, and covariances of the model.

This presentation will impact the forensic science community by helping analysts understand how to implement models based on a relevant population and statistical sampling of a database.

A simple method is presented for the calculation of LLRs for fire debris samples. The method involves statistically sampling a database of ignitable liquids and substrate pyrolysis data in proportion to the estimated class distribution in a relevant population. The sample of ignitable liquid and substrate pyrolysis data will form the basis for calculating class means, variances, and covariances to be use in a kernel density estimate of the LLR for a fire debris sample. The multivariate kernel density is calculated from a limited feature set comprised of the scores for the first four principal components, which are derived from principal component analysis of the statistically sampled data. Multiple models are prepared by repeated sampling of the database. Bootstrap validation was calculated for each model and the average area under the Receiver Operating Characteristic (ROC) curve was taken as a measure of model performance.

Ten models were prepared for each of three population distributions. Each of the models had an average area under the ROC curve of greater than 0.9. The models were used to estimate the evidentiary value for a set of laboratory burns. One set of burns involved polyester carpet and padding with added gasoline. Samples were burned for time periods of 0.5 minute, 1 minute, 2 minutes, 3 minutes, and 5 minutes. A set of samples containing polyester carpet and padding with added medium petroleum distillate were burned for the same time periods. A set of polyester carpet and padding samples without added ignitable liquid were also burned for the same time periods. The base 10 log of the LLRs for all samples were in the range of 2 to -2, which are considered reasonable. The LLR values for the samples containing ignitable liquid were observed to be positive at early burn times and proceeded to become negative at longer burn times as the solvent was weathered and pyrolysis products were produced in the burn.

The method discussed is considered suitable for casework samples. The method allows the analyst to report the evidentiary value of fire debris samples as numerical (LLR) values.

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Fire Debris, Likelihood Ratio, Database

B155 The Utilization of Receiver Operator Curves (ROCs) for the Evaluation of Fire Debris: The Influence of Population Distributions on Classifier Performance

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After attending this presentation, attendees will understand how diverse populations can alter the covariance matrices that affect the performance of classification modeling in fire debris analysis. An introduction to the use of subpopulation distributions and their influence on Likelihood Ratio (LR) calculations will also be explored.

This presentation will impact the forensic science community by discussing the development of "relevant" populations and their impact on the evaluation of fire debris evidence.

An important aspect in fire debris analysis is determining whether a sample contains an ignitable liquid residue. In this work, fire debris samples are assigned to two classes, those containing Ignitable Liquid (IL) residue and those containing only Substrate (SUB). One consideration in the development of classification models for fire debris is defining a population. A population is comprised of SUB and a subpopulation of IL classes drawn from the eight American Society for Testing and Materials (ASTM) E1618-14 IL classes in a defined distribution.[1] The challenge of defining a relevant population is attributed to the lack of known ground-truth ASTM E1618-14 IL class distributions in casework samples. This work examines the effect of diverse population distributions on LRs calculated from a one-level multivariate normal classification model.[2] The one-level classification model only takes into account between-sample variances. Diverse population distributions were generated computationally, as previously reported.[3]

Receiver Operating Characteristic (ROC) curves for model performance were generated based upon the results of LR calculations. Models were built based on each distribution and were tested on data from all other distributions. An Area Under the Curve (AUC) was calculated to evaluate classifier performance from the ROC curves. The change in the population distribution altered the mean and covariance of SUB and IL classes. Changing the covariance matrices modifies the LRs used to generate the ROC curves and assigns a different ordering of ground-truth labels. The population models that demonstrated the best performance consisted of significant SUB contribution and subpopulation comprised of representatives of each ASTM E1618-14 IL class. Population distribution models that contained a minimum SUB contribution and lacked representations from an ASTM E1618-14 IL class resulted in poorer performance.

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Fire Debris, Likelihood Ratio, Population Distribution

B156 A Combined Approach of Gas Chromatography/Mass Spectrometry (GC/MS) and Chemometric Strategies for Fire Debris Investigation Purposes

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After attending this presentation, attendees will better understand how to combine GC/MS data with multivariate data analysis strategies for fire debris investigations.

This presentation will impact the forensic science community by illustrating that, even though in a preliminary stage, this study seems to emphasize the employment of multivariate strategies targeted at potentially helping the interpretative process of fire debris investigations.

In fire debris investigations, a largely debated issue deals with the possibility of recognizing whether or not the collected evidence is related to the occurrence of an arson.[1] In practice, data from GC/MS analyses performed on collected fire debris may be compared to those from ignitable liquids (e.g., that found in possession of a suspected arsonist). With the goal of evaluating the use of gasoline as a fire accelerant, several gasolines sampled from different gas stations located within the area of Turin, Italy, were analyzed by Solid-Phase Microextraction (SPME) -GC/MS.[2] Fresh and weathered samples were analyzed and compared to standard mixtures and protocols, namely American Society for Testing and Materials (ASTM) 1618.

The collected mass chromatograms were subsequently interpreted using a variety of targeted and untargeted approaches of multivariate data analysis, using both raw and semi-quantitative data. A data set including 150 GC/MS analyses relative to 30 gas stations was used for multivariate analysis. Gasoline samples were analyzed both as pure liquids and as mixtures of fresh and weathered mixtures at different percentages (i.e., 25%, 50%, 75%, and 95%). Several multivariate data analysis procedures were tested and their results were compared. The use of chemometric strategies allowed the building of explorative, classification, and likelihood ratio models, indicating the probabilities that fire accelerants have actually been employed. Once the chromatograms had been collected, different chemometric approaches were tested on both raw and semi-quantitative data. Principal component analysis located the fresh gasoline samples within a scores plot according to their origin (i.e., the different gas stations). *N*-way strategies were tested to determine how multivariate strategies were able to assess the occurrence of fire accelerant in an arson scene. To this purpose, whole tridimensional GC/MS data collected in scan mode were compared with those obtained from different gas station gasoline samples. Similar investigative approaches have been used with the integration of Bayesian's logic. Further development of multivariate feature-based and score-based likelihood ratio models built on the collected GC/MS spectra are in progress. In particular, principal component analysis, Self-Organizing Maps (SOM), and partial least squares – discriminant analysis, as well as *N*-way models, proved successful with the goal of identifying the usage of fire accelerant.[3,4]

Even if in a preliminary stage, this study seems to emphasize the employment of multivariate strategies aimed at potentially helping the interpretative process of fire debris investigations.

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Fire Debris Investigation, GC/MS, Multivariate Data Analysis

B157 The Detection of Toxic Adulterants in Seized Drug Exhibits in Kentucky and Vermont

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After attending this presentation, attendees will be able to describe the number and variety of adulterants encountered in seized drugs in Kentucky and Vermont in the United States.

This presentation will impact the forensic science community by providing data on the prevalence of toxic and non-toxic adulterants in drug evidence seized from March 2015 through May 2017.

Cutting agents are constantly changing over time, increasing the risks to the user's health caused by the compound's interactions. Many laboratories in the United States perform seized drug analysis by an acid/base extraction of drug evidence. This may result in toxic adulterants being removed before the analytical phase. Also, most laboratories will report on only controlled substances in the Drug Enforcement Administration (DEA) list (Schedules I to IV) or per their state regulations. Both factors lead to under-reporting of other substances that may contribute to the adverse effect profile of illicit drug use.

Two hundred aliquots from seized drug exhibits from Kentucky and 315 samples from Vermont were received dissolved in methanol or ethanol and were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS), followed by Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) after dilution (1:100) of the neat sample in mobile phase. The samples were received deidentified, but were assigned an in-house identifying number, linked to the date of receipt for analysis by the originating lab and the zip code or county of origin.

Overall, the most prevalent toxic adulterant found was caffeine (31.0%), followed by quinine/quinidine (24.7%), levamisole (11.6%), acetaminophen (8.2%), and procaine (8.2%). In Kentucky, levamisole (18.0%) was the most prevalent toxic adulterant detected, followed by caffeine (15.0%), diphenhydramine (14.0%), quinine/quinidine (11.0%), and lidocaine (8.0%), while in Vermont, the most prevalent was caffeine (46.2%), followed by quinine/quinidine (37.7%), procaine (16.0%), carisoprodol (15.0%), and diltiazem (10.3%).

The seized drugs of abuse identified overall were as follows: heroin (117 samples), cocaine (85 samples), methamphetamine (61 samples), fentanyl and analogs (40 samples), MDA/MDMA (4 samples), and U-47700 (4 samples). Heroin was primarily adulterated with caffeine (50.4%) and quinine/quinidine (41.8%), as well as fentanyl and analogs (caffeine in 74.0% of the samples, quinine/quinidine in 51.6% of the samples). Cocaine was primarily adulterated with caffeine (35.2%) and levamisole (28.2%). Caffeine was also the main adulterant identified in U-47700 samples (75.0% of the samples). The methamphetamine samples were less adulterated in general; the most prevalent adulterants identified in those samples were diphenhydramine and caffeine in 6.5% and 4.9% of the samples, respectively. The four MDA/MDMA samples identified in this batch were not adulterated.

In Kentucky, the majority of the samples were methamphetamine, of which some were adulterated with diphenhydramine (6.5%) or caffeine (4.9%), and cocaine mostly adulterated with levamisole (33.3%), caffeine and diphenhydramine (22.2%). By contrast, in Vermont, most of the samples were heroin adulterated with caffeine (48.9%) and quinine/quinidine (41.6%), and cocaine adulterated with caffeine (58.0%), quinine/quinidine (29.0%), and procaine (25.8%).

Knowledge concerning the toxic adulterants is important for the management of acute intoxications and also in criminal investigations, helping in the identification of routes of trafficking; however, common reporting practices frequently do not provide information regarding the prevalence of these toxic adulterants.

Seized Drug, Cutting Agents, Toxicity

B158 An Investigation into Darknet Markets: Their Use in Predicting Emerging Drug Trends and the Correlation With Discussion on Surface Web Drug Forums

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After attending this presentation, attendees will understand the information that darknet drug markets and surface web drug forums can provide in predicting ever-changing trends in drug use.

This presentation will impact the forensic science community by highlighting the role vendors, buyers, and forum users on darknet markets and drug forums play in driving developing drug trends. This research seeks to bring to light the changes in drug trends over time through the discussions found on both drug forums and darknet markets.

Darknet markets, also known as cryptomarkets, are online marketplaces that are hidden from normal web browsing and can only be accessed through the use of privacy software such as The Onion Router (Tor). Surface web drug forums are found through normal search engines and are communities in which drug users and enthusiasts can discuss their drug habits as well as their experiences with various illicit substances. Both locations offer their members a place to openly converse on the new substances they are ingesting to create a euphoric effect; however, darknet markets allow for the open selling of the illicit substances, while the drug forums are restricted to discussion only. Despite the added anonymity Tor software provides, most darknet market drug forum discussions are focused on vendor reviews rather than discussion of the euphoric experiences.

The correlation between the drug trends and the discussion contained within the forums and markets was analyzed via different means. The information contained on the surface web forums was collected using a forum crawler, while statistics were generated for the drug listings on the darknet markets. In addition to total listings for the major drug classifications, vendor and buyer information was browsed to look for location-based differences in drug popularity. An open source forum crawler was modified for this research to collect the data found on popular drug forums. The data was then analyzed using data mining techniques to search for popular keywords that were previously unknown. A lexical repository was created to store the information found.

Due to the constant flux of popular drug use and the increased law enforcement activity targeting darknet markets, the up-to-date results will be reported during the presentation. Multiple darknet markets have been investigated throughout the course of this research to provide a broader collection of the illegal drug trade. The well-established prevalent opioid use on the East Coast flowing in from European vendors and the fact that the West Coast suffers from a high influx of amphetamine derivatives supplied by Asian vendors was verified through this research, allowing for the validation of the methodology.

Darknet Markets, Drug Forum, Drug Trends

B159 Predicting the Origin of Heroin by Analysis of Inorganic Elements and Isotope Ratios of Strontium (Sr)

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The goal of this presentation is to inform attendees regarding the efforts to profile heroin by means of inorganic trace element compositions as well as Sr isotope ratios 87Sr/86Sr.

This presentation will impact the forensic science community by demonstrating the potential for heroin sample matching and origin determination by examination of radiogenic Sr isotope ratios and trace elemental composition and the potential for this application to other forensic matrices.

The effort to understand the dynamic nature of drug production and distribution has lead intelligence and forensic experts to increasingly rely on analytical chemistry for solutions. In an attempt to aid assignment of origin to seized shipments, heroin samples of known provenance have been dissolved by microwave-assisted acid digestion and studied by three varieties of Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) for elemental composition and isotopic ratios $^{87}Sr/^{86}Sr$ whenever possible. For the elemental analysis of heroin, inorganic composition was quantitatively determined by quadrupole ICP/MS and high-resolution ICP/MS. For the precise measurement isotope ratios of strontium, samples were prepared by affinity resin chromatography and analyzed by multi-collector ICP/MS. To predict a provenance assignment, the results of the analysis have been analyzed by single-variable and multivariate statistical analysis. This is the first large-scale study with 40+ samples from each of the major source regions to investigate provenance determination through inorganic trace element composition and radiogenic strontium isotope ratio analysis.

The utility of light-element, stable isotopic ratio values has been investigated in heroin for provenance studies.[1-3] Geochemical studies have demonstrated that $^{87}Sr/^{86}Sr$ values found in plants and animals can be directly correlated to those found in the geological material upon which the organisms live (and feed). The natural variation in $^{87}Sr/^{86}Sr$, which is primarily resultant from spatial and temporal differences in geological formation, forms the basis of the hypothesis for this study. One of the few forensic studies into strontium isotope ratios reveals that natural-product drugs (marijuana, in this example) can be used for origin determination.[4] In the case of semi-synthetic drugs, such as heroin in this study, it is believed that batches of heroin that are cultivated and processed in a particular location will share similar radiogenic Sr ratio values. It is assumed that strontium contamination is possible during the processing of opium (morphine) into heroin as well as subsequent packaging and handling; therefore, it is expected that there may be some bias to the heroin $^{87}Sr/^{86}Sr$ values.

Despite this anticipated uncertainty, it has been estimated that ≥70% of unknown samples can be correctly assigned based upon the described methods.[5] As expected, inorganic methods of analysis do not currently possess the discriminating certainty of published methods for organic analysis; however, this research was conducted in order to provide an orthogonal means of analysis to support existing, successful heroin-profiling techniques.[6-8] This has been the first known study to profile heroin by a fusion of inorganic element composition and heavy-element isotopic ratio data.

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Heroin, Trace Elements, $^{87}Sr/^{86}Sr$ Ratios

B160 The Material Effects of Commercial Swabs on the Extraction of Multiple Drugs Using Microfluidics and Mass Spectrometry

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After attending this presentation, attendees will understand how different materials of commercially sold swabs can affect extraction and analysis of multiple New Psychoactive Substances (NPS) using a microfluidic extraction device in combination with High-Performance Liquid Chromatography (HPLC) and low-resolution Mass Spectrometry (MS).

This presentation will impact the forensic science community by providing material effects of commercially sold swabs for decisions on better trace evidence collection of NPS and drugs of abuse.

This specific Microfluidic Device (MFD) was developed for the extraction of dyes from fibers and is now being fully tested for other areas of forensic science.[1] This research is a continuation of a presentation given at the 2017 AAFS annual meeting on the comparison of three instrumentation methods for the identification of 70+ drugs. The method developed of collecting the extraction in a micro-vial is used because it extracted, separated, and identified the most compounds, efficiently allowing a valid comparison of material effects.

How materials of commercially sold swabs affect the extraction of certain compounds is the primary goal of this study. This enables forensic specialists to understand what swabs are better for specific NPS for evidence collection. A variety of swabs with tips of materials such as polyurethane foam, cotton, polyester, rayon, and nylon were procured from Puritan Medical Products®. These included a variety of tip types such as flocked or pre-wetted, as well as Popule swabs, which are swabs that contain a small amount of solvent or water in their shafts to saturate the tip for cleaning. To determine material effects, swabs of each material were saturated in a standard solution of 70+ compounds that contain various Differential Optical Absorptions (DOAs) such as amphetamines, cannabinoids, opiates, and barbiturates. To determine trace effects, 10µL of the solution were pipetted onto the swab tips and sampled. Sample preparation for the MFD involved removing a small piece of the swab tip doped with the solution, placing it in the cavity of the microfluidic chip, placing a glass slide on top, and inserting it into the MFD. Extraction commences via the user-friendly interface that controls the parameters and is collected in a micro-vial for HPLC-Triple quadrupole (QqQ) analysis using a method previously developed with Shimadzu Scientific Instruments®, a collaborator on this project. All electronic conditions were the same for ionization and detection parameters, and the QqQ was operated in Multiple Reaction Monitoring (MRM) where multiple product ions are detected for precursor ion identification. An NPS was accurately identified if at least two product ions were detected for the precursor, the signal-to-noise ratio for these ions in the mass spectrum is three or more, and the area of the mass-to-charge ratio signal increases when compared to a blank spectrum collected from the MFD and analyzed.

Results indicate that polyester and polyurethane foam are the most effective at releasing a larger variety of NPS when the tips are fully saturated. Ionization bias caused by electrospray ionization is decreased with this method because the extraction is first separated via HPLC. The lesser amount of NPS identified is simply due to the material effects. For the second method for trace analysis, smaller sized, flocked tips were better since the swab had less depth to absorb the 10µL of solution. This kept the NPS in the same location; thus, the piece removed for extraction and analysis contained most of the drugs. Samples taken from larger swabs rarely contained even half of the compounds as the solution had too much area to spread. This can be improved by sampling multiple areas of one swab. Wetted versus dry Popule swabs revealed that more drugs were released when the Popule contained a solution of 91% Isopropyl Alcohol (IPA) and 9% Deionized (DI) water. Limited variation occurred between dry and wetted swabs when the Popule contained sterile water.

Overall, this work provides forensic analysts with new knowledge for determining swabs to use for evidence collection of trace samples. The MFD adds a simplified extraction step with reduced human error and bias since no analyst interaction is needed during the extraction process. It shows versatility for extraction of drugs from a variety of materials, where only the effects of the materials cause different NPS to be identified. This work benefits the forensic community by fast, automated extraction, simple transfer into the HPLC/MS system, facilitated identification with a low-resolution MS instrument by MRM, and simplified confirmation of identification via comparison with the blank spectrum of the MFD.

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Multiple Drug Analysis, Material Effects, Microfluidic Mass Spectrometry

B161 Low-Field Nuclear Magnetic Resonance (NMR) Applications in Forensic Drug Analysis

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After attending this presentation, attendees will understand the potential utility of low-field NMR spectroscopy in the qualitative and quantitative analysis of drugs. This presentation will focus on the use of low-field NMR as a substitute for high-field NMR for differentiating structural isomers of New Psychoactive Substances (NPS) that can be difficult to distinguish with other methods. The use of low-field NMR to provide accurate quantitation will also be discussed.

This presentation will impact the forensic science community by suggesting an alternative to current methods employed by forensic laboratories that have limitations for confirming specific positional isomers. This presentation will demonstrate that low-field NMR can be a suitable technique by using examples from several drug classes. Limitations of the technique will also be discussed.

Forensic laboratories commonly receive new synthetic cannabinoids, cathinones, and opioids that are difficult to report. Slight changes to chemical structures (e.g., shifting the position of functional groups, such as methyl groups or halogens, around an aromatic ring) can cause problems with identification using traditional methods. Classic gas chromatography/mass spectrometry cannot easily distinguish between certain positional isomers.

NMR is a powerful tool used to elucidate the structure of these isomers using 1D and 2D spectra from ^{1}H, ^{13}C, ^{19}F, and ^{15}N nuclei probes to assign elements to their specific position on the molecule. High-field NMR is typically used for these determinations but is not practical in many forensic laboratories due to the cost of the instrumentation as well as cryogen, facility, and staff requirements. In contrast, low-field NMR is less costly, has a smaller footprint, does not need cryogens, and requires little maintenance; however, this comes at the cost of spectral resolution and sensitivity.

This study evaluated the use of ^{1}H NMR on low-field benchtop NMR (60 MHz) and 600 MHz systems to differentiate positional isomers of several classes of NPS. The positional isomers were readily differentiated on both instruments, as expected. The use of quantum mechanic spin system modeling of ^{1}H spectra on the high-field system for portability of experimentally observed spectra to other magnet field strengths was also investigated and successfully demonstrated by transforming 600 MHz spectra to 60 MHz. This capability suggests that the generation of field-strength independent spectral libraries may be feasible and would facilitate data dissemination across instruments without the need to acquire a large collection of reference spectra.

Quantitation based on NMR is unique in that it can be performed relative to a distinct reference compound without requiring a calibration curve derived from the analyte of interest as utilized commonly in chromatographic methods. Purity determination for certain drugs (e.g., methamphetamine) can affect sentencing guidelines and can also be used by law enforcement for intelligence purposes. This study also explored the use of low-field NMR for quantifying methamphetamine in commonly encountered sample mixtures to mimic casework.

Low-Field NMR, New Psychoactive Substances, Methamphetamine

B162 Separation and Identification of Drugs of Abuse by nano-Liquid Chromatography/Electron Ionization/ Mass Spectrometry (nLC/EI/MS)

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After attending this presentation, attendees will understand the merits of nLC/EI/MS for the analysis of drugs of abuse when compared to Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/ Electrospray Ionization/Tandem Mass Spectrometry (LC/ESI/MS/MS) methods.

This presentation will impact the forensic science community by introducing a viable method for the separation and identification of thermally labile drugs of abuse, as well as the advantages of nLC/EI/MS when compared to GC/MS and LC/MS/MS.

GC/EI/MS and LC/ESI/MS/MS are two commonly used analytical techniques in the forensic analyses of drugs and explosives. The primary advantage of GC/MS is its extensive library-searching capability, which allows identification of unknown compounds; however, many compounds used in forensic sciences are thermally labile and decompose in the GC injector, which typically operates at high temperatures. LC/MS has been useful in addressing the issue of thermal degradation by utilizing an atmospheric pressure ionization source, such as ESI, eliminating the need for a high-temperature injector and, therefore, vastly decreasing or eliminating fragmentation.[1] By using an MS/MS, compounds can be fragmented; however, no good library for LC/MS/MS currently exists.

LC/EI/MS is an ideal analytical technique for the analysis of thermally labile drugs because it combines the advantages of the LC for sample introduction at low temperatures with the EI/MS ionization, fragmentation, and library-searching capability.[2] Many Novel Psychoactive Substances (NPS), including synthetic cathinones and synthetic cannabinoids, are thermally labile and, therefore, are difficult to detect and identify using GC/MS. Moreover, analysis of these compounds by LC/MS/MS is hindered by the lack of a universal library searching of the MS/MS spectra. There is also a need to develop a field-portable analytical technique for on-site real-time confirmatory analysis of illicit drugs, especially the synthetic drugs that are flooding the illegal markets. To address these issues, a field-portable nLC/EI/MS is being developed by using a field-portable Easy 1000 nanoLC™ in conjunction with a field-portable Viking 573 mass spectrometer, on loan from the Federal Bureau of Investigation (FBI). Development of the nLC/EI/MS also allows the analysis of thermally labile compounds or compounds that are difficult to analyze by GC/MS. NanoLC flow rates are in the range of 100nL-500nL/min, allowing the sample to be introduced directly into the MS ion source. Analytes are then ionized and fragmented using electron ionization, producing fragmentation patterns similar to the conventional GC/MS. An nLC flow rate of 300nL/min was used with a mobile phase of H_2O and acetonitrile each containing 0.1% formic acid. First, the method parameters such as flow rate, column length and inner diameter, mobile phase composition, and injection volume were optimized for maximum sensitivity using caffeine, methamphetamine, and morphine. After determining the optimum parameters, a nano C18 column was attached to the nLC for separation of drug mixtures. Good separation was achieved for a methamphetamine and caffeine mixture, as well as for a morphine and cocaine mixture. These compounds were identified using the Viking National Institute of Standards and Technology (NIST) EI library. Application of this new technique to the separation and identification of novel psychoactive drugs, such as synthetic cathinones and synthetic cannabinoids, will be discussed. Once method development is completed, it is planned that the nLC/EI/MS will be tested in the field.

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Portable Nano-LC, LC/EI/MS, Drug Analysis

B163 A Collaboration for Forensic Student Success: Bridging the High School-to-College Transition

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After attending this presentation, attendees will be knowledgeable regarding a new approach to forensic science students transitioning from K-12 studies into undergraduate programs. Attendees will learn about this transitionary program through a pilot research-focused internship conducted between a high school and a primarily undergraduate institution.

This presentation will impact the forensic science community by providing a model of how to prepare high school seniors for undergraduate forensic science programs on two different aspects: forensic science educational expectations and campus life expectations. Research has shown that a gap exists between secondary and post-secondary education due to a lack of consensus between K-12 graduation requirements and higher education curriculum requirements.[1] Although a tight collaboration between a high school and college forensic science program cannot endeavor to change requirements, the pilot internship provides a first glance at the skills and critical thinking needed to succeed in a forensic science undergraduate program. With the increase of interest in forensic science, universities and high schools have responded by developing summer programs, outreach programs, and college-level classes for students.[2] While these programs provide opportunities to promote the many sciences rooted in the forensic discipline, the transitionary program offers an advantage. Students have the ability to work on original forensic research, analyze samples via instrumentation, present their results, and explain how their studies impact real-world situations. The pilot program's hands-on experience with forensic research prepares students for the skills and critical thinking expected in forensic science.[3]

Any life transition is difficult, and the first year of college transition is oftentimes considered the hardest a young person's experiences. During this time, students may experience personal and emotional problems while struggling to be successful in the classroom. Many colleges and universities have created programs and courses to help ease the stressors encountered during this transition (e.g., first-year experience groups, orientation sessions, and individual counseling). Faculty and administrators also continually work toward addressing deficiencies in the classroom with tutoring, small class sizes, and individualized student support. The approach presented in the pilot internship addresses the deficiencies of college-bound young adults by creating an opportunity for students to begin this transition during their senior year of high school. The program allows the students to experience campus life through dorm housing, social activities, off-campus trips and field work. These experiences help to ease the transition of high school students to campus life.

The pilot program partnered a Philadelphia, PA, suburban high school with a rural Pennsylvania college. In the early summer of 2017, Keystone College hosted seven high school seniors with varied interests in the biological and physical sciences. Three students were interested in the forensic sciences and are the focus of this program assessment. The goal of the program was to immerse high school seniors in original forensic science research through a week-long internship program in which they live and learn on campus as college students. At the completion of the week, students gained laboratory, critical thinking, presentation, and research skills to carry into college. These newly acquired or enhanced skills will help them be successful in their high school-to-college transition. Original research focused on trace evidence analysis and the postmortem submersion interval was conducted and results will be presented. Data and evaluation through surveys of the students during the program and during their first term as college students will also be presented.

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Education, K-12, College Transition

B164 Using Forensic Cases to Improve Ethical Reasoning Skills

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After attending this presentation, attendees will understand how and why an ethical reasoning curriculum was created for forensic science students.

This presentation will impact the forensic science community by highlighting the importance of improving the ethical reasoning skills of forensic science students. This presentation will explore the ethical reasoning modules created and the classroom results. Particular focus will examine the way real-life cases are incorporated in the problem-based learning format.

The misconduct of Fred Zain, Annie Dookhan, Phillip Mills, and Sonja Farak still haunt the forensic science community. The misconduct performed by these individuals highlights the need for improved education focused on ethical reasoning and decision making. A pro-active educational approach provides students with the skills necessary to navigate ethical challenges in the workplace.

A national higher education organization, the Association of American Colleges and Universities (AAC&U), lists ethical reasoning and action as an essential learning outcome. Ethical reasoning skills are imperative for the successful navigation of career challenges. Ethics education is beyond right and wrong. Traditional ethics education is often recall-based. Students study theories and professional codes, but skill development is missing. Students need to be equipped with the skills to make ethical decisions.

A curriculum was designed to improve ethical reasoning skills for forensic science majors. Examples from pop culture along with real-life criminal cases highlight ethical reasoning in forensic science. The reasoning process and ethical dilemmas presented in the curriculum simulate real-life work. The curriculum is built as a developmental trajectory from understanding reasoning to activities that simulate decision making in real-life cases. This is a problem-based learning focus. Developing a problem-based learning curriculum in a module format engages students in an active learning process. Additionally, creating the modules in an online system allows for more detailed data analysis and expansion to a broader audience beyond a single classroom.

Multiple modules exist in the current ethical reasoning curriculum. First, the student is exposed to a brief philosophical background that provides a basic understanding of an individual's belief system and how new beliefs are created when confronted with genuine doubt. The next module explores the different types of reasoning methods. Students identify the three forms of reasoning in a variety of circumstances (i.e., text, video, and case descriptions). The next module further delineates the types of reasoning into multiple modes. Another module specifically focuses on the ethical theories and principles surrounding justice, privacy, and the common good. The role of these ethical principles as they relate to forensic science are explored. The modules use pop culture examples, from shows such as Monty Python and Sherlock Holmes, to introduce concepts before real-life examples are incorporated into the modules. The modules progress from simple to complex case examples. The use of real-life case examples is imperative for students to understand the impact of the forensic practitioner's actions. The module integrates the previous materials into full case studies that are completed by individuals or groups. These full cases present information at different times in the analysis in order to simulate how information is obtained in a case.

Results specifically from the module focused on reasoning types demonstrate students' abilities to understand and identify the three reasoning types. Detailed data analysis further illustrates questions in which students struggle. Additionally, specific skills tied to each assessment question indicate the level of student learning. The data analysis tools associated with the online system allow for detailed evaluation of student learning and provide constructive feedback for improved iterations of the modules.

Ethics, Reasoning, Education

B165 Teaching Ethics in Forensic Science: A Laboratory Approach

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After attending this presentation, attendees will be informed of other methods by which a traditional lecture-based and discussion-based subject may be presented in an alternate format that emphasizes creativity and laboratory skills that may be applied to novel applications. Suggestions for laboratory practicals involving a variety of true-to-life ethical dilemmas that one may face in a laboratory environment will be presented, in addition to suggestions for active learning.

This presentation will impact the forensic science community by examining real-world ethical dilemmas and constructing a case scenario around them that would lead to guided discussion, creative problem solving, and laboratory-based principles to help resolve some of the issues addressed. Such an approach will involve looking at underlying motivation, methods for accomplishing deceptive practices in forensic practice, and utilizing methods to uncover them.

This proposed class is designed to integrate the teaching of ethics into a forensic chemistry major at the undergraduate or graduate level. Expanding on an existing one-unit special-topics class dealing with ethics in a forensic science setting to a three-unit semester class will involve laboratory and case study sessions along the lines of: (1) production, examination, and detection of forged fingerprints and footwear lifts; (2) detection of altered and fraudulent photographs in scientific journals; (3) detection of misrepresentation in photographs; (4) questioned document examination of altered laboratory notes, such as examination of interlineations, examination and decipherment of obliterations, altered ink entries (Infrared (IR) luminescence and fluorescence), and Electrostatic Detection Apparatus (ESDA) methods for the examination of indented writing); (5) development of protocols for the detection of planted crime scene evidence; (6) preparation of simulated historical documents accompanied by artificial aging methods; (7) evaluation of court displays and other demonstrative evidence; (8) obtaining background information on opposing expert witnesses — evaluation of resumes; (9) critical examination of court transcripts; (10) dry labbing — work flow, time management, supervision issues, and quality assurance methods to aid in its detection; and, (11) ethics tool kits — establishing guidelines for ethical decision making, personal and general, and the development of decision-making processes for different ethical dilemmas.

In addition to the lab practical's, the course will incorporate homework and discussion topics centering around ethical case studies and common ethical situations that may occur in a laboratory setting. Using creative problem-solving methods, students will brainstorm and develop methods to falsify evidence, detect various types of altered evidence, and develop methods to challenge questionable evidence.[1]

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Education, Ethics, Applied Learning

B166 Supplementing Forensic Science Services With Research, Training, and Mentoring: Employing Quality Management Personnel to Meet an Organization's Continuous Education Program Goals

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After attending this presentation, attendees will have a reference model of quality management staff employed to manage continuous education programs in a forensic science organization.

This presentation will impact the forensic science community by providing knowledge of a system to meet the continuous education and scientific research needs of a combined medical examiner and crime laboratory institute.

With a focus on casework, backlogs, and turnaround times, many forensic science laboratories are unable to commit to the development of research projects or programs to mentor future forensic scientists. Time is a limiting factor to consider if an organization strives to fulfill an objective of maintaining a connection between forensic science practitioners and the broader scientific community.

The Harris County Institute of Forensic Sciences (HCIFS) is an integrated medical examiner office and crime laboratory located in Houston, TX. The HCIFS organizational structure includes an independent Quality Management Division, which oversees the multiple quality systems throughout the entire organization. Based on a strategic goal to establish an academic environment for training in forensic science, HCIFS created a full-time Training and Development Manager position that reports directly to the Quality Director. HCIFS demonstrates dedication to diversity in the education of current practitioners, future forensic scientists, and forensic pathologists, while fostering a research culture through a variety of programs and also maintaining seven accreditations, including two dedicated to the education and training of forensic pathologists. The Training and Development Manager coordinates the activities in each of these programs and assesses training needs, organizes and administers professional development opportunities, coordinates training and research programs, and creates training programs to support the continuing education of the large percentage of staff who hold a certification in each of the diverse forensic disciplines.

Accredited by the Texas Medical Association for Continuing Medical Education (TMA-CME), HCIFS offers continuing medical education opportunities year-round, specifically designed to meet the unique knowledge, competence, and performance needs of forensic pathologists. HCIFS trains up to two forensic pathologists per year through their comprehensive and rigorous forensic pathology fellowship program, which is accredited by the Accreditation Council for Graduate Medical Education (ACGME). Post-doctoral fellowship programs designed for advanced research and method development are maintained in three additional disciplines at the Institute — forensic anthropology, forensic genetics, and forensic toxicology. Additionally, medical students and medical residents can participate in a rotation through the forensic pathology division for education and training.

HCIFS offers an annual summer internship program through which students from United States academic institutions can apply to be an intern in a variety of different disciplines. Internship opportunities are offered with each of the forensic disciplines in the Institute, including: forensic anthropology, forensic entomology, forensic toxicology, drug chemistry, forensic genetics, trace evidence, firearms identification, forensic investigations, forensic emergency management, forensic imaging, and histology. The Institute also offers internship opportunities in the administrative branch, such as systems support and finance. The summer internship program offers a structured program where students apply their scientific knowledge to a structured research project, provide presentations of the results of those projects to HCIFS management and staff, and participate in professional development. Year-round internship opportunities are maintained through partnerships with local universities in the victim's assistance section and the histology laboratory.

A unique opportunity for international medical examiners and forensic science practitioners is also available at HCIFS. Practitioners in the field of forensic science affiliated with academic or governmental institutions outside of the United States can apply to observe HCIFS methods and procedures as an information-sharing partnership.

The HCIFS hosts an annual "Topics in Forensic Sciences" (TIFS) conference. The TIFS conference is a multidisciplinary forum for information sharing with the medical, forensic science, legal, and law enforcement communities. During the TIFS conference, research is presented, procedures are illustrated, and best practices in methods are discussed.

The HCIFS supplements forensic science services with comprehensive training and maintains connection with the broader scientific community. By employing a Training and Development Manager in the Quality Management Division, the organization is able to maintain and coordinate training programs, research projects, and mentoring programs while complying with the standards set forth by multiple accreditation bodies.

Continuous Education, Quality Management, Training Programs

B167 Validation of a Portable Direct Analysis in Real-Time Mass Spectrometry (DART®-MS) System for Trace Explosives Detection in the Laboratory or in the Field

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The goal of this presentation is to provide performance characteristics of a portable DART®-MS system for the rapid analysis of trace explosives.

This presentation will impact the forensic science community by providing a validation framework for the use of a portable DART®-MS system in the detection of trace explosives and smokeless powders.

The detection and identification of trace explosives is critical to counterterrorism efforts as well as the forensic analysis of post-blast residues. While techniques such as Ion Mobility Spectrometry (IMS) are capable of rapidly screening for explosives with high sensitivity, benchtop instruments with a mass spectrometer detector are preferred due to their increased specificity. Forensic science practitioners are hopeful that the recent advances in the field of Ambient Ionization Mass Spectrometry (AIMS) will provide analysts with a robust analytical solution to meet the demands of both the laboratory and the field. AIMS techniques allow for the direct analysis of samples, or surfaces containing residues, without the need for sample preparation, extraction, and/or separation, thereby increasing sample throughput and reducing backlogs issues. The most popular and promising ambient MS technique for forensic applications is DART®-MS. Forensic laboratories around the country have already applied DART®-MS to the analysis of forensic evidence such as illicit drugs, pharmaceuticals, lotions and lubricants, and chemical warfare agents. Ionsense® (in collaboration with Waters Corporation) has recently released the DART® QDa, a mobile miniaturized mass spectrometer with a DART® source. This type of instrument has the potential to improve routine forensic analyses in the laboratory as well as be a reliable tool for first responders in the field by providing rapid and accurate detection.

Before a novel analytical technique can be fully adopted by forensic laboratories for casework, a thorough validation study must be completed to establish performance characteristics. This work was completed to address many of the components required for a thorough validation study. Initial studies utilized a Design Of Experiments (DOE) to identify the parameters most influential to method optimization for the analysis of mixtures, real-world samples, and unknowns. To accomplish this, two separate studies were completed, one examining source/desorption effects and one examining mass spectrometer effects. In both instances, a two-level full factorial DOE was implemented. Results identified the relative impact that instrument parameters, such as thermal desorber temperature, DART® ionization gas, and DART® grid voltage, have on explosive response.

After completion of the DOE studies, analytical figures of merit such as Limit Of Detection (LOD), linear dynamic range, repeatability, and reproducibility were determined. In order to accurately obtain these figures, inkjet printed standards were used. Inkjet printing technology allows for the precise deposition of traces of material on a surface with great reproducibility (<1% Relative Standard Deviation (RSD)).

Explosives studied included military explosives (RDX, TNT, PETN), peroxide-based (TATP, HMTD), inorganic explosives (AN, PC), and smokeless powder components (ethyl centralite, diphenylamine, dibutyl phthalate, nitroglycerin, and n-nitrosodiphenylamine). These compounds were analyzed both as pure compounds and in complex mixtures to better understand competitive ionization effects. For most explosives, performance was comparable to laboratory-based DART®-MS systems, with LODs in the sub-nanogram range, and minimal interferences from complex matrices.

Explosives, DART®-MS, Smokeless Powders

B168 Chemical Imaging of Cyanoacrylate-Fumed Fingerprints Using Mass Spectrometry Imaging

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After attending this presentation, attendees will understand how mass spectrometry imaging is compatible with some current forensic practices, such as cyanoacrylate fuming, and could therefore be a valuable technique in the forensic field.

This presentation will impact the forensic science community by drawing attention to compatibility issues that are inhibiting the adoption of new technologies. In addition, new insight into the polymerization mechanism of cyanoacrylate fuming is presented.

Introduction: Chemical analysis of fingerprints using mass spectrometry is a method for gaining valuable information about potential suspects. In order for the technique to be realistically integrated into an investigative procedure, it must be compatible with current forensic techniques. As much of current practices are focused on visualization of fingerprint ridges for a database match, procedures to enhance the quality of the fingerprint are common. Cyanoacrylate fuming is one such technique in which latent fingerprints are exposed to gaseous cyanoacrylate, forming a visible polymer on the surface of fingerprint ridges. In this work, the compatibility of cyanoacrylate fuming and Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS) is investigated as well as the cyanoacrylate polymerization mechanism.

Methods: Several fingerprint samples were prepared both with and without development by cyanoacrylate fuming and with different matrices for MALDI-MS. They were analyzed using a MALDI-linear ion trap-Orbitrap™ MS coupled to an Nd:YAG laser. Unique background peaks present in only the negative mode spectra of cyanoacrylate-fumed samples were further analyzed and structural information was obtained with Tandem Mass Spectrometry (MS/MS). Standard solutions of several common fingerprint compounds were used to study the polymerization reaction, including a triacylglycerol (glyceryl tripalmitate), several fatty acids (oleic, stearic, and palmitic acids), cholesterol, squalene, and an amino acid (isoleucine). The solutions were pipetted onto two slides, one of which was fumed with cyanoacrylate, while the other was not. The level of polymer formation was compared between different analytes.

Results: Dihydroxybenzoic Acid (DHB), α-Cyano-4-Hydroxycinnamic Acid (CHCA), iron oxide nanoparticles, and silver nanoparticles were tested as matrices, which are necessary to absorb the laser energy and desorb/ionize analytes. All endogenous compounds were still present at their expected masses with no significant change in their signals. The images of endogenous compounds in the fumed fingerprints were of equal quality to the images from the non-fumed fingerprints. Likewise, exogenous compounds appeared at their expected mass; however, one class of ionic compounds had suppressed signal in fumed fingerprints. Other groups have attempted similar analyses of fumed fingerprints on MALDI-Time Of Flight (TOF) without much success. It is proposed that the instrument used in this study, MALDI-linear ion trap-Orbitrap™, is superior to MALDI-TOF for MS imaging of cyanoacryalte-fumed fingerprints because there is minimal influence from electric field inhomogeneity on the MALDI plate surface.

Several peaks were observed in fumed fingerprints in negative mode that were not present in non-fumed fingerprints. These peaks were thought to be related to the cyanoacrylate polymer and were further analyzed by MS/MS. Based on the MS/MS as well as elemental composition from accurate mass, these peaks were determined to be cyanoacrylate dimer and trimer derivatives. A mechanism for their formation and polymerization is proposed based on their structure.

Varying amounts of polymer formed on the surface of different standard compounds. For example, some, such as triacylglycerols and squalene, had little-to-no polymer formation, while others, especially fatty acids, had significant polymer formation. This result indicates that fatty acids may play a more important role than other endogenous compounds in the polymerization process. In addition to the visual increase in cyanoacrylate polymer formation, signal intensities for the cyanoacrylate compounds also increased.

Conclusion: Cyanoacrylate fuming and MALDI-MSI of latent fingerprints are very compatible techniques. Important chemical information can be gained from fingerprints that have been cyanoacrylate fumed without sacrificing signal intensity. No compounds studied so far have been chemically modified by the fuming process, so data analysis is straight forward. In addition to the compatibility study, valuable insight into the mechanism for polymerization of cyanoacrylate on fingerprints was also achieved. Cyanoacrylate dimers and a trimer were identified in the mass spectra and the importance of fatty acids in the polymerization process was revealed.

Polymerization, Cyanoacrylate Fuming, Mass Spectrometry Imaging

B169 Lifestyle Determination From Chemical Identification in Fingerprints

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After attending this presentation, attendees will better understand Mass Spectral Imaging (MSI) applications to latent fingerprint analysis. Specifically, this presentation will focus on the importance of all exogenous compounds present in latent fingerprints.

This presentation will impact the forensic science community by improving understanding of the usefulness of MSI for the identification of chemical compounds in latent fingerprints. Most importantly, the exploitation of all exogenous compounds will allow the forensic science community to compile the lifestyle of an unknown fingerprint donor.

We are surrounded by chemicals, many of which are influenced by the lifestyle of an individual, and can be studied by metabolomics tools.[1,2] This lifestyle characterization approach can be extended to latent fingerprints. MSI has been applied to chemical imaging of fingerprints and visualizing endogenous and exogenous compounds. Previous work for exogenous compounds has focused on drugs and explosives, but little has been done to exploit other compounds that can be used as lifestyle markers.[3,4] The focus of this work was to compile potential lifestyle markers of a fingerprint and develop a method that can efficiently analyze a broad range of exogenous compounds.

Consumer products were applied per product instructions, and a fingerprint was then deposited on a glass slide pre-cleaned with methanol. Citrus fruits, alcohol, and food oil samples were touched to mimic consumption, a spill, or the handling of foodware before making a fingerprint. Gold and silver targets were sputter coated for matrix deposition at 20mA for ten and five seconds, respectively. A linear ion trap-Orbitrap™ mass spectrometer with a Matrix-Assisted Laser Desorption/Ionization (MALDI) ion source was coupled with a 355nm Nd:YAG laser. Imaging and profiling of fingerprint samples was conducted with a 100µm raster step, ten laser shots per raster step, and a 30µm laser spot size over the m/z range of 50-1,000. For multiplex imaging, a spiral raster step was employed for fragmentation of known precursor masses.[5] Positive and negative ion modes were employed for profiling, imaging, and multiplex imaging.

In the analysis of latent fingerprints containing bug spray and sunscreen, the variations in active ingredients allowed brand differentiation. The differences in the spectra of three bug spray brands were easily correlated to the active ingredient in each. While most sunscreens can be differentiated based on the active ingredient list, two brands containing the same active ingredients were isolated based on the relative abundances of each compound. The spectra of Coppertone® and Neutrogena® sunscreens were dominated by octocrylene, but avobenzone was only abundant in Neutrogena®. Octocrylene, avobenzone, and octinoxate proved to be key compounds in distinguishing sunscreen brands.

Human fingerprints naturally contain Triacylglycerols (TGs), secreted from sweat glands, but those from plants have distinct patterns of saturation on fatty acyl chains. As expected, three cooking oils and a vegetable spray revealed distinct TG patterns distinguishable from human TGs. The most abundant TG species in olive and canola oil is TG 54:3 as a sodiated adduct at *m/z* 907, and TG 54:4 at *m/z* 905 in sesame oil, which are present only in minimal abundance in natural fingerprints. Olive oil had a narrow unsaturation pattern, whereas sesame and canola oil exhibited very broad unsaturation patterns. Vegetable spray is easily identified in fingerprints due to multiple unique TGs and the presence of phosphatidylcholines.

Fingerprints contaminated with alcohol were commonly identified based on the presence of sugars, ethyl palmitate, ethyl myristate, and glycerol. The analysis of wine proved to be the most informative in negative mode, based on the presence of gallic, tartaric, succinic, malic, and galacturonic acids. Fingerprint spectra containing beer were dominated by various sugars, including a unique malt starch, from the malting and brewing process.

The chemical compounds in mandarins, lemons, and limes were explored. Citric acid was present in all three, but consistently present at higher relative abundance in the lemons and limes. Mandarin fingerprints also contained malic acid, naringenin, tangeretin, and nobiletin, which were not present at detectable levels in the lemons and limes.

This study proves that a broad range of exogenous compounds can be detected and confirmed in latent fingerprints using MSI. A multiplex imaging method was applied to the analysis of exogenous compounds in latent fingerprints. Each compound in the latent fingerprints was identified by accurate mass and Tandem Mass Spectrometry (MS/MS). The compilation of the detected exogenous compounds could lead to a lifestyle determination of the unknown fingerprints source.

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MALDI-MSI, Fingerprints, Lifestyle

B170 The Identification of Prohibited Treatments on Racing Tires by Solid Phase Microextraction (SPME)

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The goal of this presentation is to provide attendees with an understanding of SPME as applied to the detection of racing tire treatments.

This presentation will impact the forensic science community by highlighting the area of trace tire treatment analysis in automotive racing.

In the realm of automotive racing, many competitors will do whatever it takes to win a race. In several racing leagues, it is prohibited to alter tires in an attempt to increase the "grip" or ability of the tire to maintain contact with the track. The most common way that competitors will attempt to alter tires is by applying "tire treatments," mixtures of chemicals that either swell or soften the rubber to increase the surface area in contact with the track. Tire treatments are commercially available and are allowed in some racing leagues. The commercial treatments fall into two categories — "plasticized" and "petroleum distillate." Many of the products used in leagues that prohibit them are labeled as "undetectable," implying that they would not be found by testing post-race. Some competitors will use their own mixtures that are typically based on petroleum distillate products and can resemble or contain common ignitable liquids.

Tire samples provided by the United States Auto Club (USAC) were prepared for analysis by placing a cut piece of tire into a 20mL headspace vial. The sample is then heated to 40°C and volatiles are extracted using a Poly(DiMethyl)Siloxane (PDMS) SPME fiber for five minutes, followed by analysis using Gas Chromatography/Mass Spectrometry (GC/MS). Thus far, qualitative analyses have been sufficient to determine treated tires from untreated tires. The "plasticized" treatments typically give off compounds such as diethyl pentanedioate and 2-ethyl-1-hexanol. Tires treated with "petroleum distillate" products typically give off complex chromatograms that resemble those of common ignitable liquids. Tires have been seen to have been treated with gasoline as well as medium-heavy aromatics, among others.

Since the beginning of this project, 201 samples have been analyzed. Of those samples, 23 have been flagged as having been treated. A small number of those flagged appeared to have been soaked in gasoline. The minimally destructive nature of the testing has allowed repeat analyses of samples in certain instances. Unless rules change, detection of tire treatments will continue to be an area of interest. SPME is, and will continue to be, a powerful tool within this area of analysis.

This work was funded by Indiana University–Purdue University Indianapolis (IUPUI) and the USAC.

Solid Phase Microextraction, Tire Treatments, GC/MS

B171 Decreasing the Uncertainty of Peak Assignments Using Multidimensional Ultra-High Performance Liquid Chromatography (UHPLC)

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After attending this presentation, attendees will understand the potential for multidimensional UHPLC to significantly reduce peak assignment uncertainty based on retention time.

This presentation will impact the forensic science community by demonstrating the utility of multidimensional UHPLC as a separation technique for drug analysis. Screening and identification of emerging drugs can be achieved with significantly decreased uncertainty using multiple dimensions compared to one-dimensional LC. Identification can be accomplished through retention times obtained in the first and second dimensions.

Chromatographic techniques, which are commonly employed in forensic analysis, utilize retention time as an identification parameter. Conventional single-dimension chromatographic techniques, such as Gas Chromatography (GC) and LC, inherently lack the separation power required to resolve the multitude of combinations possible when analyzing emerging drugs. The use of multidimensional chromatography, which significantly increases the resolving power, is a viable means to increase the utility of retention time measurements for compound identification.

One-dimensional UHPLC separations were conducted on mixtures of controlled emerging drugs and mixtures of positional isomers of certain of these solutes for either synthetic cannabinoids, synthetic cathinones, or phenethylamines in order to determine the most orthogonal combination for multidimensional chromatography. The separations utilized several stationary phases for both Reversed Phase Chromatographic (RPC) separations and Hydrophilic Interaction Liquid Chromatographic (HILIC) separations. All separations utilized 2.1mm x 100mm or 2.1mm x 50mm columns with 1.7µm or 1.8µm particle sizes, with either a 10min acetonitrile or methanol gradient (hold up to 5min) with a pH 2.3 formic acid or ammonium bicarbonate pH 11.6 additive, or identical isocratic mobile phases containing acetonitrile and water with an ammonium formate additive for up to 5min HILIC separations. HILIC and high pH separations were only applicable to the basic cathinone and phenethylamine solutes. Peaks were identified through their obtained Ultraviolet (UV) and Mass Spectrometry (MS) spectra. The retention times obtained for each separation were used to determine correlation coefficients (R^2) for two columns, which were then used to determine the Neue selectivity factor (S^2)[1], a measure of the orthogonality for multidimensional chromatography. The peak capacity (n_c) was determined for each separation and the theoretical peak capacity ${}^1n_c * {}^2n_c$ for a multidimensional separation that assumes full coverage of the possible separation space, which is difficult to obtain in practice. Since the actual separation space can be approximated by the S^2 value, the actual peak capacity can be estimated by the following equation: ${}^{2D}[n_c]_{actual}={}^1n_c[1+S^2({}^2n_c-1)]$ with $[1+S^2({}^2n_c-1)]$, which represents the gain factor in going from a one-dimensional separation to a multidimensional separation. In this work, the actual multidimensional peak capacity was also used to measure peak assignment uncertainty. Based on the one-dimensional separations performed, it was determined that a combination of a C8 and a PFP column produced the highest S^2 values, and were thus more orthogonal, for both the controlled synthetic cannabinoids and the JWH-018 positional isomers. For the above column combination, the peak capacity for the controlled synthetic cannabinoids mixture showed an increase from 69 for one-dimensional LC to 3352 for multidimensional chromatography. The gain factor was determined to be approximately 50; thus, the peak assignment uncertainty was decreased by 50x with the use of multidimensional chromatography. Likewise, the JWH-018 positional isomers, which elute in a narrower separation space, showed a peak capacity increase from 5 to 53 and a gain factor of 10. For synthetic cathinones and phenethylamines, it was determined that the best combination would be a C8 and PFP column operated in the RPC and HILIC modes, respectively. For these mixtures of controlled substances, similar peak capacities as synthetic cannabinoids were obtained in the first dimension, with gain factors of nine and five for the synthetic cathinones and phenethylamine, respectively, in going from one-dimensional to two-dimensional separations. Similar to the synthetic cannabinoids, smaller gain factors were obtained for positional isomers of synthetic cathinones and phenethylamines.

Examples of multidimensional separations will be presented. Through the use of multidimensional UHPLC, uncertainty in peak assignments would be significantly reduced, leading to increased accuracy in the identification of seized drugs. The number of chromatographic runs performed can also be reduced as only one chromatographic system would need to be employed to obtain orthogonal separations.

This project was supported by an award from the National Institute of Justice, Office of Justice Programs, and the United States Department of Justice. The opinions, findings, and conclusions expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.

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Emerging Drugs, Multidimensional, Chromatography

B172 Assessing the Forensic Utility of a Silicone Rubber Passive Sampler for the Detection of Pollutants in Aqueous Environments

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The goals of this presentation are to introduce the field of environmental forensics to attendees, provide historical context of passive samplers, illustrate the advantages and disadvantages of silicone rubber as a sampling medium, and discuss these attributes as they relate to a forensic investigation. The findings of the silicone rubber assessment will be presented, and future characteristics that require additional experimentation will be discussed.

This presentation will impact the forensic science community by illustrating how improving the methodology used to detect organic pollutants in aqueous systems will directly benefit the National Enforcement Investigations Center, the forensic laboratory of the Environmental Protection Agency (EPA). By implementing a passive sampler system, the EPA can more efficiently investigate suspected criminal activity, monitor high-risk areas, and increase sample throughput while decreasing costs. These benefits can be extended to environmental regulatory laboratories as well. Most importantly, effectively prosecuting environmental crimes will prevent the continued circumvention of the law, protecting our aquatic ecosystems and primary sources of drinking water. This study seeks to equip investigators with a powerful tool to prosecute environmental crimes by conclusively determining the effectiveness of a silicone rubber sampler for detecting organic pollutants in fresh water systems.

Environmental forensic investigations often center around the illicit release of toxic chemicals into rivers, lakes, and streams. The nature of illegal dumping into these aqueous environments leads to the evidence quickly dissipating and vanishing beyond detection. Organic pollutants are easily transported over large distances through waterways, and due to their highly lipophilic nature, are readily absorbed by the ecosystem.[1] Currently, to determine the presence of a suspected pollutant, analysis begins with "grab" or "active" sampling. This method is performed by repeatedly collecting large amounts of water in glass jars over the course of a predesigned sampling period.[2] The disadvantages of this traditional method are numerous, presenting the forensic chemist with unique sampling challenges. A novel approach to environmental forensic sampling is required to adjust to the concealed nature of the crime. Passive samplers have the potential to fill this role. This study determined if silicone rubber is a sampling medium adequately suited to be utilized by environmental forensics as an investigative tool to uncover clandestine dumping of chemical pollutants into aqueous systems.

To assess the performance of the silicone rubber sheets, the sheets first needed to be prepared for deployment. This procedure was optimized in the initial phase of experimentation and was set at five 2½-hour wash steps. The first three washing steps were in a 1:1 mixture of ethyl acetate and hexane, and the final two were in 1:1 mixture of ethyl acetate and methanol. This washing method was developed by O'Connell et al.[3] Once the commercial-grade silicone rubber was cleaned, it was ready for deployment. Initially, the sheets were tested in a controlled laboratory setting. A known amount of 83 target compounds was added to three liters of deionized water. After a predetermined amount of time, either one day, three days, five days, or seven days, the sheets were removed. Following deployment, the sheets were extracted by Soxhlet for eight hours in a 1:2 mixture of acetonitrile and methanol. This extraction was then solvent-exchanged to methylene chloride and blown down to one milliliter with a nitrogen stream. This condensed extract was analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). Following successful laboratory testing, the silicone rubber sheets were field tested in nearby mountain streams and in the Denver, CO, Metro Wastewater's effluent system to determine resistance to environmental factors. The sheets withstood this harsher testing scenario.

The silicone rubber sampler absorbed a wide array of semi-volatile analytes commonly targeted in environmental analysis during laboratory testing. In field testing, the silicone rubber sampler was also capable of absorbing a range of compounds, including pesticides, polycyclic aromatic hydrocarbons, and various other compound classes at estimated concentration levels below the levels typically observed in environmental investigations. This preliminary testing is encouraging and suggests the silicone rubber sample is well suited for forensic use in investigating environmental crimes.

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2. Namiesnik J., Zabigala B., Kot-Wasik A., Partyka M., Wasik A. Passive Sampling and/or Extraction Techniques in Environmental Analysis: A Review. *Anal Bioanal Chem*. 2005; 381: 279–301.
3. O'Connell S., Kincl L., Anderson K. Silicone Wristbands as Personal Passive Samplers. *Environmental Science & Technology*. 2014; 48(6): 3327-3335.

Environmental Forensics, Semi-Volatile Organic Compound, Passive Sampler

B173 An Investigation of the Correlation Between Human Age and Aspartic Acid and Asparagine Racemization and Isomerization of the Eye Lens Crystallins Proteins

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After attending this presentation, attendees will be able to recognize the use of proteomics in the age estimation of human eye lens proteins using high-resolution nano-liquid chromatography (nanoLC) in conjunction with high-resolution high-mass accuracy tandem mass spectrometry. Moreover, attendees will understand the mechanisms of the post-translational modifications (racemization and isomerization) of eye lens proteins and how these modifications can be used as potential biomarkers of aging.

This presentation will impact the forensic science community by demonstrating another method for determining the age of an individual at the time of death.

Isomerization of aspartic acids and asparagines results in the formation of several optical and structural isomers, such as the conversion of L-α-Asp to L-β-Asp, D-α-Asp, and D-β-Asp via succinimide intermediate. Identification of these complex isomers requires a high-resolution separation technique to differentiate between these optical and structural isomers of the tryptic peptides. Since these isomers all have the same *m/z*, their identification requires the use of synthetic peptides. Eye lens samples from 40 patients have been obtained and 28 of them have been analyzed by high-resolution nanoLC in conjunction with a high-resolution mass spectrometer. The samples include both male and female ranging in age from 45 years old to 87 years old. Eye lens proteins were extracted based on their solubility in water and 8M urea solution. The proteins were digested with trypsin and analyzed using a 50cm-long nanoLC column with 0.75µm ID.

To identify and quantify the tryptic isomers, three synthetic peptides (TVLDSGISEVR, IQTGLDATHAER, and DVTIQHPWFK) were prepared and analyzed. Baseline separation of the isomers for each of the peptides were achieved for both the synthetic peptides and for the tryptic digests of the lens samples. These isomers were identified by comparing their retention order with the synthetically prepared peptides containing the four isomers. Preliminary data demonstrated the ability to use high-resolution separation for the identification of aspartic acid and asparagine isomerization. Analysis of eye lens crystallins from individuals with varying ages indicated significant differences in relative intensities of the four isomers for these peptides. Work is underway to correlate the relative intensities of these isomers to the age of the individuals. This study will be expanded to include other tryptic peptides containing single aspartic acids or asparagine.

Human Age Estimation, Eye Lens Proteins Isomerization, High Resolution Proteomics

B174 Experimental Approaches to Study the Degradation of Messenger RNA (mRNA) in Dried Bloodstains Subjected to Storage in the Laboratory

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The goals of this presentation are: (1) to help define the useful roles for the molecular analysis of RNA in evidentiary body fluid stains; (2) to help attendees understand the role of stochastic effects on quantitation of RNA species; and, (3) to educate attendees regarding the molecular mechanism by which RNA degrades once outside the environment of the body.

This presentation will impact the forensic science community by explaining that, when a criminal act is discovered, the role of the investigative process is to answer the "who," "what," "where," and "when" of the crime. Of these questions, the "when" is often the most difficult to pinpoint, especially in identifying how long a sample has been at the scene or when someone has died.

Studies on the degradation of transcripts in dried body fluid stains hold potential for correlating transcript disappearance with the passage of time. If such a relationship could be established confidently, RNA degradation could be used to estimate the length of time since deposition of a body fluid at a crime scene or help estimate the time of death. Quantitative Polymerase Chain Reaction (qPCR) assays directed against one transcript whose abundance changes with time and a second that is stable presumably allows for normalization of results from different qPCR reactions and the delta Ct results (i.e., Ct for changing transcript minus Ct for the stable transcript) can be plotted as a standard curve relating transcript degradation with time. Transcripts from housekeeping genes as well as ribosomal RNA (rRNA) species have served the role of the "stable transcript" for comparative purposes; however, housekeeping transcripts and rRNA also slowly disappear over the time course and, moreover, stochastic effects that manifest in results from one or the other, or both, transcripts during reverse transcription and subsequent qPCR amplification cause unacceptable levels of variability in the quantities of all but the most abundant transcripts.

RNA sequencing data, produced using Ion Torrent™ Next Generation Sequencing (NGS) technology, from the transcriptomes of dried body fluid stains stored for varying periods of time identified collections of transcripts that disappeared from RNA-seq data with differing rates and also revealed the sequencing read depth of nucleotides in the 5' ends of numerous transcripts decreased from sequencing results faster than the read depth for nucleotides at the 3' end.[1] This observation raised questions of how mRNA transcripts degrade in dried body fluid stains and suggested that assessing transcript degradation based on the apparent differential disappearance of the 5' and 3' ends of **a single transcript** would allow for the results to be expressed as ΔCt (i.e., 5' Ct minus 3' Ct) and also perhaps minimize stochastic effects on data reproducibility, thereby improving the overall reliability of time estimates using the transcript degradation curves. Thus, rather than comparing Ct values produced during qPCR for two different transcripts (either or both susceptible to stochastic effects during reverse transcription), this method quantifies the opposite ends of a single transcript. Reported here are findings of this approach with several transcripts present in bloodstains that were aged at room temperature for periods of up to two years. Results suggest that mRNA in dried bloodstains degrades from the 5' end of the transcript and the difference in amplicon abundance from the 5' and 3' ends of several transcripts produces degradation curves that are more consistent and reproducible and thus may more accurately estimate the age of the stain.

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RNA Degradation, RNA Quantitation, Sample Age

B175 The Characterization and Persistence of Vaginal Bacteria Under Fingernails

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The goal of this presentation is to inform attendees regarding the usefulness of bacterial flora in determining the biological source for vaginal contact in sexual assault investigations.

This presentation will impact the forensic science community by demonstrating that bacterial flora could be characterized according to their genera and evaluated to determine if there was vaginal contact in sexual assault investigations, which could provide more weight to sexual assault biological evidence.

For sexual assault cases involving digital penetration, probative DNA evidence from the victim's vaginal fluid could be under the suspect's fingernails. This type of DNA evidence can establish a direct link from suspect to the victim; however, the suspect could argue that the accumulation of the victim's DNA under the suspect's fingernails was due to casual or daily contact with the victim. A solution to this problem is to identify the body fluid as vaginal fluid using the presence of *Lactobacillus* bacteria species that are found natively in the vagina. Previous research has shown that it is possible to identify vaginal fluid using the 16-S ribosomal RNA (rRNA) gene of *L. crispatus, L. iners*, *L. gasseri,* and *L. jensenii.* Also, 16-S rRNA can be used to identify bacteria that are associated with a specific part of the human microbiome, such as the human skin. Resident flora that naturally occur on hands include *Staphylococcus*, *Proteus*, *Klebsiella*, and *Acinetobacter*. Further research has shown Next Generation Sequencing (NGS) technology can aid in 16-S rRNA sequencing and classifying bacteria associated with the vagina or skin. The goals of this project are: (1) to characterize the normal bacterial flora found underneath fingernails, in the vagina, and underneath fingernails following digital penetration; and, (2) to study the persistence of vaginal bacteria underneath fingernails following digital penetration.

In this study, 80 samples were collected from four couples (AAAB, BABB, CACB, and DADB) at designated time points (baseline, 0hr, 6hr, 12hr, 18hr, and 24hr) from underneath fingernails after digital penetration. The DNA was extracted, then the 16-S rRNA gene was targeted and amplified. The samples were then analyzed by NGS. A statistically significant difference in *Lactobacillus* frequencies when comparing source (experimental vs. control) was observed using a paired *t*-test (p=<0.0001). Higher frequencies of *Lactobacillus* were observed in experimental samples ranging from 0%-99.6% with a mean of 63.8%. In two couples, AAAB and CACB, the *Lactobacillus* populations were on average 6-11 times higher in experimental samples than control samples. The results were replicated in couple CACB and the experimental samples from that couple were predominately *Lactobacillus* even up to 24 hours ranging from 63.2%-99.6%. Also, a statistically significant difference in *Staphylococcus* frequencies when comparing source (experimental vs. control) was observed using a paired *t*-test (p=0.0046). Higher frequencies of *Staphylococcus* were observed in control samples ranging from 0%-97% with a mean of 41.9%. In two other couples, conversions from a predominately *Lactobacillus* population to predominately *Staphylococcus* population were observed after 6-12 hours.

Based on the results of this small study, it is possible to detect *Lactobacillus* at high frequencies after 24 hours; therefore, *Lactobacillus* could be used a biomarker to determine vaginal contact since a *Lactobacillus* population of a minimum frequency of 27% can suggest vaginal contact; however, a failure to detect *Lactobacillus* does not indicate the absence of prior vaginal contact.

Vaginal Bacteria, Sexual Assault Investigation, Next Generation Sequencing

B176 A Molecular Assessment of DNA Methylation Profiling For Body Fluid Identification in a Forensic Application

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After attending this presentation, attendees will better understand the use of epigenetics and methylated DNA in forensic practice as a tool for the identification of different biological fluids (blood, saliva, semen, and menstrual blood).

This presentation will provide navigation through the roles of epigenetics and DNA methylation in forensic applications and illuminate their uses in the identification of different bio-fluids. This presentation will impact the forensic science community by offering new epigenetic markers that are proven to be of value in this area and will help forensic science practitioners use a simple tool for the identification of blood, saliva, semen, and menstrual blood in different age and gender groups.

Background: Since 1985, when DNA analysis was applied to solving forensic problems, its foremost applications have included criminal investigation, personal identification, and paternity testing. DNA methylation is emerging as an attractive marker in forensic genetics that can provide investigative leads to help solve crimes. Natural roles of DNA methylation in mammalian systems include imprinting, X chromosome inactivation, heterochromatin maintenance, developmental controls, and tissue-specific expression controls.

DNA methylation plays a critical role in normal cellular processes and serves as a mechanism that turns off gene expression. Aberration in DNA methylation has long been linked to diseases such as cancer and is exploited as diagnostic biomarkers, but in forensic analysis, the use of DNA methylation as a tool is still in its infancy; however, there are many advantages to using DNA methylation as a forensic tool, with one of the most important being the stability of the marker, unlike protein or RNA markers that are quickly degraded, thus allowing for quantitative analysis of older samples.

Forensic applications of DNA methylation include: (1) the identification of body fluids; (2) differentiating Monozygotic (MZ) twins; (3) DNA methylation in age estimation; (4) the determination of paternal origin of allele; (5) the determination of cause and circumstances of death; (6) sex determination; and, (7) the authentication of DNA samples.

Goal: This study will explore the different applications of DNA methylation in forensic science with reference to the results of a study on Egyptian DNA methylation profiles of four markers (LINE-1, MT2A, MGMT, and FGF7) in blood, saliva, semen, and menstrual blood in attempt to assess the potential of those markers for identification.

Methodology: For this purpose, 52 samples from female participants and a similar number of samples from male participants were gathered. To explore age variation, each sample was divided into two groups of 26 each, one group more than 30 years of age and the other less than 30 years of age. Each sample was subjected to the following procedures: DNA extraction and amplification of the collected samples, then a bisulfite treatment of the amplified DNA samples. The bisulfate-modified DNA was used as a template for fluorescence quantitative Polymerase Chain Reaction (qPCR) assessment.

Results and Conclusion: The MGMT locus exhibited a differential methylation pattern in blood compared to semen, saliva, and menstrual blood. Therefore, the MGMT marker exhibited significant differences in its methylation patterns for the identification of blood when compared to the other fluids. MT2A was presumed to show a differential methylation pattern in saliva as it displays hyper-methylation state, but hyper-methylation is also seen in semen. No significant difference was seen between menstrual blood and other body fluids. These factors render MT2A not useful in differentiating between body fluids. The FGF7 marker displayed a differential methylation pattern in semen. Methylation values were greater in semen relative to blood, saliva, and menstrual blood. The methylation profile of LINE-1 successfully differentiated saliva from the other three examined biofluids. This marker displayed hyper-methylation in all saliva samples and hypo-methylation in all blood, semen, and menstrual blood samples. Regarding the use of these markers in differentiating between males and females, as well as between the different age groups (less than and more than 30 years of age), highly statistically significant differences were obtained.

DNA Methylation, Biological Fluid, Identification

B177 Can Detection of Testosterone With Anti-Testosterone Antibody Be Used to Identify Male Cells?

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After attending this presentation, attendees will better understand the potential of targeting testosterone to presumptively identify male cells on evidence items and at crime scenes, as well as its potential use for cell separation techniques prior to DNA profiling.

This presentation will impact the forensic science community by introducing a novel screening method for male cells that has the ability to decrease the time spent examining evidence items in the lab and, thus, have a potentially significant impact on casework backlogs.

Currently, research on fluorescent molecules as a screening tool for sex-specific cells has been primarily limited to sperm cell antigens and to Y-chromosome DNA (e.g., Fluorescent *In Situ* Hybridization (FISH)). Given that testosterone is normally present in most non-sperm cell types of males and is at approximately ten times the level of that found in females, it is a promising candidate probe molecule for identifying male cells. Therefore, the goal of this study is to test for the preferential labeling of male versus female cells using fluorescently-tagged anti-testosterone antibodies. The development of a potential screening tool to identify male cells in a biological mixture is the ultimate goal. Initial tests of several fluorescent reporter molecules with common laboratory alternative light sources showed the green CF514 dye (excitation and emission wavelengths, 516nm and 548nm, respectively) was clearly visible up to a 1:10,000 dilution. Based on these findings, an anti-testosterone antibody conjugated to a CF514 dye was investigated along with a Fluorescein Isothiocyanate (FITC) -tagged anti-testosterone antibody (excitation and emission wavelengths, 495nm and 519nm, respectively).

CF514-linked antibody hybridization was performed with buccal and epithelial skin cells of both male and female origin. Male and female buccal cells displayed no clear differences in fluorescence intensity when viewed with a fluorescence microscope after incubation with the anti-testosterone antibody. Epithelial skin cells were then tested because they are a known target tissue of testosterone action. Pressure was applied when retrieving the skin cells to ensure that not just the most outer layer of keratinized cells was collected. An aliquot of the epithelial cells was removed, the DNA extracted and quantitated, and the presence of DNA detected, suggesting the possible presence of deeper epidermal cells.

Due to this finding, antibody hybridization was performed with the epithelial skin cell samples fixed to glass slides. Slides that were dried after the antibody incubation without a coverslip displayed a visible difference in fluorescence between male and female cells and compared to the negative control; however, slides that were hydrated with water after antibody incubation displayed no noticeable difference in fluorescence between sexes. Antibody specificity testing with purified testosterone and estradiol indicated that CF514-linked and FITC-linked anti-testosterone antibodies preferentially bind to testosterone. Therefore, the difference between dry and hydrated cell labeling may be due to other molecular or optical dynamics, which might appear as fluorescence in the hydrated cell samples.

Testosterone may be successful in identifying and resolving male components in forensic casework; however, testing with a more sensitive instrument, such as a confocal laser scanning microscope, may prove to be more informative. Moreover, samples may need to be incubated with a reaction mix that contains multiple antibodies in order to enhance the fluorescent signal and assessed using the Fluorescence Activated Cell Sorter (FACS).

Anti-Testosterone Antibody, Male Cell Screening, Fluorescence Microscopy

B178 Toward Implementation of Improved Body Fluid Identification Methods

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After attending this presentation, attendees will better understand the considerations involved in evaluating various novel body fluid identification methods for implementation into the forensic biology workflow. Attendees will be aware of the pitfalls of current serology practice and the benefits of several emerging methods while gaining insight into the challenges associated with operationalizing techniques for which few guidelines exist.

This presentation will impact the forensic science community by highlighting the results of at least three collaborative research efforts that resulted in methods to improve body fluid identification. In addition, this presentation will impact the field by: (1) describing the hurdles that must still be addressed for these methods to be used routinely; (2) proposing potential solutions; and, (3) prompting collaborative progress toward the establishment of guidelines and standard samples for their use.

While DNA technologies have advanced substantially over the past several decades, methods used to determine the presence of a particular body fluid have remained stagnant. Outdated techniques limit testing to three fluids — semen, blood, and saliva — via chemical, immunological, or histological methods. Though generally robust and easy to perform, these methods require a separate test for each fluid, have limited sensitivity, and consume a portion of the biological sample. Often, multiple sequential tests are used in combination to confirm the presence of a fluid, further adding to overall time, cost, and sample consumption. Additionally, known false positives and subjective interpretations hinder the definitiveness of an examiner's conclusions and testimony.

To this end, several emerging methods for body fluid identification are being explored at the Defense Forensic Science Center (DFSC) that could complement or replace these traditional techniques. These new methods simultaneously test for biological markers or signatures that indicate the presence of multiple body fluids, including two for which laboratories cannot routinely test at present: vaginal fluid and menstrual blood. Advanced reporting offers quantifiable results that can be reviewed by other examiners prior to court testimony as part of routine quality assurance measures. Initial results indicate a false positive rate for at least one of these methods of less than 1%.

Despite these benefits, integration into existing laboratory workflows is not straightforward. Additional instrumentation may be required, and an increase in processing time might drive decisions on how best to use the additional information provided by these methods. Though current guidelines exist for the validation of traditional serological and DNA techniques, these methods have not yet been operationalized in United States laboratories, and standard reference samples that allow both meaningful evaluation and subsequent quality control procedures do not exist. Potential strategies to begin addressing these concerns will be described and discussed.

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Serology, Body Fluid Identification, mRNA

B179 Victim Sexual Assault Evidence Kits (SAEKs) — Teamwork Between a Crime Lab, Special Victim's Unit, and District Attorney's Office

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After attending this presentation, attendees will better understand the impact of analyzing all victim SAEKs in one jurisdiction.

This presentation will impact the forensic science community by providing data on how to approach the analysis of victim SAEKs to obtain viable DNA typing data, the use of the analytical results by the Special Victim's Unit (SVU) investigators, and the prosecution outcome.

When California passed the "Sexual Assault Victims' DNA Bill of Rights" in 2003; it was the first law of its kind in the nation. The bill addresses the importance of timely DNA analysis of sexual assault evidence. On January 1, 2016, an amendment to the Section 680 of the California Penal Code encourages law enforcement agencies to submit sexual assault forensic evidence to the crime lab within 20 days after it is booked into evidence. The bill also encourages the crime lab to process that evidence, create DNA profiles when able, and upload qualifying DNA profiles into the Combined DNA Index System (CODIS) within 120 days after initially receiving the evidence.

In May 2014, the Oakland Police Department (OPD) Criminalistics Laboratory developed a plan for analyzing all victim SAEKs as soon as practicable. The laboratory defined this plan as the Contemporary Victim Kit Program. The Program goals are to analyze all victim SAEKs, enter eligible DNA profiles into CODIS within ten business days, and provide reports to the investigators within 20 business days. This Program requires the support of the county hospital Sexual Assault Response Team (SART), OPD investigators, OPD property room staff, Forensic Biology Unit (FBU) scientists, and Alameda County District Attorneys (DA). The Program includes the analysis of all evidence within the SAEK, including clothing. As such, the Program is not considered a Rapid Turnaround DNA program as defined in Assembly Bill (AB) 1517.

The FBU tracks victims' SAEKs submitted to the OPD Property and Evidence Unit weekly. The FBU then communicates to the SVU a list of submitted kits for the week. SVU investigators determine which victim SAEKs are eligible for analysis. Currently, all kits will be examined except those in which the crime occurred in a different jurisdiction or a crime has not been committed.

Currently, the FBU has enrolled 571 victim SAEKs into the Program. The FBU analyzed more than 550 victim SAEKs and, when present, the 95 corresponding suspect SAEKs. Most of the CODIS-eligible DNA profiles originated from the vaginal swab; however, eligible profiles were obtained from oral contact sites, clothing, and other orifice swabs.

The FBU, SVU investigations, and DAs evaluated the efficacy of testing and charging all victims' SAEKs. A subset of the analyzed cases revealed approximately 56% of the victim SAEKs contained CODIS-eligible DNA profiles of which 38% resulted in a named individual association. In addition, a small percentage of cases resulted in an association of one assailant to more than one victim (serial assailant).

Approximately 29% of the analyzed victim SAEKs were submitted to DA's office for charging purposes. The prosecution status of this subset falls into four categories: charged, convicted, requires additional investigation, or will not be charged. The remaining 71% cases not submitted to the DA's office are either under OPD SVU investigation or did not have a CODIS-eligible DNA profile.

This data illustrates the collaboration of three entities to ensure all viable victim SAEKs are analyzed, investigated, and prosecuted in a timely fashion, thus providing justice to survivors, creating safer communities, and convicting serial rapists. The OPD Contemporary Victim Sexual Assault Kit Program will prevent future backlogs by proactively testing kits in a timely manner.

SAEK, Contemporary, DNA

B180 The Mapping of Drug Users in Philadelphia, PA, by Creating DNA Profiles From Trace DNA Evidence Collected From Drug Paraphernalia

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After attending this presentation, attendees will recognize the potential of trace DNA evidence on syringes that were used to inject illegal drugs. Attendees will also learn about the drug epidemic issue in Philadelphia, PA, which is of great concern to the forensic and public health communities.

This presentation will impact the forensic science community by demonstrating a possibility of distinguishing homicide from suicide in drug overdose cases.

In Pennsylvania in 2015, there were more than 2,000 reported deaths due to drug overdose.[1] These drug overdose deaths are usually considered accidental, or, in some cases, the providers or the drug dealers are charged with murder. In a case in which a second party administers a drug to a user that causes an overdose, the case is closed using circumstantial evidence or confessions from the second party. Many times, there may not be a second party found who will confess to the administration of a drug, causing the case to be deemed accidental. Charging the second party with murder seems to be a gray area in the legal community because, most of the time, the second party is an addict himself/herself without any malicious intent and most likely was not in his/her right mind when injecting the drug; however, in a case in which malicious intent is present, DNA evidence may prove to be valuable. This presentation focuses on the benefits of performing DNA analysis on syringes used to inject drugs.

Improperly discarded syringes were collected from the streets of Philadelphia. These syringes were then brought to the laboratory for DNA collection. Two areas of the syringe were swabbed for DNA extraction. The first area swabbed was the tip of the needle, as it should contain blood, which can allow development of the user's DNA profile. The second swabbed area was the barrel and plunger of the syringe. If the DNA profile obtained from this area is consistent to the one from the needle of the syringe, then the drug was likely self-administered. If the profiles are inconsistent, the drug may have been administered with assistance of a second party. Finally, on a map of Philadelphia, a correlation was made between the area where the syringes were collected and the profiles obtained. This was conducted to see if a particular area is home to many drug users or only a few users. If various profiles are seen on more than one syringe it can be hypothesized that users go to this area to get help from a second party to inject their drugs.

Reference(s):

1. Metropolitan's Death Statistics. *Science.* 126.3266 (1957): 201-02. Report on Overdose Death Statistics 2015. Web.

Trace DNA, Drug Overdose, Homicide/Suicide

B181 The Complexities of Interpreting DNA Evidence Obtained in an Uncontrolled Social Setting

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The goals of this presentation are: (1) to demonstrate how DNA transfer events may impact the analyst's understanding of the DNA evidence at the crime scene; and, (2) to illustrate the complexity in predicting handling order and mode of transfer (primary, secondary, or tertiary) in an uncontrolled social setting situation.

This presentation will impact the forensic science community by adding to a growing body of knowledge regarding transfer DNA.

Recent advances in DNA typing technology have allowed for the detection of DNA transferred to various everyday objects. As sensitivity in commercial Short Tandem Repeat (STR) kits increases, so does the potential for detecting and amplifying extraneous DNA. A thorough understanding of DNA transfer, including both the active and passive processes that can lead to DNA deposition as well as the various variables that can impact DNA transfer, can assist in the interpretation and explanation of DNA found on evidentiary items. As forensic DNA laboratories continue to see a rise in the number of transfer DNA samples submitted for analysis, studies of transfer DNA that aim to identify the limits of DNA transfer, that investigate the multitude of variables that impact transfer, and that further understanding regarding DNA data interpretation in various real-world settings add to expanding scholarship that can benefit the forensic science field.

This study expands upon an investigation of DNA transfer in a social setting conducted by Goray and van Oorschot.[1] Four participants handled previously sterilized plastic objects (a communal jug and four cups) with their dominant hand to simulate a social gathering involving beverages. The order in which participants handled each object was recorded to test the following null hypotheses: (1) secondary DNA transfer will not occur and will not be detected on dominant hands; (2) tertiary DNA transfer will not occur and will not be detected on the cups; (3) a mixed DNA profile from all participants will not be found on the jug handle; and, (4) the order of handling the objects cannot be discerned from the DNA data. Each of the objects and the dominant hands of the participants were swabbed to test for evidence of primary, secondary, and tertiary DNA transfer. The samples were amplified with the GlobalFiler™ Polymerase Chain Reaction (PCR) Amplification Kit and analyzed on an Applied Biosystems® 3130xl genetic analyzer. The Mixture Analysis Tool within GeneMapper® ID-X version 1.5 was utilized to facilitate data interpretation.

DNA was detected in 41 of the 45 samples (92%) collected in this study. Only 15 samples produced profiles that were suitable for comparison utilizing the laboratory's current interpretation guidelines. Of these, 80% were mixtures containing DNA from two or more individuals and 60% had identifiable major and minor contributors. In the set of samples taken from an individual's hand, where a major profile could be identified, the major profile was consistent with the individual. In the set of samples collected from objects, there was no discernable correlation between the profiles detected and the timing or the length of contact with the object. In other words, the major profile detected did not consistently correlate with either the last person to touch the object or the individual who maintained contact with the object for the longest period of time.

These results illustrate the complex nature of interpreting DNA transfer in a social setting. In this study, the objects were pre-sterilized, the order of handlers and length of contact was recorded, and the handlers' profiles were known; however, the order of handling could not be reconstructed from the DNA profile information. This study demonstrates the ease of DNA transfer in social settings, illustrates the difficulty in predicting mode of DNA transfer based on the DNA typing results, and highlights the difficulty in interpreting the genetic data when multiple transfer events have occurred.

Reference(s):

1. Mariya Goray and Roland A.H. Van Oorschot. DNA transfer during social interactions. *Forensic Science International: Genetics Supplement Series 4*. No. 1, (2013) doi:10.1016/j.fsigss.2013.10.052.

Forensic Science, Transfer DNA, DNA Mixtures

B182 Practical Ways to Address Cognitive Bias in Forensic DNA Decision Making

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After attending this presentation, attendees will better understand the impacts of cognitive bias and balanced approaches that may be employed to reduce their effect in the context of a forensic DNA workflow.

This presentation will impact the forensic science community by allowing attendees to consider the possible effects of cognitive bias in forensic DNA testing and by providing a roadmap of general pathways to address the influences of cognitive bias on forensic decision making.

Cognitive bias, an inherent part of human intelligence, may influence decision-making and even the workflow processes of individuals and their laboratories. Seven sources of bias have been identified that may affect forensic decision making; they are related to human nature, environment, culture, and experience, as well as case-specific information. While bias in itself may not cause error, the outcome of cognitive bias, if not monitored and addressed effectively, is that analyses and interpretations may be compromised. Nevertheless, practitioners that are unaware of their inherent bias have generated reliable interpretations of DNA results; however, there also are well-documented examples in which bias has indeed negatively impacted decision making and the interpretation of forensic DNA analyses, such as working and reasoning backwards, from the suspect to the evidence (i.e., fitting the DNA profile from the known reference sample to the DNA evidence profile(s)). Through education and training, forensic scientists can be better prepared to reduce error that may be caused by cognitive bias; for example, by using Linear Sequential Unmasking (LSU).

Factors that impact bias cannot be managed by simply listing them or by individual responsibility. In addition to cognitively informed education and training, other mechanisms should be considered. A cognitively informed risk assessment of the entire DNA workflow can explore which procedures are most efficient in reducing the influences of cognitive bias. As an example of an assessment related to DNA technical review, a hierarchical approach has been advocated as a quality assurance practice to resolve disputes between analysts and reviewers. Alternatively, cognitive research indicates a hierarchical approach may introduce a variety of biasing factors that question the process, such as base rate expectations or favoring one individual's opinion over another. Mitigation could perhaps be better addressed by an independent blind review process that documents all opinions. A conclusion can then be determined, including the possibility of reporting the sample as inconclusive.

Context management of irrelevant information is another area to explore to determine how best to provide suitable information to a casework analyst without presenting information that could lead to bias during testing, analysis, or interpretation. The LSU approach requires first determining what information the examiner needs and making sure he/she receives all information required, but making a reasonable effort to minimize exposure to irrelevant information (especially the potentially more biasing irrelevant information). Second, the LSU approach requires managing the time and sequence in which relevant information is provided to the examiner. For example, avoiding working backward from the known suspect to the evidence by first evaluating the evidence from the crime scene, and only subsequently, sequentially, and linearly presenting the known suspect. Alternatively, a strong and effective technical review process could reduce the effects of cognitive bias on the final outcome in a systems-based approach.

This presentation will cover the important need for proper training and education concerning cognitive bias, practical suggestions on methods to control irrelevant task information, interpretation strategies to minimize bias, and suggestions for quality assurance procedures. Proactive mechanisms for quality enhancement are always preferred to reactive approaches to address error that may arise; however, the choice of approaches to improve quality by controlling the effects of cognitive bias must be balanced based on the overall impact on quality, realities, and the constraints of working in a forensic DNA laboratory, the importance of communication with investigators, proper decision making for analysis of samples, and cost-benefits to the system. Procedures that handicap the workings of the crime laboratory or add little value to improving the operation are not advocated, but simple yet effective measures are suggested.

Forensic DNA Decision Making, Cognitive Bias, DNA Workflows

B183 Forensic DNA Errors: Lessons From Innocence Network Cases

Greg Hampikian, PhD, Boise State University, Biology Dept, 1910 University Drive, Boise, ID 83725-1515*

The goal of this presentation is to highlight avoidable errors that can result in wrongful convictions.

This presentation will impact the forensic science community by increasing awareness that higher-sensitivity DNA testing, the demand for high-throughput work, and conflicting mixture interpretation methods have created new opportunities for accidental injustice. Simple procedures will be discussed that can be used to reduce common challenges to DNA evidence in court and reduce the chances of wrongful convictions.

One area that was at first insulated from scrutiny by the Innocence Movement was forensic DNA; however, the report by the President's Council of Advisors on Science and Technology (PCAST) in 2016 critically analyzed certain aspects of DNA analysis, including complex mixture analysis. The PCAST report cited several works and criticized the use of some common forensic methods, such as the Combined Probability of Inclusion (CPI) in complex DNA mixtures. "In summary, the interpretation of complex DNA mixtures with the CPI statistic has been an inadequately specified — and thus inappropriately subjective — method. As such, the method is clearly not foundationally valid."[1]

The study cited by PCAST was published in a 2011 peer-reviewed paper.[2] That study demonstrated that DNA analysts using the same data could reach conflicting conclusions. It was reported that 17 analysts at a single crime lab, given the same DNA data from an actual case in another state, came up with all three possible conclusions concerning a suspect (excluded, cannot be excluded, inconclusive). Most striking was that only 1 of the 17 analysts agreed with the original crime lab's conclusion that the suspect was included in the mixture. Several studies since then, including the Mix13 study by the National Institute of Standards and Technology (NIST), have shown that DNA mixtures can be a serious source of erroneous conclusions.[3] The use of probabilistic genotyping in addressing DNA errors will also be demonstrated by case examples.

A new case report concerning a Y-chromosomal Short Tandem Repeat (Y-STR) coincidental match that led to an exoneration will also be discussed.[4] In that case, a man was convicted of participating in a multiple-perpetrator sexual assault, based on a Y-STR mixture inclusion using a 17-locus kit. The conviction was eventually overturned through collaborative work involving the paper's authors from Boise State University, the Taiwan Association for Innocence, National Chiao Tung University School of Law, and the Taiwan Criminal Investigation Bureau.

This presentation will present problems with coincidental DNA matches, DNA contamination, and serology interpretations that can be addressed through proper processing, analysis, and testimony.

Reference(s):

1. President's Council of Advisors on Science and Technology. Report to the president Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods. Washington DC, 2016 (page 78).
2. Itiel Dror and Greg Hampikian. Subjectivity and Bias in Forensic DNA Mixture Interpretation. *Sci Justice.* 2011 Dec;51(4):204-8.
3. See presentation http://strbase.nist.gov/pub_pres/Coble-ABA2014-MIX13.pdf.
4. Greg Hampikian,Gianluca Peri, Shih-Shiang Lo, Mong-Hwa Chin, Kuo-Lan Liu. *Case report: Coincidental inclusion in a 17-locus Y-STR mixture, wrongful conviction and exoneration.* Forensic Sci Int Genet. 2017 Aug 7;31:1-4.

DNA Errors, Wrongful Conviction, Forensic Error

B184 2018 Update From the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)

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The goal of this presentation is to provide the seized-drug community with the most up-to-date information, resources, and documents being developed by the SWGDRUG.

This presentation will impact the forensic science community by increasing awareness of the latest developments in the seized-drug discipline, as well as new documents and resources that can enhance practices.

The SWGDRUG was formed in 1997 in a joint effort between the United States Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). The SWGDRUG works to improve the quality of the forensic examination of seized drugs and to respond to the needs of the forensic community by supporting the development of internationally accepted minimum standards, identifying best practices within the international community, and providing resources to help laboratories meet these standards. This presentation will provide attendees with information on the SWGDRUG activities during the past year.

The year 2017 marked the 20th anniversary of the SWGDRUG. As a commemoration of this significant anniversary, the first edition of the SWGDRUG Bulletin was launched shortly after the group's annual meeting, which was held during the week of June 12-16 in St. Louis, MO. The bulletin is intended to provide members of the seized-drugs community with a short summary of the SWGDRUG's activities.

Core committee members are currently working on revisions to the SWGDRUG Recommendations. Part IIIB (Drug Identification) is being revised to clarify the rationale behind the categorization of techniques, with emphasis on the development of robust analytical schemes applicable to multiple jurisdictions. A supplemental document is being developed that will include numerous examples of analytical schemes. This presentation will include discussion of some of those examples and their applicability.

Core committee members are also working on revising Part IVB (Validation of Analytical Methods) of the Recommendations. Revisions will include additional background information and clarifications on the performance characteristics to be evaluated during the validation of both qualitative and quantitative methods. Examples of method validation schemes for routinely used techniques, such as color test, Gas Chromatography/Mass Spectrometry (GC/MS), and Infrared (IR) spectroscopy will be included in this presentation and will also form part of another supplemental document to be published in the near future.

This presentation will also summarize recent updates on the SWGDRUG resources, such as the MS library, IR library, and Drug Monographs. Among the recent developments, the SWGDRUG has partnered with the National Institute of Standards and Technology (NIST) to verify the quality and reliability of the SWGDRUG MS Library, as part of ongoing efforts to provide valuable resources to the community. Drug Monographs continue to be added and disseminated via the SWGDRUG website (www.swgdrug.org) and this often-used resource has recently been enhanced to allow searches and sorting by name, nominal mass, and base peak.

The SWGDRUG core committee includes representatives from federal, state, and local law enforcement agencies in the United States, Canada, Brazil, Great Britain, Germany, Austria, Switzerland, Australia, and Singapore. The following forensic organizations are represented: the European Network of Forensic Science Institutes (ENFSI), the Academia Iberoamericana de Criminalistica y Estudios Forenses (AICEF), the Asian Forensic Science Network (AFSN), and the United Nations Office on Drugs and Crime (UNODC). Core committee members also include forensic science educators and representatives from forensic science organizations across the United States, the American Society of Crime Laboratory Directors (ASCLD), the American Society for Testing and Materials (ASTM), and the NIST.

SWGDRUG, Drug Analysis, Criminalistics

B185 What's in the Bag? Screening Trace Drug Contamination in Baggies by Thermal Desorption Combined With Direct Analysis in Real Time-Mass Spectrometry (TD/DART®-MS)

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After attending this presentation, attendees will better understand how TD/DART®-MS can be used to analyze the trace contaminants present on the outside of bags to identify the drugs present within.

This presentation will impact the forensic science community by providing a method for rapid presumptive screening of evidence for the presence and identification of narcotics.

As the presence of increasingly dangerous and toxic narcotics enter forensic laboratories, analysts must be aware of the hazards they may present. Because most evidence looks similar in nature, either pills or powders, it is often difficult for an analyst to know to what degree the evidence must be treated as a hazardous substance. Knowing the contents of the container is crucial to understanding the level of personal protection that is required. Currently, the package (typically a baggie) must be opened to retrieve a small amount of unknown evidence for testing. Spectroscopy techniques, such as Raman spectroscopy, have the ability to analyze the contents through the bag, but struggle to interpret mixtures. This work proposes a new analytical scheme through which a presumptive identification of the compounds inside the bag or container can be made without opening the evidence container.

The theory of trace contamination is well rooted within forensic science. When someone handles a material (in this case, a powder or pills) a trace amount of residue will be transferred onto the person's hands, then onto other surfaces that are subsequently touched. Furthermore, when a powder or pills are poured into a bag, a small amount of fine particulate is suspended in the air and can potentially resettle onto the outside of the bag. This work seeks to take advantage of these principles to establish whether the trace contamination on the outside of a bag or container can provide reliable information as to the constituents of the evidence inside.

In this work, samples of the trace contamination of simulated and real-world samples (bags or containers containing suspected narcotics) were obtained by swiping the exteriors with a meta-aramid wipe. These wipes were subsequently analyzed with TD/DART®-MS. TD/DART®-MS providing a rapid analysis (less than five seconds) with no sample preparation. It has been shown to have sub-nanogram detection limits and can readily handle complex multi-component mixtures. The compounds identified from the exterior of the bag were then compared to compounds identified by analyzing the actual powders or pills by TD/DART®-MS and/or Gas Chromatography/Mass Spectrometry (GC/MS). The ability to correlate the exterior signature to the interior signature was then established.

To date, screening results from swiping the exterior of the bags have matched the TD/DART®-MS and GC/MS results of the interior of the bag. While the sample set is small, current work is focused on expanding the number and variety of samples being tested. Additionally, quantification of the amount of trace contamination on the exterior of bags is also being investigated to establish the level of narcotics present.

DART®-MS, Drug Analysis, Screening

B186 Cannabinoid Vapor Pressure Measurements and Predictions by Porous Layer Open Tubular-Cryoadsorption (PLOT-Cryo)

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After attending this presentation, attendees will be able to apply knowledge of thermophysical properties of cannabis plant compounds Cannabidiol (CBD) and Δ9-Tetrahydrocannabinol (THC) in the design and development of vapor phase, cannabis detection devices.

This presentation will impact the forensic science community by providing insight into how the complex chemistry of cannabinoid compounds complicates breathalyzer development for cannabis detection.

Cannabinoids are large, polar molecules that react with oxygen; thus, thermophysical property data (upon which to base sensor design) for cannabinoid compounds are difficult to measure and find in the literature. Additionally, there are several aspects of THC that make collecting and analyzing it in bodily fluids complex. For one, THC is rapidly metabolized in the body, is excreted in the urine as a glucuronic acid conjugate, and a small portion of THC is stored in adipose tissue and released slowly over long time periods (hours, days, or weeks). Thus, not only is it difficult to measure the thermophysical properties of these compounds, but it is also a measurement challenge to detect the trace amounts of cannabinoids found in exhaled breath.

In this study, the vapor pressure and enthalpies of association of THC and CBD were made possible by the use of the ultra-sensitive, quantitative, trace headspace analysis technique PLOT-cryo.[1-4] The mass collected in the vapor phase will be presented in the form of a van't Hoff equation plot, which expresses the concentration collected as a function of temperature. A linear relationship of the recovered mass as a function of inverse collection temperature reveals the predictive capability of the methodology employed here. The measurements of the vapor pressure data and predictions (based on these measurements) of the Normal Boiling Temperatures (NBTs) and the critical constants will be presented. Additionally, predictions for the vapor pressures at closer to ambient temperatures will be presented. Comparisons of the vapor pressures of both ethanol and n-eicosane will also be discussed.

In conclusion, this study demonstrates that the vapor pressure of cannabinoids at close to ambient temperatures are predicted to be approximately eight orders of magnitude lower than the vapor pressure of ethanol, the rather simple chemical compound that is measured to determine alcohol intoxication. These measurements and predictions lay the foundation for understanding the partitioning of cannabinoids from the blood into the breath, and ultimately pave the way for the design and advancement of breathalyzers in the context of illicit cannabis detection.

Reference(s):

1. Lovestead, T.M. and T.J. Bruno. Determination of Cannabinoid Vapor Pressures to Aid in Vapor Phase Detection of Intoxication. *Forensic Chemistry.* 2017. 5: p. 79-85.
2. Lovestead, T.M. and T.J. Bruno, Detection of poultry spoilage markers from headspace analysis with cryoadsorption on short alumina PLOT columns. *Food Chemistry.* 2010. 121(4): p. 1274-1282.
3. Lovestead, T.M. and T.J. Bruno, Detecting gravesoil with cryoadsorption on short alumina PLOT columns. *Forensic Sci. Int.* 2011. 204: p. 156-161.
4. Lovestead, T.M. and T.J. Bruno, Trace Headspace Sampling for Quantitative Analysis of Explosives with Cryoadsorption on Short Alumina PLOT Columns. *Analytical Chemistry.* 2010. 82(13): p. 5621-5627.

Cannabis, Vapor Pressure, Breathalyzer

B187 The Identification of Various Controlled Substances by Headspace Chemical Analysis Using Headspace Solid-Phase Microextraction (HS/SPME) and Gas Chromatography/Mass Spectrometry (GC/MS)

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After attending this presentation, attendees will be familiar with a solventless HS/SPME method with the option of simultaneous headspace derivatization for the analysis of a variety of controlled substances.

This presentation will impact the forensic science community by demonstrating the utility of this method for testing microgram sample levels, reducing the time and number of steps for extraction and/or derivatization, reducing the consumables required, and reducing or eliminating issues arising from "dirty" samples, such as edibles and botanicals.

Controlled substances come in a variety of forms that require a variety of extraction methods to properly isolate analytes of interest. Liquid-Liquid Extraction (LLE), Solid-Phase Extraction (SPE), and dilute and shoot extractions combined with chromatography and mass spectral analysis are the most common techniques for identifying controlled substances. Some samples, including edibles and botanicals, are notorious for causing instrument problems, even when cleanup procedures are utilized. Dirty samples can cause sample carryover during instrumental analysis, requiring additional blanks or cleanups before another sample can be analyzed. In some cases, instrument components may need to be replaced to maintain separation efficiency or eliminate contamination. Furthermore, some substances require derivatization for separation and stability. This often requires caustic reagents, heating blocks, additional chemical cleanup, and significant time. Each additional step in the process presents an opportunity for error, and retesting is not always possible.

Dried analytical controlled substance standards and seized case specimens were tested. Case specimens included crystals, powders, tablets (pharmaceutical and clandestine), liquids, botanicals, and edibles. Sample sizes ranged from micrograms of material to intact tablets. Samples were placed directly into headspace vials and sealed. Using an oil bath and a Polydimethylsiloxane (PDMS) SPME fiber, this study optimized incubation time, extraction temperature, and fiber exposure time for HS/SPME from a sample's headspace. Initial analysis was performed using a GC/Flame Ionization Detector (FID) with a modified injection port to establish retention times, recovery amount, and peak quality. The information from the GC/FID runs was used to program optimal parameters for GC/MS testing. A concurrent study of the viability of headspace derivatization was performed on samples whose analytes would benefit from the process, including the synthetic cathinones, psychedelic mushrooms, and marijuana. Derivatization was achieved by placing a small insert filled with a measured amount of solvated or dry derivatizing agent directly into a headspace vial with a sample. Physical contact of the sample with the agent was not allowed; that is, the entire derivatization process occurred in the headspace during the incubation period. Results from the new method to those from the samples extracted with standard procedures were compared.

It was found that a variety of controlled substances in a variety of forms can be extracted with HS/SPME. The incubation temperature proved to be the most significant variable. Most compounds extracted well at 150°C, but some appeared to need higher temperatures that are beyond the capability of an oil bath. Incubation times ranging from five to ten minutes, with one-minute fiber exposure, proved sufficient for extraction and derivatization. The technique, for the most part, provided similar results; however, sometimes additional compounds not found in standard extractions were able to be extracted. Though not always controlled substances, the presence of these other compounds could serve as unique identifiers for revealing formulation trends, synthesis methods, growing regions, and other information that could be of interest to multiregional agencies. This method also demonstrated that derivatization does occur in the headspace, turning it into one-step process. This technique is simple and, for the most part, non-destructive. No sample required cutting, crushing, scraping, or dissolving, and most exhibited little or no change after testing. The initial work is promising and further study for the optimization of this new technique will be discussed in this presentation.

Headspace/SPME, Solventless, Derivatization

B188 Recent Seizures at the Canadian Border: New Fentanyl Analogues and Other Novel Psychoactive Substances (NPS)

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After attending this presentation, attendees will better understand the work performed by the Contraband Drug Analysis (CDA) section of the Canada Border Services Agency (CBSA) laboratory in identifying unknown drugs intercepted in shipments crossing the Canadian border.

This presentation will impact the forensic science community by providing recent information regarding NPS, including newer fentanyl analogues, intercepted at the Canadian border. Attendees will be presented with casework derived from both import and export shipments, including shipments crossing the Canada-United States border. Attendees will also be presented with various methods of concealment, analytical challenges in the analysis, and a comprehensive overview of the observed drug trends as seen by the CDA section.

It is increasingly important to be aware of drug trends involving shipments investigated at the border. Often drugs are imported as a pure chemical, which can later be processed into a dosage form prior to being sold on the street. Understanding the trends of drugs being imported can be used to signal law enforcement partners of potential drugs that may appear on the streets in the coming months and assist with their identification.

CBSA delivers a variety of programs and services designed to aid travelers and facilitate legitimate trade, while also focusing on enforcing laws and regulations to ensure the safety and security of Canada and its borders.

Border Service Officers investigate shipments crossing the Canadian border in many routes of entry, including via international mail centers, air cargo, courier, truck, marine, rail, and travelers at airports and land crossings. Shipments which are suspected to be or to contain unknown drug substances are sent to the CDA section within the CBSA, and the results of the analysis may be used for enforcement-related activities, such as controlled deliveries, search warrants, fines, investigations, and possible prosecution.

Exhibits analyzed by the CDA section typically contain controlled drug substances, prescription drug substances, unregulated drug substances, drug-related substances (such as cutting agents and precursors), and non-drug substances (including products of legitimate international trade).

The CDA section has the capability of complete structural elucidation of unknowns. Methods used within the CDA section for the analysis of unknowns include Fourier Transform/Attenuated Total Reflectance/Infrared (FT/ATR/IR), FT-Raman, Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography (LC) Orbitrap/MS, Nuclear Magnetic Resonance (NMR), condensed phase Gas Chromatography coupled with Vapor Phase Infrared Detection (GC/IRD), Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS), and Energy-Dispersive X-Ray Fluorescence (EDXRF).

In 2015, the CDA section identified more than 40 drugs not previously seen by its lab, including U-47700, W-15, butyryl fentanyl, and N,N-dimethylpentylone. Again in 2016, the CDA section identified more than 40 drugs not previously seen by its lab, including dibutylone, N-ethylpentylone, W-18, carfentanil hydrochloride, furanylfentanyl hydrochloride, furanylfentanyl citrate, 4-fluoroisobutyrfentanyl hydrochloride, and 4-fluoroisobutyrfentanyl citrate.

So far in 2017, the CDA section is on pace to identify an even greater number of drugs not previously seen in its exhibits. Some of the new compounds already identified in 2017 include N,N-dimethyl-lanicemine, propylone, U-51754, benzoylfentanyl, 2-methoxy-furanylfentanyl, 2-methyl-furanylfentanyl, 4-chloro-furanylfentanyl, 2-isopropyl-furanylfentanyl, methoxyacetylfenanyl citrate, and cyclopropylfentanyl citrate, with more to come.

Fentanyl, NPS, Import/Export

B189 The Analytical Profile of Fluorobutyryl Fentanyl Isomers

Kelly Song, BS, DEA, 99 10th Avenue, Ste 721, New York, NY 10011; and Adriana M. de Armas, BS, DEA, 99 10th Avenue, Ste 721, New York, NY 10011*

After attending this presentation, attendees will be familiar with approaches for effectively differentiating positional and structural isomers of fluorobutyryl fentanyl. Attendees will better understand the challenges that forensic laboratories may encounter with new fentanyl-related compounds.

This presentation will impact the forensic science community by providing analytical techniques for differentiating fluorobutyryl fentanyl isomers efficiently and effectively. This presentation will also impact the forensic science community by providing different approaches for other similar fentanyl substances forensic drug laboratories may encounter.

Fentanyl, a Schedule II synthetic opioid, was first synthesized in the late 1950s by Paul Janssen and is used licitly to treat severe pain. Presently, there are numerous fentanyl-related compounds.[1] Examples of such compounds include acetyl fentanyl, butyryl fentanyl, furanyl fentanyl, and acryl fentanyl. The identification of isomers of substances presents a challenge to the forensic drug analyst; new compounds are constantly being illicitly synthesized, such as fluorobutyryl fentanyl. The molecular formula of fentanyl is $C_{22}H_{28}N_2O$, whereas fluorobutyryl fentanyl is $C_{23}H_{29}FN_2O$. With an addition of a fluorine and a methyl group, the structure changes from fentanyl to fluorobutyryl fentanyl. Moving the position of the fluorine on the benzene ring allows for three different positional isomers of this compound. Furthermore, substituting the butanamide with isobutanamide allows for a different structural isomer. This study focuses on the identification of various isomers of fluorobutyryl fentanyl (meta-fluorobutyryl fentanyl, ortho-fluorobutyryl fentanyl, para-fluorobutyryl fentanyl, and para-fluoroisobutyryl fentanyl).

Techniques used in the identification of controlled substances must be both selective and sensitive. Sometimes, confirmatory techniques are not selective between isomers; hence, other approaches must be explored. In this study, various instruments were used to analyze four isomers of fluorobutyryl fentanyl, including Gas Chromatograph/Mass Spectrometry-Low Thermal Mass (GC/MS-LTM), Gas Chromatograph/Flame Ionization Detector (GC/FID), Gas Chromatograph/Flame Ionization Detector-Low Thermal Mass (GC/FID-LTM), Fourier Transform Infrared Spectroscopy with an Attenuated Total Reflectance (FTIR/ATR), ion trap (Tandem Mass Spectrometer or MS/MS), and Direct Analysis in Real-Time Mass Spectrometer (/ART®-MS).

Preliminary results demonstrated that GC-FID is an effective instrument in differentiating the two structural isomers of fluorobutyryl fentanyl. Depending on purity, FTIR/ATR can also be a suitable technique in differentiating the four isomers. Due to their very similar structures, these compounds fragment similarly; therefore, the use of GC/MS, DART®-MS, and MS/MS alone has proven to be insufficient in distinguishing isomers of fluorobutyryl fentanyl.

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1. Vardanyan, R., Victor J. Hruby. Fentanyl-related compounds and derivatives: current status and future prospects for pharmaceutical applications. *Future Med Chem.* 6(4). (2014): 385-412. www.ncbi.nlm.nih.gov. Web 19 July 2017.

Fentanyl, Fluorobutyryl Fentanyl, Isomer Determination

B190 Separation of Fentanyl Analogues, Homologues, and Positional Isomers by Ultra High-Performance Liquid Chromatography (UHPLC)

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After attending this presentation, attendees will be familiar with methods of separation for fentanyl analogues, homologues, and positional isomers, using UHPLC.

This presentation will impact the forensic science community by enhancing the collective expertise in the separation of fentanyl-related compounds using UHPLC. Positional isomers, which have the same mass and similar structure, are particularly difficult to separate using gas and liquid chromatographic methods.

Fentanyl is a Schedule II narcotic opioid that is an agonist at the μ-opioid receptor. While it mimics the effects of morphine, it is structurally different and is 50-100 times more potent. Some analogs of fentanyl, such as carfentanil, can be thousands of times more potent. Given their higher potency, there is a higher risk of overdosing. This poses a significant threat to opioid users and users of street drugs. Many street drugs, most commonly heroin, are being laced with fentanyl or its derivatives, leading to a significant public health problem. Derivatives of fentanyl are designed to avoid legal prosecution, as they are not scheduled. One of the main issues in the field of forensic drug analysis is that they are being produced faster than they can be identified.

Derivatives of fentanyl, including positional isomers, have substituents of varying degrees of polarity, hydrophobicity, and size. The substituents of the derivatives are expected to interact differently with different stationary phases and modes of chromatography. The particular challenge lies in separating the positional isomers, as they have the same substituents attached to different locations on the molecule.

A total of 12 fentanyl derivatives, including positional isomers, were subjected to analysis by UHPLC-time of flight/mass spectrometry. A variety of different 150mm x 2.1mm x2.7μm columns (SPP C18, SPP PFP, SPP Phenyl-Hexyl, and Hydrophilic Interaction Liquid Chromatography (HILIC)) were used in both the Reversed Phase Chromatographic (RPC) and HILIC modes and results were compared. The mobile phase conditions were optimized for each column to obtain the best possible separation.

For RPC, gradient conditions were preferred while for HILIC, isocratic conditions were utilized. Varying performance was found when analyzing positional isomers on different columns. For example, when using the C18 column, despropionyl para- and ortho-fluorofentanyl were separated with a resolution better than 1. Isomers such as ortho- and meta-fluorofentanyl were not separated, resulting in overlapped peaks.

These results both enhance the collective forensic knowledge and may aid forensic laboratories dealing with the influx of fentanyl-related cases by facilitating the identification process. This methodology may also aid in helping in the scheduling of fentanyl derivatives.

Fentanyl, Positional Isomers, UHPLC-TOF/MS

B191 Using Quadropole Time-Of-Flight (qTOF) Liquid Chromatography/Mass Spectrometry (LC/MS) to Distinguish 2- and 3-Furanyl Fentanyl and Other Fentanyl-Related Compounds

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After attending this presentation, attendees will better understand the analysis of 2- and 3- furanyl fentanyl and the importance of being able to separate and identify positional isomers.

This presentation will impact the forensic science community by providing LC/MS methodology capable of separating and identifying heroin, fentanyl, fentanyl-related compounds, and common adulterants.

The presence of fentanyl and fentanyl-related compounds in drug evidence has been on the rise recently. Two of these fentanyl-related compounds include 2-furanyl fentanyl(N-(1-phenethylpiperidin-4-yl)-N-phenylfuran-2-carboxamide), known colloquially as furanyl fentanyl, and 3-furanyl fentanyl (N-(1-phenethylpiperidin-4-yl)-N-phenylfuran-3-carboxamide). Differing only by the attachment site of the furanyl ring, these two compounds are difficult to distinguish chemically. Effective November 29, 2016, 2-furanyl fentanyl was placed into Schedule I federally; however, because 3-furanyl fentanyl was not specified in the final order, distinguishing between these two positional isomers is essential during analysis.[1]

The separation and identification of these two positional isomers can be accomplished via conventional Gas Chromatography (GC) methods. Standard screening conditions on the Gas Chromatography/Mass Spectrometry (GC/MS) provides a 0.1-minute separation between the isomers; however, the mass spectra of the two compounds have a very similar fragmentation pattern, with the most obvious difference being the presence of the 212m/z ion in the mass spectrum of 2-furanyl fentanyl. Thus, using standard GC/MS methods for identification of these compounds is not considered reliable as the similarities in mass spectra and slight drifts in retention time make their identification difficult. Gas Chromatography coupled with Vapor Phase Infrared Detection (GC-IRD) can provide confirmatory data to distinguish between the two positional isomers as it is apparent that the difference in site attachment of the furanyl ring leads to response differences in the Infrared (IR) fingerprint region.

LC/MS is an alternative methodology for both the separation and the identification of 2- and 3-furanyl fentanyl. Using this method, 2- and 3-furanyl fentanyl are baseline resolved from each other with a calculated resolution of 4.01 between the two compounds. The qTOF LC/MS also provides the exact mass of the analytes. Note that the positional isomers have the same molecular formula and therefore the same exact mass, thus requiring column separation to detect the presence of a mixture of 2- and 3-furanyl fentanyl or to distinguish which positional isomer is present. Confirmatory structural information was obtained through the subsequent fragmentation of each analyte's precursor ion. With an applied collision energy of 30eV, 2- and 3- furanyl fentanyl have similar fragmentation patterns. Therefore, using the combination of the chromatographic separation, molecular weight, and fragmentation patterns, the presence of 2-furanyl fentanyl and 3-furanyl fentanyl can be confirmed in a mixture.

Other fentanyl-related compounds may also present similar analytical challenges, such as their existence at low concentrations in drug evidence. To compensate for these difficulties, the LC/MS methodology was extended to include heroin and a variety of fentanyl-related compounds.

Reference(s):

1. United States Drug Enforcement Administrator. Final Order. Schedules of Controlled Substances: Temporary Placement of Furanyl Fentanyl Into Schedule I, 21 CFR Part 1308. *Federal Register 81*, no. 229 (November 29, 2016): 8 5873.https://www.gpo.gov/fdsys/pkg/FR-2016-11-29/pdf/2016-28693.pdf.

Furanyl Fentanyl, Fentanyl, LC/MS

B192 A 12-Year Study of Brake Dust From 123 Vehicles for Particles Similar to Gunshot Residue

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After attending this presentation, attendees will better understand the nature and composition of brake dust and its significance for Gunshot Residue (GSR) examinations. Attendees will also learn methods for discriminating brake dust from particles of GSR.

This presentation will impact the forensic science community by documenting clear differences between brake dust samples collected in Virginia over a 12-year period and previous reports of brake dust collected in Europe that contained particles of lead (Pb), barium (Ba), and antimony (Sb). The results suggest that the chance of misidentifying brake dust as GSR can be considered remote.

It has been previously reported that brake dust can contain particles similar in composition to particles characteristic of GSR.[1-3] This is a concern for examiners who may receive samples from people with a profession or hobby that puts them in frequent contact with brake dust. The American Society for Testing and Materials (ASTM) E1588-17 includes as characteristic of GSR: particles with a spherical or molten appearance that contain the elements Pb, Ba, and Sb.[4] This study sought to look at samples of brake dust from vehicles in Virginia to determine if particles with compositions considered characteristic of GSR could be found. An effort was made to include vehicles of the same makes and models previously reported in Europe to produce particles containing Pb, Ba, and Sb, namely: Audi®, Volkswagen®, and Land Rover®.[1,3]

Samples were collected and analyzed over a 12-year period using half-inch-diameter aluminum stubs covered with double-sided carbon tape. Sampling was performed by repeated dabbing of the stub on the brake disc and wheel of the vehicle. Information about the vehicle make and model was recorded and the Vehicle Identification Numbers (VINs) were used to determine the year of the car where possible. Samples were then analyzed using a Scanning Electron Microscope with Energy-Dispersive X-ray Spectrometer (SEM/EDS) including automated GSR software detection capability. An analysis was used that mimicked the analysis used for the identification of GSR in casework samples. From this analysis, particles were either accepted or rejected as being characteristic of, or consistent with, GSR as outlined by the most current ASTM 1588 document and laboratory protocols in use at the time of analysis.

Many particles were placed in classifications during the automated analysis that might be of interest as possible GSR; however, after manual review, none of the particles met the criteria to be considered characteristic of, or consistent with, GSR. The most common reason for exclusion was the presence of high sulfur and the absence of lead in particles that were classified as containing lead. Other reasons for exclusion included high levels of sulfur and iron in particles containing barium and antimony, the presence of disallowable elements, and morphology that was not spherical or molten. The samples were also examined for particles containing barium and aluminum (a type of particle consistent with GSR). Where aluminum was found in association with barium, it was typically at low levels in conjunction with high levels of sulfur. These particles were usually irregular in shape and were not classified as particles consistent with GSR.

After the analysis of samples from 123 vehicles and more than 300,000 particles, no particles were found that could be considered characteristic of, or consistent with, GSR. The exclusion of particles from being classified as characteristic was not only by morphology, as had been previously reported in some European studies, but also because of an absence of lead.[1,3] These results, in addition to a recent study from Australia, primarily on brake pads, suggest that the chance of misidentifying brake dust as GSR can be considered remote.[5]

Reference(s):

1. Bruno Cardinetti, Claudio Ciampini, Carlo D'Onofrio, Giovanni Orlando, Luciano Gravina, Francesco Ferrari, Donatello Di Tullio, Luca Torresi. X-ray mapping technique: a preliminary study in discriminating gunshot residue particles from aggregates of environmental occupational origin. *Forensic Science International.* 143 (2004) 1-19.
2. L. Garofano, M. Capra, F. Ferrari, G.P. Bizzaro, D. Di Tullio, M. Dell'Olio, A. Ghitti. Gunshot residue: Further studies on particles of environmental and occupational origin. *Forensic Science International.* 103 (1999) 1-21.
3. Carlo Torre, Grazia Mattutino, Valentina Vasino M.D., Carlo Robino M.D. Brake linings: a source of non-GSR particles containing lead, barium, and antimony. *Journal of Forensic Sciences.* 47(3) (2002) 494-504.
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Gunshot Residue, Brake Dust, Primer Residue

B193 A Framework for the Assessment of Gunshot Residue (GSR) Evidence: Transfer, Contamination, and Preservation

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After attending this presentation, attendees will better understand the factors that could be considered in the assessment of GSR evidence in order to strengthen the value of this evidence when presented in court.

This presentation will impact the forensic science community by reporting the results of a number of surveys for GSR, including an assessment of prevalence within a random population and on the hands of firearms-carrying law enforcement officers, as well as an assessment of the extent of transfer from police to suspects during arrest. Applying the data gathered from these surveys to the assessment of GSR evidence will permit examiners to provide a more informed judgement of the significance of a GSR test result.

A quantitative model for the transfer of GSR and evaluation of the evidence has been elusive thus far, and extensive specific data that can be used to inform casework evidence evaluation in the context of background GSR prevalence and secondary transfer scenarios are lacking.[1,2] To ensure a GSR test result can be given an appropriate and rigorous weighting in court, the deposition, retention, and distribution of GSR in the aftermath of a firing event must be better understood as well as the likelihood of GSR being present on a suspect due to incidences unrelated to the event (e.g., cross-contamination or background prevalence). The goal of the research discussed in this presentation relates to the latter consideration.

In this research, several surveys were conducted in order to determine the prevalence of GSR particles in a wide random population; samples were collected from volunteers in public spaces in order to reach a determination.[3] Further, the prevalence of GSR particles on the hands of police officers is relevant. Even if police officers do not fire a firearm while arresting a suspect, GSR present on police officers' hands as a result their general activities might represent a source of particles capable of being transferred to the suspect. To determine the magnitude of this source, the hands of serving police officers in non-firing situations were sampled for GSR. Although the abundance of GSR particles on police officers' hands is important to determine, it is of greater relevance to determine the extent of transfer of these particles from officers to suspects during arrest. Accordingly, studies of direct transfer of GSR during mock arrests were conducted. Typical hand stub samples were analyzed using Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) coupled with automated GSR analysis software (GSR Magnum™). Particles were confirmed as GSR by manual review. Data were then analyzed and interpreted with a view to contributing to a framework for the evaluation of GSR evidence.

This study explores the hypothesis that understanding a GSR test result in the context of a range of alternate scenarios by which GSR might end up on the suspect is essential in interpreting evidential value.

Reference(s):

1. R. Gauriot, L. Gunaratnam, R. Moroni, T. Reinikainen, and J. Corander. Statistical challenges in the quantification of gunshot residue evidence. *Journal of Forensic Sciences.* 58 (2013): 1149-1155.

2. B. Cardinetti, C. Ciampini, S. Abate, C. Marchetti, F. Ferrari, D. Di Tullio, C. D'onofrio, G. Orlando, L. Gravina, and L. Torresi. A proposal for statistical evaluation of the detection of gunshot residues on a suspect. *Scanning.* 28 (2006): 142-147.

3. N. Lucas, H. Brown, M. Cook, K. Redman, T. Condon, H. Wrobel, K. P. Kirkbride, and H. Kobus. A study into the distribution of gunshot residue particles in the random population. *Forensic Science International.* 262 (2016): 150-155.

Gunshot Residue, Criminalistics, Evidence Assessment

B194 A Review of Gunshot Residue (GSR) Evidence in Suicide Cases

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After attending this presentation, attendees will understand the role of GSR as evidence in the investigation of suicide cases. This will occur through a review of suicide investigations, including both medical-legal and law enforcement agency settings.

This presentation will impact the forensic science community by reviewing the application of GSR as a form of evidence in the investigation of suicides.

Suicide investigations involving firearms, both witnessed and unattended, can prove difficult for investigators and medical examiners. Several factors can complicate the investigation, including lifesaving efforts and disorganized crime scenes, emotions and demands of loved ones, and civil proceedings such as life insurance considerations. It is key to note that the presence or absence of GSR on the hands of a suicide victim will not resolve the manner of death.

GSR analysis is intended to provide information to an investigation by associating an individual with the discharge of a firearm; it is not beneficial in crime scene reconstruction. GSR cannot conclusively identify a shooter. GSR on a suicide victim's hands may indicate antemortem activities, such as firing a weapon, being in close proximity to a firearm during discharge of a weapon, or handling a firearm, a fired cartridge, or some other surface bearing GSR. A negative GSR result is not evidence of not firing a weapon and a positive test for GSR cannot confirm a suicide. GSR testing is most probative in cases in which an individual claims to have not been in the proximity of a firearm during discharge — something a suicide victim cannot claim. Individuals are not expected to have GSR during everyday activities. For these reasons, GSR findings often do not provide additional details for the investigation of a suicide. Investigators seeking evidence of a suicide must consider GSR within these parameters. With these factors in mind, some laboratories elect not to analyze GSR from suicide victims.

This presentation will provide a review of GSR cases between 2010 and 2015. This review can then be used to assist laboratories in establishing acceptance and analysis criteria as well as to provide information for the significance of the evidence when presented in a court of law. Finally, advantages and disadvantages of performing GSR analysis on suicide cases will be presented.

In conclusion, this presentation will provide a review of the analysis of gunshot residue evidence on cases investigated as suicide between 2010 and 2015.

GSR, Suicides, Gunshot Residue

B195 Novel Capabilities for Forensic Gunshot Residue (GSR) Analysis Through Exploitation of Glass Found in Primer Mixes

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After attending this presentation, attendees will be aware of glass-containing GSRs and be able recognize and identify this particle type in casework. The value of advanced analytical techniques for GSR analysis will be demonstrated and attendees will be made aware of the potential to associate these particles with a firearm source.

This presentation will impact the forensic science community by alerting GSR examiners of a new type of particle that demonstrates strong association with a firearm source. In particular, this presentation illustrates that a high probative value can be attributed to 0.22 caliber GSR and also demonstrates a new approach that can be used by GSR examiners to link GSR with unfired ammunition or spent cartridge cases; this capability is very valuable, but traditional approaches suffered from a relatively high degree of uncertainty.

The highest evidential level of the current American Society for Testing and Materials (ASTM) guidelines for identifying primer GSR involves detection of the characteristic elements Lead/Antimony/Barium (Pb/Sb/Ba) or Lead/Barium/Calcium/Silicon/Tin (Pb/Ba/Ca/Si/Sn) in individual particles by Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS).[1] In Australia, 0.22 caliber weapons are common and rimfire ammunition is most often used in firearms offenses and suicides. Up to 84% of 0.22 rimfire ammunition has a primer that does not include all of the elements required to produce characteristic particles; in particular Sb, Sn, or Ca are often absent; however, the majority of 0.22 caliber ammunition manufacturers include ground glass as a frictionator in their primer formulations.[2] This glass becomes encrusted with Pb and/or Ba when the ammunition is fired, producing particles of glass-containing GSR (gGSR).[3]

The research described in this presentation proposes that Pb/Ba/glass gGSR be considered for acceptance as a member of the characteristic GSR particle class. Evidence will be provided demonstrating that this particle type could be as probative as Pb/Ba/Sb in identifying a particle's source. The gGSR particles also have the potential of adding extra value to GSR analysis, as glass frictionators from different ammunition brands have been shown to often have different elemental signatures, and due to the stability of glass during the firing process, it may act as a means of linking samples of GSR together, to a cartridge case, or to a source ammunition.[4]

The research that will be presented has two primary objectives: (1) to assess the probative value of gGSR particles with regard to their association with a firearm origin; and, (2) to investigate the chemical variation inherent in glass frictionators from different ammunition manufacturers and the scope for linking gGSR with suspected source ammunition or spent cartridges.

Objective 1 was accomplished by using SEM/EDS to compare glassy GSR to known non-firearm sources of GSR-like particles, such as fireworks and brake pad dust. With the exception of other cartridge discharge residues, such as from some older industrial nail guns, no sources of particles indistinguishable from gGSRs were found.

Objective 2 was investigated using SEM/EDS, Time-Of-Flight/Secondary Ion Mass Spectrometry (TOF/SIMS) and Sensitive High-Resolution Ion Microprobe (SHRIMP). First, a survey of the variance of the elemental composition of frictionators across the ammunition market was conducted. Second, experiments were performed to determine whether the elemental composition and stable isotope ratios of the frictionator are preserved during ammunition discharge and whether the composition could be used to link residues to their ammunition source. The application of statistical and chemometric techniques were used to explore variation across the ammunitions available on the Australian market, where it was shown that the total combined pairwise discrimination power of TOF/SIMS, SHRIMP, and SEM/EDS was 97.8% and the discrimination power of TOF/SIMS alone was 94.1%. It was shown that the composition of glass is conserved through firing, and that post-firing samples could often be unambiguously matched to pre-firing samples with the assistance of advanced chemical techniques.

This presentation will alert GSR examiners to a new type of particle that displays strong association with a firearm source. In particular, it reveals that a high probative value can be attributed to 0.22 caliber GSR. It also demonstrates a new approach that can be used by GSR examiners to link GSR with unfired ammunition or spent cartridge cases; this capability is valuable but traditional approaches suffered from a relatively high degree of uncertainty.

Reference(s):

1. ASTM E1588-17. Standard practice for gunshot residue analysis by scanning electron microscopy/energy dispersive X-ray spectrometry. *ASTM International.* West Conshohocken, PA., (2017), https://www.astm.org/Standards/E1588.htm.
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Glass-Containing GSR, Characteristic Particles, Advanced Chemical Analysis

B196 The Determination of Gunshot Residue (GSR) Settling Velocity

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After attending this presentation, attendees will have a preliminary understanding of the approximate rate at which GSR settles to the ground.

This presentation will impact the forensic science community by providing information regarding the potential GSR contamination of those entering the crime scene following the discharge. This information can be used for crime scene reconstruction and could help explain the presence of GSR on a bystander.

GSR is a type of trace evidence that can be used in any type of forensic case involving a discharged firearm. GSR consists of all particulates expelled from a firearm during discharge. This presentation focuses on primer GSR. During primer GSR analysis, the three main elements of interest are barium, lead, and antimony. Most GSR particles have smooth spherical morphologies and are micron sized. GSR is produced when the firing pin hits the cartridge, which activates the primer. The primer then ignites the gunpowder (propellant) that causes a pressure buildup. This pressure then pushes the bullet down the barrel at a high velocity and the particles are released as a vapor through various openings in the firearm. GSR primer residues are typically analyzed using Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDS). The instrumentation works by irradiating the sample with electrons. The interaction between the sample and the electrons can identify the morphology of the particle and X-rays are emitted to determine the particle's elemental composition.[1]

Previous research has been completed to determine how fast GSR particles settle after discharge. This research used both pistols and revolvers and collected the particles by stacking petri dishes on top of each other.[2] Specific petri dishes were removed at designated time intervals to develop an overall rate.[2] The results demonstrated that the settling velocity ranged between 1.5 to 8 minutes after discharge, depending on the firearm.[2] While this research can be very informative, the mechanism of collection is not advantageous. Contamination may easily occur when entering or leaving the closed room to remove the petri dishes. Also, the stacked petri dishes do not provide a large range in heights that can be used to determine an overall rate because they were only a few inches apart. This research used a more advantageous collection method in determining the GSR settling velocity. The collection method was unique when compared to previous research; GSR samples were collected onto 0.4-micron polycarbonate membrane filters that were held above the ground by metal stands to provide greater height differences. The filters located inside the shooting room were connected to air pumps that were located outside the shooting room; the ability to control all the filters from outside the room limits particle contamination. The filters were placed at various heights above the ground. Once the firearm was discharged, the filters were turned on at different time intervals to determine the settling velocity. The filters were transferred onto SEM stubs and analyzed using the Aspect 75 Scanning Electron Microscope and Zeppelin Energy Dispersive X-ray Spectroscopy Software with a RJ Lee Group Detector. The preliminary data has shown that the particles fall at considerably lower rates than expected. The results revealed that a substantial amount of particles were still in the air after three hours.

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1. The Scientific Working Group on Gunshot Residue (SWGGSR). 2011. Guide for Primer Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry. 11-29-11. 1-100.
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Gunshot Residue (GSR), SEM/EDS, Forensic Science

C1 The Walls Are Listening: A Forensic Study of Home Personal Assistants

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After attending this presentation, attendees will be more aware of the types of forensic artifacts that can be recovered from devices connected to the Google® Home™ and Amazon® Echo™.

This presentation will impact the forensic science community by providing a method for the digital investigation of the Google® Home™ and Amazon® Echo™ in a controlled environment. Since these devices have only recently been developed, not a lot of forensic research exists. Recently, law enforcement tried to gain access to Amazon® Echo™ cloud data to help solve a murder. It is believed that these home personal assistants store data in places other than the cloud.

Home personal assistants, like the Google® Home™ and Amazon® Echo™, are increasing in popularity around the world. These devices are always on or always listening, waiting for the user to say the wake phrase. This research had three goals: (1) to determine exactly when the Google® Home™ and Amazon® Echo™ were recording an individual by recovering forensic artifacts from the devices that are connected to them; (2) to determine if the wake phrase was necessary for the home personal assistant to be recording and remembering an individual's conversations; and, (3) to see if these home personal assistants are sending data to third parties.

Data collection required a new Google® Home™ and Amazon® Echo™, along with two Android™ phones and two iPhones®. Two user identities were created; each user had an Android™ and an iPhone®. The Androids™ were connected to the Google® Home™ and the iPhones® were connected to the Amazon® Echo™. Two different types of scripts were created, a script in which the user talked directly to the device and a script in which the user talked indirectly to the device. Using a controlled environment, the scripts were read, ensuring the devices heard nothing that was not scripted. The phones were then imaged using Magnet Acquire™, processed with Axiom Process™, and examined with Axiom Examine™. Personal advertisements were checked on each phone on two different occasions by going to four websites that are well known for having a substantial amount of advertisements. The advertisements were counted and the content was noted. A control phone was also checked to make the process more objective. Chi-square statistics was used to find significant differences between categories of personal advertisement.

Artifacts from the Amazon® Echo™ were visible on both iPhones® when viewing the image on Axiom Examine™; however, the artifacts were only from the Alexa application. No artifacts could be recovered from the Google® Home™ application on the Androids™, but several artifacts from other applications that had been used, such as IFTTT™ and Evernote™, could be extracted. The devices showed no sign of recording when they were not being directly spoken to, based on the online history. A significant difference was found between the ads on the Androids™ and iPhones® during the second trial.

Home Personal Assistant, Forensic Artifact, Advertisements

C2 Observer Agreement in the Identification and Quantification of Dorsal Hand Traits From Digital Images

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After attending this presentation, attendees will understand the limitations of relying on dorsal hand traits (e.g., scars, moles, freckles, skin creases) to identify individuals from digital images.

This presentation will impact the forensic science community in terms of competency and performance by calling attention to the underlying reliability of dorsal hand traits in order to minimize the likelihood of false identifications.

Improvements in web-based technologies, in addition to increased human trafficking in the sex tourism industry, have increased the frequency of child pornography cases. Recent studies have demonstrated the potential utility of dorsal hand traits in the identification of perpetrators and victims of these criminal activities through photographic comparison. The qualitative nature of this method prevents it from meeting *Daubert* standards, as there are no known error rates associated with rates of identification of these traits from visual media. Recent studies have attempted to develop likelihood ratios for dorsal hand traits, but there have been few studies of the specific underlying reliability of visual identification and quantification of these traits. The objective of this pilot study was to explore this gap in the literature by conducting an analysis of observer agreement associated with scars, moles, freckles, and knuckle skin-creases, with the hypothesis that all traits would exhibit remarkable levels of intra- and inter-observer error. The study also asked observers to define regions of the hands independently, rather than note all features of the hand holistically.

For this study, digital images of the left dorsal hand of one individual were macroscopically analyzed by three trained examiners and three novice analysts from the Forensic Audio/Video and Image Analysis Unit of the Federal Bureau of Investigation. All images were examined at two time intervals separated by a minimum of 24 hours. One image presented the dorsal aspect of the hand encompassing the second through fifth digits (i.e., fingers). The other image captured only the dorsal aspect of the hand that included the first digit (i.e., thumb). A second set of images was taken of these same portions of the hand, but at different levels of exposure. To maximize the visibility of traits, all images were converted to CYMK in Adobe® Photoshop® CC. The full-color image was then compared with the isolated yellow channel. To examine the amount of variation observed in the data, coefficients of variance were computed for each trait. Afterward, a paired-samples *t*-test was performed for all traits with time set as the grouping variable in order to evaluate intra-observer error. To evaluate inter-observer error, an Analysis Of Covariance (ANCOVA) was performed for the same traits with experience and exposure as the covariates. The threshold for statistical significance for both analyses, which were performed in R (v. 3.4.1), was set at α=0.05.

Overall, the results of this study provided variable support for the expectation that all traits would exhibit remarkable levels of intra- and inter-observer error. Calculated coefficients of variance indicated high levels of data dispersion among scars (cv=1.206), moles (cv=1.546), and freckles (cv=1.270). Coefficients of variance calculated for counts of knuckle skin-creases on each digit suggested comparatively lower levels of dispersion (cv_1=0.419; cv_2=0.404; cv_3=0.450; cv_4=0.530; cv_5=0.354). Tests of intra-observer error indicated a statistically significant difference in mean counts between first and second observations of freckles (t=-2.43, df=11, p=0.034) and knuckle skin-creases on the second digit (t=-2.80, df=11, p=0.017), but not for any other traits observed. All computed ANCOVAs yielded statistically insignificant results for exposure; however, trained examiners and novice analysts significantly differed in their observations of scars (F=17.173, df=1, p=<0.001) and knuckle skin-creases on all five digits of the hand (F_1=4.366, df=1, p=0.049; F_2=9.854, df=1, p=0.005; F_3=24.670, df=1, p<0.001; F_4=25.266, df=1, p<0.001; F_5=14.371, df=1, p=0.001).

This exploratory study found that most traits exhibited statistically minimal intra- and inter-observe disagreement. There is considerable intra- and inter-individual variation in the specific observations made by participants, which calls into question the reliability of dorsal hand traits as suitable points of interest for photographic hand comparison. These findings are consistent with recent studies that show support for qualitative methods of identification and have implications for current efforts to develop quantitative methods based off the traits investigated here.

Identification, Photographic Comparison, Dorsal Hand

C3 United States Army Criminal Investigation Command (USACIDC) Digital Forensics: A Program Overview

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After attending this presentation, attendees will have gained information on the organizational structure and capabilities of the USACIDC's current digital forensics programs.

This presentation will impact the forensic science community by providing an overview of the services offered to assist criminal investigations involving Digital and Multimedia Evidence (DME) within the United States military.

As the United States Army's primary criminal investigative organization and the Department of Defense's (DoD's) premier investigative organization, the USACIDC, commonly known as CID, is responsible for conducting criminal investigations in which the United States Army is, or may be, a party of interest. The mission of the USACIDC is to investigate and deter serious crimes in which the Army has an interest. The USACIDC collects, analyzes, processes, and disseminates criminal intelligence; conducts protective service operations; provides forensic laboratory support to all DoD investigative agencies; and maintains Army criminal records. The USACIDC also provides criminal investigative support to all United States Army elements and deploys on short notice in support of contingency operations worldwide. The USACIDC Special Agents primarily investigate felony-level crime across the Army and provide investigative support to field commanders. They conduct a wide variety of investigations to include deaths, sexual assault, armed robbery, procurement fraud, computer crimes, counter-drug operations, and war crimes. The USACIDC agents also provide counter-terrorism support, criminal intelligence support, force protection, forensic laboratory investigative support, and protective services for key DoD and senior Army leadership.[1]

Within the hierarchy of the USACIDC, there are two individual organizations that share the responsibility for processing and examining DME to assist in Army criminal investigations: the USACIDC Computer Crimes Program (CCP) and the United States Army Criminal Investigation Laboratory's (USACIL) Documents and Digital Evidence (D2E) Branch.

Along with an overview of the organizational structure of the two USACIDC programs, attendees will be provided with much more information regarding each organization's capabilities, specializations, and examinations. This presentation will describe the types of examinations typically conducted in addition to an outline of the typical types of crimes investigated. This presentation will also include an overview of the anticipated future of the programs, current and anticipated evidence-processing issues, and a potential shift from reactive dead-box forensics to more proactive roles in investigations involving Internet Crimes Against Children (ICAC) and the National Center for Missing and Exploited Children (NCMEC).

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, the Defense Forensic Science Center (DFSC), the Office of the Provost Marshal General (OPMG), the Department of the Army (DA), or the DoD.

Reference(s):

1. United States Army Criminal Investigation Command. 2017. Accessed August 09, 2017. http://www.cid.army.mil/.

Army, Military, Program Overview

C4 Scientific Working Group on Digital Evidence (SWGDE) and Its Current Efforts in Digital Forensics and Forensic Audio — Part 1

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After attending this presentation, attendees will expand their general understanding of the mission and work of the Scientific Working Group on Digital Evidence (SWGDE), with recent activities and updates in the sub-disciplines of digital forensics and forensic audio.

This presentation will impact the forensic science community by providing an introduction to the SWGDE and their recent activities, with specific concentration on information relevant to best practices, guidelines, and standard developments in the digital evidence sub-disciplines of digital forensics and forensic audio.

This is the first of two presentations that will provide background on, and introduce the efforts of, the SWGDE. This Part 1 presentation will introduce the audience to SWGDE, its current organization, members, efforts, and will provide updates on the efforts of two of its five committees: the Forensics and Forensic Audio Committees.

SWGDE was formally established in 1998 to bring together organizations representing law enforcement, academia, legal, and commercial communities actively engaged in the field of digital and multimedia evidence to foster communication and cooperation. SWGDE's primary efforts include the development of cross-disciplinary guidelines and standards for the recovery, preservation, and examination of digital and multimedia evidence in order to ensure quality and consistency with the forensic community.

SWGDE provides guidance to the digital forensic community through the publication of standards, guidelines, and best practices with a concentration on public outreach and feedback acceptance. SWGDE also encourages a number of its published documents to be used by standard developing organizations in the creation of national and international standards for digital and multimedia evidence. SWGDE is often asked to address various issues from local and national bodies to form consensus feedback and position statements.

SWGDE is currently comprised of over 80 forensic examiners, scientists, and managers from more than two dozen federal, state, and local law enforcement agencies, as well as representatives from the academic, private, and research communities. The membership primarily concentrates its efforts addressing issues within five main technical sub-disciplines – Digital Forensics, Audio, Imaging, Video, and Photography.

This Part 1 presentation will provide informative updates from two of the five standing committees -- the Digital Forensics and Audio Committees. Committee representatives will provide updates regarding the current efforts of each committee, their work products, and the feedback that has been received during public comment periods. These two committees have the following mission statements and the presentations provided will discuss efforts specific to these missions:

Audio Committee: represents the forensic audio community by developing and promoting best practices related to the examination, analysis, comparison or evaluation of audio, evaluating current research, and assessing the forensic impact of new technology.

Digital Forensics (formerly Computer Forensics) Committee: addresses topics and issues facing digital forensics practitioners by developing forensically sound techniques through the assessment of current and emerging technologies. The committee supports the Digital Forensics community by the establishment of core competencies, best practices, forensic methodologies, and through participation in original research.

The opinions or assertions contained herein are the views of the SWGDE member participants and are not to be construed as official or as reflecting the views of the author's employer.

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SWGDE, Digital Evidence, Audio

C5 Current Efforts in Video Forensics, Image Analysis, and Photography as It Relates to Digital Evidence From the Scientific Working Group on Digital Evidence (SWGDE) — Part 2

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After attending this presentation, attendees will expand their general understanding of the mission and work of the Scientific Working Group on Digital Evidence (SWGDE), with recent activities and updates in the sub-disciplines of video and image analysis, as well as general photography.

This presentation will impact the forensic science community by providing an introduction to the SWGDE and their recent activities, with specific concentration on information relevant to best practices, guidelines, and standard developments in the digital evidence sub-disciplines of video and image analyses, as well as the field of photography as it intersects the realm of digital evidence.

This is the second of a two-part session that will provide a continuation of presentations discussing the activities and efforts of the SWGDE. In the first session, titled "Scientific Working Group on Digital Evidence (SWGDE) and Its Current Efforts in Digital Forensics and Forensic Audio -- Part 1," an in-depth introduction to the SWGDE organization was provided. The audience learned how SWGDE was established in 1998; about its membership and structure; that its mission is to bring together organizations representing law enforcement, academia, legal, and commercial communities actively engaged in the field of digital and multimedia evidence to foster communication and cooperation; and that SWGDE intends to provide guidance to the digital forensic community through the publication of standards, guidelines, and best practices with a concentration on public outreach and feedback acceptance.

Beginning with the Part 1 session, preceding this one, updates from standing committee members representing digital and multimedia subdisciplines were provided from two of its five standing technical committees -- Digital Forensics and Imaging. This Part 2 session is intended to be a continuation of information provided in the first session by continuing with updates from the remaining standing technical committees. Committee representatives will present the current efforts of the remaining subdisciplines of Video, Imaging, and Photography. An update on current activities, discussion topics and documents, as well as works in progress from each committee will be provided. The mission statements of the remaining technical standing committees are:

Imaging Committee: responsible for producing documents that pertain to the best practices for the examination and evaluation of imagery. This may involve opinions as to the provenance of an image or its content, including photogrammetric analysis, comparative analysis, and/or authentication.

Photography Committee: charged with addressing photographic and imaging issues as they intersect the realm of digital evidence. The committee develops best practices for processes ranging from field photography to the compression and archiving of those images to aid the law enforcement community.

Video Committee: focused on the development of best practices for video recovery and analysis, as well as training guidelines.

One additional non-technical committee, the Quality Standards committee, provides overarching guidelines for the Digital and Multimedia Sciences communities on how to develop and implement a quality management system including topics such as validation, testing, and accreditation to support laboratory competence and continuous improvement.

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Digital Evidence, Video, Image

C6 Testing Digital Forensic String Search Tools

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After attending the presentation, attendees will be aware of test data for string search tools and of some of the limitations and constraints of testing computer forensic string search tools. Test data and test method documentation is available via the Computer Forensics Tool Testing (CFTT) and Computer Forensic Reference Data Sets (CFReDS) websites (www.cftt.nist.gov and www.cfreds.nist.gov).

This presentation will impact the forensic science community by increasing awareness of tool test strategies and the ability of tool testing to reveal anomalies in string search tool behavior. This presentation will aid the forensic practitioner in recognizing the limitations of testing string search forensic tools and in being aware of the implications of choices of what to test or not test. The goal of testing forensic tools by a forensic laboratory is not to prove the software is always correct but to show evidence that the software is appropriate for the task at hand.

The CFTT project at the National Institute of Standards and Technology (NIST) develops methodologies for testing digital forensic tools. Currently, there are CFTT methodologies for testing the following: disk imaging, write blocking, deleted file recovery, file carving, forensic media preparation, and mobile devices.

A variety of forensic tools in each of these categories have been tested and observed flaws have been documented and reported by the Department of Homeland Security (DHS) and the National Institute of Justice (NIJ). These results can be used as a basis for identifying the types of likely failures that occur in forensic tools.

At an abstract level, string searching involves the following: (1) something to search with (i.e., a search engine); (2) someplace to search (i.e., an image file or a digital storage device); (3) something to search for; and, (4) search results (presented in a useful way).

A search engine implements a search algorithm that performs the search. A digital forensic string search tool provides an interface between a user and a search engine. The search tool interfaces with at least one search engine, but may interface with additional search engines. Most tools make a pass over the data and construct an index of strings that might be searched for or scan the entire data set for each search. Other tools may allow a user to select the search algorithm to use.

A search tool uses text strings to identify files relevant to an investigation. In addition to active files, a search tool may also need to search deleted files and unallocated space.

In the simplest case, the user is looking for a match to a target search string. Sometimes the tool user has a case-specific list of search terms. In other cases, the user wants the tool to find social security numbers (i.e., groups of nine digits). This can be specified as a regular expression (i.e., a pattern) such as [0-9]{9,9} (a string of nine digits with no separators). In addition, the user might need to search for text that is not represented in ASCII, such as searching for a Chinese word. There are multiple possible encodings for the characters (e.g., UNICODE, GB, Big 5, SHIFT JIS, etc.).

Forensic search tools often have a rich set of search parameters that could be tested. In the design of this test method and test data, this study focused on what seemed to be the most useful features in general. For an individual laboratory, other selections might be a better fit. Some common search parameters addressed in the NIST search data sets include: whole word versus substring; match case versus ignore case; character representation — ASCII versus UNICODE; active file versus deleted file versus unallocated space; exact match versus pattern match; clear text (.txt) versus formatted (.doc or .html); and, indexed search versus live search.

In summary, this presentation will describe a publicly available data set for testing forensic string search tools, including search features that can be tested, how to create test data, and the results of applying the test data set to several commonly used forensic tools.

Digital Forensics, Tool Testing, String Search

C7 Validating Mobile Forensics Tools in Your Lab With the National Institute of Standards and Technology's (NIST) Federated Testing

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After attending this presentation, attendees will be aware of a tool that can help test mobile forensics tools in a lab using the Computer Forensic Tool Testing (CFTT) Federated Testing Forensic Tool Testing Environment developed by the NIST.

This presentation will impact the forensic science community by increasing awareness of the capabilities of Federated Testing when applied to tools capable of extracting data from mobile devices and associated media (e.g., Universal Integrated Circuit Cards (UICCs)/Subscriber Identity Modules (SIMs)). This presentation will provide examples of using the Federated Testing Mobile Devices Test Suite to test mobile forensic tools in the same fashion as a digital forensics laboratory would conduct validation testing. In addition, by documenting the resource commitment required to perform the tool testing, forensic practitioners will be able to estimate the cost in time and effort to test mobile forensic tools in their laboratory. This presentation will aid the forensic practitioner choosing to use Federated Testing by providing examples of using the Federated Testing Mobile Test Suite to test actual mobile forensic tools, much as a digital forensics laboratory would conduct validation testing.

The CFTT project at NIST develops methodologies for testing digital forensic tools. Currently, there are CFTT methodologies for testing the following: disk imaging, write blocking, deleted file recovery, file carving, forensic media preparation, and mobile devices.

A variety of tools in each of these categories have been tested and observed flaws in the tools have been reported by the National Institute of Justice (NIJ) and the Department of Homeland Security (DHS). These results can be used as a basis for identifying the types of likely failures that occur in forensic tools. Currently, CFTT has implemented testing disk imaging, hardware write blocking and mobile forensics tools into Federated Testing.

Using Federated Testing has several advantages: (1) it relieves a forensic laboratory of the task of developing a test plan for tool testing because Federated Testing generates a test plan based on selections made by the user describing how the laboratory uses the tested tool (a list of test runs; detailed procedures for documenting and populating mobile devices with known active and deleted content; detailed procedures for performing essential and optional test runs; and tools to generate a skeleton test report that can then can be prepared in the style favored by the laboratory); (2) the test reports can be shared with other laboratories; and, (3) completed test reports can be submitted to CFTT for administrative review and, if no issues are found, the report is passed on to the vendor for comment. The final report is published by the DHS, Science & Technology Directorate, Cyber Security Division.

Certain trade names and company products are mentioned in the text or identified. In no case does such identification imply recommendation or endorsement by the authors or the authors' employers, nor does it imply that the products are necessarily the best available for the purpose.

Digital Forensics, Tool Testing, Mobile Forensics

C8 An Audio Enhancement Framework for Forensic Purposes

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After attending this presentation, attendees will better understand digital audio enhancement processes, limitations, and principles in order to inform a framework that will provide optimal results when performing audio enhancement for forensic purposes.

This presentation will impact the forensic science community by demonstrating that although there are many processes available to the forensic audio analyst, the order in which they are executed is vital in achieving the best results possible, as each tool used is used in isolation and modifies the signal in a unique way based on algorithms applied. The input of the next processor in a sequence is determined by the output of the previous, and this chain has a cumulative effect that provides differing enhancement results. A hypothesis that there is an optimal sequence of operations to produce the best results when performing audio enhancement is central to the research conducted for this presentation. There are currently various papers available detailing the individual methods of audio enhancement, but no document exists that provides a framework for the interaction of these processes.

The methodology proposed is agnostic, endorsing no specific software. It is based on scientific principles to future-proof the research from software developments and to allow the framework to apply to a range of individuals, including those who use free open source tools to those who work within agencies that have access to the latest, high-end software.

The goal of a forensic audio enhancement is to improve intelligibility and/or listening quality while maintaining the subjective speech interpretation and/or facilitating scientific measurements (e.g., gunshot, phonemes).[1] For this to occur, it is of the utmost importance that the desired signal is unchanged. To understand how this can be achieved, an investigation in the form of critical listening and Fast Fourier Transform (FFT) signal analysis is first performed to create a strategy that can be followed during the enhancement procedure to produce optimal results.[2] For this to be possible, a scientific review of techniques was performed and a critique of how various processes can be applied to specific issues present in forensic audio recordings was created. A logical framework was then devised, based on the knowledge of how various techniques alter the input of a processor and the signal information that is required by future processes in the chain.

The enhancement processes available to the audio analyst generally fit into six categories: source separation, distortion repair, filtering, noise reduction, de-reverberation, and amplitude correction.[3] Experimental results applied to the same input signal reveal that when several processes using identical settings are applied, the output varies in areas such as speech-to-noise ratio, artifact reduction/accumulation, intelligibility for transcription, and subjective audio quality. As each recording is infinitely different, the structure of the framework is not designed to be strictly adhered to step by step. There may be occasions when some steps are unnecessary due to the inherent character and noise present on each recording, but this will likely be discovered during the analysis stage.

This presentation will illustrate the scientific reasoning behind the proposed framework, results of the experiments, and, finally, an enhancement framework demonstration, from signal analysis to the audio output stage. Best practices such as note-taking, manipulation vs. enhancement, and evidence deliverables will also be highlighted, with the goal of attendees leaving with the practical know-how for optimal enhancement of forensic audio within the working environment.[4]

Reference(s):

1. Bruce E. Koenig, Douglas S. Lacey, and Steven A. Killion. Forensic Enhancement of Digital Audio Recordings. *Journal of the Audio Engineering Society.* 55, no. 5 (2007): 352–371.
2. Anthony T.S Ho and Shujun Li. *Handbook of Digital Forensics and Multimedia Data Devices.* (UK: John Wiley & Sons, Ltd, 2015).
3. C Grigoras and JM Smith. Audio Enhancement and Authentication. *Encyclopedia of Forensic Sciences.* Second Edition (Elsevier Ltd, 2013), 315–26.
4. SWGDE. Best Practices for Forensic Audio. October 8, 2016.

Forensic Audio, Audio Enhancement, Methodology

C9 Time Domain Analysis of Lossy Compression Decoding Artifacts

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The goal of this presentation is to disseminate important findings related to the behavior of various MP3 decoding libraries and software as they relate to time domain forensic audio analyses and analytical results.

This presentation will impact the forensic science community by providing the results of a study that impacts laboratory performance and best practices in the handling and processing of forensic audio, especially MP3 files.

This presentation reports a preliminary study on the artifacts left by different audio decoders. Lossy compressed files are common in real forensic cases, can be produced with different digital audio hardware and software systems, and their forensic analysis and/or authentication can end up being crucial in the courtroom or other extrajudicial investigations. This study reports on the time domain artifacts introduced by some of the most common freeware and commercial tools (e.g., Adobe® Audition 3.0.1; FFmpeg N-85604-g207e6de; LAME 32/64bits version 3.99.5; MATLAB® 2016a/2017a using the audioread built-in function; mpg123 1.11.0; and QuickTime® Pro 7.7.9 on original MP3 files created with 16 different OLYMPUS® digital audio recorder models: DM-520, DM-620, VN-713PC, VN-722PC, WS-550M, WS-560M, WS-600S, WS-700M, WS-750M, WS-760M, WS-802, WS-811, WS-821, WS-822, WS-823, and WS-853). Extending a previous paper presenting the zero-level sample padding problems of various MP3 decoders, the following table provides examples of the materials and results collected in this study.[1]

OLYMPUS®	Adobe® Audition		FFmpeg		LAME		MATLAB®		mpg123		QuickTime® Pro	
	median	std	median	std	median	std	median	std	median	std	median	std
MP3 Recordings	234	155	22	143	49	180	239	162	0	82	0	95

Table 1. The number of zero-level samples after decoding

The preliminary results of this study indicate that different decoders pad with a different number of zero level samples at the beginning of the Pulse Code Modulation (PCM) decoded files. The tested versions of mpg123 and QuickTime® Pro decode the original .MP3 files with minimum zero padding while Adobe® Audition, FFmpeg, LAME, and MATLAB®, using the built-in audioread function, pad with greater numbers of zero-level samples at the beginning of the PCM converted files. The artificially introduced zeros affect results of time domain processes and measurements like Quantization Levels (QL), energy, power, and Direct Current (DC) component. This can also lead to inconsistent results when the same analysis of the same acoustic event is conducted by two different scientists using different decoding tools (e.g., butt-splice detection) that will produce results at different counters. Another challenging problem can be found in forensic video where the transcoding can introduce an offset between audio and video streams. With these findings, this study proposes the use of different decoding libraries and settings that are cross-verified in forensic analyses and presented in accompanying reports.

Reference(s):

1. Berman, J. (2015) *Analysis of Zero-Level Sample Padding of Various MP3 Codec.* MSc Thesis, National Center for Media Forensics, University of Colorado Denver.

Audio Forensics, Multimedia, Digital Evidence

C10 Analyzing Video Evidence in Officer-Involved Shooting Cases

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After attending this presentation, attendees will understand how enhancement and analysis of video evidence, in conjunction with a computer animation, led to the acquittal of a police officer who was criminally charged in a controversial high-profile officer-involved shooting.

This presentation will impact the forensic science community by demonstrating how video evidence becomes more prolific in police use-of-force cases through the increasing use of body cameras, cell phones, and surveillance systems. This presentation will indicate how to properly analyze such evidence and understand its limitations.

On September 16, 2016, a White female police officer shot and killed an unarmed Black man while he was standing next to a Sports Utility Vehicle (SUV) in the middle of a road in Tulsa, OK. The incident led to protests in Tulsa and the case received national attention when video of the event, taken from a helicopter and police dashcams, was released to the media by the Tulsa Police Department two days later. On September 22, the Tulsa district attorney filed first-degree manslaughter charges against the officer involved, a charge which carries a maximum penalty of life in prison. After a seven-day trial, in which the video evidence was carefully considered, the officer was found not guilty.

At approximately 7:30 p.m., Officer Betty Shelby encountered an abandoned vehicle in the middle of a two-lane road, blocking on-coming traffic. After stopping to check for people inside the vehicle, she walked back to her own vehicle where she was approached by an incoherent Black man, later identified as Terence Crutcher. The man was mumbling and reaching into his pockets despite repeated commands to keep his hands out of his pockets. Officer Shelby radioed for help stating, "I have a suspect not showing his hands." The man walked around the rear of Shelby's police cruiser as she followed, stopping at various points and reaching into his pockets. The helicopter video of the event begins as the man starts walking toward the SUV with his hands raised. Officer Shelby stated that she pointed her gun at Mr. Crutcher and ordered him to stop and get down on the ground. Mr. Crutcher ignored her commands and kept walking, eventually stopping next to the driver-side window of the SUV. At this point, another officer arrived on scene and stood next to and slightly behind Officer Shelby, pointing his TASER® at Mr. Crutcher. Both officers stated that Mr. Crutcher dropped his left hand and reached into the open window of the vehicle, at which point both officers fired their weapons simultaneously. The single shot fired by Officer Shelby entered Mr. Crutcher's right chest and damaged his heart and lungs, resulting in his death. The autopsy revealed Mr. Crutcher to be under the influence of PCP. No weapon was found inside the SUV.

The helicopter and dashcam videos became the central evidence in the case, although neither was clear enough to be definitive as to whether Mr. Crutcher reached into the window under normal viewing. The helicopter Forward Looking Infrared Radar (FLIR) camera provided the best viewing angle of the event; however, it only produced a standard-definition interlaced recording. Due to the motion of both the camera and helicopter, the individual interlaced video frames lacked clarity.

Using forensic video analysis software, the helicopter video was de-interlaced, stabilized, clarified, and enlarged in order to facilitate a frame-by-frame analysis that showed that Mr. Crutcher's arm was inside the window opening at the time he was shot. Audio from the dashcam video recorded the sound of a single shot, confirming that the TASER® and gun were fired simultaneously.

Since the video evidence only revealed the end of Shelby's encounter with Crutcher, her defense team looked at ways to tell the story of how the event unfolded. A synchronized video narrative was produced, tying together initial 911 calls, radio transmissions, multiple dashcam recordings of responding units, and the helicopter video into a single timeline. A computer animation was created showing the various locations Officer Shelby and Mr. Crutcher prior to the beginning of the helicopter video. These videos, together with the enhanced helicopter video, will be presented in addition to a detailed discussion of how they were created.

Shootings, Video Analysis, Computer Animation

C11 Video Datasets for Developing Image Forensic Techniques

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After attending this presentation, attendees will better understand two newly constructed video datasets that will accelerate the development of various image forensic techniques, as well as contents and applications of the datasets.

This presentation will impact the forensic science community by announcing newly constructed datasets to evaluate image forensic techniques, especially for suspicious activities detection and forgeries detection. The fact that novel datasets were constructed will be beneficial information for forensic image analysts.

Improvement of video and image analysis techniques have provided significant benefits for information analysis in various fields such as prevention and investigation of crimes and terrorism. In order to drive development of such analyzing techniques that assist public safety and security, this study constructed two types of video datasets so practical technique evaluations and comparisons can be made. The datasets presented are: (1) a dataset of crowds and suspicious activity videos; and, (2) a dataset of forgery videos. These were created based on factors such as experiences with a variety of forensic image analyses. It is thought that these datasets are helpful for furthering research in this area and for the efficient social implementation of various novel video and image analysis techniques. It is planned that these datasets will become available for researchers by the conclusion of a joint research contract. A brief overview of each dataset is described in the following sections.

The first section is a set of videos containing crowds of people and a few suspicious activities happening in the crowds. This set seeks to drive the development of techniques for detecting suspicious activities from videos. Five situations, including a sightseeing spot, a sports stadium, and a meeting place such as a station, are assumed and up to 100 extras appear in the videos. In the videos, nearly all people in the crowds behave typically, but some are engaged in suspicious activities. Each scene was shot by three to four High-Definition (HD) -resolution cameras and one spherical camera from different angles simultaneously. Suspicious activities are defined based on comments from police officers and embedded into the scenarios. Annotation data is also provided.

The second section is a set of forgery videos. This set contains material videos that are used to make forgeries as well as six types of forgeries. The goal of this set is to further development of techniques for detecting forgeries in videos. In the videos, for example, removing a person or tampering with a traffic light color are performed as forgeries. One of the remarkable points of this set is that approximately 40–50 seconds of each video is tampered with. Furthermore, as the quality of forgery videos is significantly affected by the technical level of the forger, each forgery type of this set contains tampered videos with three different levels of technical quality from expert level to amateur level of forgery using the same materials.

Suspicious Activity Detection, Forgery Detection, Video Dataset

C12 Another Forensic Image Data Set (AFIDS)

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The goal of this presentation is to raise awareness and interest in a new and available research data set that will be composed of real-world forensic images from around the world. This increased awareness of AFIDS is primarily targeted at the Digital & Multimedia Sciences Section of the American Academy of Forensic Sciences due to its diverse member population across industry, academia, and law enforcement/government/military arenas.

This presentation will impact the forensic science community by providing a real-world, reasonably safe, and unique dataset to aid researchers in tool development and testing of forensic tools and related capabilities.

In 2009, Garfinkel et al. made the case for standardized forensic corpora to foster scientific approaches and progress in digital forensics.[1] Of the datasets that resulted from this effort, the Real Data Corpus (RDC) was by far the largest and most representative of the variety and complexity of data encountered during digital forensic investigations. Its use in the development and de-bugging of widely used tools such as bulk extractor and The Sleuth Kit testify to the success of the project; however, though this dataset is still actively maintained, challenges relating to cost as well as institutional policies have prevented the addition of new drive images for several years. Consequently, new applications and operating systems are absent, and the versions of software represented are long out of date. The current RDC continues to suffer from entrenched problems related to policy, operations, and oversight.

The development of AFIDS is therefore proposed. The intent is to define a community resource to enable the advancement of digital forensics research while incorporating from the outset lessons learned from previous work on large-scale restricted datasets such as the RDC and the National Software Reference Library (NSRL). AFIDS can leverage Amazon® Web Services GovCloud infrastructure to reduce costs of long-term storage and to allow controlled access by researchers without the additional privacy risks associated with distributing copies of the data. AFIDS can provide a managed interface by which researchers can submit "queries" to the dataset (ranging from simple functions to custom forensic analysis tools) that will be assessed in terms of their risk of exposing personally identifiable information before being run across the dataset via Cloud technologies. It is expected this risk assessment will facilitate Institutional Review Board oversight and reduce researcher overhead. Query results, moreover, can be cached and made available, preventing the need to repeat expensive computations.

In conclusion, a study to identify improvements over the current RDC is already being conducted; the plan is to bring AFIDS online and make it available for researchers from industry, academia, and law enforcement/government/military. The current status of AFIDS will be provided to attendees.

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1. Garfinkel, Simson, Paul Farrell, Vassil Roussev, and George Dinolt. Bringing science to digital forensics with standardized forensic corpora. *Digital Investigation.* 6 (2009): S2-S11.

Real Data Corpus, Forensic Images, Research

C13 Digital Image Recompression Analysis: Twitter®

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After attending this presentation, attendees will have gained insight into the interpretation of embedded data that can be found within the construct of images shared and downloaded via Twitter® and, with this newfound knowledge, be better equipped to analyze and understand differing image file structures as they pertain to particular social media networks.

This presentation will impact the forensic science community by illustrating that, because of the ubiquitous use of social media in this day and age, a higher understanding of data attributed to individual social media networks is necessary for the digital forensic analyst. Research of information provided by and attributed to Twitter® is a stepping stone to understanding how social media networks compare to, as well as differ from, each other in terms of the metadata associated with and attributed to each network.

This presentation offers insight into the identification of the effect of mobile and desktop uploading and downloading of images shared through Twitter®. A hypothesis statement that "useful information pertaining to the location and interpretation of embedded data encoded within digital images can be of aid to forensic digital image analysis" is central to the research conducted in this presentation.

The functionalities and prolific use of social media networks have evolved over time, constantly changing how people connect to and communicate with other individuals around the world. Within the numerous variations of social media networks, a constant function is apparent throughout the uploading and sharing of images to be viewed and, oftentimes, re-shared by friends or those who subscribe to a particular feed.

This research follows a method of looking at metadata, binary data, and quantization tables of image types on image files before the upload process to Twitter®, then looking at the same data on images after the download process. A comparison of the changes between the original and Twitter®-downloaded files will be discussed, as well as the effect on image dimensions throughout the process.

Throughout each instance of submission pertaining to images being uploaded online or through a social media network, some forms of compression are needed in differing sorts of ways. This research seeks to identify as well as interpret the embedded data encoded throughout the compression process within digital images associated with Twitter®. Knowledge gained through this research can be added to a growing database of information attributed to different social media sites.

Image Analysis, Social Networks, Twitter®

C14 Deep Learning With Camera Identification

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After attending this presentation, attendees will better understand the limitations and possibilities of camera identification.

This presentation will impact the forensic science community by providing deep learning techniques that can be used for a variety of databases. It appears that the right clustering was found for only for a few cameras based on the Photo Response Non-Uniformity (PRNU) pattern.

In this presentation, a camera model identification using a deep learning technique is introduced. The PRNU noise pattern, the fingerprint of the camera, is extracted to classify and identify the camera model. Deep learning is a subfield of machine learning, which trains the computer as a human brain to recognize similarities and differences by scanning in order to identify an object. In forensic science, it is important, especially for child pornography cases, to link a photo or a set of photos with a specific camera.

Each different camera has a different noise pattern, the so-called PRNU noise pattern. Each camera has an imaging sensor that converts the light into an electrical signal. Charge-Coupled Device (CCD), Complementary Metal-Oxide Semiconductor (CMOS), Junction Field-Effect Transistor (JFET) and Foveon® X3 are some of the popular imaging sensors, with the CCD being most common.

For the experiments, NVIDIA® DIGITS, an interactive deep learning Graphics Processing Unit (GPU) training system, is used for the implementation of the project. DIGITS applies deep learning to the database that is uploaded, then classified. In the next step, the appropriate network is chosen depending on the size of the dataset. During the second step, the model is trained in order to extract features and find similarities and differences between the categories. There are three given networks: LeNet, AlexNet, and GoogleNet. Furthermore, a graph is provided informing the user of the success of the training. The most important aspects are the accuracy of the trained model and the loss of data during the process of training and validation. In the last step, the user uploads a single image and the program provides the top five predictions.

By modifying the AlexNet, the results improved, providing material for further research. The accuracy rate was high (80%-90%); however, DIGITS was able to successfully identify only 3 camera models out of 17 from the database. Furthermore, individualization of a camera was unsuccessful. In a database containing more than one camera of the same model, the accuracy rate was extremely low. In both cases, the problem was expected to be the imaging sensor. Manufacturers use the same imaging sensors, which result in similar PRNU noise patterns. As a result, DIGITS cannot distinguish the similarities and the differences of the images. It is important for future research to create a much larger database, as the one used contained 200 images per class. A larger database will feed the program with much more information.

Deep Learning, PRNU, Camera Identification

C15 Curating Forensic Image Collection Using Machine Learning

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After attending this presentation, attendees will understand the opportunities afforded by machine learning for automatic classification or detection of forensically relevant features in images documenting human decomposition.

This presentation will impact the forensic science community by introducing new approaches to automate the annotation of digital collections and by discussing how this can transform research on human decomposition.

Some areas of forensic anthropology have accumulated large collections of digitized data. Despite the curation approaches of traditional collections that involve manual and painstaking labor analyzing individual samples, digital collections offer a tantalizing opportunity to automate some of the most tedious parts of curation work through the application of modern machine learning. These opportunities were investigated using a collection of one million photos documenting human decomposition collected at the Anthropology Research Facility (ARF) at the University of Tennessee, Knoxville. The research was conducted in stages, beginning with checking the feasibility of the approach and ending with object detection and classification.

Experiment 1 was conducted to verify the feasibility of using machine learning for images of human decomposition. While such tools are highly advanced in certain domains, for example, for recognizing faces of living individuals, they are not likely to work well on images depicting decomposing bodies. Image analysis algorithms that can detect forensically relevant features are not well known. The specific goal of this experiment was to accurately classify images annotated with tags indicating body parts by an expert. The expert used a web-based tool to interactively highlight an interesting forensic feature within an image and then provide a tag indicating body part (e.g., left foot) and a forensic feature (e.g., egg mass). A Convolutional Neural Network (CNN) structure was used to perform binary classification of these subimages based on body-part tags. A CNN structure was made of several convolutional layers, pooling layers, and dropout layers. In the model training, the loss metric, evaluation metric, and optimizer were binary cross entropy, accuracy, and Stochastic Gradient Descent (SGD), respectively. It was implemented using the sequential model in Keras, an open source deep learning Python library. Ten-fold cross-validation was used to avoid overfitting, measure performance, and select suitable parameters for training. The classification yielded high accuracy, for instance, 91.02% when classifying eye and left foot. For particularly challenging cases, such as eye versus mouth, it was lower at 62.43%. These results indicate that machine learning is capable of accurately classifying images with different shapes.

Experiment 2 was conducted to investigate the ability of machine learning algorithms to detect relevant features, such as body parts, in a full image that may include several (or none) of the body parts. This is a much harder (and more practical) problem, since the ability to automate the annotation of the body parts could be a powerful curation aid. Keypoint identification algorithms were used to discover areas of an image that represent certain high-level features (objects). Oriented Function Analysis System Technique (FAST) and Rotated BRIEF (ORB) algorithms from OpenCV were used. Keypoint approach is appealing because of its clear interpretation and extensive use in object detection. Meanwhile, it does not require that the input images have the same input size. The effectiveness of using keypoint approach was demonstrated by classifying annotated body parts with clustered keypoints. The k-medoids were used to cluster keypoints, some of which may be spurious as not all keypoints are necessarily representative of the classification label. To address that, each image was treated as a mixture of keypoint proportions within each cluster. With the labeled class, logistic regression was used to train the classifier. For example, when classifying eye and left foot with 100 clusters of useful keypoints, a high accuracy of 94.43% was reached. In more challenging cases, such as mouth versus eye, the number of useful keypoints in each image may be too small and not enough features will be detected. Overall, the approach works well if the image contains relevant features of the body parts. As further research, these algorithms will be applied to detect forensically relevant features, such as “larvae,” “scavenging,” and “marbling” and apply the approach to a large collection of images stored with the Forensic Anthropology Center. The results suggest clear avenues for further research toward automation of the curation for large collections of forensic images.

Human Decomposition, Image Analysis, Forensic Databases

C16 Source Camera Comparison Using Photo Response Non-Uniformity (PRNU) on WhatsApp

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After attending this presentation, attendees will be aware of the possibilities and limitations of PRNU on WhatsApp.

This presentation will impact the forensic science community by illustrating how the use of PRNU on images transmitted with WhatsApp can be used even if the file is converted. Depending on the version of the operating system and app, the PRNU will be filtered and a weaker conclusion can be given.

In digital footage, different types of noise, such as dark current, reset noise, circuit noise, and PRNU, are present. PRNU is caused by the imperfection of the camera sensor created during the manufacturing process. Not every pixel of the sensor is identical and will, therefore, respond differently to the same amount of light. This is called the non-uniformity of pixels. When the camera sensor, for example, will be illuminated equally, not all pixels will measure the same amount of light. This is caused by the difference of the sensitivity of pixels to light. Over the sensor surface, this creates a pixel non-uniformity pattern which is called the PRNU pattern. Because the PRNU pattern originates from the camera sensor, the pattern will be present in all footage taken with this camera sensor. The PRNU pattern is specific for a camera sensor and is also called the fingerprint of a camera.[1-3]

To determine if a video is created with a specific camera, the PRNU patterns from the disputed video and the disputed camera are needed. Using software, the PRNU patterns can be extracted from a video. The first step of the extraction is to average groups of individual frames of the video. A parameter, the frame averaging rate, can be set to determine how many frames per group will be averaged. For example, a video with a thousand frames will be averaged with a frame-averaging rate of ten. Per ten frames, an averaging will be determined that delivers a hundred averaged frames in total. The second step is to extract the noise per averaged frame. Research has shown that better results were made with the second order (FSTV) filter.[3] With this filter, the noise will be removed from the footage. By subtracting the averaged frame without the noise from the averaged frame with the noise, the noise pattern will be obtained. Then, as the third step, the noise patterns from all the averaged frames will be averaged. With this step, noise that is not present in every frame, like the PRNU pattern, will be restrained and the PRNU pattern becomes clearer. Due to electronic imperfections, some rows and columns of pixels can be systematically brighter or darker. This noise can be removed with step four by using a zero mean filter.[4] The last step in the extraction method is to remove artifacts created due to compression. Groups of pixels that share information to reduce data size create a pattern over the surface of the footage. To remove this pattern, a Wiener filter is used. Finally, the PNRU pattern is as clear as possible.[4]

To obtain the PRNU pattern from the disputed camera, a reference video is needed. These reference videos are called flat field videos. A flat field video is taken by moving the camera over a gray surface. By moving the camera over a grey surface, the camera sensor is illuminated as equally as possible and no objects will be present in the video. This causes the PRNU pattern to be as clear as possible.

When the PNRU patterns from the disputed video and the disputed camera are extracted, the two PRNU patterns can be compared. This can also be accomplished by using PRNUCompare. PRNUCompare compares the patterns and calculates the Peak to Correlation Energy (PCE).

In this research, cameras of different Android® brands as well as iPhones® were used. The PCE is lower after transmission with WhatsApp. With several phones from Samsung™ and Huawei, the likelihood ratio is low. With other cameras, a higher conclusion in likelihood ration can be drawn. The video material is re-encoded by WhatsApp and, with some cameras, the PRNU is no longer available with the method used. With the Apple® iPhone®, the PRNU pattern remained and a conclusion could be drawn if the video was created with the same source camera.

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WhatsApp, PRNU, Source Camera Comparison

C17 Forensic Identification of the Source Smart Phone Camera From a Digital Image

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After attending this presentation, attendees will understand how an image suspected to be captured through a mobile phone camera can be linked though the source mobile phone and the processing required to complete the linking process.

This presentation will impact the forensic science community in terms of an extensive study over a database containing 4,950 images captured through 30 mobile handsets of various makes and models, proposing a novel classifier-based mechanism for the image-linking process. This presentation will further provide an insight to the community to further carry out research in this domain.

Currently, a smart phone is more than a calling device. Other than making telephone calls, taking photographs is the most popular task performed by a smart phone. A recent study revealed that 90% of people have taken a photograph for the first time in their lives with the help of a camera phone. The increasing use of the camera phone has also resulted in the use of images captured through it for criminal intent. In such a scenario, one of the most relevant facts that has to be proved in a court of law is the source camera through which the digital image was taken. Regarding connecting a source camera to a specific image, only a few systematic studies have been reported in this domain. Gul and Avcibas proposed their work on smart phone camera identification based on Singular Value Decomposition.[1] Further, Sandoval et al. proposed a wavelet-based technique over sensor imperfections of mobile devices.[2] Recently, Biney and Sellahewa proposed smart phone model identification based on image features.[3] In spite of the available literature, there is a lack of extensive study in this domain.

In this present study, a modified feature set based on Photo Response Non-Uniformity (PRNU) has been proposed for the identification of smart phone cameras with the query digital images.[4] An extensive study has been conducted on more than 4,950 images captured through 30 cameras of different make and models. Moreover, several attacks were made on the metadata to explore the efficacy of commercially available tools for source camera identification based on metadata information. Results of the proposed scheme are quite encouraging.

In conclusion, this study provides a reliable method for source camera identification as the data from the metadata can be easily modified to make most of the commercially available tools intended for source camera identification foolproof. In such a scenario, image analysis-based method as proposed here results in more reliable results.

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Metadata, Photo Response Non-Uniformity, Image Analysis

C18 A Case Study: Ransomware Containing Child Pornography Observed on an Android™ Mobile Device

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After attending this presentation, attendees will expand their general understanding of malicious software through the presentation of a case study involving ransomware observed on an Android™ mobile device.

This presentation will impact the forensic science community by providing an overview and example of forensic analysis performed on ransomware discovered on a mobile device.

Malicious software that is designed to cause harm to an electronic device (computer, mobile device, etc.) may be considered malware. There are multiple types of malware, such as programs to allow one user to control another user's device, programs to allow one user to spy on another, programs to display advertisements on an infected machine, and programs designed to disrupt a device's ability to function normally.

Ransomware is a type of malware designed to threaten the user unless a ransom is paid. Examples of ransomware include the WannaCry ransomware attack of May 2017, which affected several electronic systems worldwide. Ransomware threats may come in the form of data hijacking, data encryption (such as the WannaCry attack), or unwanted data disclosure (such as revealing private records or potentially embarrassing information to either the public or a specific individual).

This presentation follows the examination of a cellular phone submitted for examination to the United States Army Criminal Investigation Laboratory (USACIL). The device was owned by a soldier accused of downloading and possessing child pornography utilizing his Android™ cellular phone. Examination of the device indicated the soldier was actually the victim of a ransomware attack.

The particular ransomware located on the soldier's device was found within Android™ Package (APK) files typically associated with Android™ application installations. Forensic software allowed for unpacking the APK files to explore the contents more thoroughly. In this case, the APK files contained two Hypertext Markup Language (HTML) files (web pages). One of the files displayed obvious child pornography to the user of the device. The second displayed a fraudulent notice from the "DEPARTMENT OF JUSTICE FEDERAL BUREAU OF INVESTIGATION" imposing a $500 fine for the user's "ATTENDANCE OF THE FORBIDDEN PORNOGRAPHIC SITES."

This presentation will include an overview of the case described above and explore the inner workings of the APK ransomware malware files.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army (DA) or the Department of Defense (DoD). Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, the Defense Forensic Science Center (DFSC), the Office of the Provost Marshal General (OPMG), the DA, or the DoD.

Ransomware, Malware, Examination

C19 Android™ Thumbnails: Is There More? An In-Depth Analysis of the Android™ Photo Gallery and Camera Processes Looking for Metadata

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After attending this presentation, attendees will expand their general understanding of how the Android™ mobile operating system's Gallery application interacts with the camera application/device and how to identify metadata that has being missed in current forensic software tools.

This presentation will impact the forensic science community by providing advanced forensic processing techniques intended to be used to recover critical metadata never before seen in mobile device analysis and image recovery. It will provide an in-depth understanding of the Android™ image processing subsystems as well as provide novel techniques for the manual analysis of the related data files.

The forensic analysis of mobile devices is one of the most critical components of many criminal investigations but never more so than with those involving child exploitation. With the ease of use and access to mobile devices, combined with the inexpensive availability of large data storage, cameras on mobile devices are becoming the default mechanism for picture taking by many communities, especially those which partake in matters of child pornography and exploitation.

One such case involves pictures of a missing girl assumedly taken with, and later deleted on, a Samsung™ mobile phone running the Android™ operating system. The thumbnail images were easily recovered through the use of common forensic software tools; however, since the images were recovered from the Gallery application's cache files, no metadata was recovered that was directly connected to the images in question. The question arose as to whether it can be proven that the actual camera on the cell phone took the pictures and whether the pictures could have been taken around the same time the child went missing. The answers to these questions could provide demonstrable proof of a suspect's participation with a victim at the time of a suspected criminal act.

Deleted pictures on a mobile phone are not uncommon and often only the thumbnail images remain recoverable by current forensic software tools. Thumbnail image recovery is a common technique utilized by mobile forensic analysts during typical cell phone examinations and is usually completed through the carving of graphic file formats from cached storage areas on the device, such as a thumbnail cache. On an Android™ operating system, this is usually accomplished by performing a carving process on the "imgcache" file (a common name, not literal filename) or set of files. While the cache file itself has been fairly well documented, the processes behind the creation and writing to these files and the entire Gallery cache subsystem, as well as what interactions with the device impact these files and how, have not been documented in much detail. There are multiple files that can be impacted by the Gallery application, one of which has been overlooked to date, and it may hold the clues to a missing girl's demise.

The files created by the Gallery application were analyzed for this research to determine whether metadata beyond the thumbnail image itself can be recovered. The entire Gallery process was researched, tested, and analyzed to make a more thorough determination as to the operations of the Gallery application, its interaction with the camera subsystem, and whether advanced forensic processing techniques could be used to successfully recover critical metadata never before seen. This presentation will provide the methods of testing designed and implemented to attempt to answer the investigative questions and determine whether additional metadata is available for recovery. The results of the testing processes and research will be provided, along with new defined processes that can be implemented for data recovery and extraction.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the author's employer. Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors or their employer.

Digital Evidence, Mobile Forensics, Data Recovery

C20 An Analysis of Apple® Mobile Devices to Support or Refute Claims of Spoliation of Data

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The goal of this presentation is to present forensic analysis and case studies of Apple® iOS® mobile device data in connection with claims of data spoliation.

This presentation will impact the forensic science community by discussing various key forensic artifacts analysts can use to confirm or refute claims of data spoliation from Apple® mobile devices that may arise during civil proceedings.

In both criminal and civil court proceedings, the hiding or destruction of digital evidence can be an obstruction investigators and attorneys encounter. These same acts can also provide key evidence in both criminal and civil cases. Specifically, in civil cases, the intentional or negligent act of destroying evidence that is relevant to a legal matter can lead to spoliation sanctions against one party, including financial penalties and dismissal of a case with prejudice. Spoliation claims involving digital evidence have been routinely investigated on computer hard drives using standard digital forensic methods and tools. With the ever-rising popularity of mobile devices in the business world, especially Apple® iPhones® and iPads®, more and more relevant digital evidence is being stored on these devices, requiring digital forensic examiners to confirm or refute claims of spoliation in civil proceedings.

The examination of deleted data is generally performed on physical images of storage media. Ever since the release of the iPhone® 4s and the iPad® 2, Apple® iOS® technology has enabled security features that do not permit the capture of full physical images of Apple® mobile devices without first circumventing the native iOS® security layers, otherwise called "jail breaking." E-Discovery collections for civil matters do not generally involve the bypassing of iOS® security layers or jail breaking custodian devices in order to obtain a physical image. As a result, digital forensic practitioners performing data collections and forensic analysis in civil matters have had to rely on available evidence from logical file systems (as opposed to physical images) obtained through items such as Apple® iTunes® and iCloud® backups, as well as Apple® File Connection (AFC) data.

This presentation will focus on key data areas within an iOS® mobile device that can help digital forensic examiners confirm or refute spoliation claims. Analysis of various areas of mobile devices will include artifacts relating to Mobile Device Management (MDM) policies, various device reset actions, and text message retention/forwarding policies. An analysis of artifacts present on erased iPhones® will be discussed, along with a comparison of extracted data from erased iPhones® that have been restored with previously backed up data using both Apple® iCloud® and Apple® iTunes®. Case studies involving the forensic analysis of spoliation claims from Apple® mobile devices will also be presented with the analysis to demonstrate real-world practical scenarios.

Apple® iOS®, Data Spoliation, Mobile Devices

C21 Snapchat® Data Recovery Capabilities

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After attending this presentation, attendees will expand their general understanding of the methods used to recover Snapchat® content from mobile devices, comparing the ability to recover data from both Apple® and Android™ platforms.

This presentation will impact the forensic science community by providing an outline of the current capabilities for the recovery of mobile device Snapchat® data.

Forensic analysis of mobile devices is one of the most quickly evolving areas of Digital and Multimedia Sciences (DMS). With the development and release of mobile devices occurring at a very rapid pace, Digital Forensic Examiners (DFEs) and mobile forensic software companies are faced with the task of determining how to extract and interpret data from the constantly evolving hardware and software of mobile devices. As each new iteration of mobile device and/or mobile device Operating System (OS) is released, it must be determined how to not only extract data from the device, but how to convert the raw data into a format that makes sense to the end user. The use of mobile device applications, or apps, further complicates data analysis of mobile devices. Not only is the base OS of mobile devices under constant development, but individual application developers release and update apps at a surprising pace.

Snapchat® is a mobile device application that allows users to send and receive multimedia content, such as pictures and video, between specified individual contacts. The transferred multimedia is termed a "Snap." Settings within the sender's Snapchat® application determine how long the sent content will be viewable on the receiver's mobile device, from one to ten seconds. After the time limit has expired on the receiver's device, an attempt is made by the Snapchat® software to delete the data. Security features of the Snapchat® application are also designed to prevent users from taking screen captures of received content through other mobile device applications.

A previous presentation on this topic provided an overview of the examination of an Android™-based mobile device submitted for examination to the United States Army Criminal Investigation Laboratory (USACIL) in a case involving the Snapchat® application. Upon completion of the case, it was determined that traditional *mobile device* forensic software packages were at that time unable to extract any deleted Snapchat® pictures from the mobile device; however, traditional *computer forensic* software was successful at recovering the pertinent content. Further research determined that additional functionalities of the Android™ OS, such as facial recognition capabilities, may be responsible for capturing Snapchat® image data independent of the Snapchat® application on the particular device under examination.

This presentation will provide the outcomes of a research study developed as a result of the aforementioned case and the previously conducted research, comparing the ability (or lack thereof) to recover Snapchat® data from both Android™ and Apple® devices. This research takes into account the potential additional functionalities of the Android™ and Apple® operating systems that may circumvent the data deletion design of the Snapchat® application.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army (DA) or the Department of Defense (DoD). Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, the Defense Forensic Science Center (DFSC), the Office of the Provost Marshal General (OPMG), the DA, or the DoD.

Mobile Device, Snapchat®, Data Recovery

C22 Skimmer Forensics: The Identification, Seizure, and Analysis of These Problematic Little Devices

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The goal of this presentation is to educate attendees on identifying, seizing, and examining credit and debit card skimmers.

This presentation will impact the forensic science community by illustrating how an examiner must resolve the stored information on these devices to actual account numbers, otherwise there is nothing with which to charge the subject of the investigation.

When magnetic card readers are used to steal Personal Identity Information (PII) (e.g., credit or debit card numbers), they are known as skimmers. Many people still associate a skimmer as the hand-held device a waiter or waitress uses to steal a person's credit card number when paying for dinner in a restaurant, but the world of skimmers has matured and evolved. They are custom made and can be placed inside gas pumps, on top of ATMs and point-of-sale terminals, or even secreted inside door access readers. With the emergence of Bluetooth® skimmers, the suspect no longer has to retrieve the device but only has to be in close proximity to pair to the Bluetooth® and retrieve the stolen numbers. To further complicate matters, the manner in which the information is stored on the skimmer can be widely varied using different types of modulation, encoding, and encryption. So even if the odd electronic components of a skimmer, when mashed together, look "illegal," if an examiner is not able to resolve any stored information on the device to actual account numbers, there is nothing with which to charge the person who had the device.

Skimmers are created with various designs, form factors, and architectures and are intended to be hidden from the victim, so they are not necessarily easily identified. Once identified, the seizure, handling, and packaging of skimmers differ from that of other digital evidence and, given their unique nature, there is a lack of vendor tools and processes available to examine skimmers. All of these issues combined equate to one general problem — skimmers present a challenge for law enforcement and the banking system.

Due to the responsibility of enforcing Title 18, USC, Section 1029 — Fraud and related activity in connection with access devices (yes, a credit card or debit card account number is an "access device") — the United States Secret Service created a process for examining credit card skimmers. Not only does the Secret Service process these devices for examination, but educates other state, local, and federal agencies in regard to the methodology. This presentation will walk attendees through skimmer identification, seizure, and analysis. Chip-off processes, python scripting with examiner validation, and Bluetooth® module interrogation will all be discussed. Future work for encrypted skimmers and the emergence of shimmers (a skimming device designed to compromise Europay, MasterCard℠, and Visa℠ (EMV) chip cards) will also be presented.

Much of the material presented will be incorporated into updated versions of a Scientific Working Group on Digital Evidence (SWGDE) document and an American Society for Testing and Materials (ASTM) standard practice, both derived from the United States Secret Service's skimmer examination process.[1,2]

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Skimmer, Credit Card Fraud, Digital Evidence

C23 Darknet Investigation and Forensic Techniques

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After attending this presentation, attendees will be made aware of the utility of using darknet tools to conduct investigations. Attendees will be able to define the darknet in technical terms, know how to install darknet software in the lab, and be able to conduct a darknet investigation. Attendees will learn about recent darknet cases, and how investigators can make use of darknet tools to assist in their investigations.

This presentation will impact the forensic science community by increasing awareness of the darknet and the capabilities of darknet tools and investigative techniques. Further, this presentation will inform attendees regarding the use of darknet tools and investigative techniques in current cases, detailing results and conclusions as to effective techniques based on lab testing.

By reviewing several recent darknet operations, this presentation will explain how others have successfully employed digital and network evidence extraction techniques to investigate darknet cases and how one successful darknet case can jumpstart another. Understanding these darknet investigation techniques impacts the forensic science community by increasing investigator capabilities and options when facing darknet technology, illustrated by their use across several major cases.

The shutdown of online drug and contraband marketplaces AlphaBay and Hansa swept headlines in July 2017; however, prior to AlphaBay, Law Enforcement Agencies (LEAs) prevailed in a number of darknet cases. An increasing number of global darknet investigations have identified suspects and opened cases in jurisdictions across the United States, ultimately pushing arrests down to the state and local level. This presentation reviews darknet technology and techniques such as the ones deployed in these actual investigations.

In one case, unknown to the general public, in the predawn hours beginning April 4, 2015, in time zones across the world, LEAs in nearly every state in the United States and in 17 other countries started knocking on doors, surprising occupants, and handcuffing suspects in one of the most far-reaching child abuse darknet cases in history: Operation Pacifier. Over 200 prosecutions have followed in jurisdictions across the United States. Based on extensive appeals, some are still ongoing as of 2017. In the weeks that followed, defense attorneys, journalists, and the public wanted to know how investigators identified the defendants. This presentation reviews how darknet suspects may be deanonymized in theory and in actual cases.

The scale and scope of the recent and ongoing Operation Pacifier darknet cases is unprecedented. Globally, it encompasses more than 17 countries' Law Enforcement Agencies (LEAs), Europol, and, in the United States, the Federal Bureau of Investigation (FBI) and more than 200 cases in more than half of the United States state judicial systems. Operation Pacifier and other recent darknet cases, such as Silk Road and Operation Onymous, have raised myriad new technical issues, as well as legal issues and rule changes, across many jurisdictions.

While the darknet has a number of legitimate uses, it has also become a haven for criminals.[1-3] Investigators have been developing, refining, and implementing techniques to infiltrate the darknet and use them to solve cases involving narcotics trafficking, carding, identity theft, child abuse, and other illegal activities.[4-7]

Darknet cases and issues reflect that, as technology advances, so do criminal methods. Like a technological cat-and-mouse game, law enforcement has had to develop compensating online darknet tools and tactics. The darknet has spawned unique legal and technical issues, which has required a fundamental change in investigative ground rules, because it conceals suspects' identities by concealing their Internet Provider (IP) address. In order to attack and defeat darknet technology and its anonymity, LEAs have adapted, from use on the surface web, what are broadly called Network Investigative Techniques (NITs). NIT is a term covering a wide scope of investigative strategies, tools, and approaches, including scripts, server takeovers, or simply observing email header information.

Legally, NITs are generally used in searches and seizures of computers, devices, or other technology, which means they may fall under the scope of the Fourth Amendment. Because they are often performed blind with respect to the target's identity and therefore location, they can, and frequently do, involve criminal investigation searches outside the United States, including searches of non-United States citizens.

This study selected and reviewed eight related darknet investigative operations, including Operation DarkNet-Lolita City (2011), Operation TorPedo-PedoBoard (2011-13), Operation Freedom Hosting (2013), Operation Silk Road (2013), Operation Onymous-Silk Road 2.0 (2014), Task Force Argos KidClub (2014), Task Force Argos LoveZone (2014), and Operation Pacifier-Playpen (2014-15). Prosecutions in many of these cases are still ongoing today, as appeals reach higher courts and constitutional and other issues are adjudicated.

Darknet cases and evolving investigative techniques have also raised legal challenges.[8] These include Fourth Amendment search and seizure issues, challenges to the Fifth and Fourteenth Amendments, *Daubert*, and Rule 702 and evidence issues, NIT usage, the standards for scientific validity (to which the President's Council of Advisors on Science and Technology (PCAST) creates new perspective), Sixth Amendment discovery scope issues, and issues of international law, including the role of Mutual Legal Assistance Treaties (MLATs). How these issues have played out in actual prosecutions, as the law advances in response to technology innovations, are reviewed.

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Darknet, Tor, Network Forensics

C24 A Freedom Hosting Darknet Case Study: Anatomy of a Takedown

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After attending this presentation, attendees will better understand the step-by-step techniques by which the suspects in a famous darknet case were apprehended.

This presentation will impact the forensic science community by increasing awareness of the darknet, the capabilities of darknet tools, and the investigative techniques used in current cases. This presentation will detail results and conclusions as to effective techniques based on lab testing and increase awareness of criminal capabilities and how to counter them, as well as the legal issues that arise in these cases, and important implications for warrants and rules of evidence.

Using reverse engineering and other techniques, it will be demonstrated how security vulnerability exploits can help in investigating specific darknet cases. Additionally, this presentation will show how these techniques impact the forensic science community by increasing investigator capabilities and options in darknet cases, illustrated by their use in a major case — Freedom Hosting.

When the shutdown of online drug and contraband marketplaces AlphaBay and Hansa swept headlines in July 2017, United States Attorney General Jeff Sessions said: "... the forces of law and justice face a new challenge from the criminals and transnational criminal organizations who think they can commit their crimes with impunity by 'going dark.' "[1,2]

While the darknet has a number of legitimate uses, it has also become a haven for criminals.[3,4] Investigators have been developing, refining, and implementing techniques to infiltrate the darknet and use them to solve cases involving narcotics trafficking, carding, identity theft, child abuse, and other illegal activity which may be found there.[5-7] Here, one famous case, Freedom Hosting, is reviewed. This presentation will present a deep dive into technical options available and methods required to solve this, and other darknet cases, and to identify, apprehend, and arrest the suspect, Eric Eoin Marques.[8] A combination of techniques to de-anonymize darknet users will be shown.

Freedom Hosting was first targeted by the hacker collective calling itself "Anonymous" in what they called "Operation Darknet" around October 14, 2011.[9] Their attack targeted Lolita City and other child abuse websites on the darknet host Freedom Hosting. The attack, a vigilante operation, was not conducted by Law Enforcement Agencies (LEA). It disrupted but did not succeed in shutting down Freedom Hosting or its darknet websites. Its history helps illuminate the roots of later major darknet operations, including one referred to as Operation Freedom Hosting-2013, conducted by a collaboration of LEAs on the very same darknet host (Freedom Hosting) as Operation Darknet in 2011.

News of the 2013 operation came to light on August 1, 2013, when a reddit user mentioned in an obscure post that he had noticed some unusual "iframe" code on darknet internet service provider Freedom Hosting's websites.[10] On August 3, the local Dublin news service *Irish Independent* reported that Eric Eoin Marques, owner and administrator of Freedom Hosting, a website hosting company, had been arrested five days earlier in a classic phase A (website administrator) sting.[11,12] On August 4, 2013, Tor Project Executive Director Andrew Lewman confirmed to the world that a number of darknet sites had indeed disappeared from vendor platform Freedom Hosting.[13] On August 5, *Ars Technica* put together the local Dublin arrest story and the disappearing darknet sites, and the story went international.[13] We now know of this July 29, 2013, website host sting as a phase A of Operation Freedom Hosting.

What was also not publicly known for another year was that phase B of Operation Freedom Hosting was already underway. LEA deployed an investigative technique referred to as a Network Investigative Technique (NIT) for phase B on August 1-4, 2013. During this time, the Federal Bureau of Investigation (FBI) secretly controlled about 23 live Freedom Hosting sites, including some relatively innocuous sites, such as TorMail.[14,15] They displayed only an error message, while quietly deploying a NIT to catch users.[15] The sting had expanded to target not only the host's administrator, but also site users. In 2014, FBI and Department Of Justice (DOJ) warrants, complaints, and affidavits became public, confirming the operation. The press and public had been distracted with the report of Marques's arrest, not widely realizing that another, wider sting was already underway.

Documents indicate that a NIT had revealed the identities not only of child abuse site administrators, but also of site users. In phase B, the FBI seized and operated the 23 Freedom Hosting websites, deployed NITs, identified site users, and set in motion the arrest and prosecution of users. These users included David and Teri Schell, who were also Silk Road 2.0 sellers, and Grant Klein for child abuse offences.[16,17] While investigators have prevailed in this and many other darknet cases, it is sobering to note that as of February 2017, a darknet website calling itself Freedom Hosting still operates on the darknet.[18]

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Darknet, Tor, Freedom Hosting

C25 A Digital Forensics Tool for the Language-Based Analysis of Child Sex Offender Chats

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After attending this presentation, attendees will have a better understanding of the language-based differences of chats between minors and contact-driven vs. fantasy-driven internet offenders. In addition, attendees will learn about a new digital forensic tool being developed for law enforcement that automatically analyzes minor-offender chats extracted from digital devices.

This presentation will impact the forensic science community through a discussion of the language-based differences of chats between minors and different internet sex offenders. In addition, this presentation will discuss a new tool being developed for law enforcement to assist with their investigations involving internet crimes against children.

Internet crimes against children are technology-facilitated crimes committed against minors involving sexual exploitation. In 2016, the Internet Crimes Against Children (ICAC) task force and affiliates conducted more than 61,000 investigations and 77,800 digital forensic examinations resulting in the arrests of more than 9,300 offenders.[1] Not only is law enforcement swamped by the number of cases and sheer volume of data involved, but child sex offenders are not a homogenous group. Some child sex offenders are contact-driven, and their goal is to meet with the minor in the physical world for sex. Others are fantasy-driven and interested in cyber-sex rather than meeting the minor in the physical world.

It is possible to differentiate a contact-driven offender from a fantasy-driven offender based on linguistic differences in their chats with minors. Chiu, Seigfried-Spellar, and Ringenberg collected chat logs between minors and arrested contact-driven vs. fantasy-driven offenders from Ventura County Sheriff's Department.[2] Based on the preliminary findings, Chiu et al. identified language-based differences in the chats; specifically, contact-driven offenders were more likely to share their negative experiences and emotions with the minor compared to fantasy-driven offenders, which is a self-disclosure grooming tactic.

Based on these results, Seigfried-Spellar, Ringenberg, Chiu, and Rogers proposed a digital forensics tool to assist law enforcement in their ability to triage cases based on the probability of the offender being contact-driven vs. fantasy-driven.[3] Ultimately, this digital forensic tool will assist law enforcement in their ability to efficiently and effectively analyze chats between offenders and minors, possibly preventing contact-driven offences by allocating resources to the more dangerous offenders.

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Child Sex Offender, Digital Forensics Tool, Linguistics

C26 Defining a Taxonomy of Digital Evidence Artifacts Available From Small Unmanned Aircraft Systems

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After attending this presentation, attendees will better understand that data of evidentiary value exists on small unmanned aircraft systems, the affiliated devices and applications, and that a logical taxonomy of data can be observed among the data types present on the devices.

This presentation will impact the forensic science community by sharing the results of data acquisitions from a variety of consumer and professional drones and by increasing comprehension of a taxonomy of the data existing on small unmanned aircraft systems as a starting point for reporting, artifact identification, and support by tools and techniques.

In the past ten years, a technology expansion on traditional remote-controlled aircraft has emerged globally. Consumer- and professional-level drones, referred to as Small Unmanned Aircraft Systems (SUAS), resemble miniature planes or helicopters and range in weight from less than 1 pound to more than 50 pounds. Prices range from a few hundred dollars to thousands of dollars.

Data of evidentiary value exists on small unmanned aircraft systems and the affiliated devices and applications. Law enforcement agencies are already receiving SUAS devices as evidence, but limited tools and techniques exist within the digital forensics community to address this new technology. Law enforcement, forensic service providers, and forensic tool vendors are actively seeking to identify what types of data exist on SUAS systems to determine if SUAS systems should be interrogated for digital evidence artifacts.

Data is acquired from data storage locations of the small unmanned aircraft systems using logical, serial, and physical acquisitions as defined in industry best practices published by the Scientific Working Group on Digital Evidence (SWGDE).[1] Where available, data is parsed with industry-available tools or using custom development when no industry tools are available. The data artifacts are examined and classified to identify a taxonomy of what data presently exists on small unmanned aircraft systems.

This study examines the data types presently on SUAS systems and seeks to define a taxonomy for use by the digital forensics community and law enforcement agencies. The outcome of this study is a proposed initial taxonomy of data from SUAS devices that can be expanded as technology evolves and additional data artifacts are discovered.

The classification of data of evidentiary value from small unmanned aircraft systems will guide law enforcement, forensic service providers, and the digital forensics community as they encounter these new technology devices during investigations.

Reference(s):

1. Scientific Working Group on Digital Evidence. SWGDE Best Practices for the Acquisition of Data from Novel Digital Devices. SWGDE. Washington, DC, 2017.

Drones, SUAS, Drone Forensics

C27 Vehicle Forensics: A Method of Validation for Infotainment System Tracklog Maps Using Java™ OpenStreetMap Editor®

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After attending this presentation, attendees will better understand potential methods for creating and validating infotainment system tracklog maps recovered using a commercial vehicle forensics tool and the free, open source Java™ OpenStreetMap Editor®.

This presentation will impact the forensic science community by introducing a means of validation for infotainment system tracklog maps.

Traditionally, the majority of digital forensic evidence comes from personal computers and digital devices. The locations where digital evidence can be found continue to expand as technology further develops. Vehicles provide a medium for new technologies. Today's vehicles consist of a series of computers and Electronic Control Units (ECUs) ranging from navigation and communication units to automatic, rain-sensing wipers. Newer vehicles can contain more than 70 ECUs, each handling a separate task.[1] Infotainment, a portmanteau of information and entertainment, is a computer system that combines tasks such as navigation, communication, cell phone operations, and internet functions.

There are three main types of stored data found on infotainment systems: navigational data, vehicle event data, and user data.[2] This research focuses on navigational data; more specifically, tracklog maps which consist of data generated by the Global Positioning System (GPS). Tracklog maps contain a series of connected nodes that show the path the car traveled at a given moment. This can include speed, bearing, and distance traveled. Navigational data can also include recent locations, favorite places, waypoints, and trackpoints.

Research and method validation of obtaining digital artifacts from vehicle infotainment systems have been limited to this point, most likely due to the newness of the vehicle forensics field. At this time, there is only one vehicle infotainment system forensic tool that is currently available in the commercial marketplace.[2,3] This means there is currently not another tool to validate evidence obtained by this commercial tool. While this tool is still relatively new in the digital forensic field, it currently supports over 5,200 different models of vehicles from 22 different vehicle manufacturers.[3] This research provides a means of validation for tracklogs not found in any previous research regarding vehicle forensics.

This research was guided by the following questions: What third-party tools can be used to map coordinates with existing road overlays? How do tracklog maps produced by commercial tools compare to tracklog maps produced by other third-party tools? How accurate are tracklog maps created by both the commercial and third-party tools? How can a third-party tool be used to validate the commercial tool's method of creating tracklog maps?

For this study, data from a physical acquisition of a 2015 Ford® F-150 was used. An abundance of data was recovered from the acquisition, including all three types of data found on infotainment systems. Tracklog maps were produced automatically by the commercial tool for three years of the vehicle's life. Third-party tools such as Google® Maps™, Google® Earth™, and Java™ OpenStreetMap Editor® were evaluated as possible useful applications in the validation process. Google® Maps™ and Google® Earth™ did not have a convenient means of entering data to map a tracklog. Java™ OpenStreetMap Editor® was found to have the functions necessary for use as a third-party tool to validate the tracklog mapping function of the commercial tool.

Mapping tracklogs using Java™ OpenStreetMap Editor® produced route maps that were identical to the ones produced by the commercial tool's mapping function. It was found that Java™ OpenStreetMap Editor® could enhance the data obtained by the commercial tool by creating an overlay of street maps and satellite imagery. This provided a visual representation of the actual route taken by the vehicle. Lastly, a validation method was produced using Java™ OpenStreetMap Editor® as a third-party tool to validate the mapping functions of the commercial vehicle's forensics software.

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2. Coronetto A.D., LaMere B., McGee C. Vehicle System Forensics: Introducing Your New Star Witness. *US Law*. 2015 Fall/Winter.
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Vehicle Forensics, Infotainment Systems, Method Validation

C28 Is Your Home Secretly a Confidential Informant for the Police? The Potential of Smart Home Devices to Serve as Evidence in Criminal Cases

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After attending this presentation, attendees will better understand what data of evidential value is contained on various smart home devices as well as techniques to forensically acquire the data.

This presentation will impact the forensic science community by providing the techniques to acquire data of forensic value from various smart home devices.

We are living in a society where more and more of the items that we come into contact with each day are connected to the internet. This phenomenon has powered an entire technological industry known as the Internet of Things (or IoT). Nowhere can this idea be better exemplified then in the modern home. As recently as ten years ago, the average home would have been lucky to have one device connected to the internet, namely the home Personal computer (PC). Now we are seeing a diverse range of devices in the modern home that are also connected to the internet (from the light switch to the kitchen sink and everything in-between). This has given rise to the new smart home, where these devices are constantly monitoring activity and trying to help make people's daily lives easier.

This explosion in internet-connected devices (and the terabytes of data that comes with them) brings an interesting question to the forefront for law enforcement: Can these smart devices act as eyes and ears for the police and help solve modern crimes? It is already known that the Amazon® Echo™ must be continually listening to every spoken word in order to be at our beck and call, but does this mean that it can also record the voice of the home intruder, and if so, can that voice be easily recovered *without* the help of Amazon®?

The goal of this study was to determine the forensic value of several common smart home devices and the techniques to acquire the relevant data from these devices. The research focused on the Amazon® Echo™ and Google® Home Smart Hubs™, the D-Link® 965 Wi-Fi Video Security Camera, the Logitech® Logi Circle Wireless HD Video Security Camera with 2-Way Talk, and the August® Smart Lock. This study determined what data was stored locally on these devices versus stored in the Cloud and explored techniques to extract that data.

Smart Home, Smart Devices, Internet of Things

C29 Smart Home (Home Automation) Forensics: An Analysis of an Amazon® Echo™

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After attending this presentation, attendees will obtain the results of research regarding the availability of forensic artifacts maintained by smart home (home automation) devices such as the Amazon® Echo™.

This presentation will impact the forensic science and investigative communities by providing sources for data potentially relevant to criminal investigations maintained within home automation devices such as the Amazon® Echo™.

Digital Forensic Examiners (DFEs) are responsible for extracting data from a growing number of electronic devices and performing analyses on a multitude of different resultant data types. It is the responsibility of the members of the Digital and Multimedia Evidence (DME) community (DFEs and researchers) to determine how to extract data maintained on these new electronic devices, interpret the extracted data correctly, and discover if the data may be a potentially useful source of information to aid in future investigations. With the expansion of smart home devices, it has become the responsibility of the DME community to search for potentially pertinent data stored within devices such as the Amazon® Echo™.

The Amazon® Echo™ is a voice-enabled wireless smart speaker developed by Amazon.com, Inc. and released in the United States in 2015. The device consists of a cylindrical speaker with a seven-piece microphone array. A smaller version of the device was released as the Amazon® Echo Dot™. The device connects to the voice-controlled intelligent personal assistant service, Alexa™, which responds to a "wake word" of the name "Alexa." The device is capable of voice interaction, music playback, making to-do lists, setting alarms, streaming podcasts, playing audio books, and providing weather, traffic, and other real-time information. It can control several smart devices using itself as a home automation hub. In the default mode, the device continuously listens to all speech, monitoring for the wake word to be spoken. The device hears from across the room with far-field voice recognition, even while music is playing.

This presentation will consist of an overview of the Amazon® Echo™ device, a history of the device's usage in police investigations, and an overview of data types extracted from exemplar devices. This presentation will provide the results of an exploratory study into forensic artifacts left behind on the Amazon® Echo™ and explore how these artifacts may be used to aid future criminal investigations.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army (DA) or the Department of Defense (DoD). Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, the Defense Forensic Science Center (DFSC), the Office of the Provost Marshal General (OPMG), the DA, or the DoD.

Amazon® Echo™, Forensic Artifacts, Digital

C30 The Virtual Crime Scene: The Role of 3D Motion Capture and 3D Model Buildings for the Reconstruction of Dynamics and Reproduction of Settings in a Case of Murder

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After attending this presentation, attendees will better understand the role of virtual reality through the use of 3D Motion Capture (Mocap) and settings reconstruction technology.

This presentation will impact the forensic science community by demonstrating how these techniques are fundamental to accurately recreating scenes and environments in cases of murder, accidents, suicides, or altercations where it is important to determine the dynamics of the event.

The use of images is a fundamental aspect in a forensic pathologist's work. To date, photographic surveying is performed primarily by using 2D images; however, in recent years, the application of 3D technologies has gained importance. In the literature, various methods have been studied, including virtopsy, 3D printing, scanners, cameras, 3D modeling software, photogrammetry, superimposition, and satellite navigation programs. The applications were personal identification, estimation of age, sex, height, body mass, injury analysis, weapon identification, crime scene scanning, and Bloodstain Pattern Analysis (BPA). Very few studies have proposed a method for reproducing the dynamics of a crime. This study presents a virtual model created using 3D Mocap and modeling, namely the reproduction of settings. Mocap is a photogrammetric system designed to capture the movements of a person who wears tracks equipped with markers and is recorded by cameras through an algorithm to realistically reproduce movements. It also offers the ability to perform object tracking in space and head tracking to capture the person's facial movements. The recorded movements are interoperable with Motion Builder®, Max®, Maya®, Xsi®, Blender®, C4d®, Poser®, Daz® Studio, Face Robot®, and the Autodesk® Gameware product series, including HumanIK® used to create video game characters.

This presentation describes the method for producing a 3D video that reproduces the dynamics and the scene of a murder, later used in court. The device used was a stereophotogrammetric system, consisting of infrared OptiTrack® cameras capable of capturing movements up to 100 frames per second (fps). Some objects have been modeled in 3D manually, taking into account the original measurements.

A woman was found dead in her apartment with multiple injuries on her head, chest, and hands. A judicial inspection was conducted in which the planimetry of the apartment was requested. Each object was cataloged, photographed, and annotated. The analysis focused on the entrance (where the victim had been found) and the kitchen (an area to the left of the entrance where items on the ground were found). The choice of these areas resulted from the analysis of the other rooms that showed no trace of blood or signs of a scuffle. An autopsy was performed to topographically identify the injuries present on the anterior and posterior surface of the corpse, inflicted with a knife (12 lesions) and a blunt object (22 lesions). Each injury was numbered, photographed, and measured. The height and weight of the victim were recorded. During the investigation, the suspect focus was on a man. An external inspection was conducted on the man, whose height and weight were noted, and some abrasions on his face were also measured. Finally, a BPA of the house was performed. All data were compared and the dynamics were reconstructed. By comparing the data discovered at the scene, on the victim, and on the aggressor, the 3D computer technician was able to virtually recreate the environments in which two people, of the same heights and constitutions as the victim and the aggressor, mimicked the movements before the murder and the blows made during the murder. At this stage, the rendering of frames was conducted: this is one of the fundamental aspects of 3D computer graphics. It is the last stage that generates the representation of the final image, with the help of algorithms that define the color of each point. The rendering was entrusted to Supermicro® systems with multiprocessor Xeon® Quad and NVIDIA® Quadro® Plex systems for Graphics Processing Unit (GPU) rendering projects. This reconstruction allowed the forensic pathologist to give credibility to the hypothesized reenactment by making clear to the public prosecutor and to the investigators the dynamics and the cruelty of the blows. Therefore, the use of these forensic techniques is emphasized for their contribution in exploring the dynamics that otherwise would be difficult to demonstrate.

Forensic Science, Virtual Crime Scene, 3D Motion Capture

C31 Overcoming the Hurdles of Imaging, Storing, and Archiving Digital Evidence

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The goals of this presentation are to determine the best ways to increase efficiency and decrease storage space during digital imaging.

This presentation will impact the forensic science community by helping digital forensic examiners reduce imaging time and decrease the amount of storage each image takes.

Digital forensics is an integral part of the forensics field. Digital evidence is information stored or transmitted in binary form that may be introduced and relied on in court. Digital evidence can be found on a computer hard drive, a mobile phone, a Personal Digital Assistant (PDA), a Compact disc (CD), and a flash card in a digital camera, among other places. Digital evidence is commonly associated with electronic crime, or e-crime, such as child pornography or credit card fraud.[1]

When digital forensic examiners obtain a digital device to be analyzed, they first image and save an exact, bit-for-bit copy of the original storage media on another storage device, such as a hard drive. This is known as the forensic image. Forensic imaging involves two types of images: a logical image and a physical image. A logical image is a copy of all the files on a storage device, except deleted files, file fragments, and deleted space from a partition. This allows an investigator to quickly scan the contents of a hard drive. An E01 file is an example. A physical image is a bit-for-bit copy of the storage device, including the deleted files and file fragments. A RAW file is an example.

Once imaging is completed, a digital fingerprint of the media is acquired, known as a hash. The hash generation process involves an algorithm calculation of all the zeros and ones that exist across the sectors examined. Altering a single zero to a one or a one to a zero will cause the resulting hash value to be different. If the hash values match, then the image is an exact copy and is used as the working copy to analyze the data.[2]

Because of the increase of digital evidence being submitted for analysis and the length of time evidence is required to be kept, untested digital evidence and already processed evidence are accumulating. The purpose of this research was to look at ways to image, store, and archive digital data to take up less storage space and provide greater efficiency. This will help decrease the time spent to test pieces of evidence, thereby decreasing backlogs, and decreasing costs.

The following questions guided the research: (1) What is the best way to create a forensic image (E01 file or RAW file)?; (2) What is the best way to store that E01/RAW file?; (3) What is the most efficient way to process that image?; and, (4) In the most efficient, cost-effective way, how should that data be archived?

Eight different types of storage devices were imaged using two different types of files, E01 and RAW. This was performed with two forensic imager tools, AccessData® FTK® and SUMURI® PALADIN®. A comparison was performed using different paths, with and without a write blocker, and using different levels of data compression. This determined the fastest way to image digital evidence and the best way to minimize the space taken by saved evidence. Since PALADIN® has a software write blocker, the images produced using the hardware write blocker in FTK® were compared to the images without an additional hardware write blocker in PALADIN® (only using the software as write protection).

The results demonstrate that RAW images sent directly to the server using PALADIN® without a hardware write blocker was the fastest and most efficient way to image, but E01 in FTK® took up 5%–18% less storage space. Imaging E01 files in PALADIN® resulted in failure approximately 50% of the time, especially when using an additional hardware write blocker. In FTK®, adding compression did not change the amount of time or storage space taken. In PALADIN®, adding compression increased the time while decreasing space. Future research would include imaging larger storage devices, testing different software and write blockers, and adding a virtual machine.

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2. *Forensics: What is imaging?* (2009, June 27). https://whereismydata.wordpress.com/2009/06/27/forensics-what-is-imaging/.

Digital, Forensic, Imaging

C32 Measuring the Hygroscopic Capacity of Integrated Circuit Packages and Circuit Boards

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After attending this presentation, attendees will understand: (1) that integrated circuit packages (computer chips) and printed circuit boards have the capacity to absorb water they come in contact with; (2) the variables affecting the amount of water absorbed; and, (3) the implications of hygroscopic capacity when addressing electronic devices that have been exposed to or damaged by liquids.

This presentation will impact the forensic science community by providing results from direct methods experiments guided by extensive research in other science disciplines, but with little previous research applied to digital forensic science. This presentation seeks to broaden the understanding of the impact of liquid exposure to electronic devices by examining the effect on the two primary discreet parts of electronic devices.

The electronics manufacturing and assembly industry has well-defined standards regarding moisture sensitivity of electronic device components related to manufacturing and assembly of electronic devices.[1,2] These known industry standards have yet to be applied and inform the manner in which digital forensic scientists are addressing electronic devices exposed to or damaged by water or other liquids.

Significant risk exists to data stored within integrated circuit packages if specific steps are not followed when addressing electronic devices that have been exposed to water or other liquids. Liquid absorbed within the integrated circuit packages may turn into a vapor, creating damaging expansion within the chip if the device is exposed to heat.[3]

In this study, integrated circuit packages (computer chips) and printed circuit boards were exposed to a variety of liquids to understand the amount of liquid the discreet devices will absorb over a defined duration. The hygroscopic coefficient is the equilibrium when the maximum amount of liquid can be absorbed. This study sought to identify the time duration at which the hygroscopic coefficient is achieved on integrated circuit packages and printed circuit boards.

By understanding the amount of liquid absorbed in a given duration, the results may inform digital forensic science practitioners on the duration of drying that will be required to safely remove the absorbed liquid from the devices. Additionally, different liquid types (e.g., freshwater, salt water, and brackish water) may absorb at different rates, requiring different techniques based on the type of liquid exposure.

Testing for this study included the use of laboratory-grade water testing equipment and analytical balances to measure the impact of liquid and duration against the materials composition of the electronic devices.

The results of this study may impact the direction provided to Forensic Service Providers (FSP) and the practices used by FSPs when addressing evidence that has been exposed to or damaged by liquid.

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Hygroscopic Capacity, Liquid Damage, Water Damage

C33 Electromagnetic "Soundscapes" and Their Relevance in Media Forensics

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After attending this presentation, attendees will understand: (1) Electromagnetic Fields (EMF) and their origins; (2) quantification of the electromagnetic soundscapes in urban areas and in buildings; (3) the recording equipment and its susceptibility to EMF measurements; and, (4) what traces are left in audio recordings.

This presentation will impact the forensic science community by demonstrating how EMFs stemming from the Electric Network Frequency (ENF) have value for the authentication and "time stamping" of digital recordings carrying audio; however, EMFs stemming from other sources may have a value for the determination of the site of recording.

EMFs stemming from the ENF has, when accidentally recorded, proven to have a value for the authentication and "time stamping" of digital recordings carrying audio; however, magnetic fields stemming from sources other than power lines may, when present and recorded, also become useful for the forensic investigation of digital (and analog) recordings.[1] The electromagnetic signature recorded as audio may provide information of the site of recording.

This paper presents the results of an initial study in the field.

In urban planning as well as in sound art, the term "soundscape" has been applied to site-specific recordings since being introduced by Michael Southworth in 1969.[2] The analysis of the soundscape reflects incidents of the environment (i.e., noise in or around urban spaces (near or distant), buildings, traffic, natural sources, installations, peoples' activity, music, etc.). This type of analysis is also valuable for the assessment of background sounds on forensic recordings.

For years, artists have also searched for the sound of underwater locations using hydrophones, the sound of vibrating structures using accelerometers, and the sound of electromagnetic fields using various forms of coils connected to the input of audio recorders. Work of this type has now and then revealed various interesting electromagnetic signatures, which to some extent are regarded as site specific.

That said, no recording device should ever transform the EMFs into audio. The general standards (IEC 61000, CISPR 1x, etc.) for Electromagnetic Compatibility (EMC) provide a set of rules that ensures that no generator of such electromagnetic disturbances exhibits a magnitude large enough to be detected in audio recorders; however, casework has shown that site-specific electromagnetic events accidentally have been recorded/transmitted by (mobile) recording systems, such as voice recorders and cell phones. These incidents are the motivation for an initial study to indicate whether or not this a serious matter in media forensics. The magnitude of ENF-related electromagnetic fields in different (urban) environments has been previously documented.[3] This presentation discusses some of the other electromagnetic sources and some possible ways they end up as audio.

Examples of sources (Audio Frequency (AF) -range) occasionally appearing in forensic audio recordings are: (1) ignition noise (cars, bikes); (2) noise on power lines (machinery-induced noise, for instance, spot-welding devices); (3) noise in public transportation (electric trains, subways, light rail); (4) induction loops for assisted listening (spill); (5) elevators (relays and motors); Nad, (6) other sources.

Examples of recording devices are: (1) cell phones with and without a headset attached; (2) voice recorders (analog and digital) with and without an external microphone: and, (3) laptops with gamer gear.

The test was conducted by feeding a sine sweep into a power amplifier loaded by a 3.3-ohm resistor in series with a .47mH coil. The Device Under Test (DUT) was placed 10cm above the coil. The system was calibrated using a reference coil system (400mA/m ~ 0.775V). Devices were tested in EM-fields up to 1A/m, operating the unit alone with no attachments and also using the unit with external earbuds and microphone.

The general result demonstrates that modern mobile recording devices tested exhibit high susceptibility to Audio Frequency (AF) broadband electromagnetic signals; however, of course, old tape-based systems are indeed sensitive. The conclusion from this survey is that electromagnetic fields above 100Hz and with a field strength below 100mA/m are hardly detectable in modern digital devices.

In normal urban surroundings, a field strength above 100Hz and higher than 100 mA/m is rare. When audible in digital recordings, it typically concerns recording/phone units mounted with external microphones or units connected to poorly filtered power lines.

The impact on crime scene analyses is relatively small as it seldom occurs; however, one must be aware of the existence of the phenomenon.

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Electromagnetic Field (EMF), Soundscape, Site-Specific EMF

D1 Quantifying 3D Gait Reconstruction With a Single Camera

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After attending this presentation, attendees will understand the usefulness and methodology of reconstructing 3D human movements from sequential images, such as Closed-Circuit Television (CCTV), and of estimating the lengths of body segments and joint angles. Attendees will understand 3D body movement estimation using Principal Component Analysis (PCA) and the effect of the camera angle on the estimation accuracy.

This presentation will impact the forensic science community by providing relevant information on quantifying 3D gait parameters and body segment lengths from 2D images.

Gait has been studied as a biometric parameter useful for identifying individuals. Human gait is influenced by biomechanical and physiological factors such as weight and height as well as injuries associated with the skeleton or the brain. Therefore, by measuring an individual's gait, his or her personal biometrics can be estimated. Thus, it is possible to verify a person's identity using an analysis of his or her gait on CCTV even when the person's face is obscured. Currently, there have been several studies that attempt to recognize gait from single images.[1,2] Recently, 3D gait reconstructions were performed from 2D images using PCA.[3] In some studies, the results of observing 2D images may vary depending on camera angles.[2,4] Also, to reconstruct 3D gait, major joint points should be recorded; however, marking such points on every image frame is time consuming. The objectives of this study were to develop a method to reconstruct 3D body movements from sequential 2D images and to analyze the effect of camera angle on the accuracy of the gait-related movement reconstruction.

3D gait data were collected from each of the 30 recruited subjects using a motion capture system. 3D coordinates of each joint in each image frame were obtained and subjected to PCA analysis to obtain mean posture and principal component data. Following acquisition of the 3D joint coordinate data, the position and orientation of the camera could then be changed to obtain projected 2D images. 3D gait data were used to create 2D images taken from a virtual camera at 0º to 60º elevation and 0º to 180º azimuth angle. The 3D posture of each subject was reconstructed using PCA for the key frame image at each camera angle by optimizing the weight of principal components of posture and the transformation matrix. Also, the study developed a robust method using information from the phase of gait to lessen the effect of camera angle. From the reconstructed posture, segment lengths were calculated by measuring the distance between joints. Major joint angles were extracted from reconstructed full-gait cycle images through movement estimation accomplished by interpolating positions between key frames.

The results of this study confirmed that gait analysis for identification is best performed by viewing subjects from the sagittal plane rather than the coronal plane. Also, the higher the elevation angle of the gait imaging camera, the better the reconstruction accuracy. The phase of subject gait was shown to be an input parameter; consideration of this parameter reduced inaccuracies occurring from varying camera angles. This study was limited by the use of manually identified joint markers and interpolation of gait-related continual images. The results of this study support the argument that individual human subjects can be identified from analysis of their optically recorded 3D gait cycle.

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Gait Reconstruction, Human Motion, Single Camera

D2 The Advancement of the Evaluation Method of Fracture Risk by a Blow Using Computer Simulation-Calibration by an Actual Fracture Experiment

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After attending this presentation, attendees will understand the advanced fracture risk evaluation method using computer simulation.

This presentation will impact the forensic science community by providing an advanced fracture risk evaluation method using computer simulation.

To aid in the determination of intent, specifically in cases of assault, child abuse, and negligence, it is necessary to establish the relationship between the type and magnitude of external force associated with human injury. While the relationship between force and injury has been studied for many years, recent studies reveal that the presence of soft tissues, such as skin and muscles, have a large influence on this relationship, particularly those resulting in bone fracture; however, since the dynamic mechanical properties of soft tissues are difficult to measure, they are not sufficiently clarified, and thus it is currently impossible to quantitatively evaluate the role of soft tissue. While laboratory studies imposing quasi-static loading have shown a relationship between bone mineral density and bone strength, evaluation of bones resistance to high strain rate loading (i.e., fracture from high-impact, sudden intentional injury) has not been well studied. The former studies have been accepted by Japan's Ministry of Health, Labor, and Welfare, but injuries from the latter mechanism are not well understood. Computer simulation of these injuries is confounded by: (1) lack of understanding of the viscoelastic (energy absorbing) properties of soft tissue; (2) the inability to verify the results of such simulations using actual representative human bones; and, (3) immature bones from children and infants. Thus, the purpose of the present study was to quantitatively evaluate the biomechanical buffering properties of soft tissue on the maximum load-bearing properties of human finger bone.

Drop-weight tests were performed using weights, impact velocities, and soft tissue thicknesses (actual and "dummy") skin. The influence of these variables on the buffering properties was investigated. In addition, for verification targeted at advanced fracture risk evaluation using computer simulation, fracture experiments and computer simulations were performed on pig tails to simulate human fingers. Data obtained from the experiments were compared with the results of the simulations. The fracture experiments used static compression testing to quantify the force required to produce a fracture. The computer simulations used Finite Element Analysis (FEA) to estimate the force to fracture. To gain information about the force necessary to fracture infant bone, fracture experiments and computer simulations were performed on young animal bone.

As a result, in the quantitative evaluation method of fracture risk using dummy skin, it is obvious that the maximum permission load is the same when the impact energy is the same, regardless of impact velocity at the soft tissue and dummy skin of 10mm or more. For example, when the soft tissue thickness is 10mm, the relationship between impact energy E_{imp} and maximum transmission load F_{max} can be expressed as F_{max}=764.11 E_{imp}. From this, it became possible to calculate the load applied to the bone through the human skin from the impact energy calculated using the dummy skin. Moreover, in the comparison of the fracture load value obtained from the fracture experiment with the numerical analysis result by FEA, it was found that the calculated fracture load (i.e., 1,883N–2,118N) was nearly equivalent to the result of the fracture experiment (i.e., 1,940N–2,150N). These results demonstrate that the accuracy of fracture risk prediction may be improved using computer simulation. The results also demonstrate that computer simulation is an effective tool in finger fracture risk evaluation and may also be applied in future studies to understand the dynamic mechanical properties of soft tissue and how it affects bone fracture loads.

Fracture Risk Evaluation, FEM Analysis, Dummy Skin

D3 Fulgurites in Litigation

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The goal of this presentation is to illustrate the way in which a detailed microanalytical characterization of a relatively novel material, a fulgurite (amorphous silica produced by an energetic event), can provide detailed information about the origin of a fire.

This presentation will impact the forensic science community in two ways: (1) by illustrating the ways in which a novel material and creative analytical approach can provide critical scientific evidence in a forensic investigation; and, (2) by providing the community with a baseline of specific analytical data concerning the use of a fulgurite as forensic evidence.

The term fulgurite, which derives from the Latin *fulgur* (meaning "thunderbolt"), was originally intended to refer to amorphous silica produced by lightning strikes. Over time, this term has been more broadly applied throughout the literature to include amorphous silica (or related compositions) produced as a result of high-temperature or high-pressure events, which can also include anthropogenic activities.

A suspected fulgurite can be identified as such on the basis of specific analytical characteristics, which can include: morphology (a tube that can take on a dendritic shape); size (fulgurites typically range in volume from a cubic centimeter to a cubic meter); an amorphous structure; marginal zones that include transition grains in which a single grain can range from crystalline to amorphous; a hollow central channel that is often nearly circular; the presence of one or more silica high-temperature/high pressure-polymorphs (e.g., tridymide, cristobalite); gas inclusions; and flow lines captured in the quenched amorphous matrix. Such characteristics can be identified over a variety of length scales that range from field-level observations (centimeters to meters) to nanoscopic features (nanometers).

Once identified as a fulgurite, analytical characterization over this range of length scales can provide information about the mechanism by which it formed. In the context of a forensic fire investigation, the need may arise to determine whether a fire was started by a lightning strike or a power line discharge. While both events are the result of an electrical discharge of power, the magnitude and duration of such power transfers can vary dramatically. As such, certain features may be used to distinguish between the origin of this electrical discharge.

While analytical approaches will vary based upon the circumstances of a given case, the features that may be exploited to assist with such a determination include the composition of the fulgurite and surrounding host rock, the crystallography of the transition zone grains, the potential transfer of metal particles, and the macroscopical and microscopical morphology of the fulgurite. The mineral phase of certain grains as well as the microstructure (through analysis by Raman spectroscopy) can provide specific constraints on the pressure-temperature history of a sample. The above analytical data can be coupled with thermodynamic modeling of the possible scenarios. This combination of analyses and calculations can provide an independent means by which to elucidate the origin of the discharge that produced the fulgurite.

This approach may provide constraints on the cause and conditions of fulgurite formation, distinguish between natural and anthropogenic origins, and, in some cases, provide insight into the relative timing of fulgurite formation. This presentation will provide analytical and modeled data derived from the study of several fulgurite samples produced both anthropogenically and naturally, which will illustrate the ways in which fulgurites may be recognized and exploited in a forensic investigation. Ultimately, the application of such a seemingly esoteric material can provide pivotal information as to the cause of a fire during the course of a forensic investigation.

Fulgurite, Amorphous Silica, Lightening

D4 The Role of Engineering Sciences in Forensic Fire Investigation

S.B. Addison Larson, MS, Sherman, CT 06784*

After attending this presentation, attendees will better identify how current misconceptions within the forensic science community regarding the role of the engineering sciences in forensic fire investigation have improperly confined the dynamic and multidisciplinary field of forensic fire investigation and placed too much emphasis on arson detection.

This presentation will impact the forensic science community by identifying organizational guidelines, policies, and procedures that are needlessly inhibitory and by substantiating the position that forensic fire investigation may be better aligned with the engineering sciences than general investigation sciences.

In April 2017 at the Congressional Fire Services Institute (CFSI), the American Academy of Forensic Sciences (AAFS) and the International Association of Arson Investigators (IAAI) affirmed a mutual desire for "a strong and mutually beneficial relationship between the two organizations."[1] IAAI was accredited by the Forensic Specialties Accreditation Board (FSAB) in March 2015.[2] The official position statement from IAAI recognizes the authority of the National Fire Protection Association (NFPA) and the value of NFPA 921: Guidelines for Fire and Explosion Investigation which is the "foundation for its training and certification programs."[3,4] It would stand to reason that AAFS also considers NFPA an acceptable source.

NFPA 1033 Standard for Professional Qualifications for Fire Investigator lists several areas of up-to-date knowledge a (fire) investigator shall have and maintain beyond the high school level, but does not require an engineering degree. One-third of the topics listed are largely engineering specialties — thermodynamics, fire dynamics, explosion dynamics, failure analysis/analytical tools, and fire protection systems.[5] NFPA standardizes fire protection engineering but makes distinctions within the field in terms of formal education. A fire engineering technologist (or technician) is an individual who does not hold an engineering degree, but has obtained a secondary education in "fire engineering technology, fire and safety engineering technology, or a similar discipline."[6]

Fire investigation guidelines/methods (NFPA 921) are closely related with fire safety code (e.g., NFPA standards 2217, 5578,) and building codes (i.e., general design requirements, structural tests, inspections, acceptable building materials) and the application of engineering principles to fire protection. This is of interest in civil litigation due to increased liability and can be useful in all phases of building construction, industrial losses and disputes, product liability and forensic analysis, and plan review.

Among the multiplicity of forensic professions that incorporate a broad range of knowledge is the local fire marshal, the authority having jurisdiction. The fire marshal's regulatory function "often overlaps with the building official…[and] planning departments...[and] engineering departments."[9] Code interpretation is considered a "higher level activity," particularly reviewing proposals for performance-based design. The relationship between engineering and fire science is intrinsic, just as fire science is to fire investigation.

This presentation will address conflicting definitions which contrast the term "arson investigation" with the preferred term, "fire investigation" — the interpretation of fire pattern evidence which, until demonstrated otherwise, may be the result of accidental or natural causes. If the authority having jurisdiction believes the fire may have incendiary cause and origin, the investigation will become an arson investigation and may likely incorporate other forensic tools, such as toxicology and analytical chemistry, etc. An engineering approach can be useful to correctly exclude other causes, after which the investigator can work inductively to demonstrate incendiary causation through laboratory and other testing.

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4. *NFPA 921: Guide for Fire and Explosion Investigations*. National Fire Protection Association (2017).
5. *NFPA 1037: Standard on Fire Marshal Professional Qualifications*. National Fire Protection Association (2014).
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7. *NFPA 220: Standard on Types of Building Construction*. National Fire Protection Association (2015).
8. *NFPA 557: Standard for Determination of Fire Loads for Use in Structural Fire Protection Design*. National Fire Protection Association (2016).
9. *NFPA 1037: Standard for Professional Qualifications for Fire Marshal*. National Fire Protection Association (2007).

Fire Investigation, Engineering Sciences, Fire Marshal

D5 The Impact of Ventilation on Fire Damage Patterns From Room Fires in Full-Scale Structures

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After attending this presentation, attendees will better understand the cause-and-effect relationship between ventilation and fire damage inside of full-scale residential structures. This research addresses a topic that, in the past, has challenged arson investigation findings.

This presentation will impact the forensic science community by providing data and analysis from ventilation-limited structure fires and the flow path(s) within the fire structure. This type of analysis is essential for accurately determining the area of origin based on the fire damage patterns. The results from a series of full-scale fire experiments document the repeatability and development of fire patterns based on the availability of oxygen within the structures and how this aids in determining the area of origin.

During the past decade, research conducted for the purpose of examining fire-fighting tactics has brought to light the impact that changes in home construction materials, contents, size, and geometry have had on fire incidents. Today, fires are predominantly fueled by synthetic contents. The combination of energy-efficient construction and high-heat release-rate fuel loads commonly lead to ventilation-limited fire conditions. Therefore, how and where the fire receives oxygen for combustion impacts the fire dynamics and subsequent fire-damage patterns.

The experiments were planned with the assistance of a technical panel that included members of the Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF), International Association of Arson Investigators (IAAI), National Association of State Fire Marshals (NASFM), National Institute of Standards and Technology (NIST), NIST Organization of Scientific Area Committees (OSAC), and National Fire Protection Association (NFPA) 921. The test scenarios ranged from fires in the structures with no exterior ventilation to room fires with flow paths that connected the fires with remote intake and exhaust vents throughout the structures. Room-of-origin scenarios included the living room, bedroom, and kitchen.

An overview of the results from a series of 20 full-scale residential-style structure experiments examining the impact that changes in ventilation had on the fire damage inside will be presented. The test structures included a one-story ranch and a two-story colonial. The floor area of the ranch was approximately 110m^2 (1,200ft^2). The colonial had a two-story family room and open foyer with an approximate floor area of 300m^2 (3,200ft^2). Each of the comparable experiments used similar furnishings and interior finishes, providing a level of repeatability between the experiments. Each structure had more than 200 channels of instrumentation. Sensors to monitor temperature, pressure, gas velocity, and oxygen concentration were located at strategic points throughout the structures. Video and infrared imaging cameras were also used to document the experiments.

This research demonstrated how combining a basic knowledge of fire dynamics with an understanding of the flow paths of oxygen and heat through a given structure can serve as a tool for analyzing the movement of the fire through the structure as well as identifying the area of origin.

This project was supported by the National Institute of Justice, Office of Justice Programs, United States Department of Justice.

Fire, Investigation, Ventilation

D6 Steering Failure in a Triple Fatality Crash

David Hallman, MS, Hallman Engineering LLC, 14040 96th Avenue, N, Maple Grove, MN 55369*

After attending this presentation, attendees will have a basic understanding of vehicle steering systems, the role of steering system failure in this particular crash, and the interpretation of available evidence that indicated the root cause of failure in relationship to the timing and location of the crash.

This presentation will impact the forensic science community by identifying a pre-crash failure that could easily be overlooked by an investigator unfamiliar with vehicle steering systems and the evidence and interpretation of the evidence that led to the discovery of this failure.

Crash reconstruction is the investigative process of examination and evaluation of all available physical evidence related to the crash in an attempt to determine the sequence of events prior to, during, and immediately after the crash. This is accomplished through an evaluation of the vehicles, tire marks, and potential electronic crash data imaged from vehicle control modules. Collectively, this evidence can be interpreted by a trained crash investigator and used to determine crash causation. This presentation will discuss the evidence and the interpretation of that evidence, which led to the determination of the root cause of a particular steering failure-related crash.

The physical evidence evaluated during a crash reconstruction includes, but is not limited to, the involved vehicle or vehicles (physical and electronic data), the roadway where the crash occurred, injuries to the vehicle occupants, and witness statements. Roadway evidence can be subdivided into pre-crash evidence, crash event evidence, and post-crash evidence. The point of crash impact separates these three roadway evidence subsections. Roadway evidence may include, but is not limited to, debris, tire marks, fluid trails, or vehicle components. Prior recall and repair history of the involved vehicles must be examined as this information may be vital in determining causation. Post-crash examination of the vehicles will reveal evidence about contact between the vehicles and specific damage, but combining vehicle evidence with evidence observed at the collision site, either physically or through the use of scene photographs, will provide a more complete reconstruction of the crash and crash sequence.

The case presented incorporates all of these factors and involves a late model Ford® E150 van that crossed over the center line on a two-lane road and struck a late model Toyota® Sienna® van. Both vehicles were traveling at approximately 55 miles per hour, pre-impact. Little to no pre-crash braking occurred before the driver's side front of the Ford® van impacted the driver's side front of the Toyota® van. Three of the four occupants of the Toyota® van were pronounced deceased at the crash site.

A detailed examination of both vehicles occurred prior to an evaluation of the available roadway and vehicle recall and repair history. While examining the Ford® van, it was discovered that the passenger-side outer tie rod had separated. The tie rod end ball stud was intact and had apparently "pulled out" of the socket; however, the tie rod end socket appeared relatively undamaged. This is not the typical observation made in this type of failure. Generally, if the ball stud pulls out of the socket, the socket is substantially deformed or broken. Furthermore, the typical failure mode for an outer tie rod end in a collision is fracture of the stud that secures the tie rod end to the steering knuckle as it is generally the smallest section and being threaded creates a ready-made notch for failure initiation and stud fracture.

Evaluation of the pre-crash roadway evidence, particularly the pre-crash tire marks left by the Ford® van, indicated that the passenger-side front tire had left an unexpected, non-braking marking on the roadway leading to the point-of-impact. The mark exhibited indications that the passenger-side front tire had been nearly perpendicular to the travel direction of the Ford® van, first to the left and then to the right, while this van continued its approximate forward travel direction.

Crash Reconstruction, Steering Failure, Tire Marks

D7 A Forensic Engineering Review of the TAU Phenomenon (Looming) and a Cautionary Application to Crashes

Adam Aleksander, PhD, Aleksander & Associates, PA, PO Box 140558, Boise, ID 83714*

The goal of this presentation is to inform attendees of the phenomenon of looming, namely the rate of change of the image of approaching objects on the retina, and its threshold values.

This presentation will impact the forensic science community by presenting alternative arguments and pointing out the contrasts to attendees, who will benefit from this discussion by appreciating the complexity of this topic and the apparent ten-fold variability in the threshold detection level of the human visual system.

This aspect of human vision has been studied for more than 40 years, and as yet, there is no general acceptance of this theory as being a critical determinant in crashes, in particular regarding rear-end collisions.

Nevertheless, some experts opine that this phenomenon entirely explains some crashes, neglecting the messy little details that limit or at least create doubt of the theory's applicability. This study presents the general topic and an example, which includes a matrix of specific calculations pertinent to a fatal rear-end collision involving a pickup truck and a commercial truck.

The study of human vision is an integral part of human-factors engineering, which pertains to the assessment of human capabilities in the real world, and includes physical, sensory, and cognitive elements of our daily performance.

An object approaching the eye projects an image on the retina. Initially, the image size appears constant or slowly increasing, but just prior to a potential contact, the rate at which the retinal image increases rises dramatically (exponentially). The projected image is defined by the subtended angle (in radians θ), and its rate of change over time is dθ/dt. The theory suggests that this retinal image rate of change can be used by the visual system to determine the Time To Collision (TTC) and is independent of other speed-related metrics. This ability has long been observed in animal behavior, including animals as diverse as fiddler crabs, chicks, monkeys, flies, and human infants, who all try to avoid looming patterns.

It is reasonable to believe that, in part, we use this aspect of vision as we run over irregular terrain and adjust our gait, or know when to duck under a branch, or, in the case of a diving bird, when to fold the wings. This looming theory has been popularized as a quick explanation of rear-end accidents, particularly trucks. The argument is that a stopped vehicle is not apparent to a driver closing at speed until they are so close that a crash is unavoidable. This simplistic approach ignores the fact that, as drivers, we use many other cues in the visual field, such as contrast, optic flow, textures, and gradients, and that the criteria (thresholds) for looming detection are highly variable.

The discussion of TTC and looming phenomena is straightforward with respect to simple physics, but is complex, and some say questionable, in its applicability to humans and the relationship to automobile crashes. This discussion will focus on a particular case, Kovalchuck v. System Transport, a Washington state jury case in 2017. The base data for this case are as shown in Figure 1, below.

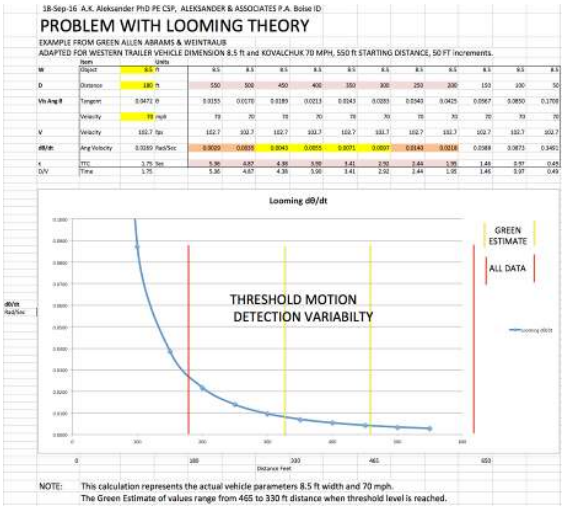

Figure 1. Base Case Parameters, Looming Threshold.

This diagram, the substantiating calculations, and alternative arguments will be presented. Contrasts will be noted for attendees, who will benefit from this discussion by appreciating the complexity of this topic, and the apparent ten-fold variability in the human threshold detection level. Findings of others will also be referenced, and a summary of work in this field will be included. This presentation will conclude with the jury finding.

TAU, Looming, Inattentional Blindness

D8 The Pursuit of a Stolen Patrol Car

Kurt D. Weiss, MS, Automotive Safety Research, 5350 Hollister Avenue, Ste D, Santa Barbara, CA 93111-2326*

The goal of this presentation is to illustrate, by way of examples, how physical evidence can be used together with video analysis to determine vehicle collision parameters in an attempted escape.

This presentation will impact the forensic science community by demonstrating how physical evidence created during a vehicle acceleration maneuver can be analyzed to ascertain useful collision parameters.

Law enforcement officers intercepted a trespassing suspect in progress. The suspect was handcuffed during a routine vehicle search. A sweep of the residence revealed no one was home, but a screwdriver was found near the front door and was presumed to have been used to force entry. The suspect was arrested pursuant to penal code 602.5 (criminal trespass) and placed in the rear of a police car. Further investigation revealed a large amount of cash, a laptop, a portable scale, a .22 caliber pistol, and 11 cell phones.

While the officers were inside the residence processing evidence, the suspect moved his hands from behind his back, around his legs, to the front of his body. The tow truck driver, who was called to remove the suspect's vehicle, was alerted to sounds of the suspect kicking the partition in his attempt to escape (Figures 1 and 2). Turning toward the noise, the tow truck driver's flashlight illuminated the suspect squeezing head-first through the partition window and into the driver's compartment.

Figure 1.

Figure 2.

As the tow truck operator attempted to alert the officers to his discovery, the suspect settled into the driver's seat and shifted the transmission into reverse, spinning the rear wheels as he backed out of the gravel driveway toward the entrance.

In pursuit, the law enforcement officers charged from the residence and sprinted approximately 184 feet toward the accelerating vehicle. At the end of the driveway, the suspect turned the steering wheel and the vehicle yawed clockwise. The brakes were jammed and the vehicle heaved to a stop on the asphalt private drive (Figure 3). The suspect discovered the tow truck and second patrol car were blocking his intended escape route.

Figure 3.

It took approximately seven seconds for the officers to reach the suspect, who was now aware that the sergeant had drawn his service pistol. The suspect ignored his repeated commands to stop, and without further hesitation, slammed the transmission into a forward gear and accelerated the vehicle. Fearing for his safety, the sergeant pulled the trigger continuously, pivoting from his standing position as the vehicle passed.

A ballistics analysis later revealed that all five rounds penetrated the vehicle. Bullets #1 and #2 shattered the driver's tempered glass window (Figures 4 and 5). One bullet passed through the suspect's left arm and exited the right front door. The other bullet tore through the suspect's large intestine and both iliac arteries, requiring extensive surgery. Bullets #3 through #5 entered the vehicle behind the driver's position yet apparently missed striking the suspect.

Figure 4.

Figure 5.

The vehicle impacted the rear of the parked tow truck, halting his attempted escape (Figure 6). The suspect abandoned his escape attempt and was once again apprehended.

Figure 6.

Analysis: Video from an on-board, forward-facing, dash-mounted video camera, photographs, and an incident scene diagram were used determine the vehicle's time-position history during the escape maneuver. It was determined that the patrol car accelerated to approximately 18mph while backing out of the driveway (77 feet long), and the vehicle impacted the tow truck at approximately 15mph.

The vehicle's Restraint Control Module (RCM) recorded a frontal impact event consistent with the subject incident having an algorithm run time of 376ms and longitudinal velocity change of -11.96mph.

Photographs taken during the investigation of the patrol car's instrument panel were compared to an exemplar vehicle to determine the forward gear selected during the escape attempt. The analysis indicates the transmission had been shifted into first gear (Figure 7).

Figure 7.

The subject patrol car was tested to measure its acceleration characteristics on a gravel surface and to evaluate the restrictions imposed on the driver while operating the vehicle wearing handcuffs (Figure 8). The vehicle was instrumented with a Racelogic VBOX II Lite Global Positioning System (GPS) -based data logger and GoPro® Hero3 video camera. Ten reverse-to-forward gear demonstrations were recorded. The reverse demonstrations revealed an average maximum acceleration of 0.252g over a distance of 43.3 feet with a 15.9mph maximum speed. The forward demonstrations revealed a mean maximum acceleration of 0.256g over a distance of 40.2 feet with a 14.4 mph maximum speed.

Figure 8.

The county law enforcement agency and four involved officers reached a settlement with the custody subject prior to trial.

Handcuffs, Coban, RCM

D9 Limit Performance and Controllability Testing of Vehicles Towing Utility Trailers

Robert L. Anderson, MS, Applied Research and Investigations, PO Box 1208, Scottsdale, AZ 85252*

The goal of this presentation is to demonstrate testing procedures for vehicles towing utility trailers and the resulting data from such tests.

This presentation will impact the forensic science community by making known the available standardized test procedures and providing data demonstrating how towing utility trailers limits vehicle performance.

An investigation was conducted to determine how the weight ratio of the tow vehicle to the trailer being towed influences performance.

Two tow vehicles were tested: a 2005 Honda® Pilot®, weighing 5,176 lbs. and a 14-foot U-Haul® Ford® F450, weighing 8,559 lbs.

Two trailers were tested: a 6'x12' utility trailer, weighing 4,008 lbs. and a 5'x8' utility trailer, weighing 2,700 lbs.

Testing instrumentation included a steering machine, cameras, accelerometers, and angle rate sensors. The testing included the following test procedures: (1) braking in a turn, consisting of a turn of either 90° on the steering wheel or 180° on the steering wheel. The speed was increased for each test until a physical limitation, such as jackknifing, occurred; and, (2) sine plus dwell involved a reverse steer of magnitude 180° (similar to a sine wave) with a dwell of one second on the second or corrective steer. The speed was increased for each test until a limit, such as jackknifing, occurred.

The conclusions include: (1) for the 90° steer and braking in a turn, loss of control in the form of extreme jackknifing was experienced at 45mph for the Honda® pulling the 6x12 trailer. Jackknifing was not experienced at that speed with the 5x8 trailer or with either trailer towed by the Ford® truck; and, (2) for the 180° steer and braking in a turn, loss of control in the form of extreme jackknifing was experienced at only 25mph for the Honda® pulling the 6'x12' trailer and was not experienced with the smaller 5'x8' trailer or the Ford® truck with either trailer.

Similarly, the Honda® pulling the 6'x12' trailer experienced jackknifing at 35mph and this was not experienced with the smaller 5x8 trailer or the Ford® truck with either trailer.

In conclusion, vehicles towing trailers in which the vehicle/trailer-weight ratio is close to unity, such as the Honda® towing the 6'x12' trailer, are more likely to lose control than vehicles towing trailers in which the vehicle/trailer-weight ratio is appreciably greater than unity. This presentation benefits the forensic science community by offering a methodology for standardized stability testing for vehicles towing trailers and provides preliminary data detailing how the vehicle/trailer-weight ratio is related to stability in highway-relevant driving activities.

Utility Trailer Towing, Limit Handling, Vehicle Testing

D10 Tow Hitch Failure

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The goal of this presentation is to introduce attendees to fundamental testing principles to validate or refute an alleged tow hitch failure. The analysis will include calculations and 3D animations of the possible events.

This presentation will impact the forensic science community by describing analytical methods that can be used to evaluate potential events in an alleged tow hitch failure.

On a rainy afternoon that also included strong wind gusts, a boat owner hitched his boat and trailer at a marina complex that performed maintenance on the boat. Personnel at the marina attached the boat-laden trailer to the owner's tow vehicle. The boat owner was cautioned against towing the unit due to the prevailing weather. Eleven miles from the marina, after driving at highway speeds over various roadway surfaces replete with construction activities, various turns and stops, the boat and trailer allegedly became uncoupled. This resulted in the driver losing control, damages to the tow vehicle and boat/trailer combination, and injuries to the driver. These injuries are alleged to be either new injuries to spinal column-based soft tissue or reinjury of such tissue from prior accidents. The loss of control allegation was attributed to improper coupling of the tow vehicle and boat/trailer, categorically implying that the receiver on the trailer was simply placed atop the tow ball on the tow vehicle's hitch. A forensic engineer opined that the physical evidence was consistent with this allegation.

To evaluate the allegation, several basic investigative protocols were employed. The tow ball and hitch were inspected in addition to the receiver located on the boat trailer. An analysis was performed to calculate the forces necessary to cause hitch failure. Analyses were also performed to determine the forces associated with the observed damage on the tow vehicle and the trailer. Additional analyses were performed with an exemplar tow combination. The accident site was extensively photographed to locate physical marks and identify them with accident scene photographs taken by the claimant. The accident was reconstructed and 3D animations were developed to demonstrate the alleged accident scenario as compared to the possible scenario developed from the evidence and supported by established physical laws.

Review and analysis of the physical evidence disproved the opinions of the opposing expert. Damage to the ball on the hitch attributed to loss of control by the opposing expert was shown to be in the wrong position. Other damage attributed to the failure of the ball was instead shown to be attributable to normal wear. The failure of the trailer was shown to be due to material corrosion at a weak location on the trailer. Review of the photographs taken by the claimant conclusively proved that his allegations regarding the cause of the accident were untrue. Testing revealed that if the allegation of the improper coupling of the tow vehicle to the trailer/boat combination had occurred, the unit would have become uncoupled at a speed change of less than five miles per hour and the drive to the accident location was impossible. In fact, the vehicle, boat, and trailer, if coupled as alleged, could not have exited the garage at the marina without having become uncoupled. The accident was proven to have been caused by fishtailing of the unit on wet roads and high winds and that the accident was staged to place blame on the marina.

Forensics, Energy, Testing

D11 The Injury Potential of Fidget Spinners

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The goal of this presentation is to highlight the injury potential of fidget spinners, in particular from sharp points or edges that can cause injury when the spinners are used inappropriately as weapons.

This presentation will impact the forensic science community by assisting attendees in understanding the key issues related to the ability of fidget spinners to create stabbing or slashing injuries and other factors that can be relevant to injuries arising from fidget spinner design and manufacture.

Fidget spinners are essentially toys that have a planar multi-lobe structure that spins around a central bearing. Common designs have two or three lobes, and the bearings are contained in a central circular pad or bearing race with a hole through it. The operator either spins the toy while holding the center pad with opposing digits or places the central hole over a finger and spins the device around the digit. These spinners are typically made of metal or plastic, and a wide range of different metal alloys have been used, including brass, titanium, die-cast aluminum, and stainless steel. The bearings at the center of each spinner are made of ceramic or stainless steel and provide exceptionally low friction. As a result, these spinners spin easily and are balanced by weights on the outside that aid balance and retain rotational speed. Fidget spinners reportedly benefit those who have difficulty concentrating or need to relieve nervous energy and/or stress. Some educational institutions have embraced the spinners for aiding attention while others have banned them. There is no scientific evidence that spinners help treat symptoms of autism or Attention-Deficit Hyperactivity Disorder (ADHD).

Fidget spinners have become incredibly popular and retail for low prices (typically <$10). The spinners are typically designed to provide the user with long spin times from an initial impulse, low vibration, and provide a distinctive sensation and noise. Some spinners contain battery-powered Light-Emitting Diodes (LEDs).

While most fidget spinners are designed and intended as toys, there are some issues with the spinners that can potentially lead to injury. Some spinners are now on the market that have sharp points and are marketed as “ninja.” The design of such spinners can be used to inflict injury.

The injury potential of fidget spinners falls into four categories, all of which are related to failure to meet European Conformity (CE) standards or their international equivalents: (1) manufacturing quality — some spinners have manufacturing defects that leave sharp metal burrs; (2) selection of materials of manufacture and the composition of paints, for example; (3) small parts and corresponding age-appropriate warnings (such as whether the spinners contain batteries that could be accessed or swallowed by small children); and, (4) sharpness of the points and blades and whether or not these can create injury.

This presentation reports on an investigation into the sharpness of the points and blades of a range of fidget spinners that are available in the United Kingdom market and their potential for injury. The spinners are marketed as “ninja shuriken” spinners or “dragon blade” spinners. The points on some of the spinners are sharper than that recommended by the European Standard BS EN 71-1:2014 Safety of toys. Part 1: Mechanical and physical properties.

This presentation will illustrate that, although the edges of the spinner blades were dull, the points of some spinners tested are sufficiently sharp to penetrate both tomato and pork skin when used with a stabbing action. Attendees will understand the key issues for fidget spinners that are being designed and marketed to appear threatening and may be used inappropriately as weapons.

Fidget Spinners, Sharpness, Penetration

D12 A Critical Assessment of Cutting — Slicing, Stabbing, Sawing, and Chopping: The Mechanisms of Separating and Penetrating Biomaterials and the Relevance of Sharpness

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The goal of this presentation is to introduce the four main categories of using edged or pointed instruments in forensic cases: slicing, stabbing, sawing, and chopping. Attendees will better understand the way in which biomaterials are divided or invaded by these implements and how this relates to implement edge and tip sharpness.

This presentation will impact the forensic science community by enhancing the competence of forensic scientists and engineers in understanding how each of these four categories of human tissue insult occurs with the various implements retrieved from a crime scene.

Knives, saws, axes, machetes, and other implements are often used in violent crime. Specifically, knives are often used in stabbing attacks in which the depth of the wound is long compared to the injury on the skin or in slashing attacks in which the length of wound on the skin is long, but the depth of the wound into the body is relatively shallow. Saws and axes may be used in dismemberment cases. Other implements (screwdrivers, chisels, etc.) are also used in violent attacks, and the challenge for the forensic scientist or engineer is to relate the particular implement used to the mechanism by which the injury is inflicted. The sharpness, or degree of pointedness, of these implements is a key variable in helping make the link between the implement and the injury. Thus, the goal of this presentation is to define the four different methods in which material can be separated, namely by slicing (or slashing), stabbing, sawing, and chopping, so that the importance of the key features of the implement being used can be investigated and the necessary or controlling forces understood. This presentation will help attendees understand the way in which biomaterials are separated by cutting and how this relates to the sharpness of the implement used.

In cases of slashing, the knife is usually used in a slicing mode in which the blade of the knife is drawn across the body. The lowest cutting force is required when a slice-push action is used. Often in slashing, the blade is presented "obliquely" to the surface being cut.

In stabbing attacks, the sharpness of the tip is important for initial penetration with sharper tips reducing the amount of force required. Once the implement has penetrated clothing or skin, the sharpness of the blade edge is also important for estimation of additional penetration depth. Another parameter that influences the amount of force required for cutting is the friction between the blade and the material being cut. The cutting forces are reduced when the size of the blade is smaller or the material is tougher.

Sawing typically uses a reciprocating motion that engages the series of narrow cutting edges typically found on each tooth of the saw. In contrast to knives, saw blades have parallel sides. Saw teeth are "set," which means the cut is slightly wider than the thickness of the blade; this helps prevent "binding" of the saw blade in the material being cut and diminution (or cessation) of the essential reciprocating motion. Saws are designed with different teeth profiles to optimally cut different materials; however, saws used in crimes are usually those of convenience, not specific design.

Chopping is typically performed by wedge-shaped implements such as axes and machetes that are designed to cut through materials by a single forceful impact rather than a steady or reciprocating push or pull. The force delivered by a chopping impact is generally of greater magnitude than the force delivered by a push. The muscles of the arm and shoulder contribute to accelerating the fall of the implement and add to the total force of impact above what would be obtained by simple free-fall. The material is divided into two parts by driving the wedge-shaped chopping implement into the material, and a crack (or split) forms at the tip of the wedge. The wedge angle of the implement, along with implement weight, velocity, and material resistance, influences the ability to form the crack. The principle motion of implements used for chopping are perpendicular to the cutting edge of the implement.

This presentation will discuss the ways in which different implements cut through or penetrate materials and identify the important characteristics of the blades and edges that are key factors in controlling the separation of these materials.

Cutting, Sharpness, Penetration

D13 Who's Driving?

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After attending this presentation, attendees will understand how biomechanics can provide useful information that helps identify operators of motor vehicles involved in accidents.

This presentation will impact the forensic science community by raising awareness that motor vehicle operator identification is sometimes obscure and by illustrating how biomechanics can be used to reduce uncertainty in efforts to identify operators of motor vehicles involved in crashes.

Operator identification of a motor vehicle involved in an accident, while always imperative due to potential criminal and nearly ubiquitous civil consequences, is sometimes obscure due to event circumstances or operator intent to eschew culpability. Correct operator identification is also necessary to prevent wrongful incrimination of innocent passengers.

First responders may report unrestrained occupants in out-of-position interior vehicle locations or outside the vehicle following crash-induced ejection. Operator identification is obscured when such occupants are unable or unwilling to disclose the facts or when uninjured drivers flee the scene before first responders arrive. Forensic investigations seeking operator identification may benefit from the application of biomechanics to analyze the relationships between vehicle damage and human injury.

External vehicle collision analysis quantifies crash force vectors and energy magnitudes. It is an essential prerequisite for application of biomechanical principles seeking driver identification. The principle direction of force is a key parameter established by vehicle collision analysis that, in turn, enables useful information to be obtained from the "second collision" (i.e., occupant collision with the vehicle interior).

Direction of occupant motion is, in the event of a single crash event, parallel to the line of action of the principle direction of force. This line of action determines the trajectory of the body of the unrestrained occupant or the limbs of restrained occupants and the vehicle interior components struck. Information regarding occupant trajectory, considered with first responder-provided data, enables estimation of pre-crash occupant position. Vehicle rollovers and successive crash impacts confound this analysis due to multiple, and sometimes unknown, crash force lines of action. Position identification may also be confounded by occupant ejection due to uncertainties regarding vehicle angle at the time of ejection, escape velocity, and body trajectory.

Consideration of crash force magnitudes may also assist driver identification because these force magnitudes are often proportional to occupant injury severity. Crash force magnitudes may be passenger compartment-specific and are most helpful when considered from two complimentary perspectives: vehicle interior interactions with the human body and human body interactions with the vehicle interior. These interactions require information concerning the mechanical properties of the struck vehicle's interior elements and striking human body components. Quantification of interior component striking force magnitudes begins with peak values established by external collision analyses; these peak force magnitudes are subsequently mitigated due to intervening vehicle structures. Mechanical properties of vehicle components are typically constants with each vehicle design and are amenable to empirical verification. Mechanical properties of human limbs, organs, or tissues are highly variable and depend upon gender, age, genetics, etc. Analysis of the multiple relationships among crash force magnitudes, vehicle interior damage (magnitude, direction, and location), and reported (emergency services and hospital records) type and extent of injury to each vehicle occupant collectively provides data regarding occupant positions at the time of the crash.

Biomechanical analyses of organ damage due to intra-body cavity impact may also provide useful occupant-relevant data. Examples include aortic isthmus injury due to blunt chest impact, femoral head-induced acetabular wall fractures due to knee-dash impact, seat belt-related liver or spleen lacerations, and cranial contusions due to B-pillar impact. Unfortunately, quantitative biomechanical analyses of these injury types are frequently limited by insufficient knowledge of the constitutive mechanical properties of the affected organs and their individual variations with gender, age, genetics, etc. Difficulties performing such analyses are substantially increased when any of the occupants are infants or toddlers.

In conclusion, biomechanical analyses of human injuries relative to vehicle damage can be a useful tool in aiding forensic investigations seeking motor vehicle-operator identification.

Biomechanics, Motor Vehicle Crash, Driver Identification

D14 An Overview of Physical Evidence to Assist With Driver Identification in Vehicle Collisions

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The goal of this presentation is to illustrate, by way of examples, how witness marks observed on vehicle interior surfaces and seat belt assemblies and the associated occupants' injury patterns may be collectively used to determine who was operating the vehicle at the time of collision.

This presentation will impact the forensic science community by providing a list of vehicle surfaces to be examined, the physical evidence commonly observed, and the general body regions and injury sites involved in collisions where driver identification is unclear. This presentation will further demonstrate how aligning physical evidence created during frontal, rear-end, lateral, and rollover collisions with observed injury patterns or types may be vital in correctly identifying the occupant's seating position. This presentation will also provide a useful list of vehicle interior components and the associated body regions regularly examined for physical evidence confirming the corresponding impacts. While this list is not intended to be complete, it presents a useful compendium upon which the forensic investigator may rely.

Vehicular collision forces and the resulting occupant motion will often produce impacts between the occupant and vehicle interior surfaces. Impact forces applied to the human body during contact with the vehicle interior may cause injury. An analysis of the resulting interfaces and location of corresponding injuries may be used to correctly identify the driver in a motor vehicle collision. Specifically, an occupant's initial motion during a vehicle collision is toward the velocity change vector. Knowing the collision type (frontal, rear-end, side, or rollover) will help the investigator correctly identify and examine the potential surfaces of interaction. In frontal collisions, these typically include forward surfaces such as the sun visor, header, windshield, roof pillars, steering wheel, airbag fabric, dashboard, glove box, knee bolster, and center console. In rear-end collisions, the surfaces exposed to occupant contact are the front seat assembly, head restraint, rear seat, rear roof pillars, package shelf or cargo area, rear window, and roof header. In side impacts, potential contact evidence may be observed on the door panels, window glass and frames, roof pillars, and center console. In rollover collisions, the headliner, passenger handles, and dome lights must be examined.

Physical evidence often observed on vehicle interior structures includes blood stains, tissue and hair deposits, makeup, fractured glass, steering wheel rim deformation, seat back deformation, and plastic trim deformation, as well as scuffs, abrasions, and cracks on various vehicle surfaces. Close-up examination of interior surfaces may reveal striations to the plastic. Striations are caused by clothing with a coarse fabric weave, such as denim. In these circumstances, knowledge of occupant clothing may be useful in the analysis.

Regions of occupant injury include feet, lower extremities, knees, hips, abdomen and upper torso, neck, head, forehead, face, shoulder, upper extremities, and hands. Commonly observed occupant injuries include abrasions, pattern bruises, lacerations, and fractures, along with a host of internal injuries. The location of these injury sites may be aligned with corresponding vehicle interior evidence of human forcible contact. Medical records may reveal notations of a knee abrasion or a femur or hip fracture. This may be indicative of forcible knee impact into the knee bolster, and this may be verified by vehicle inspection. Abrasions may also result in tissue or hair deposits. The search of the vehicle interior for such a deposit may be instrumental in evaluating the seating position of this occupant. Coloration of hair fibers in particular locations may assist in determining which occupant struck the particular vehicle area.

If the seat belt is worn during the collision, the webbing presents an additional surface of interaction. Restraining forces are applied to the occupant by the seat belt webbing and examination of such webbing may reveal tissue, clothing fiber, or metal transfers arising from the application of these forces. Bruises from the webbing are often observed, though not always expected, when the seat belt is worn during a crash. A diagonally oriented shoulder belt bruise is a telltale indicator of restraint use. The direction of these bruises, crossing upper-left to lower-right or vice versa, is helpful in determining seating position.

Injury, Seat Belt Sign, Forensic Evidence

D15 Determination of Driver Identity: Effective Scientific Investigation, Comparison and Contrast of Forensic Evidence, and Its Spoliation in Various Cases

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After attending this presentation, attendees will better understand the wide range of forensic investigation and analysis techniques employed to identify motor vehicle operators. These techniques include 3D land surveying, accurate measurement of vehicle 3D motion, determination of all significant points of vehicle impact to objects and the ground, photogrammetric analysis to allow accurate replication of vehicle position and trajectory, correct interpretation of vehicle crush and crashworthiness performance relative to occupant ejection, trace evidence recovery, and human factors testing.

This presentation will impact the forensic science community by demonstrating an effective scientific methodology utilized to investigate complex rollovers or other crashes with multiple ejections, both with and without seat belt restraints. Examples provided will illustrate how this methodology can be used to overcome the difficulties faced by forensic investigators studying cases in which driver identification is ostensibly unclear. These difficulties include: lack of witnesses, passage of time, destruction of the subject vehicle (accidental or intentional) or other pertinent physical evidence, gross misinterpretation of facts and evidence by others, and obstruction by insurance companies, tow yards, and police agencies. This presentation will benefit forensic practitioners in engineering sciences, criminalistics, vehicle safety, accident investigation, jurisprudence, and pathology.

Determination of vehicle driver identity during collisions or rollovers with vehicle occupant ejection presents unique challenges in accident reconstruction. Police or insurance company investigations are often inadequate, resulting in failure to observe, measure, and document important facts and physical evidence pertinent to driver identification. This leads to misinterpretation and incorrect conclusions; however, the demand to assign driver identity in a crash with severe or fatal injury serves as a significant motivation to reach rapid and correct conclusions, especially in criminal prosecutions. In several cases, insurance company investigators, police, prosecuting attorneys, emergency services personnel, tow truck operators, wrecking yards, vehicle occupant relatives, auto manufacturers, or other authorities have unwittingly and sometimes intentionally removed or altered critical evidence at crash sites, within vehicles, or even destroyed the subject vehicle before a comprehensive analysis was conducted by a qualified and objective investigator. Destruction of evidence may also occur at the hand of a driver who seeks to avoid responsibility for a serious or fatal crash. All of these corrupting factors can negatively influence a forensic investigation to determine driver identity.

Several cases involving severe vehicle rollovers or collisions with all occupants ejected, usually with one or more killed, and no witnesses will be presented. All of these crashes were examined with adequate time and equipment to properly identify and measure the available facts and forensic evidence and to conduct driving tests pertinent to human factors, visibility, road conditions, and vehicle handling characteristics through the last known path of the subject vehicle. Comparison of current conditions with crash scene photographs and measurements is critical because government agencies have occasionally altered roadways, guardrails, or signage after such a crash. These comparisons also require photogrammetric analysis of 3D land surveys to allow accurate replication of vehicle and occupant positions and trajectories, verification of police measurements and other reported data, determination of all significant points of vehicle impact to fixed objects and various types of ground surfaces, location of all deposited glass and vehicle wreckage components, and vaulting velocity and trajectory of vehicles and ejected occupants. When vehicles are available, analysis of occupant loading of seats, belts, and vehicle interior structures, along with identification and correct interpretation of vehicle damage and witness marks and transfers of fabric, skin, hair, etc., are also conducted.

On occasion, such investigations may not occur until years after the crash. This means that important data may be obscured during the intervening time, and such data may be exposed only by: descending cliffs or climbing trees; electronic metal detection for objects covered by soil or foliage; excavation of vehicle parts and glass fragments from swamps, roadside ditches, culverts, and crop fields; and recovering clothing, personal effects, and hair and other body parts from vehicle interiors and crash sites.

Accurate and thorough forensic examination of evidence at the crash site and subject vehicle are critically important. This analysis includes spatial vehicle-occupant position, determinations using exemplar vehicles and components, review of maintenance and repair records of guardrails and other roadside appliances, procurement and analysis of paint samples, obtaining maps and photographs of body injury sites, video, newspaper, and television reports of the accident, and obtaining all available reliable facts from witnesses and first responders, as well as human factors testing and the application of vehicle crashworthiness knowledge. These are the key elements that comprise a thorough forensic investigation that has the best chance of correctly identifying the motor vehicle operator. In several instances, vehicle safety defects were evident and became crucial factors in determining restraint use and failure, manner of ejection, ejection portal and timing, and pre-crash occupant locations. This includes failures of seat belts, seats, door latches, roof structures, tires, axles, and other vehicle components or safety systems.

Driver Identity, Vehicle Occupant Ejection, Pickup Truck Rollover

D16 Vehicle System Forensics and Determining Who Was Driving at the Time of a Crash

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After attending this presentation, attendees will have an appreciation for the vast amount of digital data stored in modern vehicles and how it can be retrieved to assist in identifying the driver of a vehicle following a traffic collision.

This presentation will impact the forensic science community by introducing a new source of data stored in modern automobiles and by illustrating how that data can be used to identify the driver of a vehicle during a collision investigation.

Electronic data being recorded and stored in modern vehicles is an established concept; however, what data are recorded, and where and how to retrieve that data, depend on many variables. Common types of data stored and regularly retrieved from modern vehicles include diagnostic data used in service and repair and crash data used in the investigation of traffic collisions. A more recently discovered source of evidence in motor vehicle incidents is the existence of stored electronic data in vehicle infotainment and telematics systems. The retrieval and analysis of this new data is now commonly referred to as Vehicle System Forensics.

Many collision investigations result in an issue of identifying the driver of a vehicle at the time of the collision. Scenarios in which this issue manifests itself include rollover collisions that have resulted in occupant ejections. It is not uncommon for collision investigators to respond to the scene of a rollover collision where a surviving party states that the driver of the vehicle at the time of the collision was ejected from the vehicle and is now deceased at the scene. There are many approaches to investigating this statement and identifying evidence that would support or refute this party's claim. Analyses involving trace evidence, DNA, biomechanics, and occupant kinematics are common; however, with many modern vehicles, there may be stored electronic data to assist the investigation of this issue.

As an area of focus for this discussion, a hypothetical scenario involving a late-model Ford® vehicle will be examined. The vehicle was equipped with the Ford® SYNC® 3 infotainment and telematics system. The collision scenario being examined is a simple one — a vehicle was driven off the roadway and overturned. One occupant was ejected and killed (Party A). A second occupant (Party B) survived and provided emergency personnel on scene with a statement that included the following claims: (1) Party A was driving at the time of the rollover and Party B was seated in the passenger seat; (2) Party A was ejected out the driver's door as it came open during the rollover sequence; and, (3) after the rollover ended and the vehicle came to rest, Party B exited the vehicle through the passenger door, which he opened.

Data recorded by the Ford® SYNC® 3 infotainment and telematics system in this vehicle can be accessed using the Berla iVe vehicle system forensics tool. This system allows the retrieval of extensive data from this type of vehicle. Example data parameters include vehicle speed, tracking logs, call logs, contact lists, connected devices, and "events," which include the date, time, and location of gear shifts, opening/closing of doors, hard acceleration, and hard braking. In the examined scenario, data related to the opening and closing of doors would be extremely relevant.

Investigators could undoubtedly examine the physical evidence and determine that the vehicle had been driven off the roadway and rolled over, with one party ejected and killed. Furthermore, both vehicle doors may have been damaged and found open during the on-scene investigation; however, finding observable physical evidence of which door came open during the rollover and which door was opened moments after the collision is unlikely. That data may have been recorded by and stored in the SYNC® 3 module. Specifically, this module can record the date, time, and location (Global Positioning System (GPS) – latitude and longitude) when the driver and/or passenger doors were opened or closed. This information could be directly compared to claims of the surviving party. Other data accessible with the Berla iVe system would certainly be useful in the overall investigation as well.

This scenario and depictions of the specific data that can be relied upon in examining the central issue of driver identification will be presented. Specific examples of this data will be demonstrated with a discussion of its availability and retrieval.

Vehicle System Forensics, Infotainment and Telematics Systems, Driver Identification

D17 If the Shoe Fits, Wear It — Using Injury Patterns, Forensic Science, and Impact Biomechanics to Identify the Driver in a Fatal Vehicle Crash

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The goal of this presentation is to raise awareness of the problems of identifying the driver of a vehicle in a fatal crash and to highlight the evidence that should be collected and examined when the identity of the driver is in question.

This presentation will impact the forensic science community by informing attendees that in high-speed motor vehicle collisions that result in one or more deaths, identifying the driver of a vehicle can be difficult in situations in which the occupants are unrestrained and ejected from the vehicle or displaced due to the dynamic behavior of the vehicle, post-crash. This presentation will raise awareness of the problems encountered in identifying the driver of a vehicle in a fatal crash and will reveal the key parameters obtained from analytical and forensic science methods that can be used to identify the driver of a head-on crash resulting in vehicle rollover, displacement of the occupants, and multiple fatalities.

In the subject collision, the left front of a Jeep® Grand Cherokee® struck the left front of a Chevrolet® Cavalier® sedan in a wrong-way freeway collision. There were two occupants (A and B) in the Jeep® (the "wrong-way" vehicle). After impact, the vehicles disengaged and the Jeep® rolled over. One Jeep® occupant was found in the right front seat and the other Jeep® occupant in the rear seat after the crash. A jury found that Occupant A was the driver of the Jeep® and was convicted on two counts of Intoxication Manslaughter.

Review of the injuries sustained by Occupants A and B was straightforward and instrumental in determining the Jeep® driver. The driver floor pan and left front hinge pillar exhibited a high degree of collapse and intrusion. The left shoe of the driver was impinged by this crush damage. There was also evidence of heavy knee impact on the left side of the lower dash.

The mechanism of a complex relative movement with multiple energy transmission is influenced by the deformation characteristic of the dashboard and the foot well space.[1] The crush deformation in the foot well can cause the feet to be fixed in the foot space (i.e., the foot as well as the tibia are subjected to bending and compression forces). Lower extremity injuries from the foot to the pelvis are common in offset frontal crashes.

In a set of 1,189 frontal crashes, 23 were identified with ankle or foot fractures of severity Abbreviated Injury Scale (AIS) $\geq$2.[2] Half of the injuries were caused by direct-force application due to entrapment of the leg by passenger compartment collapse.

Occupant A sustained lower extremity injuries, including midshaft fractures of the right tibia and fibula, a left anterior leg wound, and a closed fracture of the left hallux, distal phalanx (big toe). These injuries were photographed. These injuries and the mechanism thereof are well documented in the literature and in exemplar crash tests for drivers of vehicles involved in off-set crashes with foot well intrusion.

Occupant A also reportedly sustained a cervical fracture at the C6 level. The unrestrained driver of the vehicle would have interacted with the vehicle, such as loading of the steering wheel and head contact with the roof, top of the "A" pillar, or sun visor due to the collapse of the "A" pillar on the driver's side.

The laboratory analysis also determined that Occupant A fit the DNA profile obtained from the driver's side airbag. No blood match was obtained. This is an indicator that Occupant A interacted with the airbag during the initial crash phase and not during the rollover event.

The adverse expert speculated that an abrasion on the right-side neck of Occupant A may be related to a seat belt injury from the passenger side belt. This type of abrasion alone is not an indicator of seat belt usage, absent of any other forensic evidence such as diagonal bruising along the torso, left side rib fractures, and forensic markings on the seat belt. Crash tests with exemplar vehicles demonstrate abrasions characteristic of occupant loading on the drivers "D" ring.[3-6] The investigating officer specifically examined the driver and front passenger seatbelts for evidence of occupant loading and found none.

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Biomechanics, Crash, Injury

D18 Follow the Broken Bones — Using Injury Patterns, Forensic Science, and Impact Biomechanics to Identify the Driver in a Multi-Occupant, Double-Fatality Vehicle Crash

Billy S. Cox, Jr., Billy Cox Group, PO Box 1699, Navasota, TX 77868*

The goals of this presentation are to raise awareness of the problems in identifying the driver of a vehicle in a fatal crash and to highlight the evidence that should be collected and examined when the identity of the driver is in question.

This presentation will impact the forensic science community by outlining the crash investigation methodology, including the biomechanics used to identify the driver in a single-vehicle crash wherein one occupant was trapped in the wreckage and the other was ejected.

In high-speed motor vehicle collisions that result in one or more deaths, identifying the driver of a vehicle can be difficult. In cases where the damage to the vehicle is catastrophic, the occupants may be crushed in the wreckage or scattered along the crash scene if the vehicle breaks apart. Using excerpts from the forensic autopsy, crash scene photographs, and the seat belts from the vehicle, this presentation outlines the crash investigation and the forensic biomechanics used to identify the driver.

The example presented involves a single-vehicle crash wherein a speeding vehicle struck a tree, two persons were killed, and a third was severely injured. The front of the vehicle separated at the firewall and traveled approximately 160 feet. Damage to the occupant cabin was catastrophic. The vehicle struck the tree near the right front passenger-door mirror. The tree penetrated the vehicle approximately 3.5 feet at an angle of about 65 degrees measured clockwise from the centerline. The force of the impact crushed the occupant cabin such that the right front passenger seat was near the normal position of the driver's seat.

The crushed occupant cabin contained one deceased teenage male and one critically injured juvenile male. Another deceased teenage male was on the ground outside the driver's side of the vehicle. The investigating police agency identified the ejected male as the passenger and the deceased male entrapped in the wreckage as the driver.

The medical examiner's report revealed that the ejected teen male had a ruptured aorta and a massive, displaced skull fracture that extended from right front temporal region through the left parietal bone. The fracture pattern matched the Principal Direction of Force of the collision. There was also a deep laceration on left side of his neck. The ejected male also exhibited diagonal bruising and abrasions on his upper torso, consistent with shoulder belt use. The deceased male located in the wreckage had extensive lower extremity fractures, a basilar ring fracture, and an abrasion on right front side of his head with no skull fracture. There was no evidence of seat belt use on the deceased male located in the wreckage.

Analysis of the seat belts revealed that the shoulder portion of the driver's side seat belt was pulled apart during an extreme loading event. The ends of the belt were frayed and the fibers exhibited evidence that the heat generated during the forced separation created small, melted bubbles on the ends of the fibers. The mounting brackets on the driver's side seat belt retractor were bent. The "D" ring on the driver's side B-pillar of the vehicle displayed evidence of a heavy loading event. The passenger side seat belt was intact and hanging in the retracted position against the passenger side B-pillar. The mounting brackets on the passenger side seat belt retractor were pristine, indicating that the passenger belt was not worn. The forensic pathology identified in the postmortem autopsy, along with the understanding of fundamentals of impact biomechanics and crash reconstruction, confirmed that the ejected male was the driver.

After the independent investigation was complete, the investigating police agency changed the report to reflect these findings. The investigation was previously profiled in a Discovery Channel® program entitled *Crash Detectives*.

Biomechanics, Crash, Injury

D19 A Rollover Off a Cliff With No Witnesses, No Vehicle, and Nothing But Unreliable Information: The Use of Forensic Evidence, Vehicle Crashworthiness, and Human Factors Testing to Prove Driver Identity

Mark C. Pozzi, MS, Sandia Safety Sciences, 2 Marietta Court, Ste A, Edgewood, NM 87015*

The goal of this presentation is to demonstrate how accurate driver identification can be determined despite a paucity of physical evidence and gross misinterpretation of facts by law enforcement and other key personnel.

This presentation will impact the forensic science community by demonstrating how a complex real-world rollover collision case was solved by the methodical and thorough application of forensic science principles. This presentation will help those involved in forensic engineering sciences, forensic pathology, law enforcement, and jurisprudence gain insight into the correct manner in which to approach difficult cases, such as the case presented.

This case involves an unmodified (since manufacture) two-door compact pickup truck that ran off a highway, travelled over a cliff, and struck a tree as it rolled over on its way to the bottom of a deep canyon. Both occupants were ejected and killed. No witnesses saw the actual crash. The police investigation resulted in multiple misinterpretations of physical evidence and facts, plus gross over-reliance on unreliable statements from others at the scene. The insurance company destroyed the vehicle immediately upon its release by the police, before any investigation of the physical evidence contained within the pickup could be conducted. The insurance company hired well-known, seemingly qualified forensic engineers to determine driver identity. These engineers never went to the crash scene to examine available forensic evidence and facts, take measurements, or perform any of the other typical scientific investigative efforts that are typically made in such cases.

The lack of witnesses, destruction of the subject vehicle, and gross misinterpretation of facts and evidence by others were all difficulties that had to be overcome. Detailed measurements of evidence remaining at the scene, comparison with exemplar vehicles, analysis of available crash and static tests, proper mapping of the involved human trauma, accurate measurement of the crash site and related physical evidence, and consideration of the entire trip history of the two vehicle occupants were all critical factors that had been overlooked or ignored by the police and seemingly qualified forensic engineers hired by the insurance company.

A wide range of forensic investigation and analysis techniques were employed in this investigation, including 3D land surveying, accurate measurement of vehicle horizontal and vertical trajectories, determination of all significant points of vehicle impact to external objects and the ground, photogrammetry permitting accurate replication of vehicle position, correct interpretation of vehicle crush and crashworthiness performance data relative to occupant ejection, trace evidence recovery, and human factors testing.

In conclusion, thorough, methodical, rigorous forensic examination of the crash site, all physical evidence and photographs, coupled with a thorough understanding of crash dynamics and human factors, as well as application of vehicle crashworthiness knowledge, can allow accurate conclusions to be obtained despite the unavailability or misinterpretation of critical evidence.

Driver Identity, Vehicle Occupant Ejection, Vehicle Crashworthiness

D20 Forensic Analysis and Testing to Evaluate Football Helmet Environmental Degradation and the Effects of Repeat Impacts

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The goal of this presentation is to present a method for improved scientific evaluation and certification of football helmet head impact potential when used in common, but not currently tested, conditions of both high temperature and humidity, as well as repeat impacts.

Certification and evaluation of new and reconditioned football helmets should include impact performance evaluations under high temperature and high humidity soak conditions that more realistically replicate early season environmental use conditions. This presentation will impact the forensic science community by discussing why this type of evaluation can be beneficial in the early phases of helmet design, and in the reconditioning of used helmets, to assist in the proper selection of energy-absorbing padding that is more resistant to degradation of impact safety performance in high temperature and high humidity environments.

Forensic evaluation of football helmets requires evaluation of factors and variables that can adversely affect the impact-attenuating performance of Energy Absorbing (EA) pad materials as well as helmet shells, face masks, and retention straps. All these safety devices are needed to minimize transmission of linear and rotational forces applied to the head, so that risk of head injury is reduced.

One factor largely ignored by current safety standards is player head heat and contact sweating, which can induce high temperatures (>120°F) and humidity (100%) within a helmet system (Hot-Wet condition) even in moderate environmental weather conditions. This can result in degradation of helmet EA capacity and result in significantly increased risk of head injury.

In this study, adult and youth football helmets of various designs were subjected to a range of varying temperature, humidity, and mechanical impacts (of both varying amplitude and number of load cycles). Quasi-Static (QS) compression testing of commonly used EA materials and dynamic impact testing of full helmet systems were conducted. A long-recognized "multivariable" experimental method was utilized to demonstrate an efficient means for assessment and comparison of currently representative helmets.

The QS tests revealed that a short Hot-Wet soak time of only a few hours noticeably diminished EA levels. The EA pad types that were QS tested included: Thermo-Plastic Polyurethane (TPU) "waffle shaped" EA pad configurations; load-rate sensitive "gel" foam padding; and dual- and single-density elastomeric foam padding.

Dynamic helmet repeat-impact tests were conducted by using a pendulum-impact test device where various helmet designs were mounted to a Hybrid-III head and neck system and impacted against a non-yielding surface after being subjected to ambient and Hot-Wet conditions, at energy levels of 108J and 130J. These energy values are typical of the energy levels resulting from speeds (5.95- to 6.12-second 40-meter dash speeds) of 11-year-old youth players to high school athletes who often run a 40-yard dash in 5.2 seconds, which generates an energy level of approximately 125J. Dynamic full-helmet system testing demonstrated that the "Hot-Wet" condition tended to degrade helmet impact attenuation performance such that, depending on the size and type of EA material provided in the crush zone, head injury risk measures tended to significantly increase.

The Hot-Wet effect was confirmed by testing during a 1992 forensic study of helmet energy-absorbing padding performance and injury risk from only minor to moderate head impacts while wearing a "reconditioned" helmet. It was confirmed that increased helmet temperature and moisture significantly increased head injury severity levels. These earlier forensic studies were recently compared to newer helmet designs, which also showed the likelihood of increased injury risk. Clearly, increasing temperature and moisture in EA padding is an important variable that must be considered in the safe design of any helmet.

Finally, the use and benefits of a "multivariable" experimental method for helmet injury risk assessment, not reported on previously, is provided. QS testing is useful as an economical, effective "first step" to evaluate various EA materials under a range of environmental and loading conditions. Then, "second step" dynamic helmet testing provides reliable evaluation of helmet safety and head injury risk over a wide range of variables, or factors, through the use of only a relatively small amount of test combinations. The multi-variable analysis concept is used to demonstrate how the Head Injury Criteria (HIC) response for a specific helmet design varies over a range of temperature-moisture conditions and impact energy levels generated from "high-low" tests. This two-step test methodology using QS screening of EA loss followed by realistic dynamic test multivariable assessment of the full helmet system as it relates to meeting the defined levels of head injury risk protection provides a valid, cost-effective, and efficient scientific approach for studying the interrelated effects of many variables without resorting to a limited trial-and-error approach examining individual variables in isolation. The implications of this design for helmet testing and analysis may affect most types of helmets since head heat and contact sweat are factors present anytime a helmet is worn.

Football Helmet Testing, Head Injury Severity, Helmet Repeat Impacts

D21 Differential Protective Effects of Motorcycle Helmets Against Head Injury

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After attending this presentation, attendees will understand that motorcycle helmets provide differing degrees of protection against various types of head injury.

This presentation will impact the forensic science community by providing evidence from real-world traffic crashes that motorcycle helmets manage rotational acceleration less effectively than linear acceleration.

Background: Previous observational studies have clearly demonstrated that motorcycle helmets protect riders against head injury; however, the extent to which helmet protection differs across head injury mechanisms remains unclear. Most previous studies of real-world motorcycle crashes considered aggregate protection against head injury. Biomechanical research in cadavers, animals, and computer models has established that head injuries differ in their etiologies. Skull fracture requires a direct impact with a high-amplitude linear component, whereas concussion results primarily from angular acceleration of the head.[1-5] Injuries to the head caused by motorcycle crashes are likely to involve both linear and rotational forces.[2,6,7] This study examined helmet protection versus skull fracture, cerebral contusion, intracranial hemorrhage, and cerebral concussion in a consecutive series of motorcycle operators involved in traffic crashes in Kentucky.

Methods: Anonymized police accident reports, hospital inpatient claims, and Emergency Department (ED) claims for the years 2008 to 2012 were matched by probabilistic record linkage. The study sample included motorcycle operators with known helmet use who were not killed at the crash scene. Police accident reports were used to ascertained operator helmet use. Skull fractures, cerebral contusions, intracranial hemorrhages, and cerebral concussions were identified from the *International Classification of Disease, 9th edition, Clinical Modification* (*ICD-9-CM*) codes on the claims records. Generalized estimating equations were used to estimate the relative risks of each type of head injury for helmeted versus unprotected operators.

Results: Helmet protection against skull fracture (*Risk Ratio* (*RR)*=0.31, 95% *Confidence Interval (CI)=(*0.23, 0.34)), cerebral contusion (*RR*=0.29, 95% *CI*=(0.16, 0.53)), and intracranial hemorrhage (*RR*=0.47, 95% *CI*=(0.35, 0.63)) was substantial. The RR estimates represent the risk of each injury type among operators who wore a helmet, compared to operators who did not. RR<1 indicates a protective effect. The results for uncomplicated concussion (*RR*=0.80, 95% *CI*=(0.64, 1.01)) were inconclusive. The RR estimate (20% risk reduction) suggested a modest protective effect, but the result was not statistically significant.[8]

Conclusions: Motorcycle helmets were associated with a 69% reduction in skull fractures, 71% reduction in cerebral contusion, and 53% reduction in intracranial hemorrhage. This study concludes that current motorcycle helmets do not protect equally against all types of head injury. Efforts to improve management of rotational acceleration in motorcycle helmets should be considered.

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Motorcycle Helmet, Concussion, Skull Fracture

D22 Real-World Football Helmet Performance Versus Certification Testing, Refurbishment, and Inspection

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After attending this presentation, attendees will be familiar with a survey of the current state-of-the-art of sport helmet performance, testing, and real-world usage. Football, rodeo, and equestrian helmet performance, testing, and usage enforcement will be described. This will provide forensic scientists and engineers with new information regarding how football and other sport helmets are performing in relation to the growing field of knowledge regarding the incidence of Chronic Traumatic Encephalopathy (CTE) and other maladies related to youth, high school, college, and professional sports (especially football).

This presentation will impact the forensic science community by enabling those called upon to investigate head and neck injuries in football and other sports, as well as motorcycle helmets, to understand the effects of environmental conditions, certification testing, refurbishment, and real-world usage practices on helmet performance.

Testing and research has proven that significant degradation of football helmet energy-absorption performance occurs due to predictable environmental heat and humidity, as well as unavoidable body heat and contact sweat. New football helmets meeting minimal, antiquated National Operating Committee Standards on Athletic Equipment (NOCSAE) performance standards testing at moderate temperature and humidity repeatedly failed to perform adequately at higher, more realistic levels of heat, humidity, and impact forces consistent with predictable real-world usage conditions. Study testing proved that refurbished football helmets certified fit for safe use demonstrate even greater degradation of protective capacity.

Teams without adequate funds often fail to refurbish helmets adequately, and refurbishment companies often fail to refurbish or test helmets properly to insure reliable safety, leading to potentially insufficient protective performance. Not surprisingly, significant gaps have been discovered between intended or advertised levels of helmet protection, user expectations, and actual user protection. This disparity is likely affected by the proven degradation in protective capacity caused by real-world environmental and usage conditions discovered by this study. It is also predictable that helmet materials will degrade over time and with greater use. Football helmet refurbishment and testing has not always been performed to required standards, resulting in criminal prosecution of refurbishment companies for fraud. Since football helmets can be refurbished for ten years under NOCSAE guidelines with limited testing to determine actual protection performance of this equipment, all with little institutional oversight, this is clearly an even more significant concern.

Research conducted on football helmets to determine real-world, repeat-impact performance demonstrated poor helmet performance. Improper usage, maintenance, fit, damage and refurbishment, and adverse environmental conditions also affect helmet protective capacity. Investigative reports of inadequate helmet refurbishment, as well as evaluation of environmental conditions and inspections of helmets by officials, are described. Despite significant recent efforts to analyze and prevent football-related head injuries, there are far more opportunities for head injuries in practices than in competition because there are fewer trained medical personnel and other observers available to monitor head impacts, proper helmet usage, and potential helmet damage at practices.

In addition to concerns regarding inadequate or misleading helmet refurbishment, effective usage of helmets can be unpredictable and unreliable due to lax enforcement and inspections, poor fit, inadequate maintenance, accumulated helmet damage, basic ignorance, etc. The safety culture of a sport also influences how helmets are utilized and maintained as well as whether injuries are reported or head-injured athletes are restricted from play until medical approval is given for their safe return. Although football has widespread helmet-due-to-rules requirements, other hazardous sports (rodeo rough stock events) often see relatively little helmet use in training despite similar or even greater injury risks. Rodeo competitors in championship events are known to wear helmets without fastening the chin strap, showing a contempt for risks, safety equipment, and rules by competitors and their coaches, and a lack of effort by event officials to insure that safety equipment is in good condition and properly worn.

In contrast to the ten-year refurbishment of football helmets, other sports require that helmets be taken out of use after significant impacts. This is common with bicycle, motorcycle, and auto-racing helmets, but not with football, rodeo, and many other higher head injury-risk sport helmets.

Sport Helmets, Energy Absorption, Environmental Degradation

D23 Does Size Really Matter — Or Is How You Manipulate It More Important? A Review of Data Analysis and Presentation Tips and Tricks

John Nixon, CEng, MBA, ARC, PO Box 66, Bippus, IN 46713*

After attending this presentation attendees will better appreciate what can go wrong with scientific analyses and testimony, what enabling mechanisms are at work, what motivates practitioners to indulge in unethical and/or illegal behavior, and what strategies may be employed to avoid or minimize such problems.

As the old adage goes, "there are lies, damned lies, and statistics." Some would argue that whoever created that phrase was somewhat myopic — and that it could more correctly be stated as, "there are lies, damned lies, statistics, and government statistics." Experts, judges, and lawyers should be aware of techniques that may be used to misrepresent the facts. This is now more important than ever because in the modern era of digital photography and digital videography, combined with computerized editing, there is ample potential to misrepresent more than just numerical data.

Those indulging in misrepresentation fall into several categories and numerous subcategories. In broad terms, the purveyor of the misleading information may be the devious first-hand manipulator, an unscrupulous messenger who knowingly presents spurious data from a "legitimate and authoritative source" (or otherwise), or merely an innocent, ignorant "victim" who presents bogus data passed along from another source. The latter two categories may be difficult to distinguish — it is amazing how a genius can morph into an absolute imbecile when it comes to interpreting data generated by others. Perhaps this is why courts in some jurisdictions are loathe to allow experts to cite data from studies conducted by others, no matter how prestigious the source: California springs to mind.

When such misrepresentation occurs in the world at large, it can be endlessly debated and debunked (or not); global warming being a good example; however, when misrepresentation occurs in a court, there is less potential for debate — less chance to "get it right" — and the negative consequences may be dire. It usually comes down to one expert contradicting another, and a jury, presumably of limited education and experience in the given specialism, is left to assess credibility based upon unreliable subjective criteria (which expert appeared or sounded most convincing — not always the best indicator of data integrity).

It should be noted that experts are not solely to blame for data misrepresentation. Lawyers see it as their mission to discredit (warranted or not) and mislead. Cross-examination is often used to "debunk" established science by infusing pseudo-science or junk science into an otherwise rational debate. There must be a reason that those lawyers don't operate under oath, right?

Case studies and examples will be used to illustrate the points discussed and speculate as to the motivations of the guilty parties. It will be demonstrated that while some instances of wrongdoing are the acts of individuals, others strongly indicate conspiracy. It is concluded that these practices will continue unabated without systemic reform, and that this reform will take a concerted effort on the part of the legal system and society at large. Perhaps so many key players benefit from these practices that the motivation for change simply does not exist!

Data Manipulation, Misrepresentation, Experts and Lawyers

D24 It's a Fair System, Isn't It? Facts, Alternative Facts, and Other Litigation Influencers

John Nixon, CEng, MBA, ARC, PO Box 66, Bippus, IN 46713*

After attending this presentation, attendees will better appreciate what can go wrong with scientific analyses and testimony, what enabling mechanisms are at work, what motivates practitioners to indulge in unethical and/or illegal behavior, and what strategies may be employed to avoid or minimize such problems.

This presentation will impact the forensic science and legal communities by enabling them to identify unethical behaviors, appreciate the diversity of such behaviors, and provide them with ideas for strategies that may be employed to avoid or minimize such problems.

Expert forensic testimony is so widespread that it now often forms the backbone of criminal cases and nearly always plays a critical role in civil litigation. Some disciplines rely on conclusions derived mostly from hard data (test results or recorded observations, for example), while other disciplines rely on subjective opinion that is based upon experience, hypotheses, individual theories, and judgment. Virtually every discipline involves at least some subjective judgment, and that leaves plenty of scope for a well-intentioned process to go awry.

The continuing viability of industries, involvement of huge sums of money, and entire lives are often at stake (death penalty or life without parole). The outcome of trials changes the lives not only of the parties involved, but potentially of huge numbers of people for many years to come. With so much at stake, it is imperative that society ensures the integrity of the system; it should be beyond reproach. Unfortunately, we are falling short of that ideal. The court's role as gatekeeper and the application of *Daubert* and *Frye* standards have helped to keep unreliable expert testimony out of the courtroom, but even these safeguards fall short of desired ideals. *Frye*, for example, requires that testing protocols and underlying scientific principles be accepted by the relevant scientific community — but what objective standards are used to identify the relevant scientific community? The application of a *Frye*-type standard in 16th-century Italy resulted in the incarceration of Galileo because he said the earth was round and rotated around the sun, while the "relevant scientific community" dismissed his work as heresy. How can we be sure that similar errors are not being made in courtrooms today?

Incompetence can largely be overcome by remedial education, training, and stringent staff selection policies. Errors will never be eliminated completely but can be greatly reduced by the adoption of procedures, including independent peer review; however, there are far more sinister forces at work within the system (greed, power grabs, influence peddling, ego aggrandizement, etc.) that motivate humans to lie, commit fraud, intimidate or deceive others, and introduce bias. These can be difficult to detect and extremely difficult to eliminate.

Historically, the legal system appears to have dismissed the aforementioned acts as so rare as to be insignificant; however, a review of cases, and especially criminal cases, reveals that the problem is not as isolated as the layperson may assume. Perhaps there is more motivation to eliminate such acts in civil litigation, where huge sums of money are often involved, and less motivation in the criminal arena, where the high volume of cases and society's perception of criminal defendants as thieves, junkies, rapists, murderers, and general "street scum" results in less scrutiny. The post-conviction process does little to discourage these behaviors. The review system is geared toward errors of process, rather than errors of fact or opinion, and the attitude of the courts is often one of, "tough, the defendant should have hired his own expert and challenged this testimony first time around; he gets only one bite of the cherry." These undesirable expert behaviors have essentially been encouraged by the attitude of the legal community, by experts themselves, by the adoption of poor procedures, and by the creation of innocuous names for unethical or illegal activities. For example, generating fictitious test data (fraud) is often described as "dry labbing," and false and misleading testimony is often described as "he misspoke," even when it leans solely in favor of the hiring party.

Case studies will be used to illustrate the points discussed and perhaps identify the motivations of the guilty parties. It will be demonstrated that while some instances of wrongdoing are the acts of individuals, others indicate conspiracy. It is concluded that the systemic failures that enable these practices to continue will not be changed without significant and concerted effort on the part of the legal system and lawmakers.

Incompetence, Bias, Dishonesty

D25 Ethical Conflicts in Science and Engineering: The Theft of Scientific Records, Marginal Practices, and Breach of Confidentiality in the Peer-Review Process

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The goal of this presentation is to alert forensic professionals in the ways testing, data, and safety standards have been manipulated and multiple records of taxpayer-supported research and classified information have been intentionally suppressed, stolen, or destroyed. This loss of critical information negatively affects public safety and efforts to improve technology. It also allows defective, dangerous products to continue to be produced, placing the public at risk. None of this could happen if those involved followed the Engineering Code of Ethics.

This presentation will impact the forensic science community by increasing awareness of how automakers, helmet manufacturers, consumer product manufacturers, safety certification groups, government officials, and others who are responsible for generating and maintaining objective and reliable scientific information have failed in many instances to properly carry out this responsibility in an ethical manner. The forensic community and, ultimately, the public will benefit from this presentation.

Many authors have been involved in decades of biodynamics, safety testing, and other engineering research for both the United States Government and the private sector. Several were also associated, before his death, with the legendary Dr. John Paul Stapp of the United States Air Force (USAF) and National Highway Traffic Safety Administration. The theft of scientific records from Dr. Stapp's home and office immediately after his death was briefly described at the 2017 AAFS Annual Scientific Meeting. This presentation expands on that work by providing more detailed contributions from authors, some of whom were actual witnesses to the theft and had early roles in attempts to find the culprits. The author of *Sonic Wind*, a biography of Dr. Stapp published in 2015, later discovered the ultimate fate of these documents.

This presentation will begin with a narrative describing the events preceding re-discovery of the Stapp documents. Legal perspectives and a commentary regarding these documents will then be provided by an aerospace engineer/attorney. Finally, the presentation will conclude with examples outlining the ethical and other pitfalls associated with science and engineering.

One of these examples includes a recently experienced violation of author scientific rights involving a breach of confidentiality by supposedly unbiased, independent peer reviewers. The reviewers acted in collusion so blatant that one copied the review of the other, and submitted it verbatim to an engineering society. It was only after the authors requested and analyzed the comments that the collusion was discovered, then corrected by this engineering society. This is only the latest in a series of similar questionable practices that were encountered from individuals and groups who have lost their ethical compass in conducting science and engineering research.

Another example involves safety standards and test methods for motor vehicles. Specifically, despite objections by the National Transportation Safety Board and other safety professionals, manufacturers have successfully lobbied the United States government to eliminate long-established critical safety standards and test methodologies, with no conceivable benefit to public safety. This has allowed dangerous designs that defeat critical safety systems to be produced, in clear violation of well-established safety principles. Misleading warnings are also provided for consumer products in which, even if the warnings are fully complied with, the hazard is still present and will result in a predictable risk of permanent or fatal injury. The public is given no clue that this is the case, and many permanent disabling injuries and fatalities have resulted. This is a clear violation of well-established safety practices. None of this could happen if those involved, from both the manufacturing and the governmental safety agency aspect, had followed the Engineering Code of Ethics.

Evidently, it is becoming easier and cheaper to roll back or eliminate safety regulations than to actually follow state-of-the-art improvements in science, technology, and knowledge by corresponding safety and performance improvements in products. This is facilitated by suppressing publication of scientific research that exposes flaws in existing designs, which explains the motivation for collusion and other unethical practices in the peer-review process, as well as theft of scientific records.

Theft of Scientific Records, Breach of Confidentiality, Violation of Peer Review

D26 Forensic Microscopy in Determining Historical 1960s and 1970s Asbestos Exposures to Cutting Asbestos/ Cement (A/C) Pipe

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After attending this presentation, attendees will better understand how Polarized Light Microscopy (PLM), Phase Contrast Microscopy (PCM), and Transmission Electron Microscopy (TEM) were used to determine information regarding the historical asbestos exposure to a worker cutting A/C pipe in the days before proper protection procedures were mandated.

This presentation will impact the forensic science community by providing information to assist in the investigation of asbestos exposures that occurred before exposure monitoring was conducted and where few records exist.

Since the 1920s, A/C pipe has been manufactured from a mixture of asbestos, standard Portland cement, and silica sand. Millions of miles of A/C pipe have been sold around the world to carry drinking water, sewage, telephone duct, and cable conduit. Although not officially banned, A/C pipe is generally not currently used in the United States because of the potential release of asbestos fibers, which are a known carcinogen when inhaled. In the 1960s and 1970s, A/C pipe was cut in the field with a variety of tools, including power saws and abrasive disc cutters. Historical asbestos air sampling data during the cutting of A/C pipe is limited.

To provide more information about the historical asbestos air levels during cutting, a study for possible fiber release was conducted involving the cutting of a piece of A/C pipe in a controlled area while air samples were collected. PLM analysis revealed the pipe contained 20% chrysotile and 2% crocidolite. Air samples were collected in the breathing zone (seven feet distant) of a worker using a gas-powered pipe cutter with a new 14-inch diamond blade. These air samples were collected by a worker with pipe-cutting experience.

The study was conducted in a test chamber that was approximately 9ft high by 10ft wide by 12ft long. The study area had a High Efficiency Particulate Absolute (HEPA) air filtration device that was used to clean the area of particulates before testing activities. The HEPA unit ran at a rate of 177cfm during the testing. While inside the study area, the worker was protected by an air-line supply respirator and complete head and body coverings. The chamber and airline supply intake were monitored for levels of carbon monoxide.

Prior to the testing, an air sample was collected within the chamber. It was apparent that the gas-powered cutter (saw) had been used to cut cement previously. Testing was performed to determine whether the cement residue in the cutter contained any asbestos. An air sample was collected next to the power saw cutter while the cutter was turned on, run briefly, and turned off, and while it was being cleaned and blown out with compressed air at intervals over a one-hour period.

The asbestos level in the breathing zone of the worker during the cutting interval was 182 fibers/cc (PCM National Institute for Occupational Safety and Health (NIOSH) 7,400 value times TEM NIOSH 7,402 percent asbestos). The area sample (seven feet away) during cutting demonstrated a level of 159 fibers/cc. No asbestos fibers were detected in the blank samples or in the sample collected in the chamber before the testing began. No asbestos was detected in the cement residue material contained within the cutter when received. No asbestos was detected in the air when the cutter was running and while compressed air was blown into the cutter in pre-experiment cleaning.

Although the carbon monoxide monitor alarm did go off inside the chamber during the test, the carbon monoxide monitor outside the chamber near the supply air intake did not register above zero during the testing.

The high level of asbestos in the breathing zone of the worker cutting A/C pipe in this study are similar to a level of 170 fibers/cc published in a study of workers cutting A/C pipe in a trench in Japan. Other unpublished studies have also reported air samples more than 100 fibers/cc for cutting A/C pipe with power tools.

Microscopy, Asbestos, Exposure Evaluation

D27 A Forensic Performance Analysis of Load-Limiting Devices in Automotive Seat Belt Retractors

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After attending this presentation, attendees will better understand the design and operation of automotive occupant safety restraints. Specifically, attendees will better understand the load-limiting device found in most modern automotive safety belt restraints.

This presentation will impact the forensic science community by providing a key aspect of forensic investigations and analysis of occupant safety belt restraints involved in automotive crashes.

The primary function of an automotive safety belt restraint system is to restrain the occupant within the vehicle's occupant compartment. The primary requirements of any occupant restraint system are: (1) to prevent ejection of the occupant, both partial or total, from the vehicle; (2) to prevent or minimize severe interior impacts of critical body areas with surfaces within the vehicle; and, (3) to control the occupant's kinematics in such a way as to load the body through the strong skeletal structures of the body that are most capable of withstanding the loading.

As such, the safety belt should be designed to apply loads to the body of the occupant through the clavicle, sternum, ribs, and pelvis. By using these robust structures within the body, and by optimizing belt elongation, properly designed lap and shoulder belts have been able to strike a balance between neck loading and the potential for severe interior strikes that body movement can cause in many crashes. The introduction of frontal airbags for front seat occupants, along with pretensioner devices to more effectively couple the occupant to the vehicle, provided an additional opportunity to the safety belt designers to address thoracic injuries that can be caused by the safety belt loading and thus provide added protection for the frailer segments of the population. This led to the introduction of safety belt load-limiting devices, which were intended to reduce belt loading while still maintaining proper restraint protection. Unfortunately, the implementation of these devices in some circumstances increased the potential of injury from head impact, ejection, and submarining.

This presentation focuses on the trade-offs involved in the effective design of such load-limiting devices (i.e., the reduction in maximum loading to the occupant versus the corresponding increase in safety belt webbing and subsequent occupant movement). Obviously, if the additional webbing introduced is not controlled properly, the risk of injury to the occupant is greatly increased and could result in more frequent and severe injuries rather than the reduction intended. This presentation assesses the amount of additional webbing introduced by the activation of the load-limiting device by forensic analysis of the affected safety belt and its components involved in real-world crashes.

Crash, Restraints, Load Limiters

D28 Autonomous Vehicle Control Systems and Human Factors

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After attending this presentation, attendees will better understand the control systems of autonomous vehicles, explore what the future of Highly Autonomous Vehicles (HAVs) will entail, and gain familiarity with some of the legal and ethical issues involving autonomous vehicles. In an increasingly connected world, vehicle control technology has the potential to lead to safer vehicles, once the issues of security, reliability, and ethics are addressed.

This presentation will impact the forensic science community by helping attendees: (1) learn the current capabilities of autonomous vehicles and forecast the future of autonomous vehicles; (2) understand how forensic engineering may contribute to control systems design; and, (3) gain a better understanding of the dynamic relationship between HAV technology and related ethical issues.

The increased integration of technology in vehicles, coinciding with the exponential increase in computing power, has led to the advent of the HAVs. Computers process data substantially faster than humans, leading to decreases in automotive braking times, quicker avoidance of hazards, and generally increasing occupant safety.

In 2014, more than 32,000 people lost their lives on the road in the United States (Figure 1), according to the Fatality Analysis Reporting System (FARS) for 2014.[1] This decline is partially attributable to the numerous safety features automobile manufacturers have added to vehicles to reduce deaths from traffic accidents. The most well-known vehicle safety features are pretensioners and airbags. These two features have reduced mortality in head-on collisions by more than 80%.[2] As the level of technology increased through the turn of the century, additional safety features, such as Blind Spot Detection, Lane Departure Warnings, and even Adaptive Cruise Control, have been added to assist or alert drivers to avoid accidents. The results of a 2008 National Highway Traffic Safety Administration (NHTSA) Crash Causation Survey to Congress revealed that 75% of collisions were due to either recognition or decision errors (Figure 2). Recognition errors were defined as inadequate surveillance, distraction, or general inattention. Decision errors were defined as speeding, illegal maneuvers, following too closely, etc.

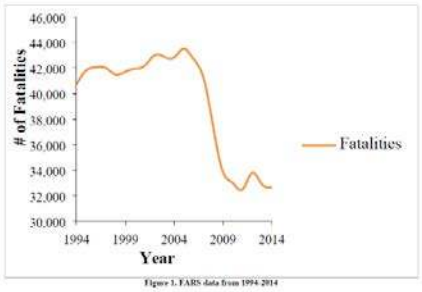

Figure 1. FARS data from 1994-2014

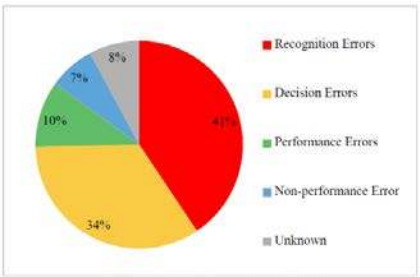

Figure 2. 2008 NHTSA Crash Causation Survey to Congress

Examination of state laws regarding the use of HAVs on public roads reveals a myriad of regulations ranging from permissive to unauthorized use. Such chaos creates issues for the smooth operation of commerce between states and could violate an individual's human rights. The NHTSA's proposal for states to adopt a Model State Policy could create uniformity and partially solve some of these issues.

A major difference between present and future vehicle accident forensics concerns the amount and accessibility of HAV-related data. Two petabytes (10^{15} bytes) of data accompanies processing and responding to the environment around one HAV annually; however, designers of HAV control systems dictate vehicle responses to these input data. This raises new ethical issues because, while human drivers may be "forgiven for making an instinctive but nonetheless bad split-second decision … programmers and designers of automated cars don't have that luxury, since they do have time to get it right and therefore bear more responsibility for bad outcomes."[3] A novel issue is thus raised regarding how will such ethical decision-making be programmed into autonomous vehicles. This will require more thorough dialogue with government, industry, academia, and the public. Other questions will likely also arise from the interface of HAV technology with society. These questions may include: (1) Can HAVs fully replace human drivers?; (2) What will be the socioeconomic impacts of implementing HAVs?; and, (3) Will HAVs disrupt the balance between individual privacy and public security?

This presentation will demonstrate how HAV electronic control systems are tested, including modeling, human simulation, and full-scale experimentation. Exciting and significant performance information will be illustrated with these modeling and testing examples.

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Autonomous, Ethics, Controls

D29 What Confidence Do We Have in Confidence Intervals?

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The goals of this presentation are to clarify the correct interpretation of confidence intervals and illustrate how sampling bias and similar factors lead to incorrect confidence limits.

This presentation will impact the forensic science community by informing attendees that confidence intervals are frequently used to ensure compliance with criteria such as maximum contaminant limits. Attendees will learn that sample bias or inappropriate application of upper confidence limits can have significant consequences in evaluating these criteria.

Confidence intervals are widely used to express the certainty with which we can establish that a set of samples exceeds or meets criteria, such as maximum contaminant limits; however, confidence intervals and upper confidence limits are often misunderstood and misapplied.

When considering small sample sets, there may be insufficient information to discern the statistical properties of the data. As a result, many assumptions have to be made regarding important properties, such as the inherent distribution of the data. The situation is further complicated by heterogeneous sample matrices, samples taken at different times, variability introduced by sampling methods, sample preservation, analytical techniques, laboratories, and similar factors that influence data sets encountered in real-world situations.

These sources of uncertainty confound environmental forensic investigations in which investigators often have little control over the data collection efforts. They may be dealing with data sets collected for an event that occurred some time in the past and they can no longer make relevant measurements, or they may be dealing with data collected by another party and they cannot make independent measurements or influence where and how the data are collected. They are simply left with a data set they need to analyze and objectively express the confidence with which they can make various claims.

Further frustrating the establishment of confidence intervals is biased sampling. In some instances, samples are only taken when there is some indication that contaminants are present; for example, staining or when a field instrument such as a photoionization detector indicates the presence of volatile organic compounds. In these instances, forensic investigators have no negative confirmation samples showing background levels. Conversely, sample sets may be biased toward background levels if most of the samples are unrelated to a problem area or there is some other flaw in the sampling protocol, such as sampling too shallow or deep, disturbing the sample, or any number of factors that could cause the samples to be biased low.

Using synthetic data sets for which the statistical properties are known, this presentation explores how these factors contribute to the difficulty of interpreting the data and establishing confidence intervals. This presentation discusses the correct interpretation of the confidence interval, explores how the sample size influences the confidence interval, and illustrates how the distribution of the underlying data is distorted by time, sampling methods, variability in the methods of analysis, and the influence on the confidence intervals. The importance of the sampling protocols is discussed and how sampling bias translates into bias in the confidence interval is demonstrated.

Statistics, Confidence Intervals, Maximum Contaminant Limits

D30 The Effect of Microscopic Surface Coatings and Residues on the Size and Shape of Bloodstains

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After attending this presentation, attendees will be familiar with how the shape of a bloodstain can be significantly altered if the blood were to impact on a microscopic coating, such as the residue oils and lipids from a latent print.

This presentation will impact the forensic science community by providing results from controlled experiments illustrating how nearly imperceptible surface coatings influence bloodstain size and shape, thereby potentially affecting the results of such analysis. This presentation extends the frontier of research in Blood Pattern Analysis (BPA) to account for alterations in bloodstains due to common coatings that may be encountered at a crime scene.

BPA uses the location, size, shape, and distribution of bloodstains to provide information regarding the events occurring at the crime scene. For example, aspects of the stain can provide insight into the speed at which the blood drop originally hit the surface, which can provide information about the cause of the bloodletting event. Furthermore, when estimating the drop trajectory responsible for a bloodstain, methods that account for gravitational and drag effects rely on an accurate estimate for the impact velocity.[1] Previous research has found a relation between the impact velocity and the number of spines radiating from the dried drop or from the stain size if spines are absent.[2,3] These relations tacitly assume that the drop shape and size would be identical on any smooth surface.[4] The hypothesis presently explored is that microscopic coatings, including residue oil from a latent fingerprint, can also have a significant effect on the size and shape of a bloodstain.

This hypothesis was tested using systematic experiments conducted in a laboratory environment. Four different coatings were prepared on glass slides having a polished non-porous surface. The first set of glass slides was cleaned and left uncoated. The second set of slides was coated with a fixed volume of vegetable oil smeared across the surface to create a uniform microscopic film. The third set of slides was coated with natural sweat secretions deposited by a finger rubbing across the slide, and the final set of slides was coated with a thin layer of soot from a flame, as might occur after a fire. Another group of slides was created using a combination of both clean and coated regions. For each type of slide coating, a single blood drop was released from a given height so that it impacted at a particular velocity. Velocity adjustments were made by changing the height at which the blood drops were released. Impact velocities varied from 1m/s to 18m/s, or terminal velocity for small drops. By recording the drop impact using both high-speed and time-lapse photography, the drop dynamics leading to the final stain could be quantified.

The results demonstrated a significant difference between the shape of a bloodstain on the clean surface and that with a microscopic coating. The size of stains formed on clean glass was larger than those on the glass surfaces with an oily residue. In addition, for the oil-smeared surface, several spines with associated satellite droplets were formed, whereas these did not form on clean glass slides under identical drop impact conditions. By contrast, on the glass surface coated with soot, the blood drop completely recoiled from the surface and, thus, did not leave behind a stain at all.

This study illustrates that commonly found microscopic coatings can alter the way a drop of blood spreads and dries on a smooth surface. This affects the final appearance of the bloodstain upon which BPA examination is based. The results indicate that bloodstain analysts may be able to further reduce uncertainty in forensic reconstructions by noting conditions that might indicate the presence of specific microscopic coatings.

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Surface Coating, Bloodstain, Terminal Velocity

D31 Forensic Confrontation in the Engineering Sciences: An International Murder Prosecution Involving American Embassy Military Personnel

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After attending this presentation, attendees will understand the principal phases of a military court-martial, the critical role of forensic engineering evidence in the pre-trial stages of the case, and their use as "back-stops" to alternative theories.

This presentation will impact the forensic science community by explaining the relationship between the forensic engineer, the military prosecutor, and the expert's ability to inform the military judge or jury.

The Uniform Code of Military Justice (UCMJ) is divided into three phases: pre-trial, trial on the merits, and sentencing (if necessary).

In cases involving serious offenses, including death, two separate entities begin investigating: (1) the law enforcement community; and (2) the chain of command. When cases occur overseas, there is also usually a separate host-nation investigation. These multiple investigations are designed for different purposes and can sometimes be counter-productive. Additionally, there is no organic forensic examiner support in the military outside of a handful of experts. Thus, civilian forensic science experts are usually hired to perform services, often long after the "evidence" has been collected.

The primary goal of this presentation is to present a representative prosecution of a case under the UCMJ involving the death of a Panamanian citizen by a United States Army soldier. The victim's body was discovered in the early morning hours on a Panamanian police training area where the officers were practicing small-arms marksmanship. The victim was the known girlfriend of the accused American soldier, who was receiving training that same day at that training area. The victim died of blunt force trauma. The defendant claimed that she jumped out of the truck and, when he stopped the vehicle, he failed to put it in park, causing it to roll back on top of her. The defendant provided few details and there was no evidence available to determine the location of the actual death. Attempts to collect digital or electronic evidence also failed.

The Panamanian government conducted an autopsy, but no X-rays were taken. At a later date, when the forensic expert was hired, the prosecution was unsuccessful in its efforts to exhume the body for radiographic examination.

At trial, the defense provided expert testimony related to the manner, cause, timing, and location of the death of the victim. The forensic expert hired by the United States Army was essential in challenging that testimony.

Driving, Fatal, Prosecution

D32 Data Center Failures and Outages

Helmut G. Brosz, BASc, PEng, Brosz Forensic Services, 64 Bullock Drive, Markham, ON L3P 3P2, CANADA*

After attending this presentation, attendees will understand the critical importance of preventing data losses in today's information society, the causes of such data losses, and the preventative measures available to avert such losses.

The presentation will impact the forensic science community by: (1) exploring the causes, mitigation, and prevention of power failures and accompanying data losses; and, (2) discussing power failures and outages and the potential for litigation involving data center losses.

Data centers are the hub of communication networks. They are powered by electricity from local utilities, back-up generators, and Uninterruptable Power Supply (UPS) systems. Despite power supply redundancies and proper maintenance, total or partial outages occur and can be a costly disaster for users. Outage costs or penalties in the one-million-dollar-per-minute range have occurred. Litigation can ensue as one or more parties invariably are held responsible. Four cases are presented.

Case 1: Defective switchgear — 480-volt switchgear and circuit breakers are essential components of a UPS distribution system. In-plant inspection at the time of manufacture and proper commissioning are important to achieve the high degree of reliability required. Good electrical connections are necessary to prevent overheating and failure. Thermography is a useful tool for detecting incipient faults and preventing power failures and data losses.

Case 2: Defective Diesel engine overrunning clutches on UPS — Overrunning clutches are used to transfer electro-mechanical power from a motor generator set to a diesel engine during a power failure. Although this transition may last only a few seconds, during this time the clutch is exposed to the harmonic vibrations produced by a large (3,000kw) reciprocating diesel engine. Some types of clutches can fail during these circumstances. This potential source of failure can be averted by proper in-plant inspection and pre-equipment purchase vetting.

Case 3: Improper Maintenance — An establishment's computer and scanners tasked with reading paper credit card receipts were continually failing. The onsite service contractor would replace various printed circuit boards in this equipment using bare-hand handling methods in a service environment that was not designed to safely discharge accumulated static electricity. Upon touching the terminals of the exposed printed circuit board, the technicians would discharge static electricity to sensitive circuit components, ultimately resulting in computer malfunction. Costly arrangements had to be made to have the credit card data read by a competitor.

Case 4: Improper grounding systems — A cold war-era communication center on a hilltop was converted to a data center. The secure 1,000-foot vertical steel well water supply pipe of this center was used as the main building ground. The soil conditions in the vicinity of the center were very dry. The 1,000-foot steel well pipe was connected to the electric utility neutrals and ground. Thus, during utility ground faults, high-fault currents flowed toward the best ground in the area, which happened to be the 1,000-foot well casing. The rise in potential caused failures and outages in the data center. Rearrangement and redesign of the grounding system was necessary.

Data Centers, Failures, UPS Systems

D33 Forensic Image Processing

Marcus Borengasser, PhD, Department of Defense, 3205 Lago Vista Drive, Melbourne, FL 32940*

The goal of this presentation is to provide an overview of forensic image processing techniques and the availability of open source software.

This presentation will impact the forensic science community by providing an introduction to forensic image processing that will raise analyst awareness of an increasingly important technology.

Surveillance video is nearly ubiquitous, from government, institutional, and commercial buildings to outdoor venues and unmanned aerial systems (drones). In many cases, the technology driving the development of video systems has far outpaced the acquisition of resources to aid the analysis of video imagery; however, familiarity with a few image processing concepts can often produce favorable results with video imagery that has been degraded by noise or improper lighting. Other image processing algorithms can be used for feature enhancement or data fusion. Also, open source image processing software is available and has many functions applicable to forensic image processing.

Digital filters are powerful algorithms that can be used for both noise suppression and feature enhancement. Low-pass filters, for example, can block high-frequency image components, such as internal noise or lighting artifacts. High-pass filters behave in the opposite manner, enhancing high-frequency components that produce an edge-sharpening effect. Most open source image processing software includes standardized low- and high-pass filters, plus the capability to design custom filters.

Another powerful image processing technique involves enhancing the pixel brightness histogram. When a video frame has been degraded from adverse lighting, too bright or too dark, pixel brightness values can be redistributed to partially correct for such adverse lighting effects. These histogram enhancement techniques, collectively referred to as a linear histogram stretch, are suitable for both black-and-white and color images. When a video frame has areas that are too dark and also too light, a non-linear histogram stretch can be applied. Almost all open source image processing software includes histogram-stretching functions. In addition to histogram stretching, there are a variety of related histogram-enhancement functions; most of them are easy to understand and implement.

Digital filtering and histogram enhancement can be considered suitable techniques for image restoration. A separate category of forensic image processing is feature extraction. While the process of image restoration is guided by an analyst, feature extraction can be automated to some degree. For example, in a case in which a video surveillance system is used to detect the presence of a feature, such as a vehicle or a person, classification techniques can process a series of video frames to detect the feature of interest. Similarly, image transformations, such as principle components, can be effective for separating image features based on their relative reflectance.

The availability of open source software, coupled with a basic familiarity with image processing techniques, enables analysts to gradually increase their forensic image processing skills. Given the abundance of deployed video surveillance systems, along with law enforcement body cams and drone video, demand for forensic image processing expertise can only increase.

Digital Video, Image Processing, Video Surveillance

E1 The Challenge of Diagnosing Sexual Abuse in Children: A Matter for Experts

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After attending this presentation, attendees will understand the normal prepubertal female genital anatomy in the absence of abuse, in order to distinguish congenital abnormalities and findings due to sexual abuse.

This presentation will impact the forensic science community by demonstrating the importance of a careful examination of the ano-genital area of children in cases of child abuse in order to avoid communicating wrong or misleading information to a prosecutor.

Female genital anatomy may have different features due to age and hormonal influence. Some authors describe the normal anatomy of the prepubertal population to identify accurate data that can be used for comparison purposes in cases of suspected child abuse.[1,2] Most studies focus on the appearance of hymen. According to Adams, "… the defect in the posterior (inferior) half of the hymen wider than a transection with an absence of hymenal tissue extending to the base of the hymen" is a definitive sign of trauma or sexual contact.[3]

The normal anatomy of prepubertal girls can also vary because of congenital pathologies that involve the urogenital organs. For medical practitioners, it would be useful to recognize these congenital abnormalities in order to distinguish them from the normal and to correctly identify definitive signs of abuse.

This study reports two cases of suspected sexual child abuse. In the first, the diagnosis of abuse was confirmed by the comparison between the findings and the normal anatomy; in the second, thanks to a careful medical examination and the use of imaging, the initial suspicion of abuse was converted to a diagnosis of congenital abnormalities.

Case 1: A 5-year-old girl was hospitalized because of seizure attacks and no response to stimuli. The mother told the doctors that in the past year, the girl had suffered from abdominal pain, genital hyperemia, cystitis, and vaginal hemorrhage. The girl began to talk about strange touches perpetrated by her uncles. The girl was examined in the local ambulatory care facility specializing in multidisciplinary evaluation (pediatrician, medical examiner, and psychologist) of suspected child abuse. The staff identified a certain sign of trauma or sexual contact: *a defect in the posterior half of the hymen wider than a transection with an absence of hymenal tissue extending to the base of the hymen*. The association of this evidence with the stories related by the girl and the clinical findings was fundamental to diagnosing the abuse.

Case 2: Due to vaginal hemorrhage and self-masturbation, a medical examination was conducted on a 9-year-old girl. No hymenal tissue was noted during the examination. Sexual abuse was also suspected because of information from her mother about the father, from whom she was now separated. The mother told the doctor that the father used to sleep with the child every night. The child underwent another multidisciplinary examination (pediatrician, medical examiner, and psychologist) regarding the absence of the hymen, and examining the hood, clitoris, and hypoplasia of the labia minora. The vaginal orifice was clearly visible. The operators diagnosed a congenital pathology. The diagnosis was also confirmed by an ultrasound examination that demonstrated the normality of the urinary tract and the pathological immaturity of the vagina, uterus, and ovaries (the latter were described as two fibrous, ribbon-shaped formations).

These two cases provide strong evidence concerning the modality of how the evaluation of suspected sexual child abuse should be handled. The examiners should be fully educated regarding the genital features that can be observed. In addition, for an accurate diagnosis, utilization of a multidisciplinary analysis based on a combination of pediatrician, medical examiner, and psychologist evaluations is preferred.[3]

This presentation should serve as a stimulus to heighten the importance of an in-depth knowledge of physiological and pathological aspects of child genital anatomy in order to reach a correct differentiation between normal findings and those associated with abuse or congenital abnormalities.[4] In fact, according to the literature, the congenital absence of the hymen is an unlikely occurrence unless there are also concomitant major genitourinary anomalies.[5] Thanks to this information in the second case, considering the absence of the hymen in association with the pathological appearance of the clitoris, labia minora, vagina, uterus, and ovaries, the diagnosis of congenital abnormalities was made.

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Child Sexual Abuse, Congenital Abnormalities, Forensic Examination

E2 Cupping Therapy Practiced on Children: Maltreatment or Alternative Therapeutic Procedure?

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After attending this presentation, attendees will have increased knowledge of cupping as one of the possible differential diagnoses of bruises in the child victim of suspected maltreatment.

This presentation will impact the forensic science community by providing information concerning the clinical presentation of skin injuries caused by Cupping Therapy (CT).

Two unusual cases, managed in the multidisciplinary unit dedicated to the evaluation of suspected abused children ("Bambi") of the Ospedale Infantile Regina Margherita in Turin, Italy, are presented as illustrations.

Case 1: Two Algerian brothers, three and five years old, were accompanied by their father to the hospital. The parents were separated and the children had previously been with their mother. Upon their return, the father noticed injuries on their backs. The medical examination found the presence of painful, round, and excoriated bruising, approximately 3cm in diameter, on the backs of both children. The patients reported that the mother had produced lesions in order to drive out bad spirits.

Case 2: A 2-month-old Chinese girl was taken to the "Bambi" unit by social workers who had noticed the presence of skin lesions. The little girl actually had a series of round- and oval-shaped reddish bruises, one on the neck, three on the chest, three on the abdomen, and one on the right forearm. The largest measured 3cm in diameter, the others measured 1cm, on average. The parents, who were both very young, did not know the Italian language and were in no way socially integrated.

The lesions observed in these three children were all roundish or oval-shaped bruises and were all produced at the same time as they were of the same color. They were attributable to the domestic practice of CT, one of the oldest traditional procedures in folk medicine around the world. A common element in all cultures that practice CT is the extraction of toxic substances from the body by creating negative pressure in a cup by means of heat or a special suction apparatus. Traditional Chinese medicine and the Arab world both include their own form of CT.[1] It is used to treat many diseases, including painful conditions, nausea and vomiting, urinary tract infections, and respiratory and rheumatic illnesses.[2] After a period of decline, CT has regained popularity over the past six decades by means of its promotion by holistic health care practitioners in China, and also in the Western world. CT was even used during the 2016 Olympic Games in Rio de Janeiro by an extraordinary swimmer who broke the world record.[3]

Some side effects have been reported, especially when practiced at home by inexperienced people; anemia, factitial panniculitis, and herpes viral infections are the most frequent adverse events as well as abscesses, post-inflammatory skin hyperpigmentation, and keloids.[2]

It is essential to be aware of this form of traditional practice and of the characteristics the lesions can leave on the skin in order to avoid the misdiagnosis of traumatic bruises due to a compressive mechanism. Moreover, in similar situations, it is necessary to thoroughly investigate the familiar, psychological, and social context of the child. While CT is configurable as an alternative therapeutic practice when used on an adult subject, it is placed at the limit of maltreatment when performed on a child who cannot consciously express consent.

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Bruises, Child Abuse, Cupping Therapy

E3 Two Cases of Acute Drug Poisoning in Children: A Form of Life-Threatening Neglect

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After attending this presentation, attendees will better understand the necessity of recognizing and promptly reporting a potential child maltreatment situation. Timely intervention may prevent further abuse of a minor and provide protection to safeguard the child's well-being.

This presentation will impact the forensic science community by highlighting that child abuse includes not only child sexual abuse and maltreatment, but also child neglect, which is the most common form of child abuse, although it is not always easy to identify or to prevent.[1]

Cases involving physical violence and sexual abuse typically receive much public attention, but in cases in which minors are not subjected to these obvious signs of injury, they may be overlooked. Without careful assessment and examination, undetected problems and the associated consequences often interfere with the child's psycho-physical development. It is often easier to identify young victims of potential abuse and to facilitate their referrals to a dedicated center for diagnosis and follow up; however, children who live in socially disadvantaged families should be supervised with greater attention by social services, health professionals, or judicial authorities. It is vital to ensure the involvement of special social services who can intervene when parents are unable to properly care for a child, ensuring their development and protecting their well-being.

In literature, child neglect does not have a unanimously recognized definition. It is typically defined as a deficit in meeting a child's basic needs, including the persistent failure to provide health care and to protect the child from exposure to any type of danger which could cause serious, long-term damage or even death. Other examples of neglect include incidents that result in significant health damage or create developmental delays in the absence of organic causes.[2] An unusual type of neglect is the lack of supervision of children who may severely endanger their lives by merely touching or ingesting their parents' drugs or abuse substances.

This presentation will describe two cases of life-threatening child neglect due to acute drug intoxication which were observed in the dedicated child abuse unit called "Bambi," located within Ospedale Infantile Regina Margherita (OIRM), a pediatric hospital in Turin, Italy. Exhibiting severe clinical symptoms, these young patients required hospitalization in the pediatric hospital. The first case involved a 9-month-old boy who was brought to the pediatric emergency unit with hyporeactivity and hypotonia. No circulatory or respiratory problems were observed. Questioning the parents led to a suspicion of the ingestion of a piece of cannabis that had fallen to the ground during a meeting with friends. This was confirmed by the detection of high levels of cannabinoid derivatives in the child's blood and urine. The parents, both unemployed, were shocked because they thought their baby had eaten a dog biscuit while crawling, and had not taken any action.

The second case involved a 4-year-old boy who was transferred from an outside hospital to the Intensive Care Unit of OIRM in a coma with a marked bilateral miosis and intubation due to acute respiratory failure. At first, he showed mild reaction to painful stimuli and hematochimics with increased transaminases. Subsequently, his symptomatology ameliorated; the lethargic baby began to awaken and was discreetly oriented in time and space with spontaneous breathing. Therefore, he was extubated and brought to the pediatric emergency unit where his clinical condition continued to improve. As the child manifested respiratory insufficiency, pin-point pupils, and coma, clinicians immediately suspected an acute opioid intoxication. It was confirmed from a detailed clinical investigation that revealed the young patient had put three transdermal fentanyl patches, used by his grandfather suffering from cancer, on his body while his parents were not present.

Through these two illustrative cases, this presentation underlines the importance of paying attention not only to child maltreatment or sexual abuse, but also to child neglect situations, which may be very dangerous or fatal for children left without supervision.

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Child Abuse, Child Neglect, Drug Poisoning

E4 Child Sexual Abuse: The Importance of the Forensic Medical Examination in the Judicial Decisions

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The goal of this presentation is to provide attendees with a perspective of the impact of the forensic medical examination in the legal outcomes in cases of child sexual abuse in Portugal.

This presentation will impact the forensic science community by revealing that medicolegal and forensic intervention significantly contribute to the judicial decision in Portugal and points out the strengths and weaknesses of both the forensic and judicial systems.

Depending on the objectives, studies about sexual offenses may focus on the alleged cases of sexual contact (the number of cases may be overvalued by false claims) or on the legal outcomes (the number of cases may be undervalued because of the filed or acquitted cases due to the lack of evidence or to the unaccountability of the abusers). It is therefore critical to determine the association of both types of studies, as well as the comparison of their results, to highlight the quality of medicolegal and forensic intervention on this subject. Such analysis will certainly help to better understand the effects of medicolegal and forensic interventions on court decisions, as well as assess their strengths and weaknesses. Since such an approach has not yet been consistently measured in both international and national literature, the general objective of this study was to contribute to a better characterization of child sexual abuse in Portugal from a medicolegal and forensic perspective to promote the professionals' abilities in early detection and diagnosis.

An analysis on the forensic medical examination reports of alleged sexual offenses against victims under 18 years of age was performed, concerning cases from the northern medicolegal services of the Portuguese National Institute of Legal Medicine and Forensic Science (INMLCF), between 2004 and 2012. Also obtained were the respective legal outcomes from Public Prosecutor's Offices and Criminal Courts (n=429). A comparison between convicted and non-convicted cases was performed.

The study revealed that 66% of the cases were filed or temporarily suspended and 31% were charged and tried, although in 81% of such cases, the abusers were convicted (26% of all cases). The average time span between the forensic medical examination and the final judicial decision was 9 months in filed or temporarily suspended cases and 14 months in prosecuted and tried cases. Convictions presented were statistically significant ($p < 0.05$) with: (1) the disclosure of the abuse by the victims; (2) the availability of eyewitnesses accounts; (3) the repetition of the abuses; (4) the detection of the suspect's genetic profile on the body and/or clothes of the victim; and, (5) the reference to diagnostic or suggestive sexual contact in forensic medical conclusions.

It therefore follows that medicolegal and forensic intervention significantly contributes to the judicial decision. Regarding the forensic medical examination, the following strengths were identified: the training of the forensic experts, who work at the INMLCF; the existence of an on-call 24/7 activity; and a national model for the forensic medical report. Weaknesses that were determined include: the delay in the disclosure/reporting of cases, which precludes most DNA studies; the limited request of toxicology and microbiology screening; and forensic psychological evaluation.

Sexual Abuse, Children, Forensic Medical Evaluation

E5 An Experimental Evaluation of Participant Recall as One Indicator of the Reliability of Infant Death Doll Reenactments

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After attending this presentation, attendees will better understand the limitations of individual recollection of the details of a death scene independent of other factors.

This presentation will impact the forensic science community by providing a data-supported evaluation of this commonly practiced infant death investigation tool.

Doll reenactment is a controversial component of the medicolegal investigation of infant death. Its potential utility to the determination of cause and manner of death is often levied against the emotional burden that it places on the family member who is asked to conduct the reenactment.

This presentation will present the results of an experimental evaluation of a key component of the reenactment process, the recollection of the reenactor. The experiment was designed to eliminate other confounding variables, including the acute stress on, effects of drugs/alcohol on, and/or the truthfulness of the person conducting the reenactment.

An empty room in the Harris County Institute of Forensic Sciences (HCIFS) facility in Houston, TX, was configured for the experiment. A portable crib was placed in the room away from other objects and from surrounding walls. An infant-sized doll was placed in the crib in addition to other items, including blankets, stuffed animals, a baby bottle, and a pacifier. A total of 46 members of HCIFS staff were asked to enter the room and to recover the decedent and bring it to the experiment coordinator. They were told the process should take approximately ten seconds and were not given any other experiment details, including the fact that they were going to be asked to conduct a reenactment. The array and arrangement of items placed in the crib varied for each participant. Photos were taken immediately prior to each recovery and immediately after each reenactment. The experiment participants were divided into two groups -- those who conducted their reenactment approximately 2 hours after their recovery and those who conducted their reenactment approximately 24 hours after their recovery. For the reenactment, the crib was cleared out and all items, including the doll, and items that may not have been in the crib during the recovery were placed in a bin next to the crib. Participants were instructed to enter the room and to place the doll and items into the crib as best as their recollection permitted.

Twenty-six binary variables were evaluated for correctness, including body position (supine/prone), direction of face in relation to the body, nose/mouth covered at recovery, and the presence and location (separately) of various items in the crib, including a bottle, a pacifier, blanket(s), and stuffed animals. There was considerable variation in the accuracy of the variables. Participants were able to accurately recall whether the doll was prone or supine (41 of 46 (21 of 23 for the 2-hour group, 91%, and 18 of 20 for the 24-hour group, 90%)). Other variables were less accurately recalled. Whether the nose/mouth were covered at the time of the recovery was accurately recreated 13 of the 23 times by the 2-hour group (57%) and 10 of the 20 times by the 24-hour group (50%).

The effect of time between recovery and reenactment on the accuracy of the reenactment was evaluated statistically by developing a “correct” reenactment score based on a subset of variables. Responses were scored as “correct” if the reenactor correctly scored the following four variables correctly: body position (prone vs. supine), direction of the face in relation to the body, whether the nose and mouth were covered at recovery, and the location of the doll in the crib (correct quadrant). Sixteen (35%) of the 46 participants achieved a “correct” score using this rubric (11 (69%) at 2 hours and 5 (31%) at 24 hours). The difference approaches statistical significance (Wilcoxon p value 0.07). A logistical regression yielded a 66% probability of a “correct” reenactment at 2 hours verses a 52% probability at 24 hours. These results indicate that after 24 hours, a person who is not subjected to the stressors and distractions that are associated with finding an infant unresponsive has a roughly 50% chance at correctly reenacting the core components of an infant death scene.

Infant Death, Doll Reenactment, Death Scene Investigation

E6 A Growth Chart Review in Sudden Unexpected Infant Death

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After attending this presentation, attendees will better understand the relationship between infant growth and Sudden Unexpected Infant Death (SUID).

This presentation will impact the forensic science community by exploring potential risk factors related to SUID, adding to the current research regarding the causes and preventive measures that may be taken.

SUID is used to describe the sudden and unexpected demise of an infant less than one year of age. The term expands on the previously common term Sudden Infant Death Syndrome (SIDS), which describes the death of an infant that cannot be explained, even following a thorough death scene investigation and autopsy, to include infant deaths of unknown cause as well as cases of possible asphyxia.[1] While the rates of SUID have declined over the past 30 years, largely attributable to a rise in safe sleep recommendations and campaigns, infant mortality still poses a problem in many parts of the country. For example, the United States national infant mortality rate is estimated to be 5.8 per 1,000 live births in 2016, but as of 2015 infant mortality rates in Shelby County, TN, remained as high as 8.23 per 1000 live births.[2,3] While socioeconomic factors likely play a large role in focally high infant mortality rates, associations with low birthweight and prematurity have been previously made. The objective of this study is to explore the relationship between infant growth and SUID by examining the growth curves of infants dying of SUID in accordance with data provided by the Centers for Disease Control and Prevention (CDC).

Autopsy reports and birth records from 85 cases of SUID from 2014-2016 were reviewed (44 females, 68 African Americans). Average gestational age was 38.64 weeks (*Standard Deviation (SD)*=1.87), excluding cases of prematurity prior to 36 weeks' gestation. Weight, length, and head circumference at both birth and death were recorded and compared to the 2000 CDC 0-36 months growth chart.[4] The average weight, length, and head circumference percentiles at birth were 31% (*z-score*=-0.67), 45% (*z-score*=-0.21), and 20% (*z-score*=-1.16), respectively. When corrected for gestational age, average percentiles were 41% for weight (*z-score*=-0.27), 57% for length (*z-score*=0.27), and 30% for head circumference (*z-score*=-0.71). Average age at death was 91 days (*SD*=56.29). The average weight, length, and head circumference percentiles at death were 45% (*z-score*=-0.39), 36% (*z-score*=-0.79), and 48% (*z-score*=0.05), respectively. When corrected for gestational age, average percentiles were 50% for weight (*z-score*=-0.09), 70% for length (*z-score*=-0.53), and 50% for head circumference (*z-score*=0.35). Of the 85 cases included, 72 were associated with an unsafe sleep environment; 50 were associated with co-sleeping, 18 were associated with covering the decedent with a blanket, and 38 were associated with prone or side sleeping of the decedent.

This preliminary review suggests that while low birthweight and head circumference at birth are associated with SUID, these values tend to normalize over time. It is possible that these associations are artifacts of prematurity, although head circumference at birth remains low even when correcting for gestational age. Additionally, when correcting for gestational age at time of death, infants dying of SUID appear on average to be reaching expected growth targets – even exceeding those targets, in the case of length. Confounding factors that should be further explored include maternal smoking during pregnancy, exposure to secondhand smoke, whether or not the infant was breastfed, and level of prenatal care. Finally, the strong association between SUID and unsafe sleeping conditions cannot be ignored. Education regarding safe sleep practices remains a vital instrument in preventing SUID.

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SUID, Growth Chart, Infant

E7 The Sensitivity of Pediatric Organ Donation: Ways to Decrease the Decline Rate From Coroners/Medical Examiners

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After attending this presentation, attendees will understand there are higher rates of organ procurement denials of pediatric cases than adults and the repercussions this has on pediatric patients on the organ waitlist.

The presentation will impact the forensic science community by providing options and process improvements to ensure a thorough death investigation while improving the "organ gap."

Each year, the number of potential organ donors increases while recipients added to the wait list increase astronomically. The "organ gap" is a major concern for all Organ Procurement Organizations (OPOs) and transplant surgeons. The main objective for Coroners/Medical Examiners (C/ME) is to complete a thorough death investigation on behalf of the decedent no matter the cause of death. Higher levels of sensitivity lead to higher denial rates for pediatric cases that are under their jurisdiction. When death investigation and organ donation cannot be accomplished simultaneously, the consequences are serious as one OPO reported that more than 40% of potential organ donors were lost in one year because of medical examiner and coroner denials.[1] An emerging subset of the "organ gap" with a substantial amount of denials are pediatric organ donors.

There are more than 117,000 recipients on the national waitlist, including 2,000 pediatric patients on the organ list.[2] There has been a steady decline in the number of organ procurements from persons under the age of 17 years. Denials in pediatric potential organ donors remain a serious issue because of the inability of many children to receive organs from adult donors.[1] After guardians, C/ME are vital to reducing the denials for potential pediatric donors. From 2014-2016, South Carolina coroners denied 6% of the pediatric organ referrals vetted at the time; this is significantly higher when 0.3% of adults were denied. The "sensitivity" usually occurs when the death is thought to be homicide by child abuse, sudden and unexpected death, or accidental death. When death occurs under these circumstances, C/ME are more likely to decline organ donations. ME/C should permit the recovery of organs and/or tissues from decedents falling under their jurisdiction in virtually all cases, to include cases of suspected child abuse or other homicides and sudden unexpected deaths in infants. It is recognized that blanket approvals may not be possible in every case and may require an "approval with restriction(s)".[3] The final decision after obtaining parental consent lies on the C/ME.

Occasionally, postponing a denial in the case of homicide or accidental pediatric deaths can be beneficial for collecting evidence for the death investigation. The main reason for the refusal is often associated with the death investigation and subsequent legal proceedings. There are very rare instances that organ donation altered the chance of prosecution. The forensic pathologist/ME/C has the responsibility of educating the attorneys regarding the procurement process and assuring them that a complete and accurate examination can be accomplished and all necessary evidence and specimens will be able to be collected.[3]

Death investigation is a priority, and establishing a set of guidelines between coroners, forensic pathologists, and OPOs based on ethics, investigation, and state statues can be effective in creating a good rapport. The "comfort index" of medical examiners, not to say prosecutors and law enforcement officials, would necessarily have to be measured and the proper groundwork laid.[4] The construct of a "comfort index" can be useful in determining approval of donation for cases of child abuse, traffic accidents, natural diseases, and anencephaly. Communication can include educational seminars that highlight the process of the OPO and include photography tutorials that will aid the coroners' investigations. Process improvement and communication will be pivotal in optimizing organ donations and increasing recovery for pediatric recipients on the wait list. Some ME/C offices currently have "zero denials" and this should be the goal of every ME/C office.[3]

A case in which the OPO gained consent from the guardians, but received an initial decline for organs and tissue from the coroner, will be presented. Documentation by the OPO while the coroners gathered witness statements and completed their death investigation was beneficial in this accidental death of a 14-month-old child. The coordination with the coroner, forensic pathologist, and OPO led to a reversal and successful donation of a heart, liver, and kidneys. It is hoped that this case can serve as an example that organ procurement does not obstruct the sensitivity of pediatric death investigation. Conferring with the forensic pathologist to ensure organ procurement will not skew the autopsy was applicable in this case and will be on future cases.

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Pediatric Organ Donation, Coroner/Medical Examiner, Organ Waitlist

E8 The Expanded Application of Forensic Science and Law Enforcement Methodologies in Army Counterintelligence

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After attending this presentation, attendees will understand the importance of forensic science as it applies to Counterintelligence (CI) investigations and that combined with standardized law enforcement methodologies, it is as critical to CI as it is in traditional criminal investigations.

This presentation will impact the forensic science community by identifying the factors involved with why Army CI operates differently in its investigative activities compared to its counterpart organizations. Addressing these factors will make it possible for Army CI to improve their overall case resolution through expansion of the investigative capabilities by adding forensic science resources, training, and personnel.

This presentation should provide insight into the inner workings of Army CI, which reflects an observed lack of investigative capabilities when compared to the other military CI investigative agencies of the Naval Criminal Investigative Service (NCIS) and the Air Force Office of Special Investigations (OSI). This study indicates that the lack of investigative capabilities in Army CI is prevalent in three key areas: (1) forensic science resources and support; (2) law enforcement methodologies and procedures; and, (3) basic investigative training. This presentation will examine this investigative capability gap that seems to exist primarily due to a false belief within the United States Army that a CI investigation is not also a criminal investigation.

This study deals with the capability areas Army CI need to improve. There appears to be a difference in the opinions of Army CI agents compared to NCIS and OSI agents in regard to the investigative capabilities within their respective agencies. The existence of such a difference of opinion would support the idea of a lack of investigative capabilities within Army CI. This study will further support or refute that opinion by utilizing information gathered from published research, professional communications, and 12 years of experience in Army CI and civilian law enforcement.

Current and recently retired special agents from the Army CI, NCIS, and OSI participated in a survey to examine this difference of opinion. The opinions of these agents were noted in the investigative capability areas previously mentioned. The data obtained will reinforce the fact that Army CI conducts investigative activities with a general lack of capabilities in the key areas already identified, creating unnecessary burdens for its special agents in the conduct of their investigations.

The survey results revealed statistically significant differences in the responses by Army CI agents versus NCIS and OSI combined. The responses were divided into three categories related to the three areas of investigative capability previously identified. An analysis was conducted comparing the mean average answers of NCIS+OSI to that of Army CI in each of these categories. In each of the categories of questions asked, extreme statistical significance was present, demonstrating that Army CI agents tended to agree that a lack of investigative capability in each of these areas was present in their organization. This did not appear in a majority of the answers of the NCIS and OSI agents in any of the categories. This statistical difference is further evidence of the disparity between these CI investigative agencies and indicates that Army CI may be less effective by comparison. The findings are consistent with published data. These results further point to a systemic problem within the current investigative culture of Army CI that could be corrected if forensic science and law enforcement methodologies are expanded in a manner similar to that which already exists in other organizations.

Counterintelligence, Investigation, Forensic Sciences

E9 Crime Fear Comparison Based on Residence Selection: Gated Communities vs. Apartments

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The goal of this presentation is to focus on the prevention of types of crime that are based on routine activities due to changes in life styles as a result of industrialization and urbanization movements.

This presentation will impact the forensic science community by focusing on the importance of private security in crime prevention in neighborhoods.

Crime has been one of the major problems of urban life. Hence, fear of crime and suspicion of urban places are among the costs of the increasing crime rate. This fear has been the driving force in the rise of private security businesses. The basic services that gated communities' private security task force supply are access controls, vehicle and/or foot patrol, extra lightening in dark corners, Closed-Circuit Television (CCTV) screening of the site and delivering briefs, and place signals at the site for emergency situations.

This research focuses on the fear of crime of people who live in different types of residences in urban areas. The sample group of the research consisted of individuals 17 to 79 years of age who reside in Istanbul. The purpose of the study was to make a comparison between the housing priorities of residents who choose to live in gated communities and those who choose to live in downtown apartments with no security personnel and/or agents. The first sample group of 100 people (78 male, 22 female; 17 to 79 years of age) was randomly chosen from a gated community where approximately 1,600 people are living in 420 households. The comparison group consisted of 100 people (51 male, 49 female; 17 to 76 years of age) who were randomly chosen from people living in non-secured apartments in 12 different neighborhoods of Istanbul.

The method used in this study, in order to measure the level of crime fear of attendants is similar to Ferraro's The Fear of Crime Scale.[1] The standard questionnaire consisted of 22 open-ended questions, divided into four different categories and grading was based on the four grade Likert scale.

The questionnaire used in this study was pre-tested on ten randomly chosen respondents and no cultural biases were observed. The questionnaires were distributed to all participants after delivering an introductory explanatory presentation and were collected one week later. The purpose of the seven-day time span was to provide adequate time and privacy to the participants to answer the questions carefully and comfortably.

The obtained data were quantitatively analyzed with SPSS. A standard *t*-test was used to analyze the data. The results obtained were scientifically impressive. The study reveals that the crime fear of the residents who live in gated communities is significantly lower than those who live in unsecured apartments. The research also reveals that the need for security is the third-most important criteria in home selection for people who live in gated communities. Surprisingly, building supportive and lasting neighborhood relations is significantly higher among these residents. Also, a significantly higher majority of unsecured apartment residents claimed that the main crime prevention method is to live in secured sites.

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Crime Fear, Crime Prevention, Private Security

E10 The Spanish Police Intervention Unit Inside the Chaos: Crime Scene Protection Cases

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After attending this presentation, attendees will better understand the concepts of rioting and police crime scene protection used by the Spanish National Police and how the evidence is protected and collected by the crime scene investigators at public disturbances and after terrorist attacks.

This presentation will impact the forensic science community by illustrating how the Spanish National Police Intervention Unit (UIP) operators are trained to protect dangerous crime scenes, thus allowing investigators to perform their job safely. This presentation will highlight a number of actual cases and explain a new way to investigate complex crime scenes.

While a number of different studies have looked into the different scenes between the military and civilians, there are few studies regarding the protection of crime scenes in civilian disorders, such as riots. The Spanish National Police UIP is responsible for protecting public safety in case of riots, crowd control, and after a terrorist attack. Also, Crime Scene Investigation (CSI) personnel must be protected in such chaotic scenarios, providing them a guaranteed environment in which to work. The UIP training provides the operators with the guidelines to work side-by-side with forensics in dangerous scenes. There will be an in-depth focus on the significant challenges faced during a number of actual UIP cases, including special crime scene issues and the urban "combat" environment.[1]

This presentation will present three high-profile investigation cases in which Spanish National Police UIP operators played a key role in protecting crime scene investigators and will explore the interaction of multiple forensic disciplines of the ForLab Project, in which UIP operators are trained to protect themselves and the crime scene.

In May 2002, before the Champions League Semifinal match between Real Madrid and Barcelona, there was a bomb attack near the Torre Europa building, forcing UIP operators to protect the football match and the work of forensic investigators while angry hooligans flew into a rage. In March 2004, several commuter trains were blown up after the worst terrorist attack on Spanish soil, and UIP operators were deployed to protect the scene as soon as the first call arrived. In November 2014, two clubs' hooligans fought at Manzanares riverside, resulting in one man dead after the clash. The ForLab Project (Forensic Laboratory for *in situ* Evidence Analysis in a Post-Blast Scenario), in which the Spanish National Police Forensic branch is involved, depend on UIP operators to seal and protect the crime scene until forensics arrives.[2]

The opinions or assertions contained herein are the private view of the authors and are not to be construed as official or as reflecting the views of the Spanish Ministry of the Interior or Spanish National Police.

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National Police UIP, Crime Scene, Evidence Protection

E11 A Usual Cause of Hospitalization and an Unusual Cause of Death

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The goal of this presentation is to illustrate how a traffic accident, which is one of the most frequent causes of morbidity and mortality in the general population, can lead to death following a microtrauma such as a metatarsal fracture.

This presentation will impact the forensic science community by informing attendees that although car accidents are a well-known cause of death, they are usually associated with multiple injuries to various organic structures, making it difficult to decide which was the most serious and mortal wound. Even a simple metatarsal fracture can act as a co-cause of death. Thus, the importance of recognizing typical and atypical patterns of injury associated with traffic accidents could provide a tailored measure for the initial assessment and management of trauma patients in order to improve outcomes.

This case dealt with a patient who came to the emergency room following a car accident. Upon arrival, he complained of right foot trauma that was treated with a temporary immobilization device; however, at the physical examination, the physicians diagnosed a respiratory and cardiac insufficiency with acute pulmonary edema in the patient who had a history of hypertensive cardiac disease and chronic atrial fibrillation in pharmacological therapy.

Due to clinical conditions, urgent hospitalization in the cardiology ward was provided with a diagnosis of respiratory failure and severe respiratory acidosis. Thus, mechanical ventilation was performed, with a subsequent improvement of the clinical parameters. The next morning, the doctor in charge was alerted because of a worsening of clinical conditions. A thoracic Computed Tomography (CT) scan was performed and documented several areas of parenchymal consolidation of the left lung base associated with diffuse fibrosis, including widespread chronic bronchopathy, no pneumothorax, no fractures. At the end of this investigation, the patient presented with a loss of consciousness and the disappearance of the peripheral and carotid pulse; a cardiopulmonary resuscitation was performed with negative results and the patient died. During autopsy, an increased heart size of 700gr was documented; the left lung, which also increased in size, weighed 850gr and the right lung weighed 610gr. The bronchi displayed the release of a foamy reddish-colored material instead of blood fluid released from the great pulmonary vessels. In addition, an incision was made on the right foot dorsal region, highlighting the presence of a hemorrhagic infarction of the soft tissues, which revealed the presence of a fracture of the fourth metatarsus. Histologic examination demonstrated trivasal coronary artery stenosis, interstitial and perivascular fibrosis, numerous necrotic contraction bands, interstitial edema, mild disarray of the right ventricle, chronic emphysema, endoalveolar edema, interstitial fibrosis, areas of atelectasis, massive embolization, and partial fat. In conclusion, the cause of death was attributed to the concatenation of pathophysiological events, following a stress-induced event (represented by the car accident), which caused a ventricular tachyarrhythmia and massive pulmonary embolization from the fracture of the right fourth metatarsal, resulting in respiratory failure from acute pulmonary edema.

This seems to be the first case in which these multiple mechanisms are described acting together as co-causes of death. A review of the literature revealed that the occurrence of Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE) after foot surgery is generally believed to be low. The National Trauma Data Bank data set (2007-2009) was used to evaluate the incidence of thromboembolism in foot trauma, identifying risk factors associated with the thromboembolic complications. Significant risk factors statistically associated and clinically relevant to both DVT and PE in foot trauma were older age, obesity, and a higher injury severity score. Each of these existed in this case. On the other hand, only one case dealing with a 42-year-old healthy male patient who suffered fractures of the calcaneus, navicular, talus, and metatarsal bone caused by an accidental gunshot wound while hunting was associated with a cerebral fat embolism.

Due to the low incidence, routine pharmacologic thromboprophylaxis might be contraindicated in foot trauma; however, careful, individualized assessment of the risk factors associated with DVT and PE is important in order to prevent embolic complications.

Car Accident, Metatarsal Fracture, Massive Pulmonary Embolization

E12 Examining the Heritability Effects of Latino Youth Violence

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After attending this presentation, attendees will be better informed regarding the relationship between genetic and environmental influences associated with behavioral traits. Furthermore, attendees will learn about cultural and/or genetic effects on Latino youth violence.

This presentation will impact the forensic science community by demonstrating how violent behavior associated with minorities — specifically Latinos — has become a problem in the United States. Moreover, violence is one of the major effects of the Latino community. The substantial heritability and environmental issues surrounding violence indicate that the underlying genetics can help explain at least some features related to these behaviors.

Human behavior is a complicated process influenced by both genetics and the social environment. A growing body of knowledge has shown the relationship between genetics and environmental influences on humans. The key to understanding the roots of criminal behavior is through the genetic method. One avenue of research for the genetic foundation to behavior is through twin studies. The twin study design allows researchers to break down phenotypic variance into three components: heritability, shared environment, and non-shared environment; however, there exists no literature considering the genetic and environmental influences on culture groups, such as Latinos, using the twin study design.

The focus of this study was to test whether there is a statistical relationship between gene and environmental interaction of Hispanic and non-Hispanic twins and self-reported violence. The sample size (N=97) derived from the first wave of data from The National Longitudinal Study of Adolescent to Adult Health.[1] This data will provide a glimpse of understanding those twins who identify as non-Hispanic male and Hispanic male participants and the differences of behavioral outcome using a Chi-square analysis.

The results suggest there exists a relationship between genetic and environmental effects that play a role in Latino youth violence. The results in the analysis revealed that, overall, there was no significant relationship between Hispanic and non-Hispanic twin youth on violent outcome; however, the identical twins had statistically significant relationships on violent outcomes and fraternal twins had no statistically significant relationships on violent outcomes. In conclusion, Latino youth violence has a significant genetic effect, while the environmental effects were insignificant.

This research concludes that culture such as identifying as Latino may be limited in having effects on self-reported youth violence. There seems to be more of a genetic effect when reporting Latino violence. This evidence provides an understanding of the complexity of human nature and behavior such as violence should be further examined with genetic models.

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Latino Violence, Genetics, Biosocial Criminology

E13 Dog Bite-Related Accidents: A New Forensic Approach

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After attending this presentation, attendees will be able to apply a new forensic approach to offending dog identification when dog bite-related accidents occur. The interaction between domestic animals and humans has not been free of conflicts, and dog attacks represent a real problem, not only for health consequences, but also for possible crimes and can affect legal or financial outcomes.

This presentation will impact the forensic science community by providing scientific data from a well-controlled experiment, thus increasing gathered information. An appropriate forensic approach should include an exhaustive analysis of the scene, the victim, and the dog.

The alarming statistics reported around the world have shown that dog attacks today represent a health hazard in cases in which prevention strategies have not always been successful.[1,2] Most dogs involved in these events are known to or belong to the victim. This study found 19 dog bite-related fatalities in Italy from 2009 to 2016 (2.37 cases per year); these data have sharply increased from a previous study that described 32 dog bite-related fatalities between 1984 and 2009 with a frequency of 1.28 cases per year.[3]

A scientific inclusion/exclusion of the involved dog is possible and recommended because of possible consequences for the animal's owner that could have civil consequences (or criminal consequences, in the most serious cases). Since dog bitemark analysis should involve different forensic professionals, such as pathologists, odontologists, veterinarians, biologists, and police investigators, a review focused on this type of evidence from a multidisciplinary point of view is presented.

There are different approaches for the identification of an offending dog when an attack on a human occurs. Forensic investigations in dog attacks usually involve the examination of bitemarks, tooth prints, the dog's stomach, and other pathological methods. For the identification of the offending dog, the best approach is to evaluate the canine Short Tandem Repeat (STR) typing in saliva traces of the dog bitemarks. Generally, it is possible to obtain a canine-specific STR profile from the dog's saliva on the wounded area, even when a high background of human DNA is present (blood).

This approach is often a problem because the surface of the wound may have already been treated (for example, by first aid workers), removing the canine cells. This approach is less successful in obtaining useful STR results than before medicolegal techniques are applied. Furthermore, dogs appear more frequently in human social life, so it is not rare for canine DNA to be present on the hands, arms, legs, or feet of humans.

A new forensic approach was described for offending dog identification beginning with dog buccal swabs: the target is the identification of a human profile beginning with this sample. An additional goal of this presentation was to determine the latency time of this biological trace in the dog's mouth.

In this study, ten different dog breeds were used to bite a beef meat sample that was previously typed (internal control profile). At different minute intervals (30, 45, 60, 90, 120, 150, 180, and 240), two dog buccal swabs were taken (a swab for each dental arch). One hundred and sixty samples were collected. Subsequently, DNA was extracted and a bovine genotyping kit was used. In all samples, a complete profile of the internal control was found for 45 minutes and a partial profile of the internal control for 150 minutes.

These data are very important to confirm the possibility of using buccal swabs to identify an offending dog. Human STR typing kits are much more specific and sensitive compared to cattle kits, so one can suppose that a complete human STR profile could be obtained from the dog buccal swab, after an aggressive act, for at least 150 minutes. This is usually enough time for a medicolegal team arrive.

This study may offer a model that could always be applied to identify offending dogs; furthermore, dog bite-related accidents can provide concrete cases, even when fatal, making it possible to develop, refine, or validate medicolegal techniques.

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Dog Attacks, Cattle Genotyping, Dog Identification

E14 Science Matters: Putting Light on Facial Approximations

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After attending this presentation, attendees will understand the fundamentals of a forensic art facial approximation. These images are created for a detective to obtain a better understanding of what a suspect may look like. Detectives and investigators often request a facial approximation from the forensic artist in their agency.

This presentation will impact the forensic science community by raising awareness of the work that forensic artists perform for detectives and investigators. A large majority of this work goes beyond drawing a simple composite of a suspect. In this presentation, adjudicated facial approximation cases will display various angles and situations that often perplex a detective who is trying to determine what someone may look like. This informative presentation will include illustrations of the lighting of the human face.

The presence of light is how we are able to see the environment around us. It is a crucial source of information in producing a facial image for an investigation. How light bounces off a face and illuminates facial planes or features is critical information for the forensic artist. Light assists with the comprehension of what is seen and illuminates facial features, bad skin, or unusual marks. Specifically, the dynamics of direct light, indirect light, highlights, and reflected light on the various features within the face can provide indications of how those structures are built. This information can reveal itself even with grainy, blurry photos or video, to an extent.

The dark — an absence of light — also has a story to tell, one to which the forensic artist pays close attention. Dark areas can mean many things — an overhanging brow with deep-set eyes, skin discolorations, or facial hair, for example. The lack of light motivates the forensic artist to search for corroborating evidence in the various stills or video provided that will illuminate that particular area of the face. Looking through stills that show that specific area, under different angles and positions, may provide more information and "fill in the blanks" that were obscured by the dark.

Understanding the environment as a contributing factor is also vital. Cast shadows can influence an image or completely obscure the information that is sought. So, looking at the environment as part of the whole picture helps to understand what is being seen. The lighting is different if it comes from a modern street lamp rather than an older vintage street lamp. Not all lighting is equal; the lighting is different in a convenience store than a private residence. All these factors assist in or interfere with the forensic artist's efforts to interpret the face. The camera angle in the environment also plays a huge part; distortion is often a problem and must be factored in. In many environments, the camera angle is from above and captures not only the face but many other factors in the scene.

Forensic art is a rapidly expanding discipline that requires specialized training, above and beyond advanced drawing skills. The forensic artist is called on for many different skill sets that assist in the identification process. Applications relate closely with those of other disciplines of the forensic sciences, especially forensic anthropology, forensic odontology, and forensic psychology.

Forensic Art, Facial Approximations, Forensic Imaging

E15 Accuracy Assessment of Korean Craniofacial Reconstructions Applying the Face Pool Comparison Method

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The goal of this presentation is to inform attendees how the accuracy of Craniofacial Reconstruction (CFR) can be assessed and how the reliability would be sufficient to be utilized in forensic identification.

This presentation will impact the forensic science community by demonstrating the procedure of evaluating the accuracy of CFR and the availability of CFR in the field of forensic sciences.

CFR is a method of rebuilding a living facial appearance onto a skull based on both scientific standards and artistic skill. This technique is used to recognize or identify an individual in the field of forensic sciences, especially when other forensic approaches are not possible or available. The accuracy of CFR has been of primary importance in maintaining the reliability of its applications to forensic investigation. Therefore, many studies have been conducted to evaluate the accuracy of CFRs. A number of methods are used to assess the accuracy of CFR; face pool comparison is considered to be one of the most reliable methods.[1]

In this study, 3D skull images from six living Korean adult subjects were taken by computerized tomography. Six CFRs employing the skull images were produced by utilizing a 3D computerized modeling program. The completed six CFRs were divided into two groups: one group of three CFRs applied out-of-date Korean average facial skin thickness data and the other group of three CFRs applied recently studied Korean average facial skin thickness data. The recognition accuracies of the six CFRs were measured by a face pool comparison method. For accuracy assessment, six face pool sets were made that targeted for each CFR. A face pool set consisted of ten frontal facial photographs, including an actual face for the target CFR. For the accuracy survey, 167 assessors were recruited who were asked to compare the target CFR with the ten faces on the face pool, and they then determined which face should match with the CFR.

The results revealed that the CFRs using the recently studied Korean average facial skin thickness data demonstrated higher accuracy (the average hit rate of 30.74 %) than the other CFRs (the average hit rate of 22.35%) using out-of-date Korean average facial skin thickness data. Additionally, the results were compared with the results from quantitative accuracy measurements by using geometric morphometrics surface comparisons.[2,3] The comparison demonstrated that the results from the geometric morphometrics analysis correlate positively with the results from the face pool comparison method.

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Craniofacial Reconstruction, Accuracy Assessment, Korean

E16 Forensic Awareness of Emergency Service Nurses in Turkey

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After attending this presentation, attendees will better understand the forensic cases most frequently encountered by emergency services staff in Turkey. This presentation will detail the necessary level of knowledge needed by the nurses intervening in these cases as it pertains to keeping records and guarding and preserving evidence.

This presentation will impact the forensic science community by examining the awareness of the emergency service nurses, who are the first medical staff encountered during forensic cases, and by emphasizing the knowledge levels of the emergency service nurses in Turkey, the deficiencies in the area, and the required improvements, thus affecting forensic studies.

Emergency services have special importance because they are involved in forensic cases and their contributions have the potential to change the course of an investigation. It is a legal obligation to provide required emergency medical care for every individual who applies to the emergency service. The acts of the emergency team, which consists of physicians, nurses, and emergency medical technicians, during this care are even more important in forensic cases. The priority of medical personnel is the care and treatment of the patient; however, it is also quite important to guard the evidence because the detection of the crime and the criminal, the relief from the victimization, and the role of the medical staff as patient's advocate are the nature of the task. The emergency team should ensure the collection and guarding of evidence in forensic cases while providing life-saving health care.[1] In such cases, the awareness and knowledge of the health care personnel will prevent failure to preserve evidence and ensure correct collection. This can only be achieved by training the medical staff.[2]

It is known that nurses cannot perform the necessary tasks in forensic cases in Turkey because of their inadequate knowledge and experience in forensic studies, although they frequently encounter such forensic cases. Proper evaluation of forensic cases will contribute to the resolution of the case. This can be ensured by the presence of emergency department nurses who have been specially trained in forensic studies.[3,4] There are forensic nurses in emergency services in many countries; however, forensic nurses who have had training after graduation cannot as yet be employed in emergency services in Turkey. For this reason, emergency services nurses perform tasks such as preserving, keeping, and recording of the belongings of the patient, which should be performed by a forensic nurse.[5,6]

Health care personnel at emergency services need training in forensic cases to ensure avoiding decay of biological materials that may be evidence and to collect them properly. Nurses with different education levels work in emergency services in Turkey. The differences between nurses cause some disadvantages. One of these disadvantages is the assignment of nurses to the emergency services who do not have adequate training support regarding forensic cases. Some nurses working at emergency services do have training in the field, through vocational training, in-service training, or certification programs, in the collection and safe-guarding of evidence, the creation of chain of evidence, and keeping records, but other nurses have no training on these issues.[1,2,6,7]

By examining studies conducted on this issue in Turkey, it is observed that some emergency nurses do not perform these tasks, and some conduct these tasks incompletely or incorrectly. Examples of such shortcomings determined in this study will be presented.

This presentation will illustrate the forensic cases most frequently encountered by staff at emergency services in Turkey and the level of knowledge of nurses intervening in these cases who are responsible for keeping records and guarding and preserving evidence.

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Forensic Nursing, Emergency Nursing, Training

E17 Forensic Science in Austria: Insights on Working Processes and Result Communication Concerning DNA, Fingermarks, and Handwriting

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The goals of this presentation are: (1) to provide insight on working methodologies and processes used to communicate forensic results in Austria, particularly concerning DNA, fingermarks, and handwriting; and, (2) to promote practice-oriented measures regarding the general appraisal of traces in inquisitorial justice systems, mainly based on expert opinions collected through anonymous surveys.

This presentation will impact the forensic science community by proposing optimized working methodologies and communication processes concerning physical traces, particularly DNA, fingermarks, and handwriting, thus promoting a better use of the information obtained by the analysis of physical traces.

Forensic science is commonly defined as the use of scientific methods and techniques to search for, collect, analyze, and interpret traces left by criminal actions to understand crime and aid justice. This scientific endeavor is interdisciplinary by nature and forensic science is thus widely considered as a "patchwork" of different scientific disciplines. This concept, as well as the privatization of many laboratories, has led to a hyper-specialization and a "sectorization" of forensic science in different specialized fields (e.g., forensic chemistry and forensic biology).[1] Forensic scientists are mostly chemists or biologists who have received some form of additional training. This lack of proper identity has grown larger during the past decade and causes the model of forensic science to be scrutinized and challenged worldwide.[2-4] Debated errors (for example, the controversial identification of Mayfield's fingerprint in the train bombing assault in Madrid in 2004) also shook forensic science to its very foundations by prompting interrogations concerning its scientific nature rand causing distrust in forensic methodologies.[3,5-8] In summary, as perfectly expressed by Roux et al., "Forensic science is at the crossroads [and] its future largely depends on if and how a consensus can emerge about its own nature."[9]

Numerous authors agree that research must be conducted worldwide on the (re)definition of forensic science.[9-10] A solid research culture must be developed through the promotion of educational programs and training for forensic experts and their main partners (e.g., investigators and justice actors). The ability to communicate about the meaning of forensic results should be particularly considered. Communication is indeed an important topic in forensic science, most particularly the interpersonal communication among forensic experts and between forensic experts and their main partners.

This study is aimed at observing, analyzing, and understanding the current application of forensic science in Austria. Particular attention was given to the identification of strengths and weaknesses of the processes used to communicate on the value of DNA, fingermarks, and handwriting results, mainly based on the opinions of forensic experts. The choice to focus on Austria has been made because it is historically interesting to study the situation in this country more than a century after the work of Hans Gross, Austrian pioneer of the discipline. Furthermore, as a country working in an inquisitorial tradition, Austria can be used as an example for most European criminal justice systems. The decision to focus on the selected traces — DNA, fingermarks, and handwriting — was taken because these traces are often encountered on criminal cases and possess characteristics allowing interesting comparisons: they are usually processed by different experts in different institutions, appraised with various degrees of confidence, and evaluated and reported using different methodologies.

In order to identify the main experts of forensic science in Austria and to understand their working processes, the Austrian police and justice systems were studied based on literature and reports, particularly concerning the three selected traces. Surveys were then developed and submitted to the identified experts to further deepen the obtained knowledge and to collect the opinions of the tenants of the discipline relating to the following concepts: (1) examination and analysis of traces; (2) usability and registration of traces; (3) comparison traces — prints; (4) evaluation and interpretation of results; and, (5) communication of results.

This research is crucial and timely for the discipline, as it allowed obtaining interesting insights into the working processes of the Austrian forensic community, most particularly concerning DNA, fingermarks, and handwriting. The obtained results, mainly based on the opinions of the experts of the selected traces, helped to propose practice-oriented measures regarding the appraisal and communication of traces in Austria, and, as an extension, in any inquisitorial justice system. This research will be further developed to include surveys and interviews of other forensic experts as well as judiciary members. This will allow expanding the scope of the proposed measures and promoting a better forensic communication, which is necessary to rehabilitate forensic science as a proper scientific discipline.

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Forensic Results, Physical Traces, Communication

E18 Raman Microspectroscopy and Advanced Statistics for Detection and Characterization of Gunshot Residue (GSR)

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After attending this presentation, attendees will have a better understanding of the recent advancements of the application of Raman microspectroscopy for GSR analysis, identification, and discrimination. This presentation will describe the development of a novel and alternative method for GSR detection and analysis.[1] The implementation of advanced statistics to differentiate experimental Raman spectra collected from non-equivalent GSR samples will be discussed.

This presentation will impact the forensic science community by demonstrating whether this method can already be used for practical purposes. This research has the potential to greatly impact the accuracy and effectiveness of shooting incident investigations. This study has generated significant interest among the scientific community and practitioners. *Journal of Analytical Chemistry*, the top journal in the field in the world, highlighted the article on the journal cover. *Chemical & Engineering News*, the top news magazine of the American Chemical Society, released press coverage. Canada Discovery Channel made a piece on the daily *Planet* program about this discovery. Several emails have been received from police detectives.

Raman spectroscopy has numerous applications in forensic chemistry.[2] Raman analysis is a technique that can obtain confirmatory class identification of analytes through low intensity laser light scattering. The technique is non-destructive, rapid, sensitive, and requires little or no sample preparation. Furthermore, portable Raman spectrometers are readily available, allowing for crime scene accessibility. Raman spectroscopy offers several advantages over the current methodology for GSR analysis. The technique has been shown to detect components from both the organic and inorganic constituents of GSR on adhesive tape.[3,4] This is contrary to current GSR elemental analysis methods, which rely solely on the detection of the heavy metals (lead, barium, and antimony). This is problematic since environmental concerns have led to the increased popularity in heavy metal-free or "green" ammunition. It has been found that in the absence of heavy metals, current elemental analysis techniques are severely hindered when making accurate identification of GSR samples. Additionally, the probability of environmental and manufacturing particles assigned (incorrectly) as being GSR has increased with the onset of "green" ammunition. Until recently, the application of Raman spectroscopy for GSR analysis was largely unexplored, although this approach is not dependent on detecting metals, and is more capable of differentiating environmental contaminants and GSR. Therefore, a Raman spectroscopic method displays numerous advantages in specificity when compared to current techniques.

The firearm discharge process could be considered analogous to a complex chemical reaction. Therefore, the chemical composition of the products (GSR particles) is directly related to the chemical nature of the reagents (firearm-ammunition combination) and the conditions of the reaction. Preliminary results reveal that Raman data collected from GSR particles originating from different firearm-ammunition discharges were successfully classified according to caliber. Using a 785-nm Raman excitation, 0.38-inch and 9mm caliber firearm discharge samples were probed. Resulting data was treated with statistical methods (performed using MATLAB® with the PLS_Toolbox), such as Principle Component Analysis (PCA) and Support Vector Machines (SVM). The results demonstrate a high probability of this method to correctly classify data from the two examined calibers. Preliminary results illustrate that the variations between non-equivalent GSR samples can be detected through this method. Since GSR is often collected from a suspect, the application of this method to forensic investigations would provide a link between GSR collected from the shooter and the crime scene.

This emerging technique illustrates the possibility for an on-scene, non-destructive identification and chemical characterization method for GSR. This method has the potential to greatly impact the forensic science community by increasing the accuracy (and discriminatory power) of GSR detection. The most direct application for this research is a method to exclude a specific firearm-ammunition combination as producing an evidentiary GSR sample. The comparison of a laboratory-generated GSR sample discharge and an evidentiary GSR sample can be made without extensive preliminary studies.

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Gunshot Residue, Raman Spectroscopy, Chemometrics

E19 Analysis of Blood Traces by Attenuated Total Reflection (ATR) Fourier Transform Infrared (FTIR) Spectroscopy for Species Identification

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The goals of this presentation are to demonstrate: (1) the advantages and disadvantages of current methods for bloodstain analysis during forensic investigation; (2) the importance of species identification from blood traces found at a crime scene; (3) the significance of a non-destructive method for examination of trace evidence at a crime scene; (4) the advantages of FTIR spectroscopy in forensic investigation; (5) the FTIR spectroscopy for bloodstain examination; and, (6) the use of chemometrics for distinguishing between human and animal blood and species identification.

This presentation will impact the forensic science community by demonstrating that a non-destructive, quick, and confirmatory method for species identification based on blood traces found at a crime scene would be of great help to law enforcement. This study reveals great potential of FTIR spectroscopy combined with statistical data analysis for differentiating between species based on bloodstains.

In a forensic investigation, biological evidence can be very helpful for identifying a victim or suspect, as well as for solving a criminal case. Blood identification is typically based on the following steps: visual examination, a presumptive assay, a confirmatory assay, and lastly, the stain can be subjected for species identification before DNA profiling is performed. Presumptive and confirmatory assays are utilized for confirming a stain to be blood. The disadvantage of presumptive tests is the amount of potential false positive reactions with some environmental contaminants. Both presumptive and confirmatory assays require reactants to initiate a chemical reaction and therefore damage the sample. Once a trace found at a crime scene is identified as a bloodstain, it can be subjected for further analysis. Determining the origin of blood is critical in forensic casework since it can streamline the investigation process by including or excluding non-human stains. Immunochromatographic assays are an example of tests for confirming human origin of a stain. The ultimate goal for analyzing blood is DNA profiling, which requires DNA extraction from the sample. Therefore, current standard methods employed for the analysis of blood samples are destructive and time consuming. Another inconvenience for all current types of blood examination, besides DNA analysis, is the limited sample size found at a crime scene. Therefore, it would be highly advantageous to implement a method which is quick, non-destructive, and requires only a small sample with little to no preparation for the identification of a species' blood at a crime scene.

In this study, Attenuated Total Reflection (ATR) FTIR spectroscopy was used as a confirmatory, non-destructive, and rapid method to distinguish blood from different species.[1] Bearing in mind forensic purposes, differentiation of human and non-human blood samples was targeted, and the Partial Least Squares Discriminant Analysis (PLSDA) model that was made demonstrated complete separation between human and animal donors. In addition, the models revealed complete distinction between blood spectra from three species, namely human, cat, and dog. The method was subjected to external validation using samples that were not a part of the training dataset. Classification predictions of unknown blood donors performed by the model resulted in 100% accuracy. This study demonstrated ATR FTIR spectroscopy's great potential for bloodstain analysis and species discrimination. Furthermore, the commercial availability of portable ATR FTIR instruments affirms the potential for the implementation of such bloodstain analyses, at a crime scene as well as in the lab.

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ATR FTIR Spectroscopy, Blood, Human Origin

E20 Universal Detection of Body Fluid Traces *In Situ* With Raman Hyperspectroscopy for Forensic Purposes

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The goal of this presentation is to demonstrate the difficulties of separating Raman spectra of a sample from that of a substrate and to present a brand new way to deal with this problem.

The presentation will impact the forensic science community by familiarizing attendees with the progress made on a new method for body fluid identification that is non-destructive, fast, easy, and can be used regardless of substrate.

Raman spectroscopy has been a boon to the field of forensic science. One area in which this technique exhibits exceptional promise is body fluid identification and characterization. Previous research has demonstrated that Raman spectra combined with advanced statistics can reliably differentiate body fluids. This is an improvement on current methods that are destructive to the evidence, specific to a single body fluid, and have numerous false positives and cross reactants; however, substrate interference remains a major impediment to its practical implementation.

The new approach outlined in this presentation has immediate application for body fluid detection and allows for a universal, automatic, non-destructive, on-field method for confirmatory identification of body fluid traces at a crime scene. To overcome substrate interference, previous research has been performed using different laser excitation wavelengths, spectral background subtraction, and statistical modeling; however, no one method was able to be used for every substrate tried. Here, an approach for the universal detection of body fluids, regardless of the substrate, is presented. This approach is based on Raman hyperspectroscopy and Multivariate Curve Resolution (MCR) and a new program called Hypothetical Addition Multivariate Analysis With Numerical Differentiation (HAMAND). These techniques were applied to datasets representing simulated semen evidence. Raman spectra of the target body fluid, semen, was first decomposed using MCR and standards were picked from the resulting components. These standards were used to train the program HAMAND. The HAMAND program was then applied to experimental spectra of a semen on substrates. HAMAND then extracted the standards from the experiment spectra, if they were present. Two experimental conditions were tried, one of semen on glass, and the other of semen on blue polyester. In every instance, the signal of the body fluid was extracted and matched to a reference spectra of semen. This approach is applicable to any analyte that is either a minor contributor or spatially distributed on a strongly interfering substrate.

Body Fluids, Raman Spectroscopy, Chemometrics

E21 Bioaffinity-Based Concepts in Forensic Serology

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After attending this presentation, attendees will understand that bioaffinity-based methods for the analysis of body fluids offer simplicity to traditional forensic analyses of such samples. In addition, attendees will also understand the concept of using such body fluids to identify originator attributes in a quick and straightforward manner.

This presentation will impact the forensic science community by providing new methods for the analysis of body fluids in order to generate essential information directly at a crime scene. Ultimately, these systems can be incorporated into field-deployable devices (similar to glucometers) or connected to hand-held Smart Devices, which will allow for the rapid analysis of body fluids that can be used and interpreted by operators with no scientific training, and thus revolutionizing the "front end" of forensic science.

The analysis of biomarkers has been used in the field of forensics for many years in the form of DNA (usually from blood) for identification purposes; however, the process of matching DNA samples is very time consuming and has caused backlogs in many states. While this is a useful tool, it may not be the best method of analysis during an active criminal investigation. There are many other biomarkers present in blood that can be analyzed in a much shorter amount of time by utilizing bioaffinity-based cascades. The lab at the State University of New York at Albany has developed and is in the process of developing more cascades for the purpose of identifying personal attributes from individuals, such as age, biological sex, and general health conditions. These cascades have been developed for both blood and fingerprint analysis. The cascades created for blood analysis have focused on the determination of the age of the originator and the time since deposition of the sample.

Fingerprint analysis has been focused on pictorial comparisons since the process was adapted for forensics. Advances in this area have only progressed to the point where automated fingerprint identification systems can be used in certain cases (with an expert checking the results). Because of this, a fingerprint may be determined to be too smudged or smeared to be of use; however, what is often overlooked is that the patterns used to match fingerprint samples are created by sweat/sebum emulsions excreted from the fingertips. Like all bodily excretions, the emulsions have their own unique chemical composition, meaning there are biomarkers present for analysis. One of the cascades developed in the lab has focused on the analysis of amino acids in the samples. The cascades developed for fingerprint analysis have focused on the determination of biological sex. There is also ongoing research aimed at the development of a larger variety of cascades able to determine other attributes from blood and fingerprint samples.

Bioaffinity, Serology, Biomarkers

E22 DNA in the Air: The Recovery of DNA Samples From Residential HVAC Air Return Filters Using the Single 4N6FLOQSwab™ Method

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After attending this presentation, attendees will be familiar with collecting DNA samples from residential Heating, Ventilation, and Air Conditioning (HVAC) air return filters and the potential of using such samples to aid in forensic investigations.

This presentation will impact the forensic science community by demonstrating that the single-swab method is effective in collecting DNA samples from residential HVAC air return filters. It explores this source of biological material as an indicator of living habits and evaluates the cumulative deposition of ambient DNA within the household.

The recovery of DNA samples from various surfaces and objects is of significant value to forensic investigations. The forensic science community has engaged in vibrant discussions and medicolegal debates over topics pertaining to "touch" DNA evidence, such as the modes of DNA transference from person to person, person to object, and vice versa. This important debate continues in the backdrop of heavier reliance on DNA data with varying degrees of complexity and, sometimes, the dangerously creative interpretation thereof. When it comes to explaining "touch" DNA evidence in particular, the foundational understanding of what is reasonable (and what is not) must be reconciled with the simple fact that DNA is all around us. Humans are constantly shedding skin cells -— thousands of them every day. Although a proportion of dead skin cells will have lost nuclear DNA content in the process of keratinization, some will still contribute nuclear, as well as extra-nuclear (free) DNA template sufficient for typing. Recent research has demonstrated that common house dust contains an appreciable amount of the occupant's DNA. This study evaluates whether the common house dust that is captured by HVAC air return filters will yield useful DNA profile data (e.g., missing person searches) and whether any observable differences exist in the representation of DNA profiles from multiple occupants in different vents distributed around a dwelling.

A three-story single-family home (basement, ground level, and second floor) was volunteered as the sample collection site. Built in and continuously occupied since 1999, the current four occupants (one male and three females) have lived in this home on a regular and continuous basis for the past four years. The home is serviced by two independent HVAC systems: HVAC #1 circulates air between the first floor (zone 1, fitted with two ceiling-mounted air return vents) and the basement (zone 2, fitted with one wall-mounted air return vent). HVAC #2 circulates air on the second floor only (zone 3, fitted with four air return vents). A set of seven HVAC filters (Filtrete® Basic, 3M®), one from each vent, was sampled after being in service for 15 days. A section measuring 5cm x 2cm on the inflow surface of each filter was swabbed for DNA using the COPAN® crime scene 4N6FLOQSwabs™ that were pre-wetted with 15uL of sterile water (single-swab method). The samples were extracted using the COPAN® Nucleic Acids Optimizers (NAO), a semi-permeable basket that retains fluid until centrifuged with the PrepFiler® Express™ on the AutoMate Express™ DNA Extraction. DNA was quantitated using the Quantifiler® Human DNA Quantification Kit. The AmpFℓSTR® Identifiler® Plus Polymerase Chain Reaction (PCR) Amplification Kit was used for DNA amplification. The amplified fragments were separated on the Applied Biosystems® 3130 Genetic Analyzer. Data analysis was performed with GeneMapper® ID-X v1.4.

Useful DNA profiles were obtained from all seven vents. Samples from wall-mounted vents revealed slightly higher DNA quantities than ceiling vents with the one exception of the zone 3 vent positioned above the work desk of one of the female occupants. Although zone 2 (basement) is the least occupied space in the house, the associated filter revealed more dust and the highest DNA quantitation value (0.037ng/ul) among all samples, possibly due to that vent being closest to the HVAC unit and drawing a larger parcel of circulating air in comparison. The allelic data reveal consistencies with dwelling/sleeping arrangements, whereas common areas show mixture profiles originating from all four occupants. No foreign alleles were detectable.

Residential HVAC DNA, Touch DNA, Air Filter DNA

E23 A Universal Method for Biological Stain Characterization Using Raman Spectroscopy: From Body Fluid Identification to Phenotype Profiling

Igor K. Lednev, PhD, State University of New York at Albany, 1400 Washington Avenue, Albany, NY 12222*

After attending this presentation, attendees will better understand the potential forensic application of Raman spectroscopy. The implementation of advanced statistics for the analysis of spectroscopic data and the evaluation of the accuracy and reliability of the conclusions made will be discussed.

This presentation will impact the forensic science community by demonstrating the accuracy and effectiveness of biological stain analysis for forensic purposes.

This presentation will report on the development of a novel, non-destructive, and confirmatory method for characterizing biological stains. The all-in-one method has a capability to identify the body fluid, determine human or animal origin, time since deposition, phenotype profile, race and sex, specifically.

Traces of body fluids discovered at a crime scene are a potential source of DNA, which is a major individual evidence in the modern forensic investigation. The application of Raman spectroscopy for non-destructive, confirmatory identification of biological stains at a crime scene, including dry traces of sweat, vaginal fluid, semen, saliva, and blood, have recently been reported.[1] The method allowed for differentiating animal and human blood as well menstrual and peripheral blood.[2,3] Most recently, the method was further developed for determining the time since deposition for bloodstains for up to two years.[4] The theory behind Raman spectroscopy is based on the inelastic scattering of low-intensity, non-destructive laser light by a solid, liquid, or gas sample. Very little or no sample preparation is needed, and the required amount of material tested with a Raman microscope can be as low as several picograms or femtoliters. A typical Raman spectrum consists of several narrow bands and provides a unique vibrational signature of the material. Typically, non-resonance Raman spectroscopy is not destructive for the sample. A portable Raman spectrometer is now a reality that should allow the identification at the crime scene.

It would be of great help for criminal investigations to develop a phenotype profiling immediately at a crime scene based on a rapid analysis of biological stains. With this goal in mind, the possibility of race differentiation based on Raman spectroscopy of blood traces has been investigated.[5] Specifically, advanced statistical analysis of spectroscopic data was used to discriminate between Caucasian (CA) and African American (AA) donors based on dry peripheral blood traces. Spectra were collected from 20 donors varying in gender and age. Support Vector Machines-Discriminant Analysis (SVM-DA) was used for differentiation of the two races. An outer subject-wise Cross-Validation (CV) method evaluated the performance of the SVM classifier for each individual donor from the training dataset. The performance of SVM-DA, evaluated by the Area Under the Curve (AUC) metric, demonstrated 83% probability of correct classification for both races, and a specificity and sensitivity of 80%. This initial work was followed by further proof-of-concept studies demonstrating the differentiation of donor's sex based on bloodstains and saliva traces, as well race differentiation based on traces of semen.[6-8] Overall, the developed method has great potential for crime scene investigation, providing rapid and reliable results with no sample preparation, destruction, or consumption.

This project was supported by an award from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

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Biological Stain, Raman Spectroscopy, Chemometrics

E24 The Rapid Online Wildlife Identification Network (ROWIN): A Bioinformatic Analysis Pipeline Developed for Wildlife DNA Forensics

James P. Creecy, PhD, University of Central Oklahoma, Forensic Science Institute, 100 N University Drive, Edmond, OK 73034*

After attending this presentation, attendees will have a detailed understanding of the issues associated with wildlife forensics and the efforts being made to combat wildlife crimes.

This presentation will impact the forensic science community by revealing greater focus on the importance of wildlife forensics.

The black market trading of illegally poached wildlife is estimated to generate $20 billion in annual revenues. The travesty associated with the illegal trade of wildlife is two-fold. First, the dramatic decline of biological diversity has resulted in irreparable damage to the ecology of affected environments. Second, the black market trade of illegal wildlife products has been directly linked to funding terrorism. For more than 15 years, the field of wildlife DNA forensics has utilized the principles and tools of conservation genetics and forensic genetics to investigate wildlife crimes. Currently, the state-of-the-art technology in forensic wildlife DNA analysis utilizes Sanger sequencing to determine the genetic sequence of "marker genes." These "marker genes" are often located on the mitochondrial genome and are sequenced and analyzed one at a time.

While current techniques are effective, advances in high-throughput DNA sequencing offers the wildlife DNA forensic community an opportunity to dramatically improve the process of forensic species identification. This improvement requires the development of two key components: (1) a single method for the extraction, sequencing, and analysis of an entire mitochondrial genome; and, (2) a database consisting of forensic vouchered reference DNA samples. The wildlife DNA forensics community is therefore offered ROWIN, the first bioinformatic pipeline developed for wildlife forensics. ROWIN utilizes DNA sequencing data obtained from mitochondrial DNA (mtDNA) -specific extraction methods. ROWIN was been developed with the wildlife forensic scientist in mind and for the specific goal of eukaryotic species determination. Forensic species identification by ROWIN is independent of Polymerase Chain Reaction (PCR) and is completely controlled by the forensic scientist submitting the data. Prior knowledge of computer programming is not required and results are courtroom-ready. Implementation of ROWIN provides the wildlife forensics community with a powerful analytic tool that will result in a reduction of time and cost.

Wildlife Forensics, Bioinformatic, Next Generation Sequencing

E25 A Different Kind of DNA Casework: When It Barks or Purrs

Teri Kun, BS, UC Davis, VGL-Forensics, One Shields Avenue, Davis, CA 95616; and Christina D. Lindquist, MS, UC Davis, VGL-Forensics, One Shields Avenue, Davis, CA 95616*

After attending this presentation, attendees will understand the application of routine forensic genetic tools to casework involving domesticated animals, specifically dogs and cats. Cases involving trace companion animal biological evidence (for example, a shed hair or piece of feces) will be presented to provide an overview of breadth of casework completed by the Veterinary Genetics Laboratory-Forensic unit (VGL-Forensics) at the University of California at Davis (American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB) -International accredited since 2010).

This presentation will impact the forensic science community by increasing awareness of the field of wildlife forensics. This presentation is intended to be part of a set of presentations from forensic science practitioners in the wildlife forensics community. Wildlife species have often been the basis for research presented at the American Academy of Forensic Sciences (AAFS); however, the work of wildlife forensic laboratories has, in the past, been underrepresented in the AAFS scientific sessions. By presenting multiple talks, highlighting the breadth of the work that is completed in this community, a valuable resource will be provided to the forensic science community at large.

While committing a sexual assault in a residential backyard, Rusfus Sito Nanez III rolled in some canine feces, which later helped link him back to the victim's home, resulting in his conviction. VGL-Forensics played a key role in the trial and conviction of this serial rapist. As the only crime laboratory in the country accredited for analysis of DNA from domestic animals, VGL-Forensics has been serving federal, state, and local law enforcement agencies, as well as the general public, for more than a decade. The laboratory receives a wide variety of cases from all over the world, with sample types and species unlike those encountered by its human counterparts. Cases range from human-on-human crimes in which dog or cat biological evidence links a suspect to the crime, to large-scale dog fighting, species identification, and animal cruelty cases. Recent case examples will be presented in addition to other high-profile and cold cases.

Because domesticated animals share such a close relationship with humans, the work of the VGL-Forensics routinely intersects with the work of human crime labs and is used as an investigative tool in traditionally "human" law enforcement cases, such as homicide or sexual assault; however, dogs and cats are not "human," which means that working with these species includes some of the same challenges as working with wildlife species in wildlife crime laboratories. In order to meet the demands of courts that are accustomed to hearing human DNA evidence, VGL-Forensics has modelled our laboratory system after that of a "human" DNA crime lab, dealing mainly in match comparison and mitochondrial haplotyping methods. Details of these methods as applied to dogs will be presented as well.

Wildlife Forensics, Canine DNA, Non-Human Forensics

E26 A Different Kind of DNA Casework: When It Has Fangs or Antlers

Kimberly Frazier, MS, Department 3312, 1000 E University Avenue, Laramie, WY 82071; and Christina D. Lindquist, MS, UC Davis, VGL-Forensics, One Shields Avenue, Davis, CA 95616*

After attending this presentation, attendees will have a better understanding of wildlife forensics at the state level. Case histories will be highlighted from several states covering a variety of species and testing methods. These case histories will include: bighorn sheep cases from New Mexico and Colorado; an elk case from Colorado; grizzly bear, black bear, white-tailed deer, mule deer, and pronghorn cases from Wyoming; and an elk case from Yellowstone National Park. These cases involve species identification using protein analysis and mitochondrial DNA sequencing, sex identification, and matching using microsatellites.

This presentation will impact the forensic science community by increasing awareness of the field of wildlife forensics. This presentation is intended to be part of a set of presentations from forensic science practitioners in the wildlife forensics community. Wildlife species have often been the basis for research presented at the American Academy of Forensic Sciences (AAFS); however, the work of wildlife forensic laboratories has, in the past, been underrepresented in the AAFS scientific sessions. By presenting multiple talks, highlighting the breadth of the work that is completed in this community, a valuable resource will be provided to the forensic science community at large.

These histories will provide background information of each case, from the law enforcement perspective to the analyses performed in the laboratory to the results of each case. This background information will include information on the discovery of the violation, how officers determined the suspect and collected evidence, and the evidentiary items that were submitted to the laboratory for analyses. For the analyses performed, a brief overview of the testing procedures and results will be provided. The presentation of the cases will also include charges filed, expert witness testimony, if applicable, sentencing, and fines.

It is hoped that these case histories will help explain the diversity of the field of wildlife forensics and while analyses, in some cases, is very similar to that in the human field, they can also be very different. In wildlife forensics, it is most often the animal that is the victim.

Wildlife Forensics, DNA, Non-Human Forensics

E27 A Different Kind of DNA Casework: When It Has Antlers

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After attending this presentation, attendees will better understand the application of forensic principles to the area of wildlife forensics. The development and application of a system for the unique identification of white-tailed deer used for the prosecution of suspects who may have committed crimes involving white-tailed deer will be presented. A multiplex Short Tandem Repeat (STR) marker system was used to confirm the identity of the stolen deer and offered in court to help prosecute an individual charged with the theft of the trophy white-tailed deer buck. The presentation of the development and validation of the white-tailed deer STR marker system will reinforce the parallels between human and wildlife forensics.

This presentation will impact the forensic science community by increasing awareness of the field of wildlife forensics. This presentation is intended to be part of a set of presentations from forensic science practitioners in the wildlife forensics community. Wildlife species have often been the basis for research presented at the American Academy of Forensic Sciences (AAFS); however, the work of wildlife forensic laboratories has, in the past, been underrepresented in the AAFS scientific sessions. By presenting multiple talks, highlighting the breadth of the work that is completed in this community, a valuable resource will be provided to the forensic science community at large.

The unique challenges of wildlife forensics will be discussed as they relate to the prosecution of the individual for the theft of the trophy buck and in general. In this case, a comparison between the DNA profile from the animal and the DNA profile from shed antlers from the original owner were presented in court and contributed to the conviction of the suspect. Methods for the extraction of DNA from typical white-tailed deer samples, multiplex Polymerase Chain Reaction (PCR), and capillary electrophoresis will be discussed. The validation conducted included processing deer samples from substrates such as deer hair and antler material, blood on rope, wood, leaves, dirt, leather boots, tarps, denim, arrows, and concrete; this will be discussed. This database of white-tailed deer DNA profiles was processed using the Cervus computer program to determine if the makers were in Hardy-Weinberg equilibrium and to identify the Polymorphic Information Content (PIC) at each locus. Allele frequencies for each marker were calculated to determine the match probability in this case.

Many other examples of the application of forensic DNA testing for the successful prosecution of suspects in wildlife crimes will be mentioned. Current efforts to produce a tetra-nucleotide STR multiplex system for Sable antelope will also be discussed. At the conclusion of the presentation, attendees should have a good understanding of the application of well-established human forensic principles to the challenging area of wildlife forensics.

Wildlife Forensics, White-tailed Deer, Multiplex STR

E28 A Different Kind of DNA Casework: When It Has Fins

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After attending this presentation, attendees will better understand the similarities and differences in the application of forensic genetics to humans and marine wildlife species. Two cases, one involving seafood fraud and the other trafficking in prohibited shark fins, will be used to illustrate the legal framework, difficulties in sampling large shipments, and genetic challenges in wildlife analyses.

This presentation will impact the forensic science community by increasing awareness of the field of wildlife forensics. This presentation is intended to be part of a set of presentations from forensic science practitioners in the wildlife forensics community. Wildlife species have often been the basis for research presented at the American Academy of Forensic Sciences (AAFS); however, the work of wildlife forensic laboratories has, in the past, been underrepresented in the AAFS scientific sessions. By presenting multiple talks, highlighting the breadth of the work that is completed in this community, a valuable resource will be provided to the forensic science community at large.

The National Oceanic and Atmospheric Administration (NOAA) Fisheries Forensic Laboratory supports the NOAA Fisheries Office of Law Enforcement (OLE) with forensic analyses. OLE upholds more than 35 statutes regulating commercial and recreational fishing and protecting endangered species, marine mammals, and marine sanctuaries. OLE also enforces laws that focus on harvest, processing, and trafficking of marine resources. As such, cases that the forensic laboratory routinely analyzes fall into two broad categories: major criminal cases of seafood fraud involving false labeling and trafficking in protected species. Both types of cases require definitive knowledge of the species in evidence to determine whether a violation has occurred. Because processing of seafood and protected species early in the supply chain often removes easily identifiable physical characteristics, identification of evidence is often via mitochondrial DNA sequencing. The first case involves large-scale mislabeling of salmon. In 2007, NOAA OLE seized more than 15,000 pounds of frozen, processed salmon fillets believed to be mislabeled as Chinook salmon. DNA analysis of samples collected from the seizure confirmed that the majority of the fillets were not Chinook salmon, but were other, less valuable species of Pacific salmon. The government estimated that the market value of the falsely labeled fish was in excess of $1.3 million. The owner of the processing company was sentenced to a year in prison and fined a $347,000 community service payment. The company has since gone out of business.

The second case involves trafficking in protected species of shark. Shark fins are highly valued for shark fin soup and are subject to a lucrative worldwide trade. Because of the fin trade and other fishing pressures, sharks have come under increased protection in an attempt to stem population declines. Before 2014, only three shark species that were rare in trade were protected under international treaty, but 2014 saw nine relatively common shark species added to this list. These new protections have led to increased enforcement efforts and resulted in several large shark fin shipments seized in the United States. This case presentation will focus on a recent United States West Coast seizure of 21 metric tons of shark fins and share some lessons learned about dealing with large heterogeneous shipments containing mixed legal and illegal product, prosecutions involving foreign defendants, and species with challenging population genetics.

Wildlife Forensics, Species Identification, DNA Sequencing

E29 A Different Kind of DNA Casework: When It Has Horns or Tusks

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After attending this presentation, attendees will understand the mission of the United States Fish and Wildlife Service (USFWS) - National Fish and Wildlife Forensic Laboratory (NFWFL) and the role that forensic scientists play in providing scientific support for law enforcement investigations of wildlife crimes domestically and internationally. This presentation will raise awareness of an active and diverse field of forensic science that focuses on crimes that involve non-human and non-domestic animals, with an overview of laboratory operations and selected case studies. Crimes that involve wildlife vary widely, such as illegal hunting and fishing violations, events that result in the harm to or death of threatened and endangered species, or activities of sophisticated illegal black markets connected to international wildlife trafficking networks. Often, the primary investigative activity in a wildlife crime case is to determine whether a crime has actually been committed as sometimes both the victim and the suspect may be animals or trees.

This presentation will impact the forensic science community by increasing the awareness of the field of wildlife forensics. This presentation is intended to be part of a set of presentations from forensic science practitioners in the wildlife forensics community. Wildlife species have often been the basis for research presented at the American Academy of Forensic Sciences (AAFS); however, the work of wildlife forensic laboratories has, in the past, been underrepresented in the AAFS scientific sessions. By presenting multiple talks, highlighting the breadth of the work that is completed in this community, a valuable resource will be provided to the forensic science community at large.

The NFWFL is a fully accredited forensic laboratory located in southern Oregon and is the science support arm of the USFWS Office of Law Enforcement, assisting more than 300 special agents, wildlife inspectors, and several K-9 teams in the United States, special agent attachés in nine foreign countries, 50 State Fish and Game Commissions, and more than 170 foreign countries who have signed the United Nation's Convention on International Trade in Endangered Species Treaty. The mission of the NFWFL is to identify the species or subspecies of pieces, parts, or products of an animal; to determine the cause of death of an animal; to help wildlife officers determine if a violation of law has occurred; and to identify and compare physical evidence in an attempt to link suspect, victim, and crime scene. The NFWFL's forensic program has been accredited through the American Society of Crime Laboratory Directors/American National Standards Institute-American Society of Quality (ANSI-ASQ) National Accreditation Board (ASCLD/ANAB) since 1997, and NFWFL forensic scientists participate in regular proficiency testing.

The NFWFL's scientific staff includes experts in morphological identification, genetic analysis, chemical analysis, and veterinary pathology. Cases processed by the NFWFL are primarily focused on compliance with criminal law, and analyses of non-wildlife cases, such as animal abuse or food contamination, are rare. NFWFL scientists conduct cause-of-death determinations, class character analyses (morphology, chemistry, and genetics), and source evaluation of individuals (genetics). Often, individual cases are processed by multiple disciplines. In 2016, the NFWFL analyzed 2,605 individual items of evidence that were part of 732 investigations. NFWFL forensic scientists provided additional support in the form of 1,525 photo identifications to assist field agents in determining if suspected infractions warranted investigation.

One example of casework conducted at the NFWFL is the investigation referred to as "Operation Crash," an ongoing nationwide criminal investigation led by the USFWS Office of Law Enforcement. The investigation focused on the illegal trade in rhinoceros horn and elephant ivory involving international poaching and smuggling syndicates. Charges against defendants included violations of the Endangered Species Act and Lacey Act, conspiracy, smuggling, money laundering, mail fraud, tax evasion, bribery, and false documentation. NFWFL scientists conducted chemical analyses to determine if evidence items were of animal origin, morphological analyses to determine if horns originated from rhinoceros and if ivory originated from extant elephant or extinct mammoth, and genetic analysis to determine the species source of rhinoceros horn (Black or White rhinoceros) and ivory (African or Asian elephant). To date, this operation has secured the arrest and successful prosecution of numerous individuals and businesses and the disruption of several smuggling networks. A total of 34 years of prison and $7.5 million in fines and restitution have been imposed by the courts.[1]

Additional examples of the diversity of forensic analyses conducted at the NFWFL will provide attendees with a greater understanding of the breadth of scientific expertise provided by Wildlife Forensic Scientists at the USFWS-NFWFL.

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1. *U.S. Fish and Wildlife Service, 2016 Office of Law Enforcement Annual Report.* https://www.fws.gov/le/pdf/2016-Office-of-Law-Enforcement-Annual-Report.pdf.

Wildlife Forensics, Wildlife Trafficking, Non-Human Forensics

E30 The Risk for Immersion Pulmonary Edema (IPE) From Scuba Diving

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The goal of this presentation is to reveal how risk factors for IPE are still poorly understood and how this study investigated a diver's susceptibility.

This presentation will impact the forensic science community by making people aware of the danger of IPE, which differs from drowning accidents.

IPE occurs when the alveolar capillary barrier fails due to the constraints on a diver's lungs during a dive in which obstructing fluid entering the lungs can resemble symptoms of drowning. IPE can induce death in 1.1% of descents, which is higher than the risk associated with decompression sickness. Some advance figures even put IPE as high as 15%. The onset of IPE generally begins 15 to 20 minutes into a dive with symptoms of dyspnea, coughing, laryngeal sounds, and a feeling of chest tightness without genuine pain. Due to these symptoms, a diver may interrupt decompression breaks during an ascent, thus increasing chances of decompression sickness and hypoxia, which is found in 15%-20% of reported cases. Divers experience sensations of suffocation, coughing up blood, or if serious enough, the diver dies upon resurfacing. This report examines two fatal accidents that occurred during cold sea diving trips.

A 55-year-old man died from cardiac arrest a few minutes after beginning his dive into cold water (5°C). Heavily weighted and swimming against a strong current, fatigue eventually occurred and the diver quickly descended to a depth of 28 meters. During his quick descent, he attempted to resurface, but failed. Another diver made an emergency descent to rescue the failing diver. When the rescuer made it to the individual, he was conscious, but quickly lost consciousness during the ascent. Upon resurfacing and after removing the diver's breathing equipment, a bloody mass came out of his mouth. Unfortunately, the diver died after attempts to resuscitate him at the hospital, where pulmonary X-rays were determined to be normal.

Another unfortunate diving incident occurred in cold water at a depth of 20 meters when a 25-year-old soldier spent 25 minutes in the water. During his ascent, his partner felt a dead weight at the end of the attached rope and noticed that his partner withdrew his regulator as a distress gesture. After resurfacing, the soldier was found unconscious and a blood mass was discharged upon resuscitation attempts. The autopsy revealed no macroscopically visible cardiac lesions, as did a pathology examination. This soldier had regular medical exams and was considered able to perform his duties.

Neither autopsy found signs of macroscopically visible cardiac abnormalities. Hearts were not found to have coronary network obstructions or calcifications and exhibited perfect permeability of the coronary artery; likewise, toxicology examinations did not find any anomalies. No failures were found upon examination of the dive equipment for the two victims.

Both individuals lost consciousness during their ascent and coughed up a bloody mass when they emerged from the water, a sign of pulmonary edema. These two accidents reveal the danger of IPE, which can happen to all divers, even experienced ones. The occurrence of IPE in diving has multiple factors. Incidence and severity increase with age and cardiopulmonary comorbidities. Forensics and the diving community should be aware of the danger of IPE, which differs from drowning accidents; therefore, these events should not be ruled as a drowning accident.

Immersion Pulmonary Edema, Drowning, Swelling

E31 A Study of Death by Firearm Injury

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The goal of this presentation is to present a retrospective study of cause of death, manner of death, and nature of death.

This presentation will impact the forensic science community by presenting the results of a study of death by firearm injury, which was conducted in a forensic medical college in Bogra, Bangladesh. The period of study was from 2006 to 2014.

In this study, cause of death, manner of death, and nature of death were examined. The total number of deaths in that period were 11. Manner of death (homicidal, suicidal, or accidental) were mentioned. Firearms (rifles or smooth bore) were mentioned. The results revealed that in these firearm injuries, causes of death were ten homicides and one suicide.

A firearm is any instrument that discharges a missile by the expansive force of the gases produced by burning of an explosive substance. Forensic examiners deal with the investigation of firearms, ammunition, and the problems arising from their use. There are generally two types of firearms: rifled weapons (e.g., military rifle, air rifle, revolvers, pistols), and smooth bore weapons, such as shotguns (e.g., cylinder bore, choke bore, breech loader, muzzle loader).

Cartridges are used in both shotguns and rifles. Cartridges consist of a short metal cylinder case that is continuous with a cardboard or plastic cylinder.[1]

Firearm wounds are identified by following the findings: an entrance wound, an exit wound, the presence of gun powder, the presence of gun powder smoke, singeing of hair, blackening of the skin around the wound, the presence of cardboard, the presence of a felt wad, the presence of a bullet, a collar abrasion in the wound, a grease abrasion in the wound, and X-ray revealing the presence of a bullet.

The character of a wound depends on the distance from which the weapon is discharged: - contact and near contact wound; close-range wound, up to 1 meter; short-range (medium) wound, 1-2 meters; intermediate-range wound, 2-4 meters; and long- or distant-range wound, more than 4 meters.[3]

Postmortem examinations of the dead bodies were conducted in Shaheed Ziaur Rahman Medical College Morgue. Prerequisites for autopsy include inquest report, Chillan, commanding certificate and requisition prepared by investigating police officers, and a magistrate sent for the autopsy. External examination and detail dissection of the bodies was conducted with the help of a mortuary attendant.

Eleven firearm-wounded, dead bodies were autopsied from 2006 to 2014. The manner of death, nature of death, age, and sex were mentioned. Bullets, cartridges, and cardboard were collected.

Distribution of death by sex. (*N*=11)

Sex	No	Percentage
male	11	100%
Female	0	0%

Distribution of death by age. (*N*=11)

Age	No	Percentage
20-49	7	63.63%
40-60	4	36.36%

Distribution of death by manner. (*N*=11)

Manner of death	No	Percentage
Homicidal	10	90.90%
Suicidal	1	9.09%
Accidental	0	0%

In this study, death due to 11 firearm wounds were deemed as ten homicides and one suicide. Homicidal firearm wounds are common in Bangladesh, whereas suicidal wounds are rare. Homicidal firearm wounds occur because of terrorism, political issues, struggle for rights, adultery, and in exchange for money and properties. Cartridges and bullets were recovered from dead bodies after dissection and given to the police. All bullets and cartridges were from shotguns. Two firearm wounds were found in the chests in two dead bodies, and firearm wounds were found in temporal and occipital regions of the head in nine dead bodies. In America, two-thirds of homicides are caused by firearm injuries; people commit suicide by firearms, drugs, and hanging; 54% of all suicidal deaths are caused by firearms.[4]

Homicidal firearm wounds are common in Bangladesh. Most are declared as an encounter or cross fire. Incidence of death due to firearm injuries are increasing gradually day by day because of terrorism and the availability of firearms without registration. Deaths were due to shock and hemorrhage resulting from firearm wounds.[2]

Reference(s):

1. Dr. K-S Narayan Reddy. *The Essentials of Forensic Medicine and Toxicology.* 29th edition, Hyderabad, India by K- suguna devi, 2011, P 187–215.
2. Krishan Vij. Text book of forensic medicine and toxicology principal and practice. 5th edition, India by Elsevier, 2011, p 234–265.
3. Parikh's Text book of Medical Jurisprudence Forensic Medicine and Toxicology. 6th edition Delhi India, CBS publishers 1999 P 4.26–4.54.
4. *Firearm Injury in the US.* Ficap @ uphs upenn edu, (version 2011).

Firearm Injuries, Homicidal, Entrance Wound/Exit Wound

E32 Revisiting the Question of Yellow Discoloration of the Skull Bones and Diabetes Mellitus in an Autopsy Population

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The goal of this presentation is to illustrate if there is an association between yellow discoloration of the skull bones and diabetes mellitus in the deceased.

This presentation will impact the forensic science community by providing conclusive results that are in line with previous research. The clinical implication for noting a yellow discoloration may guide the physician to order an analysis of insulin and glucose and/or review the patient's history.

This presentation addresses the association between a yellow discoloration of the skull bones and diabetes in the deceased.[1-5] Recognition of diabetes during the autopsy serves to guide the pathologist as to which cases require further toxicological screening to look for glucose and insulin.

The study included 77 autopsy cases, prospectively, at the Unit for Forensic Medicine in Lund, Sweden, during 2016-2017, and the bones of the skulls were evaluated for a yellow discoloration. As a secondary goal, the study evaluated if a possible observed association is confounded by age or sex, or if these characteristics have an isolated effect from that of diabetes mellitus.

The forensic pathologist who evaluated the color of the skull had no background information on the case. Cases with severe decomposition and severely burnt cases were excluded. Discoloration of the skull bones was evaluated by a forensic pathologist using a Chromascop® Shade Guide used in dentistry to evaluate bleaching of the teeth. From this shade guide consisting of 20 shades, two shades were selected, one representing white and one representing yellow.

Information regarding diabetes mellitus in the deceased was obtained either from the police report or patient charts. Diabetes was considered to be present if medication such as oral anti-diabetic medication or insulin was prescribed, or if the diagnosis was mentioned in the police report or in patient journals. Information regarding age at death and sex was collected from the police reports.

Of the 77 cases, 36 (46.8%) were considered to have a yellow discoloration of the skull, and, in total, 11 (14.3%) cases had diabetes mellitus. Of those with a yellow discoloration of the skull, there were n=9 (25%) cases with diabetes, and in those with a normal color of the skull n=2 (4.9%). Using unadjusted logistic regression models, one could observe a conclusive association between a yellow discoloration of the skull bones and diabetes mellitus: *Odds Ratio (OR)*=6.5 (95% Confidence Interval (CI) 1.3, 32.5). The overall sensitivity of a yellow discoloration of the skull bones to detect diabetes mellitus was *9/11*=81%, and the overall specificity *39/66*=59%.

The model was adjusted for sex, and the observed association between a yellow discoloration of the skull and diabetes mellitus remained: *OR*=6.6 (95% CI 1.3, 33.5). The association between a yellow discoloration of the skull bone and diabetes mellitus was still conclusive after having adjusted for age: *OR*=6.8 (95% CI 1.3, 37.2). Age by itself was also conclusively associated with a yellow discoloration of the skull bones: *OR*=4.6 (1.7, 12.8).

In this study, there was a positive association between a yellow discoloration of the skull and diabetes mellitus in a medicolegal autopsy population; however, the study suffers from low statistical power, but the results are still conclusive and in line with previous research; however, the yield of the approach needs to be discussed using the sensitivity (about 80%) and specificity (59%). The test may be useful for identifying cases with diabetes mellitus, but the specificity is low, implying that a large proportion of cases free of diabetes mellitus would not be identified as such. The uncertainty concerning the true association is large, as indicated by the wide CIs.

Reference(s):

1. Henner Krug, Hansjürgen Zschoch. Reflex-photometric studies of the yellow discoloration of the cranium in diabetes mellitus. *Virchows Archiv für patologische Anatomie und Physiologie und für klinische Medizin.* 338 (1964): 166-171.
2. Jiun-Noung Lin, MD. Yellow palms and soles in diabetes mellitus. *The New England of Medicine*. (2006) 335;14.
3. Achim Th Schafer. The colour of the human skull. *Forensic Science International*. (2001), vol 117, issues 1-2, pages 53-56.
4. Art Huntley, MD, and Rhett Drugge, MD. Diabetes in skin disease. *The electronic textbook of dermatology.*
5. Jurgen Ludwig, MD. *Handbook of autopsy practice.* 3rd edition (2002), Humana Press.

Discoloration, Skull Bones, Diabetes Mellitus

E33 Standardizing a Large-Scale, Whole Body Computed Tomography (CT) Image Database

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After attending this presentation, attendees will understand the benefits of standardizing data and limiting or eliminating free text answers in databases used in medicolegal investigation. Attendees will also be informed of the process by which standards are chosen and created.

This presentation will impact the forensic science community by exploring the benefits of standardized data formats, especially in medicolegal investigations. While data standardization meets several challenges in medicolegal studies not encountered in many other fields, standardization improves potential applications of large-scale data in epidemiology.

The Office of the Medical Investigator (OMI) is a state-wide, centralized medical examiner's office for New Mexico. In 2010, 5,249 deaths were routed to the OMI; 51% of those underwent autopsy, accounting for 35% of the deaths within the state.[1,2]

In 2010, the National Institute of Justice (NIJ) awarded the Center for Forensic Imaging at the OMI a grant to evaluate whether CT scans can supplant or supplement the traditional autopsy. To this end, every decedent that underwent an autopsy received a high-resolution, head-to-toe CT scan. Scanning has continued as standard practice at the OMI, even after the end of this project. So far, more than 11,000 whole body CT images have been created; however, as the original scanning was performed to address specific research projects, and not to create a general research resource, there are no indexes or tags associated with the images that might allow additional research.

In 2016, an NIJ grant was awarded to create a free-access Decedent CT Database, which will make these 11,000 whole body CTs available to the research public. Work is currently underway to contact next of kin and query the OMI's database to populate the new database. This query presents numerous challenges in that the majority of fields within the database are free text fields without any limitations. As a result, even sex can be recorded in multiple ways (e.g., Male, male, M, m), limiting the ability of future researchers to query efficiently. This is especially true as the complexity of the metadata increases. In order to combat this issue, the CT database being developed will utilize data standards, terminologies, and classification systems. Using a modified Delphi technique, experts from varying fields determined the 59 metadata variables to associate with each image.[3] This data is being captured from the OMI investigations database as well as next-of-kin interviews.

A step-by-step process is currently underway to determine the best standards to implement. First, Unified Medical Language System (UMLS) is searched to identify all of the standards for a particular concept (e.g., race) that exist. Each standard is then identified and compared for usefulness in this particular database. A standard can be implemented as is, modified, or all can be rejected. If all current standards are rejected for use in the database (or none are found), a new standard will be proposed and implemented.

A wide range of terminologies, standards, and classification systems may be implemented. This includes LOINC, SNOMED CT, CDISC, and multiple nursing standards. By comparing the standards and determining the best fit, we will eliminate duplication of standards, utilize current health care, and research terminologies will be eliminated.

For example, one of the variables included in the Decedent CT Database is medical diagnoses. This field is often recorded as free text in medicolegal investigation; however, this limits the value of this data in later research because the same condition could have hundreds of synonyms and abbreviations. Standards exist for recording actual data (Logical Observation Identifiers Names and Codes (LOINC), Clinical Data Interchange Standards Consortium (CDISC)), but the responses are of importance here. Systematized Nomenclature of Medicine – Clinical Terms (SNOMED CT) and the International Classification of Diseases (ICD) both record diagnoses; however, one standard is used to identify medical problems (SNOMED CT) within electronic health records, while the other is used for billing (ICD-10). Both standards require coding the response from next of kin or the information from the OMI database as a computer-readable number. Due to the vast number of SNOMED CT codes and the lack of everyday use of them by physicians and researchers, ICD-10 was chosen to represent the medical diagnoses of the decedents for this project.

The free-access Decedent Database is currently under development and is slated to be available by the end of 2018.

Reference(s):

1. OMI. Office of the Medical Investigator 2010 Annual Report. University of New Mexico: 2010.
2. US Census Bureau. *2010 US Census.* 2010 [cited 2011 January 19, 2011]; Available from: http://2010.census.gov/2010census/pdf/2010_Questionnaire_Info.pdf.
3. Berry, S. *Metadata Determination for a Cadaveric Collection. Master.* (Thesis).Albuquerque: University of New Mexico; 2014. Available from: UNM Digital Repository.

Data Standardization, Computed Tomography, Research Database

E34 Stabbing Exploration: An Essential Duality

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After attending this presentation, attendees will have learned about a stabbing case in which both a Computed Tomography (CT) scan and autopsy were essential to detect all the lethal injuries and explain the absence of defensive wounds on the victim's skin.

This presentation will impact the forensic science community by confirming the necessity of collaboration between radiologists and forensic pathologists, the importance of a systematic approach, and expanding the range of CT scan indications in stabbings.

Medical imaging investigations, such as a CT scan, have their own place in forensic medicine today, particularly in thanatology (postmortem imaging). They enable the constitution of an acceptable database of pictures for juries at the Assize Court, but are also a wonderful diagnostic tool thanks to the exploration of wounds, which are hardly accessible during a surgical autopsy.

Rouen Forensic Institute was requested following the homicide of a 54-year-old man, stabbed while he was standing in his workplace, where stigmata of an externalized hemorrhage were observed.

External examination brought to light seven cervical wounds, one of which was on the left side of the neck, facing cervical blood vessels, and six of which were on the right posterior side of the neck and were not initially suspected of being potentially lethal. No defensive wounds were visualized on the hands or the forearms, surprisingly, considering the number of blows which should have led the victim trying to fend off his aggressor.

The postmortem non-contrast CT scan, taken before the autopsy, showed a fracture of the fifth cervical vertebra's right lamina and lesions of the pedicle and vertebral body on the left side of the vertebra. The presence of air bubbles in the spinal canal was highly suspect of an associated spinal damage. Exploration of the cerebral and cervical vessels was impossible due to the absence of contrast.

During the autopsy, the left cervical wound was shown to have caused a left jugular vein injury, and one of the posterior cervical wounds was transfixing, associated with a right subclavian vein injury. Exploration of the wound, which caused vertebral and medullar injuries, was very difficult because of its location and its trajectory.

If a postmortem CT scan is actually recognized as the essential additional examination in some cases, in particular for putrefied bodies or ballistic trauma, its place in stabbing exploration is not totally defined. Indeed, a non-contrast CT scan doesn't allow the distinguishing of vascular lesions but provides a good detection of bone lesions, with the possibility of a non-invasive exploration of inaccessible areas at autopsy.

In this case, a non-contrast CT scan did not reveal the two serious vascular injuries; however, it highlighted vertebral and medullar injuries, which would have gone unnoticed or would have needed very damaging explorations during the autopsy because of their location. The absence of defensive wounds were also discovered as a result of vertebral and medullar injuries, which probably caused at least a tetra paresis with difficulty in moving the four limbs.

Thus, it seems to be crucial to systematically couple a CT scan and surgical autopsy, which are actually complementary and shall not, under any circumstances, substitute for each other, particularly in stabbings.

CT Scan, Forensic Autopsy, Stabbing

E35 A Case Report of a Peculiar Bullet Track: Trajectory Making the Defense Unfeasible

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After attending this presentation, attendees will understand an unusual case of trajectory within the skull and trajectory from the firearm to the body of the victim. This presentation will demonstrate how it was possible to determine the path and trajectory of projectile and determine the location of the shooter.

This presentation will impact the forensic science community by highlighting the importance of carefully analyzing the path of the projectile in cases in which it is not located in the body of the victim and the how the single gunshot wound can reveal the true story.

A 33-year-old man was found dead inside the garage of his house. No apparent bloodstain, gun, or projectile were found at the crime scene. The wife's version, who was accused of committing the crime, was that the victim was drunk and physically assaulted her, then pointed a firearm and threatened to kill her. They began to physically fight and the gun fired. The wife wrapped the corpse in a blanket and transported it from the bedroom to the garage, then she cleaned up the crime scene.

External inspection of the decedent revealed a man of apparent age compatible with chronological age, White, 173cm tall, and weighing 75kg. Wounds in the left frontal region, upper lip, and left arm were present.

An external head examination (after hair in this area has been shaved) revealed a gunshot entrance wound, 1.5cm in size in the right parietooccipital regions of the brain with slightly oblique angle and contusion and an abrasion ring.

Gunshot entry wound to the skull: outer table of the skull exhibited cone-shaped and fractures of the parietal and base of the skull. Bullet track and trajectory: it was envisaged that the projectile's trajectory was from top to bottom, backward, at an angle of 30 degrees, from right to left and at a distance. These data show a discrepancy between the information provided by the perpetrator of the crime and the autopsy findings.

In this case report, the external examination of the cadaver did not identify the exit wound of the projectile, which made a path inside the skull, exiting through the nasal cavity without leaving an external wound. The path of the projectile inside the skull of the victim made it possible to demarcate the trajectory of the shot, contradicting the version given by the possible perpetrator of the crime.

Penetrating Head Injuries, Wound Ballistics, Gunshot Wounds

E36 When a Man Kills a Woman — Femicide in Clark County, Nevada: An Overview of Intimate Partner Homicide (IPH) and Intimate Partner Homicide Followed by Suicide (IPHFS)

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The goal of this presentation is to demonstrate how women are more likely to be killed by their intimate male partner than by any other type of perpetrator. Attendees will gain valuable insight into the patterns of female IPH and female IPHFS affecting Las Vegas and its metropolitan environs.

This presentation will impact the forensic science community by explaining femicide -— the killing of a woman by a known perpetrator. In the United States, approximately one-forth of murders committed annually are perpetrated against women. Every week, there is at least one case in the media in which the female is killed by her male intimate partner. In 2011, Nevada ranked number one in the rate of women killed by men. This presentation seeks to highlight the impact fatal domestic violence has had in Clark County.

Data was collected from the Clark County Office of the coroner/medical examiner for all female homicides for seven years (2010 to 2016), with a total of 240 victims. The investigation reports, autopsy reports, the case notes, and case images were consulted.

Of the 240 total female homicides, 148 were intimate violence, in which the attacker knew the victim. Of these 148 intimate violence cases, 84 were perpetrated by a romantic partner, 36 by a relative, 23 by other, 2 by a friend, 2 by a roommate, and in 1 case, the relationship could not be established.

For the 84 romantic relationship victims, “romantic” was defined as the offender being the husband, boyfriend, partner, ex-husband, ex-partner, or ex-boyfriend of the decedent. All of these offenders were male.

Out of the 84 cases, 73 females killed by intimate partners were adults (18 to 65 years old), 10 were older adults (more than 66 years old), and one was less than 18 years old. Of the 84 victims of fatal romantic partner domestic abuse, 44 were White, 18 were Black, 14 were Hispanic, 7 were Asian, and 1 was Multi-Cultural.

In the current sample, most females killed by a romantic partner died of gunshot wounds (47 of 84, or 56%), followed by stabbing (15 of 84, or 17.9%), and blunt force trauma (14 of 84, or 16.7%). Additionally, most of them (66 of 84, or 78.6%) had both cranial and postcranial injuries. Of those who were shot, the majority (33 of 47, or 70.2%) also had both cranial and postcranial trauma.

Out of 84 cases, 31 (36.9%) perpetrated by a romantic male partner were homicides, followed by suicide. This study found that most female IPHFS incidents were perpetrated by White males (20 out of 31, or 64.5%). Most homicide-followed-by-suicide victims were adult White females, followed by adult Hispanic females. Twenty-eight of 31 (90.3%) of the IPHFS victims were killed by firearms, and 23 of 31 (74.2%) had both cranial and postcranial trauma.

According to the Center for Disease Control, intimate partner violence is a serious, preventable public health problem affecting millions of Americans. Violence against women has reached epidemic proportions in many societies, estimated by the World Health Organization (WHO) to account for between 5%-20% of healthy years of life lost in women aged 15 to 44 years. WHO has also stated that “the overwhelming burden of partner violence is borne by women at the hands of men.” That is why studies such as this are needed to help determine patterns and to understand them, in order to prevent, but also better help, the victims of domestic abuse.

Femicide, Intimate Partner Homicide, Homicide Followed by Suicide

E37 A Homicide by Shotgun: The Other Side of a Widespread Weapon

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The goal of this presentation is to underline how a very careful external examination of the cadaver, as well as a detailed analysis of the crime scene, can shed light on the manner of death in a case of an unusual use of a common firearm.

This presentation will impact the forensic science community by emphasizing the necessity of a careful crime scene reconstruction and a complete methodological external examination of the cadaver in an attempt to reach a certain unusual manner of death.

Shotguns are used in a large number of homicides and suicides, and gunshot wounds are widely reported in the literature. This particular case exhibits circumstances and pathological findings that brought to recognition an unusual use of a common firearm.

This case is of a 50-year-old man, murdered in the garden of his countryside cottage in Gargano, in southern Italy. The forensic team was alerted by the local prosecutor for an "evident" case of shotgun homicide. The man was found at the crime scene lying on the ground, on his right side, with his head partially submerged in a large pool of blood. Several small pellets surrounded the victim and some had targeted the wall of the warehouse. Five plastic cases and one plastic wad were found. A plastic wad was found in the anterior side of the victim's shoulder bag.

The external examination revealed an abrasion on the left side of the face that seemed to reproduce the herringbone-shaped stone floor of the garden, and, on the right zygomatic area, a small quadrangular honeycomb grid abrasion was also present. "Raccoon eyes" with bilateral bleeding from the ears and several firearm wounds on the left shoulder were observed. Postmortem radiological study conducted with a Computed Tomography (CT) scan of the total body revealed a basal skull fracture, a spider web fracture complex of the left side of skull from the frontal to the occipital bone, a fracture of the right zygomatic bone, and five metallic fragments exclusively in the left shoulder.

At the autopsy, five pellets were found under the skin and in the fat layer of the soft tissues of the shoulder. A careful observation of the honeycomb grid abrasion allowed the examiner to connect it to a specific part of the shotgun stock -— the so called "recoil pad." A massive subdural, intraparenchymal, and intraventricular hemorrhage, diffuse to the basal cisterns, was found. No histological-specific evidence was reported, except for encephalic samples that exhibited perineuronal and perivasal edema and the presence of a massive hemorrhage of gray and white matter.

Death was attributed to a traumatic brain injury as the result of repeated blows to the head by a blunt instrument; the skin on the right side of the face reproduced the pattern of recoil pad, and on the left side, the herringbone-shaped drawing of the stone floor where the man had been lying. In conclusion, the detailed and careful examination of the injuries and other autopsy findings contributed significantly to arriving at the cause and unusual manner of death due to the back side of a shotgun.

Shotgun Deaths, Firearm Homicides, Crime Scene Investigation

E38 The Characterization of Volatile Organic Compounds (VOCs) Present in the Headspace of Decomposing Animal and Human Remains

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After attending this presentation, attendees will better understand a pretreatment method used for analyzing decomposition odor and VOCs identified in human remains.

This presentation will impact the forensic science community by stressing the importance of odor in evidence.

Locating human graves is important for crime solving and archaeological purposes. The development of tools and methods that can expedite locating clandestine graves has been of keen interest in the scientific community. The efforts focused on establishing the volatile chemical signature of compounds that could indicate the presence of buried human remains where surface clues are lacking and where the content of a potential grave is unknown.

The VOCs present in the headspace above partially decomposed animal tissue samples were analyzed and directly compared with results from decomposed human tissues using established Solid-Phase Micro Extraction (SPME) and Gas Chromatography/Mass Spectrometry (GC/MS) methods. The source of variation between samples was evaluated using the Analysis of Variance (ANOVA). ANOVA can assess whether altering the controlled factor, such as flow rate or material, produces a significant difference in the amount of compound collected compared with the differences found in replicate samples.

Specifically, five class prediction methods were employed, including Partial Least Squares Discrimination (PLSD), Support Vector Machine (SVM), Decision Tree (DT), Naïve Bayes, and Neural Network. VOCs present in the headspace of different animal tissue samples (muscle, fat, and skin) from a cow, a pig, and a fish were identified and compared with human samples. Although there were compounds common to both animal and human remains, the VOC signatures of each of the animal remains differed from those of humans. Of particular interest was the difference between fish and humans, because it is economically easy to obtain fish rather than beef and pork in Korea. Five fish VOC signatures (3-Udecen-2-one, Pentadecane, 3,5-Octadien-2-one, (1) 2(3H)-Furanone, dihydro-5-(2-pentenyl)-, (Z)-(.+ -.)-, 1,4-Cyclohexanedione) were not found to be a subset of animal and human; in addition to detecting only two ((1) 2-(2-Methylpropyl)-3,5-di(1-methylethyl)pyridine, (1) Benzene propanoic acid) of eight human-specific compounds, an additional one unique VOC (1,4-Dimethyltetrasulfane) was recorded from cow, pig, and human samples, which were not present in fish samples. Best decomposition odor class discrimination outcomes have been achieved by PLSD, SVM and DT.

Identifying VOCs that are unique to humans may be useful to investigate human-specific scent and may eventually lead to an instrument that can detect clandestine human burial sites. Further studies are required to routinely retrieve forensic information that is imprinted in VOCs of human decomposition, such as a variety of part of the body, including large-scale sampling of decomposed animal and human remains, to support criminal investigations. The last step of this study is to determine which compounds are generalized decomposition by-products and which are human-specific.

Decomposition, Odor, VOCs

E39 Collaboration of Forensic Disciplines Used to Solve a 13-Year-Old Homicide

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After attending this presentation, attendees will understand the importance of having multiple disciplines available to assist law enforcement with a thorough death investigation. These disciplines assist law enforcement by allowing them to utilize their equipment, knowledge, and expertise. Attendees will also recognize that non-forensic experts should be considered in complex cases in which the skills or equipment necessary are beyond law enforcement's capabilities.

This presentation will impact the forensic science community by illustrating the value of multi-agency cooperation and is intended to encourage communication between law enforcement and all forensic disciplines.

This presentation looks at a death investigation from 2004 resulting in the excavation of a homicide victim who was reported missing in 1991. A tip led law enforcement to believe that his body was buried on property that was once owned by another man. After a warrant was obtained, several disciplines were contacted to assist with locating, excavating, and identifying the remains: the archaeologist with the Office of State Archaeology Research Center; a geologist with Geo Solutions Limited, Inc.; an anthropologist and forensic pathologist with the North Carolina Office of the Chief Medical Examiner (OCME); the Fayetteville Police Department; and the State Bureau of Investigation.

To scan the area for possible burial sites, the yard was separated into smaller zones. These zones were also searched by cadaver dogs, then by the geologist, using a multifrequency electromagnetic profiler and ground-penetrating radar, to identify areas that would be consistent with a burial site. A noticeable depression was observed beside a storage building that was erected during the time that the potential suspect owned the property. The archaeologist carefully removed soil to be sifted for skeletonized remains. He also determined that the perimeter of the grave had markings consistent with two types of digging tools. Studying the soil and chunks of cement, and with the limited number of bones found, suggested that most of the remains had been removed and the grave refilled.

The anthropologist and forensic pathologist were in attendance to assist with recognizing and identifying bones and bone fragments. All the remains were sent to the OCME for processing and identification. A portion of the maxilla was discovered within a chunk of cement and was carefully removed. Also recovered was the left portion of the mandible, containing teeth with restorations and a wire from surgery for a mandibular fracture. Several other small bones and fragments were recovered.

The teeth within the mandible and maxilla were used for identification. The bones retained a total of 11 teeth, 6 of which contained restorations. The written antemortem dental chart, which included a diagram of restorations, was compared to the decedent's teeth and postmortem dental chart. The repair wire from the mandible was also used for identification by comparing antemortem and postmortem radiographs. These findings confirmed that the remains were that of the missing man.

The suspect was charged with first-degree murder and pleaded guilty to voluntary manslaughter, claiming he shot the decedent in self-defense. On April 24, 2006, a judge sentenced him to three years in prison. The rest of the victim's remains have yet to be found.

When law enforcement establishes connections to agencies possessing a variety of specialized resources, they can request assistance with difficult cases, expediting the recovery of human remains. In this example, the thorough investigation led to the recovery of remains, a conviction, and closure for a family.

Excavation, Homicide, Cooperation

E40 Suture Embolism to the Left Superior Lobar Pulmonary Artery: A Case Report and Review of the Literature

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After attending this presentation, attendees will be familiar with the various types of exogenous emboli that may be encountered at autopsy. Furthermore, attendees will gain an understanding of the clinical situations that are associated with the presence of exogenous emboli.

This presentation will impact the forensic science community by increasing awareness of a rare and often bewildering autopsy finding. Moreover, knowledge of the mechanisms of embolization will enhance appreciation for and encourage familiarity with modern surgical techniques, which inescapably impact the forensic science community.

While sources of endogenous emboli, such as thrombus, fat, and tumor are well known, exogenous substances are less commonly encountered and, therefore, less frequently discussed. Modern surgical techniques, both invasive and minimally invasive, allow for the introduction of foreign material into the bloodstream. For example, iatrogenic emboli, such as Inferior Vena Cava (IVC) filters, guidewires, stents, and embolization coils, have been encountered in the surgical literature.[1] These types of emboli may or may not result in patient symptomatology. Other rarer causes of exogenous emboli include percutaneous closure devices, biological glue, and surgical suture.[2-6] When involving critical vessels, emboli cause symptoms and may be, as in this case, fatal.

This case report recounts the autopsy findings of a 58-year-old man with a history of prolonged surgical immobilization provoking deep vein thrombosis and bilateral pulmonary emboli. He had undergone an exploratory laparotomy following a fundoplication complicated by splenic injury and challenging vascular hemostasis. Autopsy revealed a large cavity within the splenic bed filled with turbid fluid consisting of clotted blood and surgical gel-foam. The cavity was lined by omental adipose tissue studded with surgical suture demonstrating fat necrosis. Within the pulmonary vasculature were extensive pulmonary arterial thromboemboli of varying chronicity as well as surgical suture emboli within the left superior lobar pulmonary artery.

Of the handful of existing reports on exogenous emboli, even fewer of which are postmortem evaluations, this is the first case of suture embolus to the pulmonary arterial system. Though the cause of death was not directly influenced by the suture embolus but rather the extensive pulmonary thromboemboli, the presence of the suture embolus was a surprising and an initially perplexing finding; however, an understanding of the clinical, namely surgical, situations around which previously reported exogenous substances were introduced into the vasculature elucidated the likely mechanism of entry.

In conclusion, embolism of exogenous material is a rare but potential finding at autopsy of which pathologists should be aware. A familiarity with modern surgical techniques enhances understanding of potential mechanisms of embolus formation.

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Suture, Embolus, Pulmonary

E41 The Risk of Selfie-Related Deaths: A Case Report on the Dangerous "Daredevil Selfie" Phenomenon

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After attending this presentation, attendees will understand the term "daredevil selfie" and the phenomenon of selfie-related deaths.

This presentation will impact the forensic science community by describing this phenomenon as it is publicized on the internet and represents an emulative, dangerous trend.

"Daredevil selfie" represents a trend of recent development. It deals with taking a selfie in places at high elevation (i.e., skyscrapers, roofs) or in dangerous places such as railway tracks during the passage of the train. The phenomenon gained fame on social networks and was copied by others. Contrary to its broadcasting by mass media, it is a phenomenon insufficiently described in literature. From 2014 to the first half of 2016, 75 persons died of selfie-related accidents. The medium age of victims was approximately 23 years and the 82% were male. Forensic cases of selfie-related death have not been reported in literature until recently. The last case that occurred in Italy will be discussed.

This is the case of a boy found dead on the railway track after being run over. A judicial inspection was checked in a raised iron railway bridge. On the way from Taranto to Reggio Calabria, the mutilated body of a boy was found. Photographic surveys were conducted and due to the railway police and forensic investigators, the entrances and exits were recorded. A cellphone and a left shoe were found approximately two meters from the victim. The cellphone was given to detectives to conduct an investigation of its contents. The body presented a very extended laceration with the exposition of cerebral matter and skullcap fragments spread over the point of impact. Cerebral matter fragments were also found 15 meters from the point where the body was found, delimiting the "primary impact point" where the train collided with the body.

By reconstructing the accident, information emerged that the boy impacted the iron piers of the bridge on the railway track, was dragged forward up to the point where the body hit against another iron structure and stopped. Found during the autopsy were traumatic brain injuries with fractures of the skull cap on the right of the occipital bone; multiple ecchymosis from the left mammillary region to the right haunch; fracture of the right tibia and perone; and a cut wound on the back with soft tissue exposure. A psychological autopsy was conducted to better reconstruct the motivations linked to the event. The method involved thorough structured interviews and the collection of all available information concerning the deceased. After informed consent, face-to-face and structured interviews with family members of the victim were conducted. A survey was conducted on the victim's phone to analyze messages, photos or videos, and conversations via WhatsApp and his Facebook® account. By comparing data, it emerged that the victim had his back to the train trying to take an extreme selfie by holding the phone in his hand; when the boy noticed the closeness

This case report represents the last registered account regarding the phenomenon of the daredevil selfie. Unfortunately, the practice of extreme selfies is now rampant and represents an imitative, dangerous phenomenon as it is publicized on the internet. It is important to watch minors, who represent the most affected age bracket, with the help of social, scholastic, and family assistance to inform them that this is a very dangerous phenomenon in terms of mortality.

Forensic Science, Daredevil Selfie, Death

E42 A Nunchaku Strangled Woman: A Case Report

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The goal of this presentation is to provide a multidisciplinary approach to the crime scene and the reconstruction of the facts requested. The time between death and the discovery of the body can make it difficult to understand external injuries correctly.

This presentation will impact the forensic science community by demonstrating a multidisciplinary approach to the crime scene and the reconstruction of the facts, especially when the manner of death is not clear.

This case report concerns a murder by strangling, in which the murderer tried to simulate a domestic accident. The device used was quite unusual, a nunchaku, a traditional weapon common in East Asian countries, consisting of two short wooden clubs connected by a chain or rope. This weapon usually inflicts blunt force against different parts of the victim's body but, in this case, it was used as an improvised noose placed around the neck of the victim. The macroscopic characteristics of cutaneous compression sulcus, its vital characteristics illustrated by immunohistochemistry, and the discovery of the weapon of which detailed investigations of forensic genetics were performed, confirmed the homicidal occurrence.

A middle-aged man alerted the police and the health emergency service, stating that his wife fell from a ladder a few days before while cleaning and died. The man reported to the police that, in view of his state of shock, he did not have the strength to ask for help before, so he stood next to his wife's corpse for at least two days. At the crime scene, investigators found an overturned armchair in the bedroom and many opened furniture drawers. The prosecutor ordered a detailed crime scene investigation. The body was laying in a prone position on the floor, near a metal ladder, in an advanced stage of putrefaction; however, a transverse, complete cutaneous compression sulcus, presenting multiple rectangular excoriated areas along its course, was observed around the neck. No evident cranio-facial injuries were detected. The apartment was thoroughly searched. During this operation, a homemade broken nunchaku was found, with hairs on it. The prosecutor requested an autopsy to determine the manner and cause of death. A postmortem Computed Tomography (CT) scan was performed. Bone and visceral injuries were excluded. The autopsy exhibited mild pulmonary edema, as well as the presence of white foam in the main airways. The histological examination of skin specimens taken from the ligature mark revealed intra-epidermal mild erythrocytes reactions and musculature alteration such as "Zenker's necrosis." In addition, the immunohistochemical staining method on skin samples was performed utilizing anti-tryptase antibodies, IL15 and CD15, which confirmed the vitality of the sulcus. Genetic investigation revealed a match between the DNA extracted from the hairs found on the nunchaku and the DNA of the dead woman. The cause of death was attributed to acute asphyxia due to strangulation by the nunchaku metal chain. The prosecutor interrogated the husband of the deceased, who confessed to the homicide as well as the supposed modality. The husband also confessed that he waited for putrefactive process to mask the signs of strangulation.

It seems evident that a multidisciplinary approach to the crime scene and the reconstruction of the facts should be requested, especially when the manner of death is not clear, or the time between the death and the discovery of the body can make it difficult to understand external injuries correctly.

Nunchaku, Strangulation, Homicide

E43 The American Board of Forensic Taphonomy (ABFTaph): A Multidisciplinary Approach to Decomposition

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After attending this presentation, attendees will have a better understanding of forensic taphonomy, the ABFTaph, and the benefits of joining the ABFTaph.

This presentation will impact the forensic science community by introducing an inclusive organization that helps bring the smaller forensic science disciplines together, by recognizing the need for a multidisciplinary approach to research, and by providing, in the interest of the public and the advancement of the science, a program of certification, procedures, best practices, and protocols in forensic taphonomy.

Forensic taphonomy " … refers to the use of taphonomic models, approaches, and analyses in forensic contexts to estimate the time since death, reconstruct the circumstances before and after deposition, and discriminate the products of human behavior from those created by the earth's biological, physical, chemical, and geological subsystems."[1] This includes, but is not limited to, the forensic disciplines of physical anthropology, botany, climatology, entomology, geology, pathology, and soil science.

Because there is a need to identify forensic scientists qualified to provide essential professional services for the judicial and executive branches of government and because many of the disciplines listed above have a small number of practitioners without parent organizations available for stating and verifying their competence or their scientific standards, we have established a multidisciplinary board called the ABFTaph This is a non-profit, incorporated professional board which establishes, enhances, and revises as necessary standards of qualification for those who practice forensic taphonomy, and to certify as qualified specialists those applicants who comply with the requirements of the Board. Certification is based upon the candidate's personal and professional record of education, training, experience, and achievement, as well as the results of formal examinations. There are two examinations: a general exam for entry into the organization, and a discipline-specific exam to demonstrate competence in an individual's field.

The membership tiers are based on experience and include many of the same levels as the American Academy of Forensic Sciences, including a student category as a means to educate new generations of forensic scientists in an inclusive organization that is focused on the teamwork generally involved in forensic science research and cases.

Additionally, to maintain the integrity of the organization, the Board has requirements for members to provide copies of all case reports (after adjudication of associated case) and will maintain an open database of these reports with post-hoc review as a requirement for re-certification.

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Taphonomy, Decomposition, Forensic Science

E44 The Application of Eukaryotic Community Succession on Porcine Remains for Postmortem Interval (PMI) Estimation

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After attending this presentation, attendees will understand how changes in the structure of eukaryotic communities found on decomposing remains may aid in the estimation of the PMI.

This presentation will impact the forensic science community by increasing their understanding of methodology currently in place and their awareness of emerging forensic techniques. This presentation will also provide recommendations as to how investigators may better approach crime scenes to preserve and collect evidence for use in necrobiome sequencing. Finally, this presentation will highlight how the application of next generation sequencing may change the way in which postmortem interval is determined by providing a supplemental technique to traditional estimation methods. This presentation will also highlight a novel area of research that provides a useful alternative to traditional PMI estimation techniques by employing next generation sequencing. Furthermore, this study explores the use of eukaryotic communities for such estimations, an area which has not yet been fully explored.

Every cadaver is the host to a complex mixture of prokaryotic and eukaryotic communities, collectively referred to as a necrobiome. Since necrobiomes respond to environmental changes in predictable patterns during the decomposition process, it is possible to use necrobiome succession as a "microbial clock" for PMI estimation.[1] Several recent studies have used bacterial and eukaryotic community succession on murine, porcine, and human remains for PMI estimation; however, these studies either had limited replications or were conducted in a laboratory environment.[2-5]

The main goal of this study was to determine eukaryotic community succession associated with skin of porcine remains for long-term PMI estimation (>1,500 Accumulated Degree Days (ADD) or >60 days) in well-replicated (n=6) field conditions. To accomplish this goal, six sets of porcine remains were allowed to decompose in field conditions for 62 days. Samples were collected by swabbing the surface of the skin at the lateral thoracic and lateral abdominal regions every day for the first week, every alternate day for the second week, and once every week after the second week, for a total of 96 samples. Microbial DNA was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) bead-mill extraction process. Sufficient DNA yields for all samples were determined using a Qubit® 2.0 Fluorometer.

Hypervariable region V9 of the 18S recombinant DNA (rRNA) gene was amplified according to the Earth Microbiome Project protocol (http://www.earthmicrobiome.org/protocols-and-standards/18s/) and amplified products will be sequenced on the Illumina® MiSeq® FGx platform using a dual-index strategy. Sequence data will then be used to perform taxonomic identification and applied to a statistical model for PMI prediction. It is expected that the changes in eukaryote community structure seen during decomposition on a porcine model may parallel those seen in a human model, given the similarities between the two organisms.

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Necrobiome, Postmortem Interval, Next Generation Sequencing

E45 The Taphonomic Effects of Differential Burial Practices and Environment in Recently Discovered World War II Cemeteries

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After attending this presentation, attendees will: (1) gain further knowledge concerning the postmortem taphonomic effects of differential interment practices on human remains recently discovered in a coral atoll burial environment from World War II cemeteries; (2) view and learn the taphonomic effects of field-expedient trench burials and burial box interments on human skeletal remains due to environmental factors, such as ground water fluctuations and bioturbation; and, (3) gain a better understanding of the challenges faced by archaeologists and anthropologists dealing with the two largest independent recoveries to date of missing World War II United States servicemen (*MNI*=59).

This presentation will impact the forensic science community by describing unusual taphonomic effects on human remains in variable environments associated within and between burial interment practices in the historical context of World War II. This presentation will contribute to the overall knowledge of the forensic science community by describing *in situ* field discoveries and laboratory analyses documenting unique effects of taphonomic variables on human remains in a battlefield context.

Description of the site and sample: During the Second World War, the Battle of Tarawa was a costly victory for the United States military. More than 1,100 United States Marines and Navy sailors were killed, while approximately 500 were recorded as missing and their bodies believed "unrecoverable." Due to the speed of military operations at the time, the locations of the hasty post-battle cemeteries were lost or misplaced. Additionally, a number of Army Air Corps servicemen flying missions from Hawkins Field on Betio Island, Tarawa, perished in the months after the initial invasion. These servicemen were buried in individual burial boxes and placed in burial rows at a variety of depths and were subsequently affected by various taphonomic factors. In 2015 and 2017, two of the missing cemeteries containing United States Marines, Navy sailors, and Army Air Corps servicemen were discovered and systematically excavated by History Flight, a Non-Governmental Organization (NGO). As of July 25, 2017, History Flight's efforts have led to the repatriation of more than 59 lost United States servicemen from these cemeteries.

The unique coral atoll environment creates taphonomic factors that cause differential preservation of human remains within and between cemeteries and burial interment practices. Specifically, the types of interment practices, differential burial depths, impact of a constantly fluctuating water table, proximity to military-issued equipment (particularly the rubberized canvas poncho), containment within burial boxes, and tree root disturbance contribute to diverse states of skeletal preservation. Combined with historic and modern post-burial disturbances and an absence of scavenger activity, the burial contexts from these cases demonstrate unprecedented and valuable information for the forensic science community.

Taphonomy, Burial Practices, WWII

E46 Veteran Suicides in Harris County, Texas

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This goal of this presentation is to examine patterns of veteran suicides in Harris County, TX, and compare those patterns to existing veteran suicide publications.

This presentation will impact the forensic science community by demonstrating that accurate data regarding veteran suicides is critical to the development and improvement of suicide prevention programs.

The Department of Veterans Affairs (VA) has published a series of Suicide Data Reports beginning in 2012. The data presented in these publications is in part intended to inform the development of a suicide prevention program. The VA enlisted assistance from state governments to obtain the data presented in these reports. The 2016 report estimates that, in 2014, 20 veterans died each day by suicide, and that veterans account for 18% of suicide deaths in the United States (8.5% of the population). More than 200,000 veterans live in Harris County, making it one of the largest populations of military service members in the nation. This county averages approximately 450 suicides per year, but there is no current means to readily account for the fraction of those suicides that are committed by veterans, and whether that number aligns with the data presented by the VA.

Historically, the Harris County Institute of Forensic Sciences (HCIFS) did not track the military/veteran status of its suicide (or other) cases; however, in July 2016, the HCIFS began: (1) actively tracking the military/veteran status of all reported fatalities; and; (2) comparing HCIFS suicide data to data maintained by the Department of Defense (DOD) veteran database and the Texas Electronic Death Registry. This presentation will provide descriptive statistics regarding the discord between the various agencies that either generate or maintain veteran suicide statistics and its impact on the comparison of a large urban center (Harris County) with a large veteran population to other locations, and/or national statistics.

A cross-comparison of all HCIFS suicides between 2010 and 2016 to the Texas Electronic Death Registry and the DOD veteran database yielded a total of 497 suicide cases that were identified as veterans in one or both. Of those cases, 332 were identified in both databases, the remaining 165 were listed in one or the other, but not both. Four hundred twenty-six of HCIFS suicides during the same interval were listed as veterans in the DOD veteran database; however, veteran status of 94 of these deaths was not recorded on the death certificate. Similarly, 403 cases were reported as veterans by the Texas Electronic Death Registry, 71 of which were not included in the DOD database. The total number of known veteran suicides in Harris County between 2010 and 2016 (497) represents 15% of all suicides in Harris County during that interval. The number of suicides recorded on death certificates (without comparison to the DOD data) during the same interval who are veterans is 13%. The percentage of Harris County suicides recorded on the death certificate and who are listed as veterans in the DOD database is 10%. Each of these percentages is below the national average reported by the VA. Data from other counties and states would need to be collected and verified using this method in order to generalize the findings to the larger population.

A system for accurately detecting and tracking suicides among all veterans needs to be developed. In order to assess the effectiveness and improve suicide prevention programs for veterans, a more accurate reflection of the number of veteran suicides is imperative in order to assess effectiveness of the prevention programs. This would provide the capability of analyzing more current data as well as other characteristics of veteran deaths. Although much research has been completed on veteran suicides, the statistical data measuring the number of suicides is inadequate.

Veterans, Military, Suicide

E47 A Multidisciplinary Search for Missing People: Psychology, Canine Units, and Forensic Archaeology

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After attending this presentation, attendees will understand how the search for missing people is a challenging task that could be solved with a multidisciplinary approach involving the cooperation of different disciplines.

This presentation will impact the forensic science community by presenting a case report that demonstrates the potential of the cooperation between psychology, canine units, and forensic archaeology during different steps of the search for missing persons.

Missing persons cases demonstrate several levels of criticality and the police have limited resources; if the person (or the body) is not recovered in a short period of time, protracted searches are not sustainable. These cases pose complex challenges that need to take into consideration the background of the disappearance, the physical and psychosocial conditions of the missing, and the environment in which the event occurred. Therefore, a multidisciplinary approach is crucial to improve existing methodologies and develop operative protocols to both optimize investigation time and cost and to increase the number of successful recoveries.

This study will present an exemplar case from Sardinia, Italy, that involved a 78-year-old male affected by Alzheimer's disease and diabetes. In May 2015, the individual left home in his car. The vehicle was found abandoned on a mountain road, and this location was designated as the Initial Planning Point (IPP).

The survey was organized by two associations: Ophir Criminology and the National and European Multidisciplinary Equipe for Scientific Investigation (NEMESI). The operative units have been remotely coordinated by a forensic anthropologist from Liverpool John Moores University.

Ophir Criminology is a scientific association devoted to the development of forensic sciences and the dissemination of good practices for investigation. NEMESI is a multidisciplinary team that applies archaeo/anthropological techniques for a comprehensive survey of the area in conjunction with inspection by canine units (man-trailing and cadaver dogs).

The first step of the investigation was data collection from the missing person's family. Testimony psychology was used to evaluate both the reliability of witness statements and the interviews on the period surrounding the disappearance. The collection of the preliminary information is the most critical aspect that can influence the entire investigation. A shallow approach during these early stages of the investigation can jeopardize the search for evidence, losing, in some cases, possible links to major offenses (e.g., kidnapping or murder). The most useful details must be recorded and screened according to the reliability of each witness. The acquired data were, therefore, carefully evaluated to immediately assess the risk level of the case and to plan the field search by selecting the most appropriate tools.

The first set of searches with man-trailing dogs was performed from the IPP immediately after the disappearance. According to international protocols, the possible distance from the IPP was evaluated by considering the environment (farmland vs. urban) rather than the psychological condition of the individual. In addition, the physical condition of the individual, as evaluated through witness statements as opposed to medical records, was taken into account.

According to the data processed, the first search performed with NEMESI's canine units allowed some areas to be excluded, thus narrowing the search areas to be covered by the subsequent walking surveys.

The discovery of some personal belongings allowed the team to pinpoint a large area of interest that was accordingly checked with cadaver dogs. The canine unit ruled out the majority of the area, and only a small number of zones were not processed due to environmental conditions (dense vegetation and impervious surface). In the remaining areas, an extensive grid search was planned and performed with volunteers trained in field walking. The search was successful and the remains of the missing person were discovered, demonstrating the potential of a multidisciplinary and planned search that involved psychology, forensic archaeology, and canine units.

This study proposes an innovative and multidisciplinary approach that utilizes dog/handler units in the search of a missing person in conjunction with archaeological survey and testimony psychology. Therefore, this contribution also stresses the need for training on-foot volunteers in basic field walking techniques and the correct approach to the *scena criminis* (i.e., preserving potential evidence, use of PPE) to prepare operative units supporting the judicial authority in the search for the missing.

Missing People, Man Trailing Dog, Cadaver Dog

E48 The Current State of Illicit Drugs, Counterfeit Pharmaceuticals, and New Psychoactive Substances in West Asia — Particularly Turkey

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After attending this presentation, attendees will better understand: (1) the influence of developments in the opiate market in Afghanistan on the drug control situation in West Asia, particularly in Turkey; (2) the importance of the Balkan route as one of the main trafficking routes of opiates out of Afghanistan; and, (3) the main issues of concern for the region and for Turkey.

This presentation will impact the forensic science community by providing an update on the current state of illicit drugs, counterfeit pharmaceuticals, and new psychoactive substances in West Asia, particularly Turkey.

Nearly all heroin available on the illicit drug markets in Europe originates in Afghanistan. Despite the diverse trafficking routes, the main one remains the so-called Balkan route, with Turkey serving as a starting point of the main corridor for trafficking bulk quantities of Afghan heroin via the Islamic Republic of Iran to Bulgaria and through countries in the western Balkans to Western and Central Europe, or from Bulgaria through Romania and Hungary to Western and Central Europe.

In 2014, seizures of heroin and morphine along the Balkan route amounted to 48 tons, an increase compared with the quantity seized in previous years. The largest quantities were seized in the Islamic Republic of Iran (24.4 tons) and Turkey (8.3 tons). Recent reports indicate that other routes are gaining in importance. In 2015, Turkey observed the emergence of a second route, encompassing Iraq and the Syrian Arab Republic, in addition to the countries traditionally lying on the Balkan route.

Turkey reported a significant amount of seizures involving MDMA or “ecstasy” -type substances, namely more than five and one-half million tablets in 2015, as well as seizures of LSD.

A substantial challenge with respect to psychotropic substances in the region and Turkey remains the supply of the so-called counterfeit “captagon” tablets that continue to be seized in large quantities, especially in countries in the Gulf and Middle East. “Captagon” was originally the official trade name for a pharmaceutical preparation containing fenethylline, a synthetic stimulant. The substance currently known as “captagon” are tablets similar in appearance containing amphetamine cut with multiple adulterants, such as caffeine and other substances. In 2015, Turkey reported seizures of more than 15 million tablets. There is no other synthetic drug seized as regularly in such quantities. Other countries that reported large so-called “captagon” seizures include Lebanon and Saudi Arabia.

Turkey was also one of the few countries in West Asia reporting large seizures of synthetic cannabinoids often advertised as legal replacements for cannabis (more than 500kg) in 2015.

More than 200,000 persons are estimated to be in need of treatment for drug abuse in Turkey. In 2015, Turkey reported an increase in the residential treatment of methamphetamine addicts, a slight decrease in the overall number of persons injecting drugs (mainly heroin), some increase in the use of amphetamine-type stimulants and tranquillizers, as well as opioid-containing pharmaceuticals. More than half of overdose deaths involved polydrug use, with half associated with opioid and one-third with amphetamine-type stimulants and/or cannabis use.

Illicit Drugs, New Psychoactive Substances, Turkey

E49 Medicolegal Death Investigation in the Changing Face of Drug Overdoses

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After attending this presentation, attendees will have a new understanding of the impact of the current opioid/opiate crisis on Medicolegal Death Investigation (MDI) and evolving investigative paradigms involving scene responses, decedent history evaluation (including internet and social media use), and safety concerns.

This presentation will impact the forensic science community by illustrating the rising number of overdose deaths and that the increasing potency of the drugs involved has caused a paradigm shift in MDI in Cuyahoga County, OH.[1] The basic investigative elements of history collection, scene investigation, and scene safety have all been impacted. The resulting developments have involved both the public safety and public health roles of the office.

Cuyahoga County (metropolitan Cleveland), OH, has a population of approximately 1.3 million people. The Medical Examiner's Office (MEO) investigates all sudden, unexpected, suspicious, and violent deaths, including all deaths by intoxication with drugs. In 2006, there were 250 total Drug-Related Deaths (DRDs), primarily involving cocaine and prescription opioids. Over the following years, heroin played an increasing role in overdose mortality and this trend peaked in 2014 when there were 353 DRDs, with the majority involving heroin. Following this, the drug crisis in Cuyahoga County acutely worsened with the appearance of fentanyl and the fentanyl analogues (e.g., carfentanil). Mortality reached 666 DRDs in 2016 and is currently projected to continue rising in 2017. In the majority of DRDs, the decedent is pronounced dead at the scene and an MEO investigator responds to the scene. All investigators are certified by the American Board of Medicolegal Death Investigators.

The initial telephone report is now approached with a higher index of suspicion for the possibility of a DRD. Scene details, including body position, items presently at scene, and circumstances, determine whether jurisdiction will be accepted and a scene visit is required. Apparent natural deaths require adequate follow-up questions and some jurisdictions have even adopted universal checks of the prescription drug-monitoring program data prior to releasing a case.

Scene visits have evolved in important ways too. Because of the increased prosecution of DRDs, a task force was created to process the overdose death scenes as potential crime scenes. This has required the collaboration of the MEO death investigator, narcotics detectives, and prosecutors. The handling of evidence has also been transformed with increased focus on chain of custody as well as safety concerns. The changes in drug potency have led to mandatory use of personal protective equipment at scenes (minimum of gloves and face mask), as well as investigators carrying naloxone (the opioid antagonist) in the event of an untoward exposure. These safety concerns extend into CCMEO itself when drugs previously undiscovered may place receiving personnel at risk.

History collection at the death scene has expanded. Routine questions regarding types of drugs used and routes of administration have been supplemented by inquiries regarding recent sobriety, incarcerations, or rehabilitation in a treatment facility.

Family are asked about recent purchases and shipments (especially from overseas or from the internet), social media, and cellphone data that may also provide valuable information and must be evaluated.

The role of MDI in opiate/opioid deaths is evolving and expanding. As the drug overdose crisis worsens, medicolegal investigators need to adapt practices to emerging trends and to collaborate with other investigative partners to produce optimal results.

Reference(s):

1. Gilson T.P., Stopak J., Medicolegal Death Investigator Field Guide, Investigative Unit, Cuyahoga County Medical Examiner's Office, 2015.

Designer Fentanyl Analogues, Death Investigation, Opioid/Opiate Crisis

E50 Attacking the Epidemic: Methods and Considerations for Detection of Fentanyls and Novel Psychoactive Substances (NPS) by Thermal Desorption Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

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After attending this presentation, attendees will have a better understanding of the strengths, limitations, and safety concerns of employing ambient ionization MS techniques, such as DART®-MS, to the screening of evidence for fentanyl, fentanyl analogues, and other NPS. Attendees will be presented with typical spectral signatures for these compounds, instrument optimization parameters to consider, methods for identifying the limitations posed by competitive ionization (to minimize missed detections), and considerations for analyst safety.

This presentation will impact the forensic science community by providing necessary information for the implementation, strengths, and weaknesses of ambient ionization MS techniques, such as DART®-MS, for the analyses of hazardous chemicals, such as fentanyl.

The increasing prevalence of fentanyl, fentanyl analogues, and other NPS in the community poses significant challenges to law enforcement, first responders, and forensic practitioners. Because of the significant hazards these compounds present, current methods of presumptive screening may no longer be practical. Methods such as color tests that require visible amounts of material have been deemed too dangerous by some agencies (due to inhalation exposure hazards), leaving a gap in the typical analysis chain.

One technique that may fill this gap is Ambient Ionization-MS (AI-MS). These instruments, namely DART®-MS, are being increasingly employed in forensic casework because of their ease of use, minimal sample preparation, and speed of analysis. While these instruments are not ready for deployment into the back of a police cruiser or crime scene van, they have been critical in providing rapid presumptive analyses in forensic labs.

While DART®-MS and other AI-MS techniques show promise as rapid screening tools, the manner in which sampling is commonly completed poses potential risks to examiners. Inhalation of aerosolized samples and the potential for contamination of the instrument are distinct possibilities with techniques such as DART®-MS. This study will address measures that can be taken to limit exposure of the analyst and minimize contamination of the surfaces, providing a safer means of analysis.

This study focuses on identifying the capabilities of Thermal Desorption (TD) -DART-MS, an ambient ionization MS technique, for the detection of fentanyl, fentanyl analogues, and other NPS. More than 20 fentanyl analogues and 25 additional NPS were studied. Using pure compounds and a design-of-experiments approach, a method was created to evaluate analytical metrics of the technique. As observed with many other narcotics, protonated molecular ions are the predominant ions formed, and limits of detection are commonly in the tens to hundreds of picograms. Additionally, considerations for the detection of realistic samples, which commonly exist as multi-component mixtures, will be discussed. Since real-world samples commonly consist of multiple components (i.e., multiple drugs, analogues, and cutting agents), it is necessary to understand how the presence of these compounds will affect detection of the target compounds, given their small weight percentages. Using a combination of prepared and street samples, the limitations of TD-DART®-MS in detection of these minor components and as potential scenarios when detection may not be possible (false negatives) will be presented. Work toward creating a library of these compounds based on representative spectra will also be discussed.

Fentanyl, DART®-MS, Drug Analysis

E51 Side Effects of Anabolic Androgenic Steroids Abuse: What About Necrotizing Fasciitis?

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The goal of this presentation is to illustrate how widespread Anabolic-Androgenic Steroid (AAS) consumption can lead to the development of a wide spectrum of side effects, including necrotizing fasciitis, even if it is rare. Thus, in suspected cases, an autopsy should be performed with a complete biological sampling because of the substantial role of toxicology to define the cause of death and serve justice.

This presentation will impact the forensic science community by demonstrating the impact of the widespread AAS abuse in the world. This presentation will also stress the importance of developing the knowledge of pathological processes and mechanisms of organ damage related to AAS consumption, which can lead to death. Toxicological investigations can identify AAS use in suspected patients and should be performed routinely. This allows physicians to perform tailored treatments.

Previously used by elite athletes, currently AAS use has developed among the general population. The Centers for Disease Control and Prevention (CDC), in their thirty-third report on the health status of the United States, reported that approximately two million individuals use or have used AASs during their life only in the United States. In Italy, AAS consumption began to affect social sensitivity. Last year in Foggia, the death of a sports figure led police investigations into the requisition of substances in many gyms in town.

This case reports a 31-year-old weightlifter who went to the emergency ward referencing an accidental fall with left thigh trauma. Radiologically, a hematoma was detected at the left thigh level with no fractures. The patient refused further investigation. Two days later, this sports figure went to another emergency ward for lower limb edema and hyperpyrexia (38°C). A new imaging evaluation revealed extended dermal emphysema into the left gluteal. Lab results suggested a diagnosis of Multi-Organ Failure (MOF) and septic shock from traumatic left thigh necrotizing fasciitis. Surgical removal of necrotic tissues and fasciotomy were provided. Although surgery and hyperbaric therapy were performed, the man died.

Before the autopsy was performed, a review of the literature had been conducted: (1) necrotizing fasciitis may develop in the site of skin biopsy, lacerations, insect bites, acupuncture, surgical wounds, skin abscesses, burns, closed bruising trauma, and drug extravasation zones; (2) over the past years, only a few cases of athletes who developed pyomyositis or soft tissue infections were described in association with AAS abuse; (3) during the same period, several studies evidenced a wide range of AAS side effects on organs and systems with supra-physiological doses and immunosuppressive effects; and, (4) in AAS abusers, the use of non-sterile needles or frequent injections has been related to local infections at the injection site.

Thus, the autopsy of the weightlifter was performed with a complete biological sampling for toxicological purposes. Not surprisingly, keratin matrix (hair), approximately 1cm long, showed positivity for propionate testosterone, clembuterol, stanazolol, trembolone, oxandrolone, and tamoxifen, indicative of the intake of the substances detected over a period of 4-5 to 30 days prior to the date of the autopsy. Serum samples, collected during the second hospitalization, detected positivity for stanzolol and tamoxifen; however, the multi-organ dysfunction made it impossible and difficult to quantify the rate of recruitment due to a slowdown in the metabolism of these substances and a reduction in the elimination rate. Police investigation led to the seizure of six propionate testosterone vials at the man's home; subsequent analysis showed it to be the same molecule as the testosterone keratin sample.

This scenario was reconstructed: positivity in the keratin matrix expressed a chronic use of these substances; and chronic use resulted in a state of immunodeficiency, which favored the development of necrotizing fasciitis, from the site of inoculation of the substances, confirmed by histological examination.

This analysis highlights the narrow relationship between AAS abuse and immunodeficiency and is the basis for further studies; however, it should be taken into account that among all dangerous effects produced by AAS use, necrotizing fasciitis is not an unusual consequence.

AASs, Necrotizing Fasciitis, Toxicological Examinations

E52 High Order Trace Transfers: Considerations for the Analysis of Sub-Visible and Nanoparticles

Christopher S. Palenik, PhD, Microtrace, 790 Fletcher Drive, Ste 106, Elgin, IL 60123-4755*

The goal of this presentation is to illustrate both the strengths and considerations that must be taken into account as the size of particles being analyzed decreases to sub-visible and, ultimately, nanoscale evidence.

This presentation will impact the forensic science community by providing a framework upon which increasingly smaller traces can be analyzed and interpreted.

As trace evidence analysts begins to exploit the evidentiary value in finer particles, particularly those that would be considered sub-visible (<1µm-100µm) and nanoparticles (<1µm), the traces become more difficult or impossible to visually monitor. To this end, evidence collection teams, examiners, and evidence custodians must be aware of the potential that collecting, sampling, analyzing, and even packaging may have on the transfer of evidence. In addition, those responsible for the interpretation of such traces must also be aware of the potential for secondary, tertiary, and higher order cross transfers. Maintaining this awareness becomes challenging when the particles are invisible to the unaided eye and even more challenging when the particles cannot be resolved by stereomicroscopy. The use of sample blanks, purpose-built laboratory environments, and more rigorous environmental monitoring represent a few of the factors that must be considered when dealing with smaller particles and finer features. Such considerations are presently less significant when dealing with typical trace evidence samples since current traces can be visually located and tracked throughout an analysis, using either an unaided eye or low-magnification stereomicroscopy.

As a means by which to study the transfer of subvisible particles, a commercially available fluorescent detection spray was examined. This substance is composed of 2µm-5µm particles of zinc sulfide in a light hydrocarbon oil. Zinc sulfide is a fluorescent indicator component that is used as a visual illustration of contact transfers between two objects. Such evidence of contact between objects may be visualized through illumination by long-wave ultraviolet light, which stimulates luminescence in the powder. This research provides a microanalytical characterization of the zinc sulfide particles and offers multiple independent approaches by which this powder (or other fine particles) may be detected and specifically identified in quantities from major to trace.

This definitive identification provides a means by which a field test that presently provides a presumptive result (one that is largely limited to investigative work and is based on a relatively crude visualization scheme) can be converted into a court-acceptable result by providing a specific identification. This identification process can be adapted to the detection of microscopic particles of zinc sulfide, thus increasing not only the specificity of the identification but also the sensitivity of the method. Using an adapted Gunshot Residue (GSR) sampling method and automated Scanning Electron Microscopy (SEM) analysis routine, individual particles of the fluorescent detection spray are readily detected.

While such approaches can increase the sensitivity of a test by three orders of magnitude, they also raise significant questions about cross contamination and higher order transfers (e.g., tertiary and quaternary transfers). This research will provide a demonstration of a tenth-order trace transfer using this detection powder. The potential for such transfers raises significant considerations that become particularly relevant to the analysis of increasingly smaller particles as well as the trace evidence of the type and size presently analyzed in a typical crime laboratory.

Nanoparticle, Clue Spray, High-Order Transfer

E53 Teaching Forensic Image Processing

Marcus Borengasser, PhD, Department of Defense, 3205 Lago Vista Drive, Melbourne, FL 32940*

After attending this presentation, attendees will understand the benefits of designing an online program for training in forensic image processing.

This presentation will impact the forensic science community by illustrating how surveillance video is nearly ubiquitous, but many analysts are not adequately trained for forensic image processing.

Video surveillance is widely used and it is expected to increase in the coming years. Not too long ago, only banks had video surveillance and the video was typically acquired with low-quality cameras, recorders, and media. Presently, surveillance video is widely used by many businesses and institutions, in addition to video from law enforcement body cams, Unmanned Aerial Vehicle (UAV) systems, cell phones, and more. Forensic image processing is rapidly becoming a critical skill for the criminologist.

Education in forensic image processing has not nearly kept pace with the ubiquitous video systems. Unfortunately, many criminalists and crime scene analysts have only basic skills in forensic image processing. A challenging situation is often compounded by a lack of proper software, and criminalists find themselves at a technological disadvantage.

Either online or in a traditional classroom setting, forensic image processing can be offered as a suite of specialized courses within an AA or BS program or as a certificate program. Open source software is available that is suitable for forensic image processing, relieving the educational institution of a software acquisition expense plus a wide range of logistical challenges.

As with any specialized curriculum, courses in forensic image processing would have prerequisites. The most important prerequisite is linear algebra, and courses such as undergraduate physics and computer science would be needed. With the extensive course offerings from Massive Online Open Courses (MOOCs), a student can easily satisfy prerequisites.

Like many other forensic sciences, forensic image processing is procedural and can be dissembled into a series of operations. This process can be presented in a data flow context and courses in the curriculum can be designed and offered to reflect this data flow perspective. This data flow would be based on the operations needed to correct or suppress the typical video image flaws. These flaws result from internal and/or external noise that is superimposed on a video image, improper lighting that causes saturated or dark images, and improper camera calibration and/or emplacement.

Noise suppression or removal can be accomplished with digital filtering. An understanding of this important technique is critical for a video image analyst. An entire course could be dedicated to this topic and include both theory and practice.

One of the methods for minimizing the effect of improper lighting is modifying the pixel brightness histogram. This topic, which would include a variety of techniques, is as important as digital filtering and, similarly, an entire course would be needed to ensure analyst competency.

The other major topic, suppressing or removing the effects of improper camera calibration and/or emplacement, can partially be addressed with digital filtering and histogram modification; however, a third course could be designed for specialty topics, such as feature identification, classification, sensor and data fusion, and more.

Education, Video Surveillance, Image Processing

E54 The Opportunity of a Lifetime: The Educational Outreach Program at the Defense Forensic Science Center (DFSC)

Rachel Creager, 4930 N 31st Street, Bldg 925, Forest Park, GA; and Henry P. Maynard III, MSFS, 277 Edison Drive, Stockbridge, GA 30281*

After attending this presentation, attendees will learn about the DFSC's Educational Outreach Program. This presentation will inform students of the opportunities to participate in cutting-edge research at the DFSC.

This presentation will impact the forensic science community by informing future forensic scientists about options to advance their careers while furthering forensic science capabilities at the DFSC.

The Educational Outreach Program is an integral part of enhancing current and developing future forensic science capabilities at the DFSC. The Educational Outreach Program allows students to participate in meaningful research and highlights careers in the military, while building partnerships between DFSC and academia. Students receive a wealth of experience in experimental design, test and evaluation efforts, validations, and analysis of complex data. The Office of the Chief Scientist participates in various programs that allow students to work at DFSC; including the Department of Defense Science, Mathematics, and Research for Transformation (SMART) program and the Research Associate program. This presentation will benefit prospective students as it will highlight these career-developing opportunities (a brief summary of each is provided below).

The Office of the Chief Scientist coordinates the Department of Defense-funded SMART program. The goal of the SMART program is to increase the number of civilian scientists and engineers who are employed by Department of Defense laboratories. To accomplish this, students at any level within their academic career (Bachelor's, Master's, or PhD) can apply to the SMART program to receive a full academic scholarship and employment at a Department of Defense laboratory upon degree completion.

Through the Research Associate program, undergraduate students can come to DFSC to work on research projects for a summer, a semester, or a year. The paid Research Associate program differs from traditional internship programs in that the Office of the Chief Scientist has the students participate directly on a research project under the guidance of a DFSC scientist. The Research Associate program allows students to gain valuable knowledge, skills, and abilities in experimental design, testing, and analysis from dedicated forensic mentors.

Since 2011, 101 Research Associates have worked more than 51,000 hours on 85 DFSC-led research projects and 15 SMART scholars have contributed more than 3,800 hours to 15 research projects. The Educational Outreach Program at DFSC has been growing annually and these numbers will continue to rise. Come learn more about the opportunities at DFSC and join the research team!

Education, Outreach, Intern

E55 Fingerprint Analysis: Determination of Biological Sex Via Enzymatic Assay

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After attending this presentation, attendees will understand a new bioaffinity-based method for fingerprint analysis and that fingerprint samples can be used for more than pictorial comparison. These cascade-based assays can be applied in multiple areas so other researchers could apply this methodology to their own research.

This presentation will impact the forensic science community by establishing that fingerprints can be used as a biological sample like blood and sweat. It will also introduce a more rapid method of fingerprint analysis that can one day be applicable for on-site usage. The new method presented here will also spur other researchers to place more efforts on creating techniques usable by personnel without scientific training.

Fingerprint analysis traditionally refers to the process of comparing fingerprint patterns by an expert and/or an automated fingerprint identification system. Currently, the analysis ends with this matching methodology, causing the field to be dependent on the presence of a stored matching print or a matching print from an individual that is physically present. Due to this limitation, a latent fingerprint may be judged to be too smudged or smeared to be of use. What is often overlooked is that those latent prints are created by sweat and sebum emulsions excreted by the fingertips. Those emulsions have their own unique chemical compositions for each individual, making them possible biological samples for analysis. The University at Albany's lab has developed a bioaffinity-based cascade for the determination of biological sexes from the chemical composition of the sweat/sebum left as the latent prints.

The research presented here addresses the current limitations in fingerprint analysis using a bioassay system that focuses on the components of fingerprints. Bioaffinity-based assays have been developed for the determination of biological sexes from those components. In one assay, L-amino acid oxidase was used to target the amino acids present in the sebum and sweat left on latent fingerprints. Further research has led to the testing of authentic fingerprint samples collected from various surfaces as well as the development of other bioaffinity-based assays capable of differentiating between biological sexes via less complex systems. Other bioaffinity-based assays will also be developed in the future for the determination of other physical attributes, such as age group and ethnicity.

While these assays will not be able to clearly identify a person of interest, they will be extremely useful for quickly narrowing suspect pools when identification is not possible. The assays will also be useful in cases in which there is not enough time for the process of identification (possibly via DNA) to be completed. The assays that have been developed and are currently in development have the potential to become a portable method that can be used for on-site analysis. Also, due to the ease with which the assay can be performed and interpreted, specialized training for the execution of the analysis is unnecessary, unlike most currently available techniques. These assays could become a very powerful tool for forensics.

Fingerprint Analysis, Enzymes, Bioaffinity

E56 Data-Driven Decisions in Latent Print Examination to Improve Quality

Anthony Koertner, MS, 4930 N 31st Street, Forest Park, GA 30297*

After attending this presentation, attendees will be familiar with some of the limitations of the subjective methodologies traditionally applied to latent print examinations and with a path forward to a more objective data-driven approach.

This presentation will impact the forensic science community by discussing the steps taken by the United States Army Criminal Investigation Laboratory (USACIL) to move its latent print branch from a primarily subjective-based approach to a more defensible, data-driven process using more objective methods and pre-defined criteria at many stages of the latent print examination process.

This demonstrative presentation is designed so forensic science practitioners and technical leaders can better visualize and therefore understand these practices in the flow of typical latent print casework. Attendees will be provided with the resources to utilize these tools better and more objectively inform decisions in their latent print examinations.

Latent print examination has been subject to much criticism regarding its scientific standards and validity, specifically due to the lack of objective data utilized in decision making to arrive at conclusions. Many highly publicized reports, such as the *Report to the President. Forensic Science in the Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods* by the President's Council of Advisors on Science and Technology (PCAST), continue to critique the field's shortcomings in an attempt to elicit change by moving latent print examination to a place where all decisions and conclusions are fully supported by empirical, robust, and reproducible data.[1] From reporting latent print findings without the term "identification" to a statistical model that represents the strength of an association, this presentation will introduce some of the data-driven practices utilized by latent print examiners at the USACIL to inform their decisions during casework examinations. These practices not only attempt to satisfy the criticisms set forth by these various reports, but more importantly function as additional quality control measures applied to casework. Specifically, this presentation will describe how "suitability for comparison" claims are supported utilizing empirical measurements from software designed to estimate the quality/clarity of a friction ridge impression area and other software to quantify the similarity between two fingerprints and provide a statistical estimate of the likelihood they were made by the same source rather than by different sources. Together, these methods provide a means of moving away from the previous methodology's reliance on the examiner's subjective opinion for determining the significance of latent print evidence.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the United States Department of the Army or United States Department of Defense.

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Data, Subjective, Latent Print

E57 3D Imaging Technology to Uncover Changes in Latent Fingerprint Topography in Four Dimensions

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After attending this presentation, attendees will be familiar with latent fingerprint degradation patterns and ridge topography changes in 3D, including a phenomenon known as ridge drift. Additionally, attendees will be introduced to the statistical methods used in the analysis of fingerprint aging under different environmental conditions.

This presentation will impact the forensic science community by demonstrating statistical evidence of fingerprint degradation patterns without sample manipulation.

Fingerprints have been used for identifying suspects and victims at crime scenes; however, the issue of "when" a latent fingerprint was deposited is a recurring concern in courts of law. Currently, there is no accepted methodology for estimating the time of deposition of a latent fingerprint. No method has yielded yet reliable results, neither has it been fully approved by the scientific community to be used in a court of law. To address this gap in forensic science knowledge, researchers have been studying visual methods to determine fingerprint age or to model fingerprint degradation patterns.[1] Estimation of the age of fingerprints could prove useful to crime scene investigations by excluding potential suspects if the estimated time of deposition is inconsistent with the time of the crime.

Previous studies have observed topographical changes and individual ridge movements as fingerprints aged, such as the discovery of ridge drift.[2] Other researchers, including Popov et al., have detected a "horizontal migration of components" of fingerprint ridges.[3] Dorakumbura et al. reported that individual fingerprint "droplets" vary in their adhesion to certain surfaces.[4] Merkel et al. demonstrated that 3D optical capturing devices could potentially solve the problem of determining fingerprint age.[5] In accordance with these and other previous research findings, this project investigates the causes of ridge drift that cannot be explained in 2D rendering of a fingerprint.

In 2D rendering, only the length and width of fingerprint ridges can be observed. In traditional analytical methods, fingerprints are usually manipulated using destructive or invasive procedures, such as powdering, exposure to reactive chemicals/metals, or swabbing. In this study, non-destructive 3D optical profilometry is used to examine the aging of a latent fingerprint without manipulating it. This imaging technology provides reliable information on the x, y, and z axes of fingerprint ridges. The ridge heights, widths, and lengths across a fingerprint are measured and analyzed over time using established statistical methods.

The experiment involved aging sebaceous-rich latent fingerprints from six donors (three males and three females) in various environmental conditions: "exposure to natural light" (direct sunlight and darkness); "substrate type" (glass and plastic); and "finger" (index and middle fingers). These were stored indoors and allowed to age. Exposure to light, temperature, and relative humidity were not controlled but monitored to closely mimic field conditions. Samples were randomly drawn over a period of three months and directly observed and captured using 3D imaging without any pre-treatment. The equipment used was a ZeScope™ Optical Profilometer from Zygo® Corp, which provided high-quality images and mathematical data in 3D space and time.

Over the course of the study, 1,296 fingerprint images and statistical data were collected for analysis. Each 3D fingerprint image was measured to acquire two metrics: *Sa* (average surface roughness) and *Sq* (standard deviation of the surface roughness). These values were aggregated to create a statistical model to explain the change in a fingerprint's *Sa* and *Sq* over time. Preliminary data demonstrated that both *Sa* and *Sq* measurements decreased over time, strongly suggesting that fingerprint ridges were also degrading in height.

This study proposes a methodology to track the physical changes in a latent fingerprint's ridge length, width, and height over time. This study provides evidence that degradation can be monitored and measured. Lastly, this study contributes to the ongoing research of determining the age of a fingerprint and provides a basis for future research.

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3D Imaging, Latent Fingeprint, Aging

E58 Score-Based Likelihood Ratio

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After attending this presentation, attendees will better understand score-based likelihood ratio, what it is, how to compute it, factors affecting its computation, and when and how it may be used.

This presentation will impact the forensic science community by advancing quantitative methods for assessing the weight of fingermark evidence. This presentation discusses important details that shall be considered when computing score-based likelihood ratios and provides empirical data on repeatability and reproducibly of different methods for computation of score-based likelihood ratios.

Friction ridge patterns of fingerprints exhibit vast information and are the most widely used forensic evidence for solving crimes. During the analysis, fingerprint examiners visually compare the latent marks from a crime scene with one of the retrieved candidate fingerprints from a database, or compare the latent marks with the prints from a known source. Methods to quantify the weight of evidence are useful in assisting fingerprint examiners with reaching correct conclusions with a higher confidence.

Likelihood Ratio (LR) provides one the of commonly used quantitative approaches to weigh the evidence, which, combined with prior belief, provides the posterior odds of determining the same source versus different sources. Two of the most commonly used LRs are feature-based LR and score-based LR. Whether and how to use these LRs has generated great interest in the forensic community. Feature-based LR relies on large dimensional fingerprint features and has been studied more widely than the score-based LR. The methods for computing feature-based LR are complex, difficult to interpret, and are not readily interoperable because of its dependence on accurate extraction of features and types of features. Score-based LR relies on estimating the distribution of comparison scores from fingerprint comparison algorithms and has recently brought attention to forensic scientists. Score-based LR is considerably simpler to compute because the comparison scores are one-dimensional. Moreover, it borrows strength from repeatability and reproducibility of comparison algorithms which are becoming more accurate. Computing score-based LR values require estimation of the comparison score distribution functions for mated and non-mated groups from a well-established database using an accurate fingerprint recognition algorithm.

This presentation first briefly explores the difference between feature-based and score-based LRs, explains both LRs mathematically and visually, and explains how the LRs are related to the population parameters, such as the population mean and population variabilities. This is followed by a detailed discussion on the calculation of score-based LR using parametric methods and non-parametric methods, namely kernel density estimation and logistic regression estimation. This presentation then discusses their performance as well as their repeatability and reproducibility by comparing score-based LR values generated by those methods using various sample size ratios, various total sample sizes, and various probability distributions.

This study reveals that values of score-based LR depend on sample size and the ratio of mated to non-mated groups. Furthermore, it highlights the importance of careful consideration on selecting an appropriate dataset both in terms of size and representativeness of the background information of the particular forensic case.

This study was performed using operational and laboratory-collected fingerprints and fingermarks. Mated and non-mated comparison scores were generated using automated recognition algorithms. Results are reported separately for each dataset and recognition algorithm.

Score-Based Likelihood Ratio, Repeatability, Reproducibility

E59 The Impact of DNA Swabbing Collection Methods on Latent Print Evidence

Monica J. Kupsco, MS, Defense Forensic Science Center, 4930 N 31st Street, Forest Park, GA 30297*

The goal of this presentation is to highlight a processing sequence that will optimize latent print and DNA yield on paper items of evidence. Attendees will have a greater understanding of how DNA collection methods and latent print processes on porous substrates impact one another and how these data may provide a foundation for improving laboratory policy and procedures.

This presentation will impact the forensic science community by discussing: (1) the impact of DNA collection methods (wet and dry swabbing) on subsequent latent print processing of paper items; and, (2) the impact of porous latent print processing on subsequent DNA analysis.

Currently, unless a fold or some type of creased area exists on a paper item where targeted DNA swabbing can be performed, investigators have been encouraged to choose between latent print testing and DNA testing, rather than attempting both. This decision is largely anecdotal with little data available demonstrating the impacts and significance to one another. In an effort to gain more empirical insights into this issue, preliminary research was performed using six different types of paper: manila envelope, manila folder, plain white envelope, index card, lined notebook paper, and plain white paper. Two prints were deposited on each sample and were processed in one of the following ways: latent print processing followed by either dry swabbing or wet swabbing, wet or dry swabbing followed by latent print processing, or latent print processing followed by direct DNA analysis.

The data collected indicates insignificant differences in the DNA yield or number of suitable latent prints developed between wet and dry swabbing either before or after latent print processing; however, direct DNA analysis of the impression resulted in the highest DNA yield and most success in obtaining a full profile. Wet swabbing prior to latent print development will obscure parts of the print, but oftentimes clear areas can still be used to support the latent print examination.

This study provides preliminary data related to the impact of DNA collection methods and latent print examinations. Although further research is advised using different latent print processing techniques, this data suggests that laboratories may not need to require investigators to choose one method over another.

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Latent Prints, DNA, Documents

E60 Size Variations Associated With the Different Methods of Recording Outsole Impressions of Reference Footwear for Comparisons

Anthony Koertner, MS, 4930 N 31st Street, Forest Park, GA 30297; and Jeremy J. John, MS, Defense Forensic Science Center, 4930 N 31st Street, Forest Park, GA 30297*

After attending this presentation, attendees will have a better understanding of the amount of size variation tolerances that should be given for test impressions when performing footwear comparison examinations.

This presentation will impact the forensic science community by describing and quantifying the potential size variations observed in the creation of test impressions of known shoes.

Size determinations of footwear impressions are an important aspect of the general footwear examination scheme, as significant differences in size can instantly exclude a shoe as having been the donor of a particular footwear impression. There are several common methods and procedures for recording test impressions to permit a thorough comparison of the questioned impression with a known shoe.

Across agencies, policies and procedures regarding the creation of test impressions for known shoes may not be available and/or are not very prescriptive, thus allowing the footwear examiner to resort to personal preference in how these impressions are created. Test impressions are generally created on various types of surfaces with some degree of force applied to ensure a thorough recording of the outsole characteristics. The examiner will typically create a shoe's test impressions by wearing the known shoe and walking over a substrate, which creates a known reproduction of the shoe's outsole using a transfer medium such as ink, powders, inkless chemical treatments, and oil-based mediums. Variables such as substrate composition, method of generating the test impression (e.g., walking, jumping, stomping), weight of individual, size of the foot in relation to the shoe, material composition of outsole, etc., may cause variations in the overall appearance and perceived size of the impression. These size variations may be more or less prevalent in different portions of the outsole (e.g., heel, outsole, and ball).

Although such variations are understood by the practitioner community, significant effort has not yet been directed toward quantifying the extent by which footwear impressions may vary. Thirteen new pairs of shoes that were representative of outsoles commonly observed in footwear examination casework were obtained for purposes of testing these variables. This presentation will discuss the variations observed and lay a foundation for further research regarding the significance of these findings and how they may impact footwear examination conclusions.

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Footwear, Test Impressions, Size Variation

E61 Bradford Reagent and Ninhydrin: Chemical Approaches for Biological Sex Identification From Fingerprints

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After attending this presentation, attendees will understand that fingerprints can be used as more than just a picture for comparison and can be used for identifying certain attributes of the fingerprint originator based solely on the composition of the fingerprint content. Attendees will also learn that this concept is possible even when the target analyte population is narrowed from 23 amino acids to 6.

This presentation will impact the forensic science community by providing new methods for the analysis of fingerprints in order to generate essential information about suspected individuals directly at a crime scene. This concept will provide a simple YES/NO response within minutes to confirm originator attributes. In addition, these systems can potentially be incorporated into field-deployable devices (similar to glucometers) or connected to hand-held Smart Devices, which will allow for rapid analyses that can be used and interpreted by operators -— in a manner similar to water test kits and the VOCkit system, which is a small strip that has a grid of several dozen indicator chemicals imprinted on it that is used by the Army for the detection of threat agents such as anthrax, sarin, and mustard gas -— with minimal scientific training.

It has been established that the contents of fingerprints are produced by multiple hormone-based control mechanisms and are, thus, a function of physical properties such as age, ethnicity, or biological sex. It has been demonstrated that fingerprints have the potential to generate much more information because they are samples of biological origin analogous to other body fluids. One of the greatest setbacks in fingerprint analysis is that if a matching fingerprint is not saved in a database or if the person of interest is not physically present for comparison, the print is reduced to merely exclusionary evidence, despite being stored in a separate database for future use with newly obtained fingerprints. The same can be said about DNA. Even though DNA can provide the most significant information about the fingerprint originator, DNA analysis can take weeks or months, not to mention that only a few nanograms of DNA at most can be recovered from a fingerprint as the majority is lost during collection and extraction. Ultimately, even if DNA was collected, it is possible that a matching profile may not exist. The research displayed here investigates the use of fairly well-known chemical assays for the purpose of identifying the biological sex of the fingerprint originator.

The ninhydrin method is the most well-known and widely used. Federal, state, and city crime laboratories have been implementing this technique for nearly 50 years. This method has proven to be durable and reliable because of the stability of amino acids — ninhydrin has been used to detect and develop fingerprints that are more than 30 years old. Ninhydrin is a chemical that reacts with amino acids in the fingerprint content to produce the blue-purple color known as Ruhemann's purple. Here, a modified approach to the traditional ninhydrin method for fingerprint development is combined with an optimized extraction protocol and the concept of determining biological sex from fingerprints.

Despite the success of the ninhydrin method, this group's intentions are to establish a method in which only one metabolite corresponds to one originator attribute. Multianalyte assays that target a larger number of amino acids are not completely reliable because more than one attribute can ultimately affect the output of the assay. This convolutes the intentions of the assay altogether since it would be difficult to identify which attribute is ultimately responsible for causing the difference in the assay's response. For example, if attribute A causes amino acid 1 to increase, but attribute B causes the same amino acid to decrease, the change is negated and neither attribute can be identified. To eliminate this possibility, it is important for systems to be restricted to one analyte (amino acid) or a specific combination of analytes that are correlated to the desired originator characteristics. To do so, a chemical assay involving the Bradford Reagent (traditionally used for protein quantification) used to target a small group of amino acids was developed to begin the transition toward focusing on a single amino acid.

Fingerprints, Chemical, Identification

E62 The "CSI Effect": Is It Relevant to You?

Brittany Borzych, 836 Elk Lane, Westville, IN 46391*

The goal of this presentation is to inform attendees of the controversial issue of the "CSI Effect" and the different ways it can affect each person.

This presentation will impact the forensic science community by informing attendees of how they are affected by the "CSI Effect" and what they can do to adapt to it.

The "CSI Effect" is a controversial issue in the fields of forensic science and criminal justice. It is commonly called the "CSI Syndrome." The "CSI Effect" can be defined as "the influence of media, mainly television shows, on the people in juries, studies, and the general idea about how the field operates and its environment."[1] The theory is said to affect everyone, even when they don't know it. It is closely related to two possible theories. The first is the cultivation theory. This is defined as "the theory that the more television you watch, the more likely you are to believe that the world is exactly like it is in the media."[2] The second is the "Perry Mason Syndrome." This is based on the crime show *Perry Mason* and is said to be the first show that could have sparked the idea of altering perceptions of the United States legal system among defendants and jurors.[3] These are also controversial issues and there is research that supports and contradicts the existence. This will open the eyes of many people to the obstacles they may face in the field and will give them the knowledge they need to overcome it.

The effect has become a major issue in the field and has been brought to attention due to the juries' new knowledge in courtrooms. The research presented will be of a survey conducted to a handful of citizens who are jury eligible. The results are currently inconclusive, with more research to be conducted over the next few months. After extensive research, the "CSI Effect" may exist based on opinion but most researchers agree that the "Tech Effect" has more influence on th people and may be the primary cause of the theory due to the rapidly changing technology that we experience every day. The "CSI Effect" may exist with both positives and negatives coming from the theory. In the opinion that it does exist, the benefits outweigh the negatives.[4] It becomes more beneficial to acknowledge the possibility of its existence and learn how to work with it. This presentation seeks to acknowledge the possibility of the "CSI Effect" and to help people of all interests learn and understand the ways it can affect them.

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"CSI Effect", CSI Syndrome, Cultivation Theory

E63 Comparing the Degree of Force in Infants With Suspected Abusive Head Trauma to Traffic Accidents or High-Altitude Falls Is Not Viable

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After attending this presentation, attendees will have learned to be cautious when comparing injury mechanisms in suspected abusive head trauma with little external damage to traffic accidents or high-altitude falls when testifying in court.

This presentation will impact the forensic science community by informing attendees of the few similarities between infants with suspected abusive head trauma with little external injuries and infants involved in traffic accidents or high-altitude falls. This presentation will demonstrate that when rib fractures occur, they have extensive concomitant injuries to the organs of the trunk.

In the Swedish national guidelines on infant abuse and in prolific international infant abuse literature, there are statements that have influenced professionals testifying in suspected abusive head trauma cases that the forces applied during shaking are equivalent to an unrestrained traffic accident or a high-altitude fall. In certain studies, rib fractures have been considered to be specific for abusive head trauma due to compression of the rib cage; however, the occurrence of concomitant injuries to internal organs are rare.

Infants deceased from traffic accidents or high-altitude falls have few injury characteristics in common with children with suspected abusive head trauma. When rib fractures occur in traffic accidents or high-altitude falls, there are always concomitant injuries of the internal organs of the trunk.

Infants deceased after traffic accident or high-altitude falls (3 m) between 1994 and 2016 were identified in the computer registry of the Swedish National Board of Forensic Medicine. Cases identified were scrutinized regarding mechanism of injury, external signs of injury, and internal injuries.

Twelve cases were identified in which access to autopsy protocols were possible in ten. Eight cases were involved in traffic accidents, of which three were hit by cars while in strollers, and two were involved in high-altitude falls. All ten cases had extensive skull and/or scull base fractures, sometimes with open wounds and fractures of the orbita or spine. All six infants suffering thorax/abdominal trauma had injuries of the organs of the trunk. Only two infants had rib fractures and both had extensive injuries to the organs of the trunk. One case lacked a proper description of external signs of injuries. All nine cases with information had external injuries to the head and five cases had bruising or injuries to the trunk or extremities.

Professionals should avoid comparing abusive head trauma without massive external and internal injuries to traffic accidents or high-altitude falls when testifying in court. Rib fractures are rare in infants deceased after traffic accidents and when they occur, there is damage to the internal organs of the trunk. Infants with trauma to the trunk can suffer extensive internal injuries to the organs of the trunk without contracting concomitant rib fractures.

Abusive Head Trauma, Traffic Accident, Infant

E64 Expert Decision Making and Visual Analysis: An Empirical Approach to Understand and Advance Examination in the Human Identification Sciences

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After attending this presentation, attendees will better understand how experts approach forensic tasks and analyze evidence within the laboratory, and how this can be conceptualized and translated into decision-making maps. These findings draw important parallel processes between domains previously considered to be unconnected, with implications across the forensic domain.

This presentation will impact the forensic science community by illustrating that there are gaps in the understanding of the decision-making tasks required when drawing inferences, making it challenging to fully assess expert performance.

The goal of this presentation is to demonstrate to attendees the judgments, decisions, and visual processes within two key domains of the forensic identification sciences, and how procedural similarities between these can be exploited to drive empirical research, which seeks to improve these judgements and decisions through the use of state-of-the-art technology. In order to do so, this presentation presents the decision maps of experts undertaking analysis processes within the fields of forensic anthropology and fingerprint analysis. These maps have been developed by combining independent information concerning visual comparison tasks present in both fields and the potential for exchanging knowledge on decision strategies and challenges involved.

Human decision making is a key component of the forensic science process (e.g., the recognition, collection, analysis, and interpretation of evidence) and it has been shown that factors can influence those decisions, affecting the investigative process and subsequent legal outcomes, with the potential for significant societal impact at a global scale.[1,2] It is clear that the concerns raised over expert decision making, including the vulnerabilities of cognitive processes and inappropriate weight assigned to evidence, have not only been highlighted in recent key governmental reports, but created debate and heated controversy.[3,4] Specific criticism has been directed at comparison and human identification processes, as many of the forensic methods used within the identification fields (e.g., fingermark comparison, bitemark comparison, morphological hair analysis, and forensic anthropology) are based on visual comparison tasks. Within the human identification field, the challenge of combining and interpreting analyses of different characteristics of evidence, and achieving transparency in decision-making and evidence-based conclusions, needs to be tackled. This includes a better fundamental understanding of the decisions being made and the visual processes being undertaken within the forensic identification sciences. Therefore, this study takes an empirical approach to provide a comprehensive understanding of the decisions made by expert decision makers in the laboratory, allowing for further targeted empirical studies investigating visual attention within these key decision processes common to both forensic identification fields.

Two experimental studies were designed whereby experts within the fields of fingerprint analysis and forensic anthropology were observed during casework analysis in the laboratory. Forensic anthropologists were asked to assess skeletal remains and develop biological profiles, documenting their decisions throughout this process. Similarly, fingerprint experts were asked to conduct the analysis process of Analysis, Comparison, Evaluation-Verification (ACE-V) on fingermarks recovered from crime scenes. A hierarchical task analysis approach was utilized to collect and analyze data, and decision trees were produced for each process observed. The resulting decision trees from both experimental studies were then compared and contrasted at a process and decision level.

These results will be used to discuss how the method can be applied to other forensic identification sciences, and the importance and possibility of taking both a holistic and detailed approach to understanding the judgments and decisions made during the forensic identification processes, in order to address and overcome the criticisms facing forensic visual analysis and comparison tasks.

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Decision Making, Identification, Visual Analysis

E65 Testing Unsubmitted Sexual Assault Kits: The National Institute of Justice-Federal Bureau of Investigation (NIJ-FBI) Sexual Assault Kit Partnership Successes and Lessons Learned

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After attending this presentation, attendees will better understand how federal support through the NIJ-FBI Sexual Assault Kit Partnership has assisted in investigations and helped reduce the number of Sexual Assault Kits (SAKs) awaiting testing in the nation's law enforcement agencies.

This presentation will impact the forensic science community by conveying information the Partnership has garnered with regard to the collection, processing, and testing of SAK evidence from law enforcement agencies across the country.

NIJ, the research, development, and evaluation agency of the United States Department of Justice, leads the nation in supporting the forensic sciences through research and by providing state and local crime labs with funding to help process and test evidence more efficiently. This presentation will provide information on the NIJ-FBI Sexual Assault Kit Partnership initiative designed to help inform evidence collection, processing practices, and testing protocols for SAKs. This Partnership begun accepting SAKs from law enforcement agencies in September 2015 and has been helping to address a major need in the nation's forensic science and criminal justice communities: to support state and local law enforcement agencies in their efforts to reduce the number of unsubmitted SAKs. The effort has yielded valuable knowledge related to the processes associated with the intake of sexual assault evidence into the lab, as well as screening, testing, and analysis specific to the processing of large quantities of unsubmitted SAKs. For instance, knowledge gained from this initiative has contributed to the *National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach* report developed in response to the Sexual Assault Forensic Evidence Reporting (SAFER) Act of 2013, which focuses on the accurate, timely, and effective collection and processing of DNA evidence in sexual assault investigations.[1] This initiative has supported the goal of carrying out analyses of samples from untested unsubmitted SAKs so DNA profiles can be developed and placed in the National DNA Index System (NDIS), considered one part of the Combined DNA Index System (CODIS), the FBI's program of support for criminal justice DNA databases and the software used to run these databases. The Partnership has generated hundreds of CODIS hits throughout the nation, and the upload of hundreds of eligible profiles generated from DNA analysis as a result of this research has provided direct aid in the investigation and apprehension of sexual assault perpetrators.

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Sexual Assault, Sexual Assault Kit, Unsubmitted Sexual Assault Kit

E66 A Case Study: From Maternal Instinct to Staged Domestic Homicide

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After attending this presentation, attendees will understand, through a process of comprehensive analysis of a crime scene, the criminal behavior and the driving motivation of a murder of two children perpetrated by their own mother.

This presentation will impact the forensic science community by illustrating how the criminal conduct of staged domestic homicide has some repetitive patterns or common characteristics in terms of planning and execution, regardless of location, without being influenced by sociocultural or economic factors of their executors.

In the early hours of September 20, 2010, a mother told the city police Mocoa, the capital of the department of Putumayo in southern Colombia, that an unknown individual attacked her when she opened the front door of her residence, and she was attacked until she lost consciousness. After regaining consciousness, she observed that the children were in bed in the master bedroom and, when she approached them, believing them to be asleep, noted they were wet and without signs of life.

At the beginning of the investigation, the woman´s injuries were consistent with blunt trauma and the apparent coherence of her story the authorities to think there was a perpetrator who murdered her two children and left the mother seriously injured. The high social impact produced by a case of two killed children killed and the assaulted mother activated law enforcement, at both the local and national level; the Criminal Behavior Special Unit of the Attorney General's Office was called to support the investigation through a process of crime scene analysis and possibly developing a criminal profile.

On the basis of the processes of the crime scene analysis, the bloodstain patterns found, the comprehensive study of the evidence related to the victims, the assessment of the versions provided by the surviving mother and other witnesses, and the structural characteristics and safety of the building, the investigation team re-evaluated the hypothesis of an external aggressor and redirected their attention toward the crime being committed by the mother.

This change in the case offered the team a clear vision regarding the dynamics of the events that led to the deaths of the two children. With the support of the analysis of forensic evidence at the scene, the Criminal Behavior Special Unit was forced to focus their attention of finding the motivation that drove this woman to kill her own children. Therefore, the line of investigation focused on probing deeper into all areas of the mother's life and found an intricate world of love, frustration, and abandonment from a mentally unhealthy woman, egocentric and possessive, with strong intentions to overcome any obstacle to achieve all her personal desires.

After gathering and fitting together all parts of this criminal and forensic puzzle, the prosecutors demonstrated definitively and beyond reasonable doubt that the crime was committed by the mother in the form of staged domestic homicide.

Mothers Who Kill, Children, Staged Domestic Homicide

E67 Utah Quick Kit (UQuiK): A Collaborative Program on the Sexual Assault Kit Analysis Process

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The goals of this presentation are: (1) to describe the UQuiK program to streamline the DNA analysis process in sexual assault kits; and, (2) to identify the research findings on the effectiveness and reliability of the UQuiK program. After attending this presentation, attendees will better understand the importance of collaboration and communication between forensic medical providers/Sexual Assault Nurse Examiners (SANEs) and forensic scientists on sexual assault evidence collection. In addition, attendees will learn about a novel, collaborative approach in selecting most probative DNA evidence swabs to streamline the DNA analysis process in sexual assault cases.

This presentation will impact the forensic science community by providing data on a collaborative approach uniting forensic medical providers/SANEs and forensic scientists at the state crime laboratory. This presentation will add to research as findings from a study analyzing the effectiveness of this new approach will be shared.

Crime laboratories across the United States are receiving higher amounts of sexual assault kits for analysis. The rise in numbers is likely due to more sexual assault victims reporting the crime and an increase in law enforcement submission rates of sexual assault kits. Many crime laboratories have moved to a direct-to-DNA analysis approach consistent with improved Short Tandem Repeat (STR) and Y-chromosomal Short Tandem Repeat (Y-STR) analysis methods and to streamline the analysis process, bypassing serology testing prior to DNA analysis. In an effort to additionally improve the throughput of the crime laboratory analysis process, the Utah state crime laboratory collaborated with a forensic nursing team to pilot a program entitled UQuiK.

The UQuiK program involves educating forensic nurses in identifying the most likely probative swabs collected during a forensic sexual assault examination. The forensic nurses select up to three probative swabs and a reference sample, buccal swab from the victim, and package these swabs in a separate container from the full sexual assault kit. Law enforcement in this jurisdiction also received information and education regarding the UQuiK process. Law enforcement agencies were encouraged to submit the UQuiK evidence envelope to the state crime laboratory. The full sexual assault kit was also submitted, although the evidence contained in the UQuiK envelope was analyzed first. Swabs contained in the full sexual assault kit could be analyzed following evidentiary swabs in the UQuiK envelope, if deemed necessary.

The UQuiK program shortened DNA analysis time for the forensic scientists as they did not need to open a full sexual assault kit, sort and catalog the evidentiary swabs, read the history of the assault, perform body fluid testing, and select the most likely probative swabs for DNA analysis. Prior to launching the program, a pilot study found the time was decreased from 120 days to 25 days for DNA analysis.[1] As the forensic medical providers/SANEs collect the history of the sexual assault directly from the patient/victim, they are prepared with the information to select the most likely probative swabs for DNA analysis. A training program was developed to educate the SANEs participating in the study on how to select the most probative swabs. The training program consisted of three training sessions with the SANEs where studies were shared on DNA analysis findings. The SANEs were then given scenarios and asked to determine which swabs would most likely result in a probative DNA profile.

Research on the DNA analysis results from a sampling of cases under the UQuiK program and cases prior to the UQuiK program will be shared to provide data on the effectiveness and reliability of the UQuiK approach. A key component of this program is the collaborative relationship between forensic scientists and forensic medical providers/SANEs. One study found that only 53% of crime laboratories in the United States have a communication feedback mechanism in place between forensic scientists and forensic medical providers/SANEs.[2] This program underscores the need for improved communication between the professions to improve practice and outcomes.

In conclusion, this project and research study provide information on a program based upon communication and collaboration between forensic scientists and forensic medical providers/SANEs to improve the process of DNA analysis of sexual assault kits.

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Sexual Assault, Forensic Scientists, SANEs

E68 Big Business, Big Brother, and Genetic Genealogy

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After attending this presentation, attendees will better understand the three-way relationship between Direct-To-Consumer (DTC) DNA testing companies such as Ancestry.com®, those involved in forensic identification, and the genetic genealogy community. The growing size of the DTC companies has been an exciting prospect to genetic genealogists due to an increasing success rate of finding adoptee birth parents and long-lost family members; however, this same growth has been an issue for the forensic community. Although those same databases have the potential of solving crimes, DTC DNA testing databases are unavailable for forensic identification due to Fourth Amendment search and seizure issues. There is also the concern that DTC companies wish to protect access to those databases as their primary financial assets. Genealogists, on the other hand, seem comfortable with relinquishing their rights to how their DNA can ultimately be used by DTC companies for drug development and various research purposes, while at the same time regarding law enforcement as "Big Brother," ready to exploit their DNA for who knows what purpose.

This presentation will impact the forensic science community through an open discussion of how to overcome the challenges that currently limit collaboration with both DTC companies and the genetic genealogy community. Such a discussion will lead to a better appreciation of the issues facing all three communities, with suggestions as to how they can work together toward the common goal of developing strong working relationships based on trust.

The need for this discussion is compelling. At present, there are approximately three hundred thousand Y-chromosomal Short Tandem Repeat (Y-STR) profiles posted online in thousands of large and small public genetic genealogy databases. At the same time, databases of autosomal Single Nucleotide Polymorphism (SNP) testing results controlled by the DTC testing companies have grown to include millions. The database for AncestryDNA™ has now topped four million, while the 23andMe® database has passed the two million mark. While Y-chromosomal DNA (Y-DNA) can only provide information about the direct male line of a family, autosomal SNP testing can provide information not only about the individual who was tested, but also about his immediate relatives. Therefore, the size of each autosomal SNP database is virtually much larger, considering that each person tested shares an average of 50% of his autosomal DNA with his parents and siblings. If, as expected, AncestryDNA™ will top ten million members in a couple of years, based on a typical family of four, the database could represent four hundred million virtual members — larger than the United States population. Unfortunately, unlike Y-STR databases, autosomal SNP databases are not available to the public, but are controlled by Big Business.

The potential of both Y-STR and autosomal SNP databases for solving crimes and identifying John Does must be recognized and a means found for the three communities — the DTC companies, the forensic practitioners, and the genetic genealogy community — to work toward resolution of the issues preventing collaboration. This presentation will explore ways of making this happen.

Direct-to-Consumer, Genealogy, Identification

E69 Child Abuse in Northwest Italy: A Five-Year Retrospective Analysis

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After attending this presentation, attendees will understand the characteristics of child abuse in northwest Italy through the analysis of the data collected in one of the larger specialized centers in Italy.

This presentation will impact the forensic science community by providing information about the factors useful for early identification of suggestive cases of abuse.

A survey of the Italian Childhood and Adolescent Guarantee Authority published in 2015, involving a quarter of the population of Italian children, revealed that 4.7% of minors are followed by social services.[1] Among them, a fifth are victims of some form of abuse. The prevalence of minors who are receiving social services grows directly with age. This data highlights poor development of these early-warning services as social services only become involved later when the children have already grown up.

Early identification of the cases of child abuse is crucial for health and social professionals as well as ensuring prompt reporting to the judicial authority. For this reason, a retrospective analysis of the cases managed by the multidisciplinary unit dedicated to the evaluation of suspected abused children ("Bambi") of the Ospedale Infantile Regina Margherita (Turin) was conducted.

From January 2012 to December 2016, the unit dealt with 816 cases: 46% concerned Sexual Abuse (SA), 39.7% Physical Maltreatment (PM), 4.2% neglect, 5.1% were mixed forms, and, in 5% of the cases, the type of abuse was not available. In regard to gender, females were the majority in SA (81.6%), neglect (52.9%), and mixed forms of abuse (64.3%), while males predominated in PM (52.5%). The mean age of the patients was 7.5 years; 7.6 years in SA, 7.7 years in PM, and 5 years in neglect; the age distribution was significantly lower in cases of neglect (p-value 0.00024 and 0.0025, in comparison to SA and PM). The geographical origin of the family and, consequently, the child's cultural growth environment have been taken into account. Italian children were numerically predominant (63.6%), followed by African (14.5%), east European (12.9%), Latin American (5%), Asian (2%), and European (0.2%). The family status analysis observed a slight predominance of divorced parents (28.7%) vs. not divorced (27.2%), while a small percentage of children resided in community (3.9%) or with a foster family (0.5%). In 36.2% of the cases, the visit was required by the hospital ward where the patient was hospitalized and in only 14.1% was the visit required by social workers. In most cases, sexual offenders were fathers (25%), extra-familial people (24.7%), and other components of the family (20.5%), such as the mother (3.6%) or brother (3.3%). In cases of PM, perpetrators were predominantly fathers (44.8%), then mothers (33.1%), then extra-familial people (7.9%). Neglect was associated most to mothers (64.3%), followed by fathers (44.6%). In the mixed forms of abuse, the most frequent perpetrators were fathers (41.2%), mothers (36.5%), or other family members (22.2%). The percentage of the maltreated children (70.3%) did not present any kind of injury. In the remaining cases, bruises were observed in 41.9% of the children, excoriations in 26.2%, scars in 11.2%, bone fractures in 8.4%, burns in 4.2%, non-accidental head trauma in 3.6%, lacerated injuries in 3.3%, cutting wounds in 0.9%, and gunshot injury in one case.

The criterion "localization of injuries," which contributes decisively to differentiate accidental and abuse-related wounds, was also considered. For example, injuries localized at the forehead, tip of the nose, and chin are typically due to accidental falls, while injuries involving eyes, lips, and outer ears are abuse related.[2] In this series, physically maltreated children presented injuries in locations typical for non-accidental trauma in 86.5% of cases. In the cases of confirmed SA (about two-thirds of the visits for suspected SA), 67.1% of the children presented some form of anogenital findings, non-specific (e.g., erythema of the genitals) or specific (e.g., perianal scars) for genital trauma.[3] Among the cases of unconfirmed suspected abuse, 55.1% had non-specific genital findings.

For these reasons, the clinical assessment must be performed by a health care professional with specialized training in child abuse evaluation who is able to correctly identify the specific features of the different forms of abuse. This presentation provides attendees with a greater awareness of the importance of knowing the red flags indicating a situation of child abuse.

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Child Abuse, Early Diagnosis, Physical Findings

E70 Keeping Safe: Understanding Violence Against Law Enforcement by Youth Street Gangs

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After attending this presentation, attendees will be able to understand the signs and symptoms of the ever-growing problem of gang violence against law enforcement and the forensic science community so they may keep themselves safe while investigating the deaths of youth street gang members.

This presentation will impact the forensic science community by educating and ultimately keeping the forensic science and law enforcement community safe out on the streets while investigating the deaths of youth street gang members.

Within the past 13 months, violence toward law enforcement has increased by 85%, according to the Department of Justice. In some metropolitan areas of the United States, this figure is nearly 115%. One cannot turn on the television or radio without hearing about another officer-involved shooting in this country. In Los Angeles County, CA, alone, the Los Angeles Police Department (LAPD) and the Los Angeles County Sheriff's Department (LASD) have increased helicopter patrols and 911 screenings in wake of the recent police ambushes.

Youth street gangs throughout the United States still continue to terrorize the neighborhoods they claim as their own, causing the citizens in these gang-infested neighborhoods to live in constant fear of their lives every single day; however, a new trend on the streets is making a fake 911 call, then ambushing law enforcement as they respond to these fictitious calls for help. As law enforcement responds to the location of the scene, youth gangs are now using urban style tactical warfare learned from the military, using that training against law enforcement as they respond to the scene, seriously injuring or killing officers. Whereas in the past, youth gangs would retreat when confronted by law enforcement, they are now advancing toward law enforcement while shooting, using the same tactics as the officers themselves use, such as "slicing the pie" or "button hook." There are other various forms of urban tactical warfare learned in the military and the police academy that the gang members are learning on a daily basis and using against the police.

In 2016 in Los Angeles County, there were a total of 465 shots fired at police, and between January 1, 2017, and July 1, 2017, there have been a total of 688 shots fired at police. Of those shots fired so far in 2017, 93% were "gang related," yet in the year 2016, only 88% were "gang related." This is a serious "officer safety" concern for law enforcement who respond to these gang-related violence calls on a daily basis. Why are these gang members shooting at police?

This study interviewed 150 Los Angeles gang members on the streets and in the jails between January 2016 and July 2017 as to why they would decide to shoot at law enforcement. This study identified 10 distinct manifestations of these shootings against police and 12 solutions to help keep officers safe while out on the streets investigating these gang-related shootings. A sample of the findings include: distinct cultural differences between African American, Latino, and Asian American gangs as to why they engage violently with the police; state of mind (motivation) of the various gangs; disrespect felt toward police while being questioned, detained, or arrested; covert and overt racism experienced by the gang members; a "getting even" mentality; and being male or female in the gang.

All of these findings culminate in the recent influx of violence against law enforcement by gang members. In Los Angeles County alone, there are 1,351 documented gangs with a gang membership of more than 800,000. Across the country, similar results have been seen, according to the National Gang Crime Research Center in Peotone IL. There are more than 28,800 gangs in the United States with a total gang membership of 975,000. Of those, 90% are male and 10% are female. The ethnic composition nationwide include: 47% Latino, 31% African American, 13% Caucasian, 7% Asian, and 2% "mixed race," according to the Office of Juvenile Justice and Delinquency Prevention of the United States Department of Justice.

Youth Street Gangs, Law Enforcement, Officer Safety

E71 Trauma, Cognition, and the Investigators

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After attending this presentation, attendees will be more aware of the various types of trauma that investigative personnel routinely encounter and the potential impacts this may have, not only on the individual, but on the investigation. Paths forward and the development of Evidence-Based Policy (EBP) will also be addressed, so attendees can examine what policies and assistance are available in their own agencies.

This presentation will impact the forensic science community by opening discussion on a topic that is not only often considered to be taboo, but also by highlighting the potentially different trauma experiences that investigative personnel face and how, while more attention is being placed upon first responder exposure to trauma and their subsequent resilience, this focus may mask or overlook specific needs of investigators who are likely to experience trauma for a longer duration and in a different manner.

While there has been increased discussion about trauma and resilience among first responders in the past decade, this discussion has often been limited in scope and scale. There has been a focus on those immediately responding to critical incidents, as noted by Fetterman, with advances made in how we address first responder mental health in the aftermath of a traumatic experience; however, like Henry noted in his research, different positions involved in dealing with death in particular, but ostensibly also those involved in other types of cases, experience trauma differently.[1,2] This includes a variety of factors, such as time spent at the scene, and acknowledges that repeated exposure to trauma has a cumulative, rather than additive, effect. O'Hara notes that, unlike clear critical incident-related traumas, cumulative trauma can be more insidious and difficult to treat.[3] This suggests that best practice would necessitate addressing how participating in difficult or traumatic cases, especially as an investigator or as a member of a crime scene unit, impacts those personnel and provide appropriate services. Traumatic or troubling cases may also impact investigative personnel and lab personnel that were not immediately on scene as a form of vicarious trauma, which has recently been addressed among first responders by the Office of Victims of Crime.[4] Further, cognitive implications subsequent to traumatic exposure are varied in presentation and warrant further exploration. Amnestic complications associated with dissociation, as well as shifts in cognitive schemas, following traumatic exposure have implications for both subsequent cognitive functioning and possible applications of insight-based treatment approaches.[5,6] It has been suggested that the cognitive impact of trauma can be seen in both primary and vicarious/secondary trauma exposure (e.g., peers, treating clinicians).[7] Furthermore, some research has suggested a negative impact on executive functioning and memory, which may even be compounded by repeated exposure to trauma[8-11]. Additional research, especially in order to synthesize findings across trauma research, is indicated.

This presentation draws on research conducted with various law enforcement personnel who had been involved in an officer-involved shooting, to include the shooter and investigative personnel, and clinical experience, as well as current literature and other sources discussing EBP, with the dual goal of both opening and furthering discussion on a sensitive but vital topic, as well as beginning to identify paths forward toward a balanced approach that acknowledges mental health and resilience of the investigators and other investigative personnel.[12]

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Investigator Mental Health, Trauma, Cognition

E72 A Case Study: A Comparative Analysis of Common Behavioral Evidence in Three Columbian Cases of Domestic Homicides

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After attending this presentation, attendees will understand how, through a comprehensive process of analysis of the evidence collected at the crime scenes of three domestic homicides, a reconstruction of criminal behavior was made, demonstrating common patterns of behavioral evidence.

This presentation will impact the forensic science community by illustrating how good practices in crime scene processing, and the effective interpretation of the context of the evidence, reveal that crimes can have behavioral evidence in common, as described by the specialized literature in offender profiling.

Case 1: From September 9-12, 2009, body fragments of a female were found in different vacant lots on the outskirts of the city of Ibague, Tolima.

Autopsies Revealed: Blunt force trauma killed the victim; postmortem dismemberment; disembowelment; removal of the uterus and mutilation of the genitals; and the attempted destruction of fingerprints. All these actions sought to avoid identification of the victim and to mislead investigators as to the link between the victim and his aggressor.

Case 2: Occurred on the morning of September 20, 2010, in the town of Mocoa, Putumayo; the deaths of two children were reported and their mother was seriously injured. In the family's house, the bodies of two drowned children were found and their mother had marks of blunt and sharp force trauma. There was no evidence that an external perpetrator had entered the property.

Case 3: On the morning of February 6, 2014, the police of Villa del Rosario, an upscale neighborhood of Cúcuta, received a report of a break-in in an exclusive residential area where a female homeowner was killed. The autopsy revealed that the victim died because of strangulation with a rope and mechanical suffocation caused by the obstruction of the upper airways with a plastic element that covered her face. Household appliances and the homeowner's personal items were scattered on the floor of other bedrooms and social areas of the house. The victim's husband reported that approximately ten thousand dollars (US) were missing, along with his wife's cell phone.

After a comprehensive process of analysis of the three crime scenes and the effective interpretation of the behavioral evidence in common, as described by the specialized literature in offender profiling, the prosecutors could show conclusively and beyond reasonable doubt that the crimes were committed by the husbands and the mother. All offenders were sentenced.

Crime Scene Investigation, Behavioral Evidence in Common, Criminal Behavior Analysis

E73 3D Reconstruction of Shooting Incidents Using Laser Scanning and Computer Modeling

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After attending this presentation, attendees will understand how laser scanning and computer modeling can aid in the reconstruction of bullet trajectory analysis in shooting events.

This presentation will impact the forensic science community by demonstrating how a 3D computer model based on high-resolution laser scans of a scene was used in conjunction with ballistics evidence to analyze an unusual shooting event in which a bystander was struck and killed by a deflected bullet.

3D modeling and animation software is often used to analyze shooting events because of their ability to demonstrate the interaction between objects and projectiles in terms of time and space. An accurate computer model of the scene is necessary to achieve the best results. Point clouds generated by laser scanners can be imported into the software and used as they are or as a template to create the geometry in the scene. Once a scene is constructed, mannequins are placed in the scene to represent the individuals involved. Bullet paths can be depicted as lines through space from their point of origin to the objects they strike. Bullet deflections can be calculated based on the evidence. If a person is hit, the path of the bullet through the body can be depicted with lines placed through the mannequin, the same way a pathologist places rods through a body. Those paths are typically placed according to descriptions and measurements noted in the autopsy report, as well as photos of the wounds.

In this shooting incident, sheriff's deputies responded to a report of two men in a Sports Utility Vehicle (SUV) driving around a neighborhood with the passenger threatening residents with a handgun. One person who was threatened obtained the license number of the vehicle. The deputies were unable to find the suspects, so they watched the residence that the car was registered to. Late in the evening, the vehicle with the two suspects arrived at the location. The passenger, who had been brandishing the gun, immediately exited the vehicle and confronted the deputies. The driver of the vehicle then parked the SUV in a driveway approximately 20 feet down the road, exited the vehicle, and observed the confrontation between his passenger and the deputies.

The deputies had their weapons drawn and pointed at the passenger. They noticed that the driver was standing in their backdrop and ordered him to move. As the deputies ordered the passenger to show his hands and get on the ground, they lost sight of the driver. When the passenger reached inside his jacket and started to extract a gun, multiple officers simultaneously fired multiple shots at the passenger and he fell to the ground. One bullet struck the hand with which he was holding the gun, penetrating both his hand and the handle of the gun.

After the shooting, the body of the driver was found approximately 40 feet south of the passenger's location, lying on the sidewalk behind a car in a pool of blood. The question in this case was how and why was he shot?

The autopsy revealed that the driver was struck by a single round to the left side of the neck, severing the left carotid artery and left jugular vein. Macro photography of a fragment of bullet jacket and core recovered from the wound exhibited striations and deformation consistent with striking a hard, abrasive surface.

The shooting site was laser scanned and the resulting point cloud data was imported in a 3D modeling program. The position of all the deputies and the suspect passenger were determined at the time of the shooting. The locations of bloodstains and bullet strikes were identified. The trajectory model revealed that a stray bullet fired at the suspect passenger had deflected off the concrete driveway and struck the bystander driver as he was crouched behind the front end of a parked car. The path of the bullet through the neck was consistent with this position. Also, the lack of a blood trail was consistent with him collapsing where he was shot. The computer modeling also demonstrated that in that position, he would not have been visible to the deputies at the time they fired.

Shootings, Laser Scanning, 3D Modeling

E74 Bulletproof: Two Incidents of Non-Penetrative Bullet Strikes Inflicted on Soldiers Without Armor During Operation Iraqi Freedom

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After attending this presentation, attendees will have a greater understanding of the effects of unintentional intermediate substrates on would-be victims of gunfire. The substrates were circumstantial to each incident and were not intended or designed for protective purposes or to defend against firearm projectiles. The intermediate substrates likely saved the lives of both victims in the two case studies presented.

This presentation will impact the forensic science community by providing two case studies of rare, anomalous gunshot incidents by the United States Army special agents who investigated them. The case studies presented are in a category of unique and compelling incidents that often become anecdotal "legends" within law enforcement circles. This presentation will formally establish them within the academic community as a resource for future research and possible practical application.

While serving as two forward deployed special agents in the United States Army Criminal Investigation Command during Operation Iraqi Freedom (OIF) in 2005-2006, the two agents each worked a separate, unusual firearm incident involving soldiers being struck by firearm projectiles, but with little to no injury. Both incidents occurred on United States Army bases in Iraq. United States soldiers supporting OIF were issued Individual Body Armor (IBA) at this time in the conflict; however, neither of the victims in the incidents were wearing their IBA as they were located in office or residential settings with presumably little to no enemy threat. The bullet projectiles struck both victims, but failed to penetrate or otherwise break their skin due to the intervention of substrates circumstantial to each incident. As a result, both victims suffered minimal to no injury from the gunshots.

Case 1: In April 2006, a United States Army soldier committed suicide with his rifle in his quarters. The rifle's projectile passed through the soldier, the wall of his quarters, and the wall of an adjacent quarters, striking a second soldier in the back with almost no injury.

Case 2: In December 2005, an Iraqi national interpreter attacked a United States Army intelligence soldier with a pistol. The pistol's projectile struck the soldier in his uniform's chest pocket over his left chest, where he kept his unit standard operating procedure booklet and his official badge and credentials. The bullet penetrated the booklet and lodged in his badge. The soldier was able to overcome his attacker and survived the incident. The soldier received minor bruising below the impact site, but was otherwise uninjured.

Firearm Incidents, Unique, No/Minimal Injury

E75 A Unique Capstone Experience to Assess Student Learning in a Bachelor of Science (BS) Forensic Science Program

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After attending this presentation, attendees will better understand how student learning is assessed in a BS Forensic Science degree program at Eastern Kentucky University. In FOR 499: Forensic Science Capstone, forensic faculty identify whether students have acquired the central knowledge and skills that are the expected learning outcomes of the program.

This presentation will impact the forensic science community by providing assessment measures and criterion for producing the next generation of forensic scientists who are prepared with the skills and knowledge to be leaders in the field. This presentation will add to educational research being conducted in forensic science undergraduate programs.[1]

Using a capstone experience to assess learning in an academic program first requires a set of program learning objectives that are the same as the capstone course learning objectives. Below are the program and course learning objectives:

Student Learning Objective 1: Students will employ the theoretical and practical principles of chemistry, biology, physics, and mathematics to perform the work of forensic scientists. Specifically, given a specific type of evidentiary material, students will demonstrate the ability to: (1) recognize, collect, package, and store evidence commonly found at crime scenes; (2) evaluate analytical requirements and design an appropriate analytical scheme to obtain desired results; (3) properly prepare and analyze the material to generate relevant data supported with the understanding of the role of proper quality control samples; and, (4) evaluate the data and make judgments concerning analytical results.

Student Learning Objective 2: Students will demonstrate effective written and oral communication skills, especially the ability to transmit complex technical information in a clear and concise manner. Specifically, students will: (1) write concluding statements to communicate results and decisions to law enforcement and the legal system; and, (2) defend and justify decisions orally and in writing.

Student Learning Objective 3: Upon the successful completion of the BS Forensic Science degree, students will demonstrate the attitudes, ethics, and values that enable students to reach the highest level of achievement and personal growth. Specifically, students will: (1) demonstrate honesty, reliability, punctuality, motivation, strong work ethic, and good interpersonal skills that are the hallmarks of a professional scientist; and, (2) demonstrate the ethical behavior, learning skills, and initiative needed for life-long learning.

In this presentation, the capstone assessment method will be explained. In the course, the students process, analyze, report, and testify during a mock case. Faculty evaluate the individual students and the grouped data is provided to evaluate at the program level.[2] Meaningful change is accomplished when changes are made in foundational or "downstream" courses when program learning objectives are not met. Specifically, in this assessment cycle, the following three program change needs were identified following capstone assessment: (1) students need more knowledge and practice of evidence collection and processing before final assessment in FOR 499: Forensic Science Capstone; (2) students need additional practice with testimony in the FOR 465W: Expert Witness Testimony course prior to final assessment; and, (3) students need to have decided on an area of specialization in either toxicology and drugs, trace evidence, or DNA prior to registration for the capstone course.

This presentation will further explain the assessment measures and criterion used to measure mastery of each student learning objective, allowing "closing the loop" at the program level.[3]

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Capstone, Assessment, Education

E76 Developing an Information Literacy-Intensive Forensic Science Course

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The goal of this presentation is to demonstrate a model in which forensic science educators can utilize resources at university libraries to promote scientific information literacy and lifelong learning strategies for their forensic science students that they can continue to use in their professional forensic careers.

This presentation will impact the forensic science community by highlighting the benefits of forensic science faculty collaborating with subject specialist academic librarians to produce a course that fills a gap in the education of future forensic science practitioners. This course facilitates the development of productive search strategies and explains how different types of scientific research and legal materials are relevant to various forensic science disciplines. This collaboration has demonstrated a need for promoting information literacy, specifically for open-access materials, regarding forensic science information to these students, so once they graduate, they may still acquire the valuable information necessary for their jobs. As a result of this research, it was determined there was a need to expand this information literacy beyond the classroom and National Institute of Justice (NIJ) funding was acquired to allow for the development of these materials as modules to be used by practitioners in crime laboratories to enhance available resources for their use.

Forensic science is a multi-faceted field, including expertise from a variety of disciplines. A challenge for creating a college course to address skills to develop information literacy competencies and encourage lifelong learning for future practitioners is covering those diverse disciplines. This presentation will detail how librarians at Texas A&M University developed a junior-level forensic science seminar in collaboration with a forensic science faculty member to meet the research needs of students in the forensic sciences program. The learning outcomes of this class include: teaching students to describe problem-solving principles; organizing typical operational protocols; recognizing the scientific basis and application of tools and techniques in forensic science; comparing capabilities and limitations; and summarizing and illustrating current scientific, ethical, and legal issues. This presentation will detail the steps taken to create six separate information literacy-intensive classes, including the development of the assignments, and how feedback was provided to the students. These classes include sessions on dissecting scholarly articles and case law, as well as classroom discussions to teach students how to use the structure of research articles and case law to easily evaluate information. By creating this information literacy course, instructors were able to better prepare students for their program's research-intensive courses with the amount of detail required that cannot be covered in a traditional, one-hour library instruction session. Such skills will be of value when working in forensic science fields and when the need arrives for locating similar resources in relation to casework. Additionally, this presentation will address issues raised in class, such as open access, database access, and evaluation of science and legal materials, which helps the students translate current school work to their future careers. To further this concept of information literacy, as previously mentioned, the researchers received NIJ funding to determine how forensic scientists locate and evaluate information, to create educational materials aimed at enhancing these skills, and to increase awareness of other valuable informational resources. Locating and evaluating high-quality forensic science literature will help forensic scientists engage in quality science practices.

Results from this class, which is now in its third year of evaluation, demonstrate that students who take this preparatory course are better prepared for conducting meaningful research for their writing-intensive senior-level classes, both in their abilities to find relevant materials and in how to utilize and cite these materials. Graduating students who go on to graduate or to law school have also reported back that taking this course gave them a foundation to build on in their advanced studies.

Information Literacy, Forensic Science Education, Libraries

E77 Graduate and Undergraduate Education in Forensic Sciences in Turkey

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The goal of this presentation is to provide information regarding the undergraduate and graduate educational programs in the fields of forensic science in Turkey.

This presentation will impact the forensic science community by providing information about the undergraduate and graduate forensic science programs available in Turkey.

In Turkey, evaluation of criminal evidence is achieved by government agencies and private corporations, as is the case for the rest of the world. Among these institutions are the Head of Department of Criminal Police Laboratory and District Laboratories, the Head of Gendarmerie Forensic Laboratories and District Laboratories, the Presidency of Legal Medicine and its subsidiaries, the General Directorate of Customs Enforcement's Laboratories, and the Ministry of Health's Presidency of Hifzisihha and District Laboratories. The presence of expert reports can disturb the public conscience, and unsatisfactory court results are a reality in Turkey in that these reports can arrive at different conclusions from the evidence, and a person can interpret evidence differently from when the evidence in question was analyzed. The biggest problem in Turkey is not any form of pressure directed against government agencies, nor are the custom-made reports issued by private corporations; however, the issue is highly related to the presence of inexperienced and unqualified personnel, in short, the absence of quality assurance and lack of external audit. It was deduced in the report of the State Supervisory Council of the Republic of Turkey that "there have been critical defects for training experts in non-medical disciplines" and that "the expert establishments are problematic, and no criminal expert is being trained."

Raising the level of quality in justice services in Turkey to a modern standard, enabling services provided by crime laboratories and crime scene investigation in Turkey to the maintained level, and preventing discrepancies and misinterpretations in reports is only possible by training the personnel who perform the analysis according to a scheme enabling them to become target-oriented. Courses provided to students who graduated from various science disciplines are obviously not suitable and sufficient to enable them to conduct surveys in a crime laboratory and prepare an expert report, nor are any practical courses of evidence analysis taught as a matter of fact. Thus, these people are trained under a mentor system after employment at private corporations or government agencies. Graduates of forensic science graduate programs, on the other hand, struggle to compensate for their lack of knowledge, a problem arising due to the completion of a different undergraduate program. Current graduate programs are far from training future candidates for a broad range of crime laboratories analyzing chemical, physical, and biological evidence in that these people often graduate without having prior knowledge of current trends and practices. It is also a fact that master's or PhD holders of these programs prefer academic life, rather than employment in a relevant field.

In Turkey, as with every developed country, it is necessary to instruct any interested candidate well equipped with knowledge and experience in forensic science at the undergraduate level to prepare them for conducting scientific research, for developing novel methods and instruments, or for future positions, such as private or government crime laboratories. Transforming our expert establishments, whether private or governmental, and our crime laboratories into organizations that are accredited and well-known in Europe and around the world, and also enabling the export of high technology heavily depends on the aforementioned. The Forensic Science program that is proposed to open under Uskudar University's Faculty of Engineering and Natural Sciences will be the first such in Turkey, though many types of this program exist abroad. The learning content was prepared in accordance with the Forensic Science Education Programs Accreditation Commission (FEPAC). FEPAC is a body of the American Academy of Forensic Sciences (AAFS), founded in 1948, which currently has more than 6,500 members and is the only association in the world that accredits undergraduate and graduate education.

Forensic Science in Turkey, Undergraduate Education, Graduate Education

E78 Language Use Among Forensic Professionals

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After attending this presentation, attendees will understand patterns of language use that exist among the various forensic professionals.

This presentation will impact the forensic science community by offering insight into various linguistic determinants that influence language use in forensic settings.

Understanding what constitutes proper communication is an important component of interaction for forensic science professionals. Representing a broad range of job titles, work environments, and materials analyzed, forensic professionals engage in various forms of communication among colleagues and the public. The linguistic term "register" refers to the concept that language varies with individuals and their social practices. This study explores register use among the different forensic science disciplines to determine which patterns may exist that are unique to each discipline.

Data on language use were collected using an Institutional Review Board (IRB) -approved anonymous survey that was disseminated online to AAFS membership during the summer of 2016. The survey consisted of 15 questions -— six demographic questions (including age, sex, field, education, and years of experience) and nine questions regarding language use. These latter questions were designed to elicit whether registers exist that are impacted by location or the presence of others, whether or not instruction was given, and considerations of appropriate and inappropriate speech. An open-ended format was used for some of the questions to allow participants to provide examples or further explanation to their responses. Survey responses were used to analyze and compare perceptions of language use among forensic professionals.

Nine hundred seventy-six individuals representing all sections consented to participate in the survey; however, as participants were given the option to skip any question, not all questions received the same number of responses. Participation by the AAFS sections generally reflects membership numbers, with only the Anthropology Section demonstrating a notable increase between survey responses and number of section members.

With regard to language use, 71% of participants indicated their speech was impacted by the presence of other people, with superiors (46%), peers (43%), and students (43%) listed by these participants as most likely to affect language use. The use or avoidance of technical terms was the most common manner in which speech was impacted by others (40% of respondents). Additionally, 65% of respondents indicated that location impacted their speech, with "public space" selected as the most likely location to affect discourse (40%). Alternatively, 14% of participants claimed they felt audience and not location is what influences their speech. With regard to training, 46% of participants indicated that instruction for appropriate language use was not discussed at the start of their career. If language considered inappropriate is used, 49% of participants responded that the consequences would result in a minor reprimand. When asked to provide examples of language considered appropriate for the workplace, 25% of participants responded with terms representing the concept of death and human remains, with the top two reoccurring words being "decedent" (40%) and "deceased" (25%). When asked about inappropriate words, expletive language was cited or alluded to in 37% of responses.

In conclusion, results demonstrate that patterns of how professionals use language in various forensic environments can be observed, regardless of if the speaker is conscious or unconscious of this use. Many participants revealed various levels of awareness that their choice of language was in some way dependent on situation and audience. Survey data can be used to gain insight into a number of professional scenarios of language use as individuals with different backgrounds of education and professional experience define language use in various ways.

This presentation concludes that professionals exhibit metadiscourse awareness in forensic settings.

Forensic Science, Language Use, Registers

E79 Evaluation of the Booz Allen Hamilton Tactical Forensic Device: The VAMPIRE™

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After attending this presentation, attendees will understand the value of mobile fingerprint capture and identification.

This presentation will impact the forensic science community by providing insight into the capabilities and limitations of the Booz Allen Hamilton VAMPIRE™ as a mobile Automated Fingerprint Identification System (AFIS). These capabilities include photographing developed latent and latent fingerprints, capturing known and live-scan fingerprints, comparing known to known, known to latent/developed latent, and latent/developed latent to latent/developed latent.

Fingerprints have proven to be valuable in the investigation of crimes by placing an individual at a specific location and/or providing insight into whether that individual handled specific items. The VAMPIRE™ is capable of capturing both developed and undeveloped latent prints through a simple "point and shoot" method. Once the image has been captured, the image is compared to both the onboard collection history of other data previously collected and/or a watch list of individuals the operator generates from known persons of interest. The VAMPIRE's™ algorithm provides a list of possible individual identifications leading to possible future verification. No previous research or validation of the VAMPIRE™ has been conducted to date as the device is relatively new and not widely available. By comparing the image quality, image clarity, identified number of minutiae, and image quality map percentages of the VAMPIRE™ captured images with the images more commonly acquired by a Digital Single-Lens Reflex (DSLR) camera, it will be possible to ascertain if the VAMPIRE™ is a valid tool for field and lab-based forensic work.

To evaluate the VAMPIRE™on its captured image quality, image clarity, number of minutiae, and image quality map percentages in similar fashion to Pulsifer et. al., a trio of programs were utilized: the Federal Bureau of Investigation (FBI) Criminal Justice Information Services Division's Universal Latent Workstation (ULW), version 6.6; the GNU Image Manipulation Program, version 2.8 (GIMP); and Wolfram Mathematica, version 11.1.1.[1] Thirty-two friction ridge impressions from the same individual were placed on 15 surfaces of varying textures and porosities. Three magnetic powders (blue, black, and white) and one dye-stain (cyanoacrylate fuming and Ardrox™) were used to develop the latent impressions. Each of the developed impressions was captured by both the VAMPIRE™ and a Canon® EOS Rebel XSi camera and introduced to the ULW where the image quality, image clarity, and number of minutiae identified were recorded. The ULW allowed for application of an image-quality mask. An image-quality mask, also referred to as a clarity map, provided a more standardized analysis of the size and clarity of areas within a given image.[2] Once the image-clarity map was applied, it was "darkened" within the ULW and exported.[1] GIMP was used to brighten the specific darkened colored regions. The image was imported into Mathematica, where a series of commands were written to generate the percentage of the total image that corresponded to the specific colors present. The colors of the quality map range from red (questionable ridge flow present) to the observed royal blue (ridge flow, minutiae and ridge edges are certain). The comparison of the assigned color percentages, as well as the earlier recorded values between the VAMPIRE™-captured images and the DSLR-captured images allowed for any differences between the capture methods to be established.

Preliminary statistical analysis utilizing 3 of the 15 total surfaces (aluminum foil, Styrofoam™, and white painted wood) indicated there was a significant difference between the two capture methods for the number of total minutiae identified in each image. VAMPIRE™-captured images had a significantly larger number of minutiae identified than the DSLR-captured images. No significant differences were found between the two capture methods when comparing the latent quality score of the images and the overall clarity of the images.

Further research is underway to analyze the remaining 12 surfaces. The conclusion will provide further insight into the capabilities and limitations of the VAMPIRE™ as compared to the more commonly used DSLR.

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Mobile Identification, AFIS, Biometrics

E80 Enhanced Postmortem Fingerprinting Techniques

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After attending this presentation, attendees will understand the challenges encountered when identifying human remains through postmortem fingerprinting and recognize the utility and importance of enhanced fingerprinting techniques.

This presentation will impact the forensic science community by disseminating a fingerprinting technique which reduces the overall training burden, is quickly mastered by morgue technicians and forensic investigators, is less time consuming to perform, and has proven to be a cost-effective method of obtaining quality fingerprints leading to a positive identification within a fast turnaround time.

In the United States, it is the responsibility of the many decentralized local, county, and state Medical Examiner/Coroner (ME/C) offices to investigate all violent, suspicious, sudden, and unexpected deaths that fall within their jurisdiction.[1] Beyond establishing the cause and manner of death, these offices are also frequently tasked with conclusively establishing the identity of the decedent by scientific and objective means. A majority of cases are identified visually by the legal next of kin or a friend. This common process can present complications when the death occurs suddenly or unexpectedly while unattended at home or the decedent is in a hospital for a brief duration and personal documents are of dubious origin. Due to the resulting emotional stress or visceral responses of the newly bereaved, visual identification is fraught with danger and, at times, is of limited value in cases of advanced decomposition. Despite challenges experienced in this subjective identification method, a significant proportion of the typical ME/C caseload consists of accepting unidentified individuals with little chance of routine visual identification. It is generally expected by the public at large that the offices can establish an identity; however, this expectation is at times unrealized. As of December 2015, there were more than 8,000 unidentified person records reported in the National Crime Information Center's (NCIC) Unidentified Person File database.[2] Additional data suggests ME/C offices reportedly receive approximately more than 4,000 unidentified decedents a year.

In cases involving significant criminal activity, or those likely to involve evidentiary scrutiny during trial, visual identification should be secondarily supported by confirmation alongside a scientific technique: fingerprint comparison, comparisons of dental records, imaging studies, and DNA analysis. Of all these scientific procedures, fingerprint comparison is the first and most commonly used method to positively identify an individual and is used prior to other more costly options.[3] Once fingerprints have been obtained, most ME/Cs have the wherewithal or liaison with law enforcement personnel to establish or confirm the identity of the deceased.[4]

Enhanced fingerprinting techniques are paramount in cases of advanced decomposition or mummification and are frequently used in situations in which the decedent is recovered from scenes involving fire, prolonged submersion in water, other adverse environmental effects, extensive trauma to the face, dismemberment, insect activity, or animal predation. Older techniques for recovering fingerprints in these types of cases involved a combination of injecting fluids subcutaneously or further mutilation by removing the complete finger or hand from the body. These procedures have known limitations to successful deployment and are considered to be expensive, caustic, insensitive, and arguably morally unethical.

The demonstrable fingerprinting techniques of preference have proven effective in the most challenging circumstances. The impressions of the fingerprint are made from these methods and submitted for comparison to the relevant police department. These techniques have been used in a significant sample of cases with exemplary results.

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Fingerprints, Decomposed, Medical Examiner/Coroner

E81 Is Latent Print Viability Affected by Heat (Accumulated Degree Hours) From 60-Watt Incandescent Light Bulbs?

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After attending this presentation, attendees will understand how time and temperature, as a combined variable, impact the viability of latent fingerprints deposited on 60-watt incandescent glass light bulbs.

This presentation will impact the forensic science community by directly addressing the lack of research regarding the effect of varying environmental factors on pattern and impression evidence, as discussed in the 2009 National Academy of Sciences (NAS) Report.[1] A statistical regression model (using Accumulated Degree Hours (ADH) as the independent variable) will be used to predict latent print viability and provide a time frame for when a print was deposited.

In the fall of 2016, the Henrico County Police Department in Richmond, VA, recovered a print from a light bulb in a burglary case, speculating that it had been removed to prevent suspect identification; however, the defendant asserted that he had touched this light bulb months prior to the crime, and there was no published research from which to assess the validity of this claim.[2] Current research has addressed the detection limits of certain components of fingerprint residue, such as serine, which has been detected by Gas Chromatography/Mass Spectrometry (GC/MS) after exposure to temperatures of up to 150°C.[3] Although this information is valuable, it does not address the practicality of crime scene investigators visualizing and preserving prints, nor does it use time and temperature to estimate the time since a fingerprint is deposited.

This project addresses issues not covered in this study by: (1) recovering latent prints with conventional methods; (2) evaluating fingerprint quality based on a previously established scale; and, (3) relating the combined variables of time and temperature in a regression model that can be used to estimate the elapsed time since a fingerprint was deposited.[4] Through the use of the regression model and popular fingerprint recovery methods, this study will demonstrate the persistence of latent fingerprints and provide law enforcement with additional information that could bring perpetrators of crime to justice.

Ten light sockets were mounted on five strips of shipping wood and wired in parallel to provide equal amounts of electricity to each bulb. Each light bulb was secured into each light socket with gloves. Once secured, nine fingerprints were deposited on each bulb with medium pressure. A 12-hour baseline test was performed to determine the best length of time to leave the bulbs on for subsequent testing. After the baseline test, each unit was turned on for 18 hours, 72 hours, 120 hours, and 168 hours, respectively. A thermal imaging camera monitored the units to record the generation of heat from the bulbs. After each allotted time period, the prints were enhanced with black powder and lifted with tape onto a lifting card to be examined for quality using an 11-point scale.

Latent prints were recovered through the baseline test (1,488 Accumulated Degree Hours (ADH)), so the units were left on longer. Still, latent prints were recovered through 18-hour heat exposure (2,231 ADH). Of the 81 prints recovered, 54 ranked within the top half of the 11-point scale and were identifiable. Results were similar after 48 hours (5,991 ADH) and 72 hours (8,968 ADH). During these tests, 89 and 83 latent prints were recovered, with 58 and 61 being deemed identifiable, respectively. Additionally, latent prints were persistent and proven recoverable after five days (14,875 ADH) and seven days (20,948 ADH). Prints insufficient for identification were often adversely affected by moisture in the print and movement when the print was deposited, which occurred independently of heat.

In conclusion, the analyses of latent prints exposed to heat over time could provide valuable information to law enforcement and is an important addition to the body of work in the field of pattern and impression evidence.

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Latent Fingerprints, Accumulated Degree Hours, XX Scale

E82 An Efficient Workflow to Recover Examination-Quality Postmortem Fingerprints From Human Remains

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After attending this presentation, attendees will recognize and understand the various techniques used during Postmortem (PM) fingerprint recovery for obtaining examination-quality fingerprint records from Unidentified Human Remains (UHR). The incorporation of the highlighted workflow into morgue operations may help identify many of the UHR that are being held within Medical Examiner/Coroner's (ME/C) offices throughout the United States. Not only will identification of UHR yield valuable investigative information, but it also allows for the notification of next of kin regarding the fate of their family member. The use of fingerprints for forensic identification is a rapid, reliable, and cost-effective means to identify UHR.

This presentation will impact the forensic science community by furthering the ability of ME/C offices and other medicolegal professionals to enhance operations by utilizing the PM fingerprint recovery workflow to efficiently and effectively handle UHR identification matters. The specific directions and resources needed to use the updated PM fingerprint recovery techniques will be presented.

The use of friction ridge impressions for identification is well established in forensic science; however, the most efficient workflow for PM fingerprinting is often complex and the need for advanced techniques must be determined on a case-by-case basis. The condition of the friction ridge skin on each decedent will dictate which method must be used to successfully enhance and record any valuable friction ridge information; multiple techniques may be used on each UHR. The wide range of techniques include the reconditioning of skin using tissue injection, soaking/rehydration, boiling, manipulation of degloved epidermal skin, as well as recording techniques such as ink/card, fingerprint powder/adhesive lifter/acetate sheet, fingerprint powder/castings, photography, and digital scanning. While some of these techniques are by no means new or foreign to forensics, their utilization is adapted to serve the cause of identifying UHRs. Moving forward, new potential techniques such as 2D and 3D fingerprint scanning may also be introduced into the proposed workflow. A major difference in printing the deceased versus the living is the manipulation of the fingerprinting medium (i.e., fingerprint card, ink pad, scanning platen, etc.). When printing the deceased, the fingerprint medium is being manipulated against the decedent, as opposed to the living, where the person is being manipulated against the medium. Conditions such as rigor mortis, skin slippage, and mummification can all discourage attempts to obtain PM fingerprints from the decedent. Contrary to the predisposition that a specific decedent may not be printable, ridge detail on the epidermis and the dermis can still allow for acceptable friction ridge fingerprints despite the aforementioned conditions. As a result, reconditioning techniques should be used, followed by attempting fingerprint recovery techniques using the specific workflow that will be proposed by this presentation.

Postmortem Fingerprint, Unidentified Human Remains, Forensic Identification

E83 Fire Debris Analysis

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The goals of this presentation are to demonstrate: (1) the evaporation rate of ignitable liquids poured on cotton at different times; and, (2) the comparison between four types of textiles after impregnating them with ignitable.

This presentation will impact the forensic science community by revealing that: (1) the chemical composition of the volatile fraction collected from three fuels (gasoline, kerosene, and diesel) is notably different; (2) the evaporation rate of gasoline is faster than for kerosene and diesel; (3) a Solid-Phase Microextraction (SPME) sampling technique coupled to Gas Chromatography/Mass Spectrometry (GC/MS) is a very convenient, sensitive, and eco-friendly technique for the determination of volatile fuel residues; and, (4) synthetic textiles retain fire accelerant residues longer than natural fabrics.

Arson is a dangerous crime; even a small amount of an ignitable liquid can cause considerable loss of property and even human life. The identification of the arson source is a significant forensic aspect of the criminal investigation; however, it is not always straightforward, as fire often destroys most evidence of arson. In addition to the complex nature of the accelerants and the fire itself, the various materials that are most commonly used to extinguish fires, such as water, dry chemicals, and foams may also affect or even destroy the evidence. The investigations conducted at a fire crime scene should rapidly collect and analyze the debris materials resulting from the fire, including the detection of any remaining hydrocarbons in order to identify the original ignitable liquid. This is why the extraction, isolation, preconcentration, identification, and determination of these target compounds that could be used to accelerate a fire are highly important.

Among the various methods available for extraction of volatile residues from fire debris, SPME proved to be a fast, simple, and efficient extraction technique. This procedure was used, followed by GC/MS to investigate the residues of gasoline, kerosene, and diesel impregnated on various textile materials: cotton, nylon, polyester, and wool. After impregnation, each textile sample was stored in ambient conditions. The remaining fuel residues were collected by SPME, then separated and detected by GC/MS. The obtained results revealed that the polydimethylsiloxane fiber used for SPME was capable of detecting as low as 200µL of each fuel accelerant at optimum conditions. In addition to the properties of the fire accelerant itself and the ambient conditions, the evaporation rate of the fuel-ignitable liquid also depends on the absorption characteristics of the textile substrate. This study demonstrated that the diesel fuel residues were more persistent on all tissue samples than gasoline and kerosene, even four hours after the impregnation. In the same environment, the three investigated fuels (gasoline, kerosene, and diesel) remained a longer time on nylon, then polyester, wool, and cotton. Therefore, collecting fire debris from synthetic fabric samples such as nylon or polyester should be more interesting for medium periods, to allow identification of the fuel accelerant used in the crime scene.

In conclusion, the results obtained in this work demonstrated that the use of SPME for sampling of accelerant residues in arson debris is a fast and effective method and is more convenient than traditional procedures. It allows detection and determination of the fuel constituents even at very low concentrations.

Fuel Residues, Arson, SPME-GC/MS

E84 Race Differentiation by Raman Spectroscopy of a Bloodstain for Forensic Purposes

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The goals of this presentation are to illustrate: (1) the characteristics of current techniques applied for bloodstain analysis during forensic examination; (2) the importance of human phenotype profiling from body fluids found at a crime scene; (3) the significance of a non-destructive method for examination of trace evidence at a crime scene; (4) the advantages of Raman spectroscopy for crime scene examination; (5) Raman spectroscopy for body fluid analysis; and, (6) the application of advanced data analysis for distinguishing between human races based on bloodstains.

This presentation will impact the forensic science community by demonstrating how a non-destructive and rapid method is ideal for characterizing blood donors for forensic purposes. Gathering information about the donor can narrow the search during an investigation and can exclude irrelevant traces before a stain is subjected to DNA profiling. This study demonstrates the application of Raman spectroscopy, combined with chemometrics, for discriminating between Caucasian and African American blood donors.

Human blood is the most common body fluid found at scenes of violent crimes and it is an extremely valuable form of evidence in forensic investigations. The amount of sample available for an analysis could be extremely small. Therefore, proper handling and examination of an evidence is critical to preserve the trace for further examination. The ideal method for bloodstain analysis at a crime scene would be non-destructive, while providing a substantial amount of information about the sample.

In this study, Raman spectroscopy and advanced statistical analysis were applied to discriminate between Caucasian and African American donors based on dry traces of peripheral blood.[1] Samples from 20 donors varying in sex and age were used for collecting Raman spectra. This study utilized Genetic Algorithm (GA) analysis, which helped to select the spectral regions with the largest diversity between Caucasian and African American peripheral blood. For statistical analysis, Principal Component Analysis (PCA) was used to remove outliers. To discriminate between the two races, Support Vector Machines Discriminant Analysis (SVMDA) models were created. The internal Cross-Validation (CV) revealed 71% correct classification of donors based on all spectra included in a training data set. An outer loop subject-wise CV method was also performed and served to evaluate the performance of the SVM classifier for each individual donor from the training data set. The performance of SVMDA, evaluated by the Area Under the Curve (AUC) metric, revealed 71% probability of correct classification at spectrum level and 83% probability of correct classification at donor level for both races. A specificity and sensitivity of 80% was obtained. This proof-of-concept study demonstrated a great potential of Raman spectroscopy for determining a donor's race based on the bloodstain analysis. This method provides rapid and reliable results without any preparation, destruction, or consumption of the sample. The application of Raman spectroscopy at a crime scene is highly probable due to commercially available portable instruments. Furthermore, not only can a stain be identified as blood using this technology but, by incorporating statistical analysis, more information regarding the donor can be obtained, all in a reliable and statistically confident manner.

This project was supported by awards from the National Institute of Justice, Office of Justice Programs, United States Department of Justice (I.K.L.).

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Raman Spectroscopy, Blood, Human Race

E85 Nuclear Magnetic Resonance (NMR) Spectroscopy as a Screening Agent for Designer Opioids

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After attending this presentation, attendees will understand the utility of NMR spectroscopy as a screening agent for designer opioids.

This presentation will impact the forensic science community by revealing a new, non-destructive approach to qualitatively and semi-quantitatively analyze pure opioid standards and mock street sample mixtures after minimal sample preparation.

The formulations of synthetic opioids are constantly changing to maintain their legal statuses. These new drugs, often with equivalent or higher potency compared to traditional opioids, are typically derivatives or analogues of controlled substances such as heroin and fentanyl. The lack of analytical standards and the evolving formulation make it increasingly difficult for forensic scientists to identify these new designer drugs.

In this preliminary proof-of-principle study, 16 designer opioids were analyzed with proton NMR, proton-proton Correlation Spectroscopy (COSY), and Total Correlation Spectroscopy (TOCSY). For each sample, 1mg of the drug in solid powder form was dissolved in 0.75ml of either $CDCl_3$ or D_2O, depending on its solubility prior to NMR scans. Then, 1,024 1H-NMR scans were run, lasting two hours. There were 16 COSY and TOCSY scans run per sample, lasting two hours per sample. After the standards were analyzed, mock street samples were prepared by adding caffeine, acetaminophen, and glucose to one of the standards and running proton and COSY scans. A similar method was used in previous studies to rapidly scan for synthetic cannabinoids.[1]

The preliminary results proved that several signature signals in the proton NMR spectra of designer opioids can be used for rapid screening and identification. The three potential signature signal ranges include 6.52ppm–8.28ppm, 4.59ppm–4.76ppm, and 3.02ppm–3.53ppm. Due to structural differences, some designer opioids did not produce a signature signal, which was expected and can be used to exclude certain compounds. For example, most fentanyl derivatives produced a signal in the range 4.59ppm–4.76ppm; however, opioids that are not fentanyl derivatives do not have this signature signal in their spectra. Of the 16 designer opioids analyzed, 15 produced at least one signal within the range 3.02ppm–3.53ppm. The COSY spectra and TOCSY spectra confirm the interactions between the protons on the benzene rings and alkane regions. In the COSY spectra of fentanyl derivatives, there is an interaction between the protons that produced the signals in the range 3.02ppm–3.20ppm. The opioid signature signals could still be isolated and identified from a mixture profile even in the presence of minimal interference (peaks move <.05ppm) or overlap contributed by the cutting agents. The NMR method also enhances the identification of additional isomers in a mixture.[2] Additionally, the proton NMR peaks were integrated and peak areas were utilized to provide semi-quantitative compositions of the opioids and cutting agents within each mixture.

Non-destructive NMR investigation on designer opioids will allow rapid screening of opioids, while also providing semi-quantitative analysis of the substances in mixed street samples. Subsequent GC/MS analysis can still be performed to confirm the identification. The NMR methods will potentially assist investigators in the prediction of the next designer opioid and help law enforcement keep up with new formulations of designer opioids.

Reference(s):

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2. Marino et al. Rapid Identification of Synthetic Cannabinoids in Herbal Incenses with DART-MS and NMR. *Journal of Forensic Science.s* 61 (2015): S82-S91.

NMR, Designer Opioids, Fentanyl

E86 An 11-Year Review Conducted by the West Tennessee Regional Forensic Center (WTRFC) on Deaths While in an Inpatient Rehabilitation or Counseling Center

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After attending this presentation, attendees will be able to recognize some of the most common causes of death seen in the inpatient rehabilitation and counseling center setting, in addition to recognizing a variety of other factors that play a role in drug- and alcohol-related deaths.

This presentation will impact the forensic science community by helping medical examiners recognize the most common causes of death seen in the inpatient rehabilitation and counseling center setting, in addition to providing information that will help rehabilitation centers in implementing programs to better manage the health problems that accompany drug and alcohol addiction.

Drug abuse in the United States continues to be a growing problem. According to the National Institute on Drug Abuse (NIDA), the total number of drug overdose deaths in the United States alone has more than doubled from 2002 to 2015, with deaths caused by heroin exhibiting a 6.2-fold increase.[1] Alcohol abuse also continues to be a widespread issue. The Substance Abuse and Mental Health Services Administration (SAMHSA) reports that an estimated 17.3 million Americans were dependent on alcohol or had problems related to their alcohol use in 2013.[2] With these numbers in mind, rehabilitation centers face the daunting task of managing not only their patients' addictions, but also the sequelae of those addictions. The primary purpose of this study is to present a retrospective review of deaths while in an inpatient rehabilitation or counseling center for the treatment of drug or alcohol addiction reported to the West Tennessee Regional Forensic Center (WTRFC) over a 11-year period, from 2006-2016.

There were 14 deaths of persons undergoing treatment for drug and alcohol addiction in an inpatient rehabilitation and counseling center reported to the WTRFC during this 11-year period. All individuals were either found deceased at the inpatient center where they were being treated (4/14, 28.6%) or were transported to a nearby hospital where they were pronounced deceased shortly after (10/14, 71.4%). The yearly distribution of these 14 deaths ranged from 0 deaths in 2006, 2008, and 2015, and up to 3 deaths in 2012 and 2014 (average of 1.4 deaths per year). Most of the individuals involved were male (10/14, 71.4%) and White (10/14, 71.4%). The average age was 44.5 years (age range 21-66 years). The manner of death in a majority of these cases was natural (11/14, 78.6%), while the remaining cases were classified as either an accident (2/14, 14.3%) or a suicide (1/14, 7.1%). Cardiac-related diseases, including hypertensive/atherosclerotic cardiovascular disease and cardiac arrhythmia associated with cardiomegaly, were responsible for many of these deaths (6/14, 42.9%). Other causes of death included complications of morbid obesity (2/14, 14.3%), chronic obstructive pulmonary disease (1/14, 7.1%), bronchopneumonia (1/14, 7.1%), subarachnoid hemorrhage associated with cocaine (1/14, 7.1%), methadone toxicity (1/14, 7.1%), benzodiazepine withdrawal (1/14, 7.1%), and hanging (1/14, 7.1%). Many of the decedents were being treated at their respective rehabilitation centers for polysubstance abuse (5/14, 35.7%). The remaining individuals sought treatment for either alcohol (4/14, 28.6%), cocaine (3/14, 21.4%), opioid (1/14, 7.1%), or heroin addiction (1/14, 7.1%). Among the 5 individuals being treated for polysubstance abuse, opioids were involved in 4 cases (80%), cocaine and benzodiazepines in 3 cases (60%), and alcohol in 1 case (20%). Many of these individuals also presented with mental illness, including schizophrenia (2/14, 14.3%), schizoaffective disorder (1/14, 7.1%), psychosis not otherwise specified (3/14, 21.4%), and bipolar disorder (2/14, 14.3%). Half of the 14 decedents also reported having feelings of depression upon admission to their rehabilitation center.

This retrospective study provides an initial review of drug- and alcohol-related deaths that occurred during treatment at inpatient rehabilitation or counseling centers in Shelby County, TN. These findings may help rehabilitation centers in implementing programs to better manage the health problems and natural diseases that accompany addiction and prevent similar deaths. These findings will also help medical examiners recognize the most common causes of death seen in the inpatient rehabilitation and counseling center setting.

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2. NIDA. *Nationwide Trends.* National Institute on Drug Abuse, 25 Jun. 2015, https://www.drugabuse.gov/publications/drugfacts/nationwide-trends.

Drug, Alcohol, Rehabilitation

E87 The Development of a Protein Extraction Protocol in Burnt Bone

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The goal of this presentation is to illustrate how burnt bone is a common element at forensic and archaeological scenes. It can provide evidence concerning violent acts such as traumas and, in most cases, represents the unique biological evidence recovery at the scene. The isolation of biological molecules in burnt bone remains is a challenge in forensic and archaeological context because of the damage caused by temperature to the bone. The goal of this work was to design a strategy to perform a protein extraction from burnt bone to obtain biological information about the metabolic status of the subject.

This presentation will impact the forensic science community by presenting a non-demineralized extraction method to obtain proteins from the burnt bone in a single step, which can provide information regarding the premortem condition of the subject from biological material damaged by extreme temperatures.

Bone tissue holds valuable information regarding the physiological status of individuals because the cellular components and mineralized extracellular matrix participate in metabolic events.[1] The burnt bone is a common element of forensic and archaeological scenes; this can provide evidence about violent acts such as traumas and, in most cases, represents the unique biological evidence recovery at the scene. Frequently, the recuperation of biological molecules becomes difficult because of the damage caused by temperature in bone. Although there are several kits available in the market to perform DNA extraction, the isolation of other biological molecules in burnt bone remains a challenge in the forensic and archaeological context.[2,3] The proteins represent valuable biological information about premortem events, such as pathologies, metabolic events, nutritional habits, toxicological information, or even causes of death.[4]

Human remains were obtained from individuals registered in the National Institute of Forensic Sciences (INCIFO) in Mexico City and referred to the Amphitheater Department of Medicine Faculty of National Autonomous University of Mexico (UNAM). The samples were subjected to cremation under controlled conditions at a final temperature of 800°C for two hours. Fragments of rib and skull were recovered after cremation and washed two times with Phosphate Buffer Saline (PBS). The burnt bone was pulverized and all the contained proteins were extracted under reduced conditions, precipitated, quantified by the Bradford method, and evaluated by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Blot (WB). After electrophoretic analysis and Coomassie blue staining of the non-demineralized extraction products, it was found that several proteins were revealed; meaning they support the cremation process and the non-demineralized method was feasible for recovering proteins from both bone samples. Additionally, due to the evident different electrophoretic pattern of bone samples (rib and skull), it could mean there is a specific expression of proteins between burnt rib and burnt skull. This suggests it can be possible to determine the expression of proteins in burnt bone tissue as an organ-specific method to provide information about the premortem condition of the subject.

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Burnt Bone, Protein Extraction, Proteomics

E88 The Effects of Surface Composition and Time Intervals on the Stability of Explosive Residues

Jessica Shiffert, 1713 Forbes Avenue, Pittsburgh, PA 15219; and Stephanie J. Wetzel, PhD, Department of Chemistry and Biochemistry, 600 Forbes Avenue, Pittsburgh, PA 15282*

After attending this presentation, attendees will understand how delayed collection and various surface compositions affect the stability of explosive residues.

This presentation will impact the forensic science community by allowing attendees to better understand the degradation curve of three explosive residues when deposited on three specific surfaces, which will enable the prioritization of evidence collection and analysis in order to aid in investigations of explosion scenes.

As a result of the increased terrorism occurring around the world, the attacks by Improvised Explosive Devices (IEDs) are rising, which leaves them as a constant threat. These devices are not produced for traditional use and are synthesized with home-made components, which makes them unstable and unpredictable. Therefore, additional investigative and analytical efforts are required to identify the explosive elements in IEDs. Both pre- and post-blast residues can be used to identify the explosive element of the IED. Determining the explosive element can aid in the identification of a suspect, which is vital to investigations. These residues can be found at both clandestine laboratories and explosion sites on and around the IED, and various swabbing techniques can be employed to collect them from these locations. It is known that explosive residues will degrade over time; however, it is unknown how the time before collection and different surface compositions affect this degradation. Understanding the degradation curve of explosive residues can be crucial to an investigation. Additionally, this information can allow analysts to prioritize the analysis of evidence that is more likely to yield an identification.

During this research, an alcohol wipe was used as a universal swabbing method to collect explosive residues from multiple surfaces that could be found on an IED or around a clandestine laboratory. These surfaces included galvanized steel, Poly Vinyl Chloride (PVC), and packing tape. Common explosive residues, Royal Demolition eXplosive (RDX), Trinitrotoluene (TNT), and Pentaerythrite Tetranitrate (PETN) were deposited on these surfaces in the form of a liquid standard. These samples were prepared in triplicate to ensure reproducibility, then stored in air-tight containers for the following time intervals: immediate, three days, one week, two weeks, three weeks, and four weeks. Overall, 84 samples per residue were analyzed, generating a total of 252 samples. An optimized method was developed for Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) in Atmospheric Pressure Chemical Ionization (APCI) negative ion mode to identify the explosive compounds. All samples were compared to the immediate time interval to calculate a percent recovery of the explosive residue, then the data was analyzed to see if a trend was observable in the degradation rates. The results of this experiment show the degradation effects of surface composition and delayed collection.

Explosive Residues, LC/MS/MS, Universal Swab

E89 Cytochrome P450 and Chemical Oxidation of Synthetic Cannabinoids JWH-015 and Bay 59-3074

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After attending this presentation, attendees will learn the applications of Thin-Layer Chromatography (TLC) and Ultraviolet (UV) -Visible spectroscopy to characterizing chemical oxidation of synthetic cannabinoids, and how *in vitro* systems such as human liver microsomes and recombinant cytochrome P450 enzymes can be used to elucidate metabolism of synthetic cannabinoids.

This presentation will impact the forensic science community by identifying metabolites for detection of the abuse of the synthetic cannabinoids Bay 59-3074 and JWH-015.

It is hypothesized that *in vitro* incubation with human liver microsomes will allow production of metabolites of JWH-015 and Bay 59-3074. It is also postulated that oxidation of synthetic cannabinoids with chemical reagents will produce some of the same products produced by cytochrome p450 enzymes in the course of metabolism and eventually allow production of metabolite standards.

Methods used in this research included TLC and UV-Visible spectroscopy of synthetic cannabinoids exposed to human liver microsomes, recombinant human cytochrome p450 2D6, and chemical oxidants (sodium hypochlorite and hydrogen peroxide). These analyses will be supported by Fourier Transform Infrared (FTIR) spectroscopy and High-Performance Liquid Chromatography (HPLC).

The first set of experiments determined that millimolar concentrations of the synthetic cannabinoids JWH-015 and Bay 59-3074 were detectable by TLC on silica gel 25 under UV light. The JWH-015 and Bay 59-3074 were incubated with Human Liver Microsomes (HLM) or with buffer only as controls and, after centrifugation, the products were analyzed by TLC and UV-Visible spectroscopy. As controls for cytochrome P450 enzymatic activity of the HLM, the substrates coumarin, and eosin were incubated under the same conditions. The synthetic cannabinoids were not detectable by TLC after incubation with the HLM. The UV fluorescence of the main component of eosin was unaffected, but the fluorescence of a faster-moving component was decreased in one incubation with HLM. The coumarin incubation contained a new, faster-migrating (higher Retention factor (Rf) value) blue component after incubation with HLM but not after incubation in the control buffer lacking HLM.

UV-Visible spectroscopy detected increases in absorbance at 340nm, 337nm, and 310nm in the JWH-015 after incubation with HLM compared to the control. For the Bay 59-3074, UV-Visible spectroscopy detected a decrease in absorbance at 337nm and 340nm and an increase at 310nm after incubation with HLM. An issue with the incubations of the synthetic cannabinoids with HLM is the necessity to work with relatively high dilutions of these compounds because of the low solubility in aqueous buffers and the need to minimize concentrations of organic solvents in which they are soluble to avoid denaturation of the cytochrome P450s.

The synthetic cannabinoids JWH-015 and Bay 59-3074 were treated with sodium hypochlorite to see if oxidation could be detected by TLC for future comparison of the changes to those produced by cytochrome p450 or HLM. The Rf value (or mobility) of JWH-015 on TLC was decreased after incubation of the compound with sodium hypochlorite compared to control, but that of Bay 59-3074 was unaffected. Incubations with the oxidizing agent were also performed with two control substances: eosin and coumarin. In the case of eosin, two fluorescent components were detected in untreated controls whose intensity diminished with incubation with increasing concentration of oxidant. In the case of coumarin, two components were observed under UV-light on TLC plates, the slower moving of which decreased in intensity with an increasing concentration of oxidant.

It is concluded that *in vitro* incubation with human liver microsomes reveals some promise for producing metabolites for chemical characterization for the synthetic cannabinoids Bay 59-3074 and JWH-015. This may help in detecting metabolites of these compounds in biological samples. Further work can also be conducted by using FTIR and HPLC, but Liquid Chromatography/Mass Spectrometry (LC/MS) and Gas Chromatography/Mass Spectrometry (GC/MS) approaches are likely to be most suited for characterizing these metabolites. Chemical oxidation may be further explored as a means of generating oxidized forms of synthetic cannabinoids that are similar to those produced by metabolism.

Cannabinoids, Cytochrome P450, Metabolism

E90 Optimal Headspace Extraction for the Detection of Volatile Organic Compounds (VOCs) Released From Triacetone Triperoxide (TATP) Using Solid-Phase Microextraction (SPME)

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The goal of this presentation is to provide attendees with: (1) more insight into the headspace analysis of TATP; and, (2) detailed information into the VOCs associated with this explosive.

This presentation will impact the forensic science community by presenting a precise, safe, non-explosive, volatile compound in the form of a Controlled Odor Mimic Permeation System (COMPS) that will ultimately equip canine teams with the ability to detect TATP. The dominant headspace odors associated with TATP can be used in the creation of effective canine training aids using COMPS.

Due to its high instability and ease of detonation, TATP is often difficult for canine trainers and handlers to access for regular training purposes. As a result, the majority of canine explosive detection teams are unable to reliably detect this explosive.

TATP, first discovered in 1895, has become very prevalent among terrorist and other malicious groups worldwide since the early 2000s. The infamous 2001 "shoe bomber" utilized TATP, as did terrorists involved in the 2005 and 2006 London attacks. Bombs detonated at the University of Oklahoma in 2005 and Texas City in 2006 also contained traces of the chemical. More recently, this explosive was involved in famous attacks in Paris, Brussels, and Manchester in 2015, 2016, and 2017, respectively. One of the main reasons for TATP's increased use is the fact that it can be easily manufactured using inexpensive, commercially available household items, namely acetone and hydrogen peroxide. More importantly, it is very difficult to detect by traditional security scanners since it does not contain nitrogen. Nitrogen is a common component of explosives that security scanners can successful detect; however, these same scanners are unable to detect TATP. It has been proven that explosive detection canines can successfully detect TATP; however, they are unable to train and maintain proficiency with detecting this explosive because of a lack of availability of TATP during routine maintenance training. To combat this, identification of the TATP VOCs, followed by the development of suitable mimic training aids using COMPS will be conducted.

This study will present results of a developed optimal extraction method for VOCs from TATP utilizing a headspace SPME technique on solid, as well as diluted, TATP samples to reveal those headspace components that are most likely responsible for an alert from explosive detection canines. The dominant headspace odors associated with this explosive will, therefore, be used in the creation of effective canine training aids using COMPS. These peroxide-based COMPS will fill the current law enforcement gap of not being able to train explosive detection canines on peroxide-based explosives by providing an alternative and effective training aid. By using COMPS, the signature headspace odor is housed in a permeable polymer that releases the identified odor at a known and controlled rate. This technique will satisfy the thousands of law enforcement detection canines nationwide that are currently unable to detect peroxide-based explosives as a result of a lack of availability of the material for training purposes. A large number of canine handler teams will, therefore, be able to utilize the developed peroxide-based COMPS to train their canines to recognize these signature odors associated with TATP and, ultimately, improve their detection of the actual explosive.

Triacetone Triperoxide (TATP), Solid-Phase Microextraction, COMPS

E91 The Investigation of Ancestral Origins Using Human Cranial Hair

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After attending this presentation, attendees will gain a different perspective of the amino acids that are present in human cranial hair from individuals of different ancestral origins and will recognize the vital characteristics that could potentially allow for human individualization using hair.

This presentation will impact the forensic science community as it sheds valuable insight on an unbiased method for hair analysis. This research may assist investigators in determining the ancestral origin of individuals using their hair samples on a more objective level compared to conventional forensic hair analyses.

Human hair is often collected at a crime scene, and Microscopic Hair Comparison (MHC) is typically performed, along with DNA analysis, if possible. Unknown samples are microscopically assessed and compared to those of known origin to identify distinct morphological characteristics. Although MHC is a useful screening tool, it relies heavily on the experience and knowledge of the examiner. Hence, it is more subjective and the significance of the results can easily be overstated, leading to flawed testimonies. On the other hand, DNA analysis is a confirmatory test, but results from hair are not always consistent. This is because nuclear DNA degrades rapidly during hair keratinization and is only present in the hair root. Alternatively, mitochondrial DNA (mtDNA) can be used to generate genetic profiles from samples with no root attached as it is found on the hair shaft; however, the amount of mtDNA recovered varies in each case and depends on the individual. Thus, DNA analysis using hair may not be possible in certain cases.

Unfortunately, MHC and DNA analyses are limited options that could serve as roadblocks and potentially reduce the evidentiary value of hair. Therefore, additional studies are necessary to explore other comprehensive techniques. This research utilizes a more objective approach for hair analysis, notably to assist in the differentiation of ethnic origins without the heavy scrutiny and subjective nature of MHC. Hair is mostly composed of proteins and, therefore, contains a large amount of amino acids that can provide information specific to the donor. Considering this, ratios of three amino acids -— serine, phenylalanine, and threonine -— were utilized to investigate differences in hair from donors of different ancestral origins.

With consent, hair fibers from four regions of the head were collected from three individuals of the Mongoloid, Caucasoid, and Negroid anthropological groups. All samples were washed to remove surface contaminants, ground, and digested to break proteins down into amino acids. Finally, samples were derivatized to increase sample volatility and analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). Chromatographic data was obtained using scan mode, the integrated peak areas were measured, and various amino acid ratios were calculated for all individuals. Results indicate that two ratios, serine to phenylalanine and threonine to phenylalanine, are different in all three samples. The greatest difference in both ratios was between individuals of Mongoloid and Negroid descent. These results imply that certain amino acid ratios may vary among people of different ethnic origins and that this method is potentially useful in distinguishing individuals of different ethnic backgrounds in cases in which MHC is insufficient or DNA analysis cannot be performed.

Hair Proteins, Amino Acids, GC/MS

E92 The Classification of Forensic Soil Evidences by Application of Thermally Assisted Hydrolysis and Methylation With Pyrolysis-Gas Chromatography/Mass Spectrometry (THM-Py-GC/MS) and Multivariate Analysis

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The goal of this presentation is to discuss the following findings of this study: (1) GC/MS could be applied by *in situ* methylation of low volatile Soil Organic Matters (SOM) contained in a small amount of soil without complicated preparation; (2) the variation in the amount of soil was minimized by data normalization; (3) the chemotaxonomic marker compounds could be determined; and, (4) the data could be statistically interpreted confidently for soil classification. In this study, THM-Py-GC/MS produced many compounds due to the high complexity of SOM.

This presentation will impact the forensic science community by illustrating how SOM profiling can be used as a complement to mineralogical techniques in statistically classifying soil evidences and in finding chemotaxonomic marker compounds in forensic soil classification.

The forensic classification of soil samples was performed by THM of SOM using Py-GC/MS).[1] In this work, 33 THM derivatives were detected as SOM contained in <3mg soil.[2] Soil was *in situ* thermally hydrolyzed and methylated with Tetramethylammonium Hydroxide (TMAH) in pyro-foil. The specific ions of the mass spectra were selected to separate and minimize the interference between SOM peaks. SOM data were normalized with the sum of peak areas to correct the amounts of SOM contained in the soil, and the chemometric approach based on Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Linear Discriminant Analysis (LDA) was employed to evaluate and compare the soil classification.[3] The first seven Principal Components (PCs) accounted for 94.8% of the total cumulate variance and these PCs were statistically determined by multiple comparisons (Tamhane's T2 and Dunnett's T3) for the post hoc test (p-value <0.05) and were used to construct the LDA model. It was determined that multiple comparisons (Tamhane's T2 and Dunnett's T3) were a statistically good criterion for deciding on the number of PCs for the LDA model. It was also concluded that the discrimination model correctly classified 40 soil samples into six clusters with high accuracy. Furthermore, the 11 marker compounds were investigated according to the loadings of PCs and the normalized data. It was found that six clusters are differentiated by the presence of lignin, fatty acids, and urea.

This method can provide some advantages: (1) GC/MS could be applied by *in situ* methylation of low volatile SOM contained in a small amount of soil without complicated preparation; (2) the variation in the amount of soil was minimized by data normalization; (3) the chemotaxonomic marker compounds could be determined; and, (4) the data could be statistically interpreted confidently for soil classification.

In this study, THM-Py-GC/MS produced many compounds due to the high complexity of SOM. To differentiate soils, PCA, HCA, MC, and LDA were performed to extract the information necessary to characterize soil groups. These results demonstrated that lignin, fatty acid, and urea can be used as potentially useful compounds to characterize soil samples for forensic purposes. These results represent a preliminary investigation of trace organic matter present in soil. It is believed that SOM profiling can be used as a complement to mineralogical techniques in statistically classifying soil evidence and in finding chemotaxonomic marker compounds in forensic soil classification.

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Forensic Soil, Soil Organic Matters, Multivariate Analysis

E93 A Comparison of Bioelectrical Impedance Analysis Techniques for Estimating Postmortem Interval (PMI)

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After attending this presentation, attendees will understand the use of Bioelectrical Impedance Analysis (BIA) for estimating the PMI using Accumulated Degree Days (ADD).

This presentation will impact the forensic science community by presenting developments in a new quantitative method for estimating PMI.

Methods for estimating PMI are an integral part of the medicolegal investigation. The probative value of PMI estimation necessitates eliminating subjectivity. Major confounding factors in PMI estimation include: (1) the lack of regionally specific models; and (2) the challenges associated with estimation in late stage decomposition. BIA was a novel approach proposed by Hansen et al. as a quantitative method for PMI estimation.[1] The method was based on the dielectric properties of biological tissue, which function as a resistor-capacitor circuit. Decomposition changes the circuit in ways that can be quantified using BIA. The goal of this research was to further develop BIA techniques for estimating PMI using BIA measurements and ADD.

BIA techniques using human donors were developed at the Forensic Investigation Research Station, Colorado Mesa University, Whitewater, CO. Two BIA measurement approaches, fixed-distance and variable-distance, were tested on different body segments in this study. Both approaches used hypodermic needles inserted subcutaneously. In the fixed-distance approach, the source and detecting electrodes were positioned at left and right midfemur and midhumerus. The circuit formed in tissue between the detecting electrodes and electrode distances did not vary among donors. In the variable-distance approach, larger body segments were isolated by positioning the electrodes at specific anatomical landmarks. Detecting and source electrodes were paired 10cm apart, and the distance between pairs varied among donors according to individual limb length. Three body segments were used: hand-foot, thigh-foot, and hand-shoulder. Resistance (R) and reactance (X_c) were measured using a single-frequency, 400µA, 50kHz BIA unit. An on-site weather station measured ambient temperature hourly. The daily mean temperature was used to calculate ADD. Total Body Scores (TBS) were calculated following Megyesi et al.[2] Measurements of R and X_c were derived to impedance (Z) and standardized by the distance between detector electrodes.

Linear Mixed Effects Models (LMEM) were used to estimate ADD. This statistical model permits repeated measurements on a single donor. The fixed effects were TBS^2 and Z. The random effects for both intercept and slope were Z and the individual Donor. Model estimates were evaluated by comparing observed ADD (x) versus predicted (y) ADD. Analyses were completed in Program R using the lme4 package.[3,4]

Fixed-distance BIA measurements were possible for a maximum ADD of 6,072 for the midfemur and 6,128 for the midhumerus. Variable-distance measurements were possible for a maximum ADD of 874 for the hand-foot, 874 for the thigh-foot, and 778 for the hand-shoulder body segments. Conditional R^2 for the LMEM were 0.91 for the midfemur, 0.89 for the midhumerus, 0.94 for the hand-foot, 0.95 for the thigh-foot, and 0.95 for the hand-shoulder. The observed ADD versus predicted ADD were similar to the 1:1 relationship for each body segment.

The development of BIA as a method for estimating PMI continues to show promise. Hansen et al. demonstrated that the approach can be used on human remains using gel pads.[1] Measurements using gel pads lasted 227 ADD. This study demonstrates that the use of needles with fixed-distance measurements has the potential for significantly longer PMI estimation. Both the fixed and variable measurements accurately estimate ADD, but differ in the period over which measurements were possible.

BIA demonstrates the potential to address two challenging factors in PMI estimation -— the lack of quantifiable methods and the challenge of estimating PMI in late stages of decomposition. As an objective measure of changes to body composition, BIA may be statistically combined with region-specific macromorphoscopic scoring models for more accurate PMI estimation; however, BIA approaches have not yet been subjected to blind validation tests and models are still being refined, necessitating further work.

This project was supported by an award through the National Institute of Justice, Office of Justice, and the United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation were those of the authors and do not necessarily reflect those of the Department of Justice.

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Bioelectric Impedance Analysis, Decomposition, Postmortem Interval

E94 Determination of Intent: Accident, Suicide, or Homicide? The Utilization of Social Behavioral Science Within the Medicolegal Death Investigation Process

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After attending this presentation, attendees will better understand the difficulties in the forensic evaluation of a decedent's state of mind at or near the time of death. Attendees will be more familiar with interviewing and questioning methods for elucidating accurate information that will aid the forensic pathologist in the determination of the cause and manner of death.

This presentation will impact the forensic science community by providing a more scientifically grounded process for assessing the intent of an individual who cannot be interviewed. Medicolegal death investigators, medical examiners, and coroners will be able to apply this process to specific deaths encountered in forensic death investigations.

Many field investigations are conducted each year as part of the process in determining cause and manner of death. In nearly all cases, the manner of death is dependent on the circumstances surrounding the event. Others not only depend on the circumstances but also the intent of the decedent. These situations often present a problem for law enforcement, the medicolegal investigators, and the forensic pathologist because a scientific methodology for ascertaining an individual's state of mind or intent prior to death has not been readily available.

Access, availability, and the cost associated with consulting a forensic psychologist often prohibit obtaining such a consultation. Additionally, the mental assessment conducted by law enforcement, medicolegal death investigators, and even forensic pathologists may be problematic because these professionals may have little to no formal education in the social behavioral sciences.

Medicolegal death investigations vary greatly, as do the backgrounds of the investigative professionals. The goal of obtaining information useful in determining the cause and manner of death can at times be a simple process and at other times complex. These forensic investigations can include various and differing modes that lead to death, falls, motor vehicle collisions, drowning, and aircraft crashes, to name just a few. Recognition of clues distinguishing an accidental, suicidal, or homicidal death can be elusive. Distinguishing the antemortem psychological state of an individual can prove to be even more problematic for investigators.

Inaccurate or incomplete information supplied to forensic pathologists regarding an individual's psychological state may result in a misdiagnosis regarding the manner of death. This could lead to both emotional and financial hardships for families. Also, statistical information used in the allocation of governmental funding can be adversely affected. Finally, and most tragically, is that a homicidal death goes undetected because of inaccurate conclusion.

A survey of forensic literature reveals few basic field methods for accurately gathering this needed information. This study presents a sequential social behavioral field methodology utilized in the course of a medicolegal death investigation. These include scene evaluation and the directed questioning of family, friends, and other individuals. These methods, used as part of a multidisciplinary approach, may yield more fruitful and effective forensic conclusions.

Psychological, Medicolegal, Multidisciplinary

E95 Forensic Archaeology Matters: Methods, Differentiation From and Contributions to, Other Forensic Strategies in Crime Scene Investigation

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After attending this presentation, attendees will better understand how forensic archaeology and its methodology is differentiated from general archaeology, forensic anthropology, and other forensic methods. This presentation will familiarize attendees with the unique contribution to crime scene investigations that forensic archaeology offers. In general, most crime scene investigation training does not address approaches to and strategies employed with regard to variable environmental factors, human and animal behaviors, and the impact these have on clandestine burials and body dumps. Outdoor settings present a unique set of challenges that call for a unique methodology toward reconstructing the crime scene and collecting evidence that training for indoor crime scene investigation does not typically address.

This presentation will impact the forensic science community by demonstrating the value of forensic archaeology and archaeological techniques to criminal investigations broadly. While archaeological methods have long been employed by forensic anthropologists in body recovery, the same methods utilized by archaeologists have direct applications to all crime scene examination and reconstruction. The recognition of forensic archaeology as a specialty beyond burial excavation will encourage greater incorporation of archaeologists into the forensic community.

This presentation will demonstrate that forensic archaeology is more than simply a learned skill set of technical knowledge for creating grids, excavation, and mapping a crime scene. Forensic archaeologists have an in-depth knowledge of landscapes and seasonal, and environmental changes that affect artifact deposition and distribution (which includes the body) over time in multivariable site formation processes. The value of this in-depth knowledge toward determining the proper approach to each body recovery scene and for contributing to understanding victim and perpetrator interaction in the death event and disposal cannot be overstated. Forensic archaeology emphasizes contextual relationships within an environment and among the evidence of human activity. This fundamental framework of "context" enables a holistic approach to evidence interpretation.

Forensic archaeology in concert with other forensic methodologies offers unique value toward reconstructing the crime scene and/or the deposition of the body, collection methods of artifacts that preserve evidentiary integrity, reconstructing timelines, and working with law enforcement, coroner, and medical examiners' offices *before* the human remains and its associated artifacts are delivered and analyzed in a laboratory setting. Improper handling of the outdoor crime scene can be deleterious to compiling evidence and data for courtroom proceedings and should not be left to those who do not have the academic background and knowledge of a forensic archaeologist.

Forensic Archaeology, Crime Scene Investigation, Methods

E96 Geographic Information Systems (GIS) and Predictive Modeling of Body Disposal Sites

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After attending this presentation, attendees will understand a new method of locating clandestine body disposal sites in the medicolegal context using GIS and predictive modeling.

This presentation will impact the forensic science community by presenting a new technique to assist in locating clandestine body disposal sites in the medicolegal context. Predictive modeling of body disposal sites will narrow search areas beyond the information provided by witness testimony and blind luck, which will maximize resources for law enforcement when searching for missing victims.

Homicide victims are often discovered by accident or located through witness testimony, both of which are unreliable methods.[1] Moving a victim's body from the scene of the crime to a secondary site for disposal may further complicate the discovery, delaying recovery, identification, and evidence collection. Homicides are exponentially more difficult to investigate, solve, and prosecute without a body. This current study seeks to improve search methods by creating predictive models of body disposal location, which can be used in conjunction with witness testimony and traditional law enforcement search techniques.

Predictive models were created using body disposal data collected from the Office of the Chief Medical Examiner (OCME), Connecticut, to explore the feasibility of predicting body disposal sites. Prior to creating predictive models, spatial statistical tests, including Moran's I, Kernel Density, and Ripley's *K*, were conducted to determine if body disposal sites were homogeneously distributed across Connecticut. Next, two predictive models were created: one inductive model and one deductive model, both using non-sites mimicking Complete Spatial Randomness (CSR). The final inductive model equation was determined using logistic regression and stepwise selection to remove non-significant variables. The final deductive model equation was determined using the weighted map-layer approach using only variables where site values were significantly different from non-site values. Both final equations were entered into arcGIS® 10.3 using the Spatial Analyst extension to generate the final predictive surfaces.[2]

Spatial statistical analyses confirm that body disposal locations are inhomogeneously distributed across Connecticut. Results indicate predictive models of body disposal location are 56%–59% more likely to predict body disposal site location in Connecticut than random chance. At present, the models are most successful at predicting body disposal sites in urban areas. Future modeling efforts should address predicting body disposal site location in rural areas.

Predictive models of body disposal location are not intended to replace the current methods of victim search and recovery; rather, they are intended to be yet another tool in the investigative toolkit. The results of this study indicate that predictive models of body disposal location have a real possibility of narrowing search areas and maximizing resources for law enforcement when searching for missing victims.

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Predictive Modeling, GIS, Forensic Anthropology

E97 Deconstructing Desiccation and Decomposition

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After attending this presentation, attendees will understand the importance of testing individual descriptive categories for estimating Postmortem Interval (PMI).

This presentation will impact the forensic science community by presenting the relationship between gross morphological changes in decomposition and their relationship to the environmental variables of temperature, humidity, solar radiation, and wind speed.

Documented attempts to determine the PMI based on gross morphological change date back at least to the 13th century.[1] Micozzi discussed sequential stages of decomposition, but points out that the dynamic nature of several interacting variables make it impossible to assign those stages to an absolute time since death.[2] Despite this caveat, and because of the probative value of the PMI, approximate times are frequently assigned, particularly to the early stages of decomposition (e.g., Clark et al.).[3] Based on the correlation between gross morphological change and temperature, Megyesi et al., as well as others, have proposed predictive models for estimating PMI throughout the trajectory of fresh to skeletonization.[4] Vass and Sorg and Wren added humidity to models (accumulated relative humidity days), and found that shade from a forest canopy played a role in decompositional differences.[5,6] They posit that both regional and microenvironmental differences significantly affect decomposition, supporting the need for regional decomposition sequences. Research conducted using human donors at the Forensic Investigation Research Station (FIRS), Colorado Mesa University, yielded the Total Body Desiccation Score (TBDS) within which Connor et. al. described a region-specific decomposition sequence and discussed its predicative ability in an arid environment, by adding descriptive categories useful for desiccated remains.[7]

This present study takes the general descriptive categories used in the TBDS (color, bloat, moisture, desiccation, and skeletonization) and compares the scores to Accumulated Degree Days (ADD), Accumulated Humidity Days (AHD), Accumulated Solar Radiation (ASR), and Accumulated Wind Speed (AWS). Each variable was calculated in a manner similar to ADD and AHD; a daily minimum/maximum average is successively added across all days within a defined PMI. The lower threshold for ADD is defined as 0°C (following Megyesi et al.); AHD, ASR, and AWS were not assigned a floor or ceiling threshold.[4] TBDS scores were considered both in sum, and by body segment, including: head and neck, torso, arms and legs.

Preliminary results suggest that some TBDS descriptors correlate with ADD more strongly than others (listed in order of efficacy): skeletonization (0.85), moisture (0.82), desiccation (0.70), color (0.69), and bloat (0.57). The head and neck region showed the strongest correlation with ADD (0.91). All environmental factors demonstrated strong correlations to TBDS: ADD r=0.81, AHD r=0.80, ASR r=0.83, and AWS r=0.83.

Further research will be used to strengthen the TBDS method by weighting the factors that were strongly correlated with PMI. Preliminary results at FIRS indicated that placing more weight on specific body regions (i.e., the head and neck region) and considering environmental variables such as solar radiation and wind speed in predictive models, along with temperature, has the potential to increase model robusticity.

Finally, comparative research in other geographical regions needs to be conducted. In the semi-arid west, solar radiation and wind speed were important correlates with decomposition. These additional environmental variables may be more powerful correlates for different decomposition stages and may be useful for developing predictive models. In other environments, these environmental variables may be less important and other environmental variables may be more useful. Exploring these factors will both deepen the understanding of macroscopic variation in decomposition and build stronger, microenvironment-based models for assessing the trajectory of decomposition and building analytical models for estimating PMI on human remains.

This project was supported by an award through the National Institute of Justice, Office of Justice Programs, and the United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.

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Forensic Science, Taphonomy, Decomposition

E98 A Comparison of Insect Activity on Different Carrion Types at the Anthropological Research Facility (ARF) in Knoxville, Tennessee

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After attending this presentation, attendees will better understand how entomologists and anthropologists can work together to obtain meaningful research results. Attendees will learn how the insect activity on human cadavers, rabbits, and pigs differ from one another. The hypothesis of this project was that the insect activity would differ in timing and pattern for different carrion types.

This presentation will impact the forensic science community by illustrating how entomologists and anthropologists can have a close working relationship with each other. This research is also important because having suitable substitutes for human cadavers is imperative for research projects.

If one looks into the forensic science literature, there is a wide range of human cadaver substitutes used across the world. Animals in a wide range of sizes and hair content, from rats to pigs and a variety of species in between are used.[1,2] Pigs have been studied at the ARF in Knoxville, TN, previously, but are the only animal to date that has been studied as an acceptable human cadaver substitute.[2] It is not possible for all research to be conducted on human cadavers, so projects such as this one that compare the decomposition of different animals are very important.

Five subjects of each species were placed in the same microenvironment at the ARF during three separate trials. The three trials spanned three seasons: spring, summer, and winter. Decomposition progression was measured using the Total Body Scoring (TBS) system developed by Megyesi and colleagues.[3] Additional data collected included hourly ambient temperature, daily photographs, and the presence of scavenging activity. When insects were active, adult fly and beetle specimens were caught and preserved, and fly larvae were collected and preserved.

From the daily observations and photographs, it was noted that larval activity did not maintain the same location pattern in each carrion species. While some areas of larval activity were similar (e.g., masses in the eyes and nose), the pigs had consistent large masses under the tail and the rabbits had masses internally that were not able to be observed until late stages of larval development. These location pattern differences had distinct impacts on the decomposition progression and corresponding TBS between the different subject species. This aspect of insect activity has previously not been discussed during comparative studies of various carrion species.

Insects that were collected during the experiment are being identified, and the results will be discussed during the presentation. Differences in species composition on different carrion types as well as the timing of the insect activity will also be discussed.

This project was supported by the National Institute of Justice, Office of Investigative and Forensic Sciences, United States Department of Justice. The opinions, findings and conclusions or recommendations expressed in this presentation are those of the researchers and do not necessarily reflect the views of the Department of Justice.

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Insect Activity, Forensic Anthropology, Forensic Entomology

E99 Taking the Bite Out of Requesting Antemortem Dental Records

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After attending this presentation, attendees will be able to implement methods of requesting antemortem dental records from a variety of sources and conduct family interviews to assist in the acquisition of antemortem dental information.

This presentation will impact the forensic science community by providing techniques to improve communication between medicolegal death investigators and dental offices for the purpose of requesting comprehensive antemortem dental records in forensic identification, along with methods to assist in family interviews to yield complete antemortem identification information.

Correct identification is one of the primary responsibilities of the coroner or medical examiner. Forensic dental identification is just one of the many tools in that process. Medicolegal death investigators' abilities to quickly and accurately obtain antemortem dental records can expedite this process. Without accurate antemortem dental records, a forensic dental identification is not possible. The first step in acquiring antemortem dental records is conducting a comprehensive family interview. Requesting not only the most recent family dentist contact information, but any additional previous dentists and dental specialists, whether the decedent had military service or had been incarcerated in the department of corrections, in addition to previous employers is also pertinent and can increase the probability of acquiring a multitude of quality dental records.

Basic dental terminology can assist medicolegal death investigators in requesting antemortem dental records. The decedent's previous dentist may only provide a limited amount of previous records unless otherwise specified. When requesting dental records, the most recognized request is for radiographs. Medicolegal death investigators' knowledge of dental radiographs can be very helpful, both to the decedent's previous dentist and to the forensic odontologist. The most widely used types of dental radiographs are panorex, bitewings, and periapical radiographs. A panorex is a radiograph of the entire jaw that extends upward to include the maxillary sinuses and orbital socket and downward to the mandibular jawline and chin. A bitewing radiograph or, as it is sometimes called in layman's terms, cavity detecting radiograph, includes the coronal surface of both maxillary and mandibular arch. A periapical radiograph includes teeth in their entirety including the coronal surface extending to the tip of the root and supporting structures. Along with requesting dental radiographs, it is also extremely important for the forensic odontologist to have access to the written charting, odontograms (a rudimentary depiction of the teeth), ledger, and the medical history. Radiographs only give a one-dimensional picture; having access to written charting and ledgers allows the forensic odontologist to determine restoration surfaces and materials used. The medical history can also yield pertinent decedent information, such as previous medical therapy, surgeries, and implanted devices. Given the digital and electronic age, retrieval of antemortem dental records has become more easily accessible. Entire dental records can be easily emailed to the investigator or forensic odontologist.

Forensic dental identification can be a very quick, accurate, and relatively inexpensive method of identification for the coroner or medical examiner. The accuracy of the medicolegal death investigator in acquiring quality dental records in a timely manner will only expedite this service. It is vital for the medicolegal death investigator to have training in family interviews for the purpose of gaining antemortem dental information in addition to knowledge of the variety of sources of dental records.

Forensic Odontologist, Antemortem Dental Records, Family Interview

E100 A Retrospective Study of Homeless Deaths in the County of Santa Clara, California

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The goal of this presentation is to present causes of death and other variables affecting the homeless community upon review of autopsy and investigative reports at the Santa Clara County Medical Examiner-Coroner's Office spanning the years 2011 through 2016.

This presentation will impact the forensic science community by further defining the definition of homelessness used by the Santa Clara County Medical Examiner-Coroner's office and illuminating the trends in homeless deaths over the past six years. For the years 2011 through 2016, there were 476 homeless deaths in Santa Clara County, an increase of 164%. The number of deaths affecting the homeless population continues to rise, with the most deaths observed to date occurring in 2016.

The importance of a definition of homelessness is that it allows for county-wide consistency in classification. Those included in this study's definition of homeless consist of people living on the street in indoor and outdoor makeshift living areas, including homeless encampments, parks, motor vehicles, and recreational vehicles, as well as those individuals living in homeless shelters, motels, or any type of funded or transitional housing. Also included in this study's definition are individuals who did not have a valid living address upon a public records search.

Each year was analyzed separately for the following variables: decedent demographics; cause and manner of death; location of death; medical history; the presence and use of drugs; veteran status; and motor vehicle-related deaths. The yearly data was then compiled and compared to demonstrate trends across the six-year period. These data seek to present the homeless death rate in the United States and report any trends that may help in creating preventative measures to help the homeless at the county, state, and national levels.

All autopsy and investigative reports of homeless deaths in this time period were critically reviewed. The highlights of these data include: the increase in the number of homeless deaths from 2011 through 2016; the consistent use of alcohol and methamphetamine; the increase in heroin-related deaths corresponding with the national opioid epidemic; the location of death; and the rising number of elderly homeless deaths.

There were 50 homeless deaths in Santa Clara County in 2011, followed by 62 in 2012, 78 in 2013, 69 in 2014, 85 in 2015, and 132 in 2016. Between 2011 and 2016, the number of homeless deaths increased by 164%.

Alcohol and drug abuse continues to claim a significant number of homeless deaths each year, with alcohol and methamphetamine being the most commonly abused drugs; however, of interest is the increase in heroin use among the homeless decedents.

Each case was analyzed to determine location of death to illustrate where the greatest number of homeless deaths occurred in Santa Clara County. The most common locations of death comprising all six years were hospitals, emergency rooms, and nursing facilities. The second most common locations were outdoor makeshift living areas. These data demonstrate that the majority of the homeless decedents in Santa Clara County were not dying in shelters or emergency housing.

The average life expectancy for a homeless individual is far younger than the general population (64 years and 78 years, respectively).[1] As the baby boomer generation ages, so does the homeless population, further exacerbating their risk for illnesses. Santa Clara County saw a 320% increase in the number of homeless decedents aged 65 and over between 2011 and 2016.

The increased number of homeless deaths exemplifies some of the risk factors faced by this population. Housing alone will not completely solve the homeless problem; rather, more resources for medical and mental illness, drug addiction, and the elderly population need to be provided. By presenting these data, the goal is to encourage this type of change through policy reform at the county, state, and national levels.

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Homeless Deaths, Transient Deaths, Santa Clara County, CA

E101 Chemical Characterization of Tattoo Inks to Aid in the Identification of Highly Decomposed Remains

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After attending this presentation, attendees will understand the chemical composition of common tattoo inks and the ways in which the ink composition can be used for identification of decomposed remains.

This presentation will impact the forensic science community by describing analytical methods that can be used to determine the elemental and organic composition in common tattoo inks and by demonstrating the identification of ink components in decomposed tissue samples.

The popularity of tattoos is increasing, with recent figures estimating that 24% of the population in the United States currently has at least one tattoo. The depth to which the inks are injected into the skin causes tattoos to remain intact even after extensive tissue damage. Due to the relative permanence of the ink, tattoos are often used to identify victims of mass disasters, based on visual assessment of the tattoo; however, for remains that are in an advanced state of decomposition or that are otherwise severely degraded, visualization of tattoos in this manner is limited.

The purpose of this initial work was to investigate the detection of tattoos based on the elemental and organic composition of the inks. As these inks are not heavily regulated, the first step was to chemically characterize a set of common tattoo inks, then to demonstrate the presence of these components in decomposed tissue samples. A total of 30 tattoo inks were included in this initial study. Inks were blotted onto separate filter papers and dried in a desiccator for 24 hours prior to analysis. Each ink was analyzed by Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) spectroscopy to determine the organic composition and by X-Ray Fluorescence (XRF) spectroscopy to determine the elemental composition.

The IR and XRF spectra were first assessed visually to determine the possible pigments present in the inks. For example, the IR spectra of two blue inks indicated the presence of N-Cu-N absorptions at 1,088 cm^{-1} and C-N-C absorptions at 781cm^{-1} and 756cm^{-1}. The XRF spectra of the same two inks indicated the presence of copper and titanium. The IR absorptions combined with the presence of copper indicated copper phthalocyanine as the major pigment in the inks. Titanium is used as a whitening agent and, hence, further distinction of the inks was possible with the lighter blue ink containing a significantly higher intensity of titanium.

Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were then applied to the spectral data to more readily visualize similarities and differences among the inks based on both the elemental and organic composition. The XRF and IR spectral data were combined and PCA was initially applied. Inks of similar color were positioned closely on the scores plot and distinctly from other inks using the first four principal components, which accounted for 80% of the total variance in the data set; however, the lighter-colored inks that essentially differed only in the titanium concentration were not readily differentiated using PCA. Using HCA, similar groups of inks were observed as in PCA, although the lighter-colored inks were more readily distinguished.

Following characterization of the tattoo inks, the detection of ink components in decomposed tissue samples was investigated. In this initial study, only elemental composition was investigated and Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM/EDS) was used due to the imaging capabilities offered in addition to the elemental composition analysis. A subset of red, blue, and black inks was analyzed using SEM/EDS and the elemental composition of the inks was compared to that generated previously using XRF spectroscopy. After demonstrating correspondence between the two techniques, SEM/EDS was used to analyze tissue samples that had been tattooed with red ink and allowed to decompose. After 19 days, the carcass was in a state of advanced decomposition, with skin discoloration preventing visualization of the tattooed area. Nonetheless, iron, silicon, and magnesium, previously identified in red ink, were detected at significant levels in the tissue samples. Although preliminary in nature, these results demonstrate potential for the detection of ink components in decomposed tissue samples.

Tattoo Inks, Chemical Characterization, Decomposition

E102 A Five-Year Retrospective Study on Suicide and the Use of Antidepressants in Washington, DC

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After attending this presentation, attendees will: (1) understand the need to examine suicidal data; (2) realize the responsibility of death investigators and health agencies to discuss depression and suicide; and, (3) be aware of the trends in drugs taken at the time of death.

This presentation will impact the forensic science community by raising awareness of the impact of prescribing antidepressants and whether this has affected the suicide rate in Washington, DC. This presentation will also open a much-needed discussion regarding depression and other mental illnesses.

Suicide is a major public health concern because it takes the lives of more than 40,000 people nationwide and is the tenth leading cause of death in the United States. According to the National Institute of Mental Health, the suicide rate increased 24% from 1999 to 2014.[1]

Interestingly, while the suicide rate in the United States is increasing, Washington, DC's, rates have decreased 24.6% from 2014 to 2015.[2] In fact, Washington, DC, has one of the lowest suicide rates in the country.[3] The suicide rate in Washington, DC, is significantly less than nearby Virginia and West Virginia. The precise reason for this difference is unknown and warrants investigation. The difference could be due to differences in drugs taken by the decedents, social services available, and/or the recognition of suicidal ideation and public awareness.

Knowledge regarding correlations can give rise to prevention and decreased suicide rates and helps to open discussions about depression and other psychiatric disorders. This work centers on examining the number of deaths by suicide in the past five years and the presence of antidepressants at the time of death.

In 2015, the Office of the Chief Medical Examiner in Washington, DC, investigated 52 suicides, of which 51 decedents were tested for the presence of drugs. The most prevalent drugs found included ethanol, morphine, cocaine, fluoxetine (a Selective Serotonin Reuptake Inhibitor (SSRI)), and bupropion (not an SSRI). The leading cause of death from suicide in Washington, DC, is by firearms, which increased 25% from 2014 to 2015 (versus death by hanging, which decreased from 32% in 2014 to 23% in 2015). Ethanol was found in ~30.8% of suicide deaths in 2015.[2]

In contrast to the popular SSRIs, bupropion is atypical and acts as a Norepinephrine-Dopamine Reuptake Inhibitor (NDRI). There are many antidepressant medications being prescribed today, with SSRIs being the most popular. These SSRIs change the chemicals in the brain that may be unbalanced in people with a major depressive or anxiety disorder. It works by blocking the reabsorption of serotonin, thereby increasing serotonin levels. Fluoxetine and citalopram are examples of SSRIs. SSRIs have been shown to decrease violence toward others, but may also lead to increased suicidal ideation and behavior.[4] The Food and Drug Administration (FDA) requires all antidepressants to include a warning stating that antidepressants may increase the risk of suicide in persons younger than 25 years of age. This warning, put into effect in 2007, is based on two reports of a 2-fold increase of the suicidal ideation and behavior in children and adolescents, and a 1.5-fold increase in the 18- to 24-year age group, a slight decrease for those over age 24, and a much lower risk in the 65+ age group.[5]

Mental health is talked about more openly now than in the past, but talk regarding depression and suicide still too often remains off-limits. Both depressed people and society at large are still uncomfortable with these topics. This fear, along with a lack of support, often prevents people from seeking treatment. Analyzing data and promoting discussions about this difficult subject allows us to get one step closer to decreasing the suicide rate. Death investigators have access to analyzable data that may help reduce the number of suicides.

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5. *Antidepressant Medications for Children and Adolescents: Information for Parents and Caregivers.* National Institute of Mental Health. U.S. Department of Health and Human Services, Web. 27 July 2017.

Suicide, Antidepressants, Death Investigation

E103 Efforts at the Centers for Disease Control and Prevention (CDC) to Improve Fatality Management and Mortality Reporting Practices During Mass Fatality Incidents at the State and Local Levels

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After attending this presentation, attendees will understand several activities undertaken by the CDC, in collaboration with the forensic science community, to improve state and local public health preparedness and response to support mass fatality incidents. Attendees will learn how these activities address known gaps in the collecting, recording, and sharing of mortality information after mass fatality incidents.

This presentation will impact the forensic science community by demonstrating how strengthening fatality management, including data sharing with public health, can result in a more effective response to a mass fatality incident at the local, state, and federal levels. This presentation will describe and demonstrate the usefulness of CDC tools to address gaps and to improve the accuracy and timeliness of mortality data at different stages of a public health emergency response. Attendees can use what they learn to examine and update their existing mass fatality plans and exercises in their jurisdictions, if necessary.

Attendees will learn how these activities address known gaps in the collecting, recording, and sharing of mortality information after mass fatality incidents. Attendees will be briefed on the 2017 updates to CDC's *Public Health Preparedness Capabilities: National Standards for State and Local Planning,* specifically to the Fatality Management and Information Sharing Capabilities and any potential considerations for their mass fatality plans.[1] Other developed tools, *Reference Guide for Certification of Deaths Associated with a Disaster (in press) and Disaster-Related Death Scene Data Collection Forms,* will offer attendees up-to-date resources to incorporate into their mass fatality management planning.[2] Finally, attendees will learn about an opportunity to engage with CDC on a new project to develop a fatality management toolkit for state and local jurisdictions.

This presentation will provide a background on the CDC's Public Health Preparedness Capabilities and common gaps in local mortality data management identified by CDC. This presentation will explain the process taken to develop the tools and the purpose and content of each resource. Suggestions on how attendees can use these tools to update their plans will be highlighted using emergency events, for example, events such as tracking heat-related deaths during a heat wave or deaths attributed to a mass fatality incident from a tornadoe across several jurisdictions.

In conclusion, when planning for and responding to incidents with fatalities, it is the responsibility of local and/or state fatality management professionals (e.g., medical examiners, coroners) to ensure the respectful and orderly management of deceased persons, while tracking and sharing mortality data in a timely fashion with public health officials. These data can inform public health practitioners when developing targeted messaging during an event and for refining strategies to prevent deaths from future disaster events.

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1. *Public Health Preparedness Capabilities: National Standards for State and Local Planning.* CDC, accessed July 19, 2017, https://www.cdc.gov/phpr/readiness/capabilities.htm.
2. *Death Scene Investigation after Natural Disasters or other Weather-Related Events.* CDC, accessed July 19, 2017, https://www.cdc.gov/nceh/hsb/disaster/default.htm.

Mass Fatality Planning, Mass Fatality Incident, Disasters

E104 Disaster-Related Deaths and Data: A New Toolkit and Training to Enhance Death Scene Investigations After Disasters

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After attending this presentation, attendees will understand how disaster death scene data can improve public health emergency preparedness, response, and recovery efforts. Attendees will be familiar with the new toolkit, Death Scene Investigation After Natural Disaster or Other Weather-Related Events, designed to help death scene investigators investigate deaths that occurred during natural disasters or other weather-related events. Attendees will learn how information collected at the death scene can enable death certifiers to more accurately and consistently attribute deaths to disasters, and how this in turn helps public health departments identify risk and protective factors associated with disaster-related deaths and refine their strategies to prepare for, respond to, and recover from future disaster events.

This presentation will impact the forensic science community by highlighting the toolkit and new training resources available to assist death scene investigators in collecting data about disaster-related deaths and reporting this information to their Medical Examiner/Coroner (ME/C).

The Centers for Disease Control and Prevention (CDC) contracted with the non-partisan and objective research organization referred to as NORC at the University of Chicago and collaborated with state and local ME/Cs, forensic pathologists, death scene investigators, forensic anthropologists, and epidemiologists to create a new toolkit to assist death scene investigators with disaster-specific data collection. NORC presented an overview of the toolkit at the 2017 AAFS Annual Scientific Meeting; the final toolkit was released in July 2017. This presentation will focus on the forthcoming training resource.

The new toolkit contains supplemental forms and checklists to ensure event-specific data are collected at death scenes of commonly occurring natural disasters or weather-related events (e.g., hurricanes, tornadoes, extreme heat exposures). In addition to their routine data collection form, investigators can use the supplemental forms or checklists to aid in collecting important disaster-specific data. Both the forms and checklists prompt investigators to collect information specific to the disaster or weather-related event that is often available only immediately after the event. Examples of disaster-specific data include information about the decedent (e.g., engagement in activities related to disaster preparation or clean up) and the disaster or event itself (e.g., weather conditions or ongoing alerts).

To supplement the toolkit, NORC and CDC are developing a training resource to assist ME/Cs in preparing their staff to investigate disaster-related deaths. Using a variety of educational approaches and presentation techniques, the training resource provides information about disaster-related mortality data, such as the importance of timely and accurate mortality data in measuring the effect of a disaster as well as the number and type of agencies that benefit from these data after a disaster. The training resource is designed for both new death scene investigators and the continuing education of advanced death scene investigators. NORC is partnering with the American Board of Medicolegal Death Investigation (ABMDI) to pilot and disseminate this new training.

Disaster, Investigation, Training

E105 The Search and Recovery of 43 Victims From the Oso Mudslide in Washington State

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The goal of this presentation is to educate attendees regarding the devastating landslide that took the lives of 43 people and how recovery efforts were conducted.

This presentation will impact the forensic science community by teaching attendees about: (1) the challenges presented in the recovery of human remains from a natural disaster in mud and water; (2) the use of multiple resource types; and, (3) the requisite support and time needed to recover remains buried under deep, saturated mud.

A small community was devastated without warning in Snohomish County, north of Seattle, WA. On the morning of March 22, 2014, a massive landslide occurred that took the lives of 43 people, destroyed sections of State Highway 530 and a rural neighborhood in a matter of seconds. This was the deadliest mudslide in United States history. The search area was a massive 3.24km^2 (800 acres) with debris estimated to be 7.6 million cubic meters (~270 million cubic feet). Confounding the search and recovery efforts was the fact that the slide crossed and dammed the Stillaguamish River, making it an area of mud estimated to be up to 12m (40 feet) at the time of the emergency response. The last body was reported located exactly four months after the slide occurred. The Snohomish County Medical Examiner's Office reported cause of death for all victims as multiple blunt force injuries and manner of death as Accident.

At the time, the Snohomish County Emergency Management office had ten people on staff, who were suddenly in the position of coordinating and overseeing what became a response that included 240 agencies. The Emergency Operations Center (EOC) was activated for 40 days. The enormity of the devastation quickly overwhelmed local and in-state agencies. On April 3, 2014, President Obama declared the mudslide a major disaster. More than 600 personnel, including more than 160 volunteers, 77 of which were canine handlers, worked on the recovery operations.

A first-hand account of the search and recovery efforts will be presented. It was the use of K9s working alongside heavy equipment that enabled every individual to be located and recovered, even when buried under mud and submerged under water. Volunteer search, rescue, and recovery canine teams located most of the victims in the first 11 days. The challenges of working an area of that size, with resources coming from across the United States, working in cold, wet, and dangerous conditions, without power and limited communication access will be discussed. Physical hazards, such as debris, presented one set of challenges, while chemical and biological hazards presented other challenges. Transportation within the search area required specialized equipment. Coordination between agencies was conducted using a joint command under the Incident Command System (ICS). Not all canine teams were effective, but those with the proper training for the deployment and handlers prepared for such conditions were able to complete search assignments safely and effectively. An on-scene veterinary station was established at the IC as well as the forward base of operations. The United States Army National Guard established an efficient decontamination station including hot and cold running water for canines. A separate decontamination station was established for humans. Lessons learn from this catastrophic natural disaster have and will continue to inform those involved in recovering human remains from mudslides.

Recovery, Cadaver K9, Natural Disaster

E106 Rescue the Living, Find the Missing, and Identify the Found: The Dentify.Me App

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After attending this presentation, attendees will have acquired a deeper understanding of how a new smart phone application (app) named Dentify.Me can become a valuable tool in mass disaster management. Each day, humans are subjected to catastrophic events. Horrifying mass fatality incidents have been witnessed in every corner of the planet, and the world mourns for victims of natural disasters, war, and violent acts.

This presentation will impact the forensic science community by increasing awareness of how Information Technology (IT) solutions can be used as a resource in mass fatality victims management.

In such incidents, all efforts should be coordinated to rescue living victims, find missing ones, and identify those found. First, all injured victims need to be located or reported so they can be provided with first aid paramedic services. Second, a manifest of missing victims needs to be created so rescue and/or Disaster Victim Identification (DVI) efforts can be utilized efficiently to find the missing; however, the creation of a missing persons' manifest is a time-consuming and often inaccurate process, especially in the early stages of an incident as it is constantly evolving as victims are found and others are reported missing. Deceased victims who have been recovered need to be identified to bring closure to their loved ones as well as receive justice in cases of homicide; however, rescue teams rely on victims and eyewitness reports created manually or on post-active systems for receiving emergency calls. Moreover, all pre- and post-disaster data, also known as Antemortem (AM) and Postmortem (PM) data, respectively, is collected and matched manually by DVI teams for reconciliation. There are no wide-ranging proactive systems today on the IT market to address all three issues at once.

The Dentify.Me app is a proactive application that utilizes a victim-centered solution that automates the process of notifying authorities with an S.O.S., locating victims, generating missing persons' manifests, and collecting and delivering AM data to authorities. Using a semi-structured interview process and document analysis, the software has been optimized to collect essential AM data.

The Dentify.Me app engages with eyewitnesses to automate the incident detection and identification process. It uses hard system's methodology to automatically identify and locate potentially affected victims, alert the rescue team to reach out for the injured, generate a list of the missing, and collect and deliver AM data to the DVI team to assist in the matching process. It is hoped that this proof-of-concept proposed solution, when implemented, will have a great impact on mass fatality victims management by *rescuing the living*, *finding the missing*, and *identifying the found,*to efficiently bring justice to the victims and peace of mind to their loved ones.

DVI, Antemortem Data, IT Solution

E107 Forensic Science Capstone Experience: The Thesis, the Review, and the Practicum

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After attending this presentation, attendees will learn how students' personal and professional skills are encouraged through academic capstone experiences.

This presentation will impact the forensic science community by highlighting the three capstone experience options available to University of Ontario Institute of Technology (UOIT) Forensic Science undergraduate students. An overview of the curricula, assessment methods, and learning outcomes will be discussed.

In the UOIT Forensic Science Program, a capstone course is a required curriculum component prior to graduation. The course is delivered using active learning strategies emphasizing experiential learning and independent study. Combining interactive curriculum with traditional lecture material promotes a deeper understanding and a more engaged learner.

A capstone experience may take the form of an honors research thesis, a literature review, or a mock crime scene practicum project. A thesis project provides students an opportunity to conduct novel research by identifying a hypothesis and working through the methods, results, and significance of their work. The literature review course focuses on independent library-based scholarly research. Students synthesize information and provide a critical appraisal of experimental principles where necessary. The mock practicum course provides students with the opportunity to investigate a simulated crime scene and participate in all aspects of the investigation, from crime scene to lab, culminating in expert witness testimony in a mock court setting.

Each capstone project is conducted under the supervision of a forensic professional and allows the student to integrate and synthesize the knowledge gained throughout their program of study. Students' personal and professional skills are encouraged through this academic capstone experience. What's more, career paths and personal goals are fostered by creating a personalized course experience.

Emphasis is placed on developing students' practical and theoretical science skills. These *hard skills* are considered to be the foundational aptitudes that students acquire through lecture and laboratory content. *Soft skills* are also identified and nurtured. Otherwise referred to as interpersonal or social skills, these may include communication, self-motivation, problem-solving, time-management, leadership, and decision-making skills. Both oral and written communications are practiced with a strong focus on scientific report writing. Students are assessed on scientific validity, organization, completeness, and overall style and grammar. Finally, importance is placed on the student's ability to address relevant hypotheses and how the results address the latter.

Both hard and soft skill-sets are assessed in various capacities and all capstone experiences culminate with the submission of a written thesis or report as well as presentation at the year-end Annual Forensic Science Research Day.

Education, Capstone, Experiential Learning

E108 Minimum Education Requirements for Crime Scene Investigators (CSIs): The Missing Link in Forensic Science

Mary Juno, MSc, SJSU Justice Studies Department, Forensic Science Program, One Washington Square, San Jose, CA 95192*

After attending this presentation, attendees will be aware of the low minimum-education hiring requirements for crime scene investigators and the standard on-the-job training a new CSI receives. Further, attendees will understand the potential for error at a crime scene and conceptualize a link between low hiring standards and error.

This presentation will impact the forensic science community by proposing that crime labs and police departments attempt to establish error rates for CSIs and potentially re-evaluate their agencies' minimum education requirements for new CSIs. The community is invited to consider the question: Should crime scene investigation, like other forensic disciplines, be professionalized?

The 2009 NAS Report, *Strengthening Forensic Science : A Path Forward,* rightly criticized many of the subdisciplines of forensic science, causing professionals and researchers to "get hot" on the tasks of tracking and minimizing error and strengthening the scientific underpinnings of their fields; however, the NAS Report hardly mentioned crime scene investigation or any potential problems with it, even though CSIs are at the front end of forensic science where the introduction of error is arguably most critical. Errors committed by the CSI at a crime scene can include failure to: locate and recognize evidence, interpret a screening test correctly, prevent contamination, collect sufficient samples and controls, document evidence adequately, preserve evidence using appropriate packaging and handling, and maintain chain of custody, with limitless variation. No amount of forensic wizardry in the lab can make up for these errors.

Few agencies require new CSIs to possess any formal training in the sciences at the time of hiring. Currently, the most common minimum educational standard for obtaining a position as a civilian CSI in the United States is a high school diploma or General Equivalency Diploma (GED). Once hired, a CSI typically attends a short course on photography and fingerprint lifting and completes a period of supervised field training. At that point, a new CSI may know *what* to do but may not completely understand *why* he/she is doing it.

This presentation examines hiring standards and minimum education requirements for CSIs across the United States as part of a larger study that will explore quality assurance mechanisms in crime scene investigation (or the lack thereof) and the relationship between crime scene investigator error rates and education levels. This study argues that the combination of low education minimums and brief on-the-job training are inadequate. A four-year degree is proposed as the new minimum education requirement for CSIs.

Education, Minimum, CSI

E109 Group Experiential Learning in the Forensic Science Classroom

John A. Williams, PhD, Western Carolina University, Anthropology and Sociology, 101 McKee Hall, Cullowhee, NC 28723*

After attending this presentation, attendees will better understand the application of experiential learning in the forensic science classroom.

This presentation will impact the forensic science community by demonstrating the ways in which group experiential learning can be approached within the standard classroom lecture setting.

Collaborative or group experiential learning in higher education is by no means novel. It is (and has been) practiced in various forms and in various disciplines for most of the past century.[1] Group work has been shown to easily excel individual learning in all manners of pedagogy, from problem-solving to verbal skills. The interspersing of small group activities in a standard lecture format is perfectly acceptable and can provide sufficient student value.[2]

The inspiration for this study of small-groups experiential learning is a course on bone trauma. This course serves both forensic anthropology and forensic science majors. It began as a standard, straight-lecture format (Trial 1). In this iteration, static displays of various bone traumas were (and still are) used to illustrate how various events such as fractures occur. Human bone is too precious to experiment with and bones of the white-tailed deer were shown to be a useful alternative.[3] A few excess deer bones were used in class to demonstrate trauma. Student interest in a hands-on application was quite clear. The problem was a lack of sufficient bones for a more in-depth student experimentation. Four years ago, a local source of deer bones acquired from local hunters was established. Beginning that year, students were given a group-based project to simulate a form of bone fracture (Trial 2). Each group was given some degree of latitude in approaching their task. The final projects were, as expected, quite varied in their design and results. The concern was that so much time was spent on the experimental design that the pedagogical goal of the project was lost — understanding how bone responds to trauma. In the next iteration, each student group performed the same experiment and used the same experimental design (Trial 3). It was discovered that, not surprisingly, some groups were better than others at conducting the experiment. Some groups experienced the so-called "Aha!" moments, while others simply performed the experiment as instructed.

All science of teaching and learning, or pedagogical research, is focused on student learning. Within that context, three goals were established for this study of experiential-group learning: (1) connect course content to the real world; (2) reinforce learning through application; and, (3) assist students in making the internal connection of the "how" and "what" with the "why." This project has placed much of its emphasis on this third goal. If they have mastered this ability, when faced with a new circumstance (the "how" and "what"), this skill set should make it possible for them to reach a conclusion (the "why"). As this course continues to evolve, how to apply experiential-group learning to maximize these goals remains an ongoing process.

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Education, Experiential Learning, Bone Trauma

E110 A Study of the Forensic Science Education Programs Accreditation Commission (FEPAC) -Accredited Graduate Forensic Science Programs' Curricula

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After attending this presentation, attendees will be able to define how FEPAC-accredited forensic science graduate programs have implemented FEPAC's Graduate Curriculum Standard.

This presentation will impact the forensic science community by identifying the scope of any differences or unique characteristics accredited forensic science graduate programs may exhibit in their curricula, in addition to any inconsistencies. Each graduate program has fulfilled the FEPAC Graduate Curriculum Standard in order to receive accreditation; however, the manner in which they fulfilled these standards may vary among programs. This study sought to understand how each graduate program fulfilled the FEPAC Graduate Curriculum Standard by analyzing the programs' websites and self-study documents.

The National Institute of Justice and the National Academy of Sciences recommended that forensic science training shift from on-the-job training to formal education; however, the reports cited inconsistencies in the curricula of the forensic science degree programs as an impediment to this.[1,2] The FEPAC Standards were created to address this issue; however, no studies have been conducted to determine how the accreditation standards have been implemented by the FEPAC-accredited graduate programs. This study evaluated the self-study responses (n=11) and website information (n=17) specific to FEPAC's Graduate Curriculum Standard to determine how the accredited graduate programs fulfilled the FEPAC Graduate Curriculum Standard. This study also determined to what extent inconsistencies or consistencies exist among the accredited graduate programs' curricula.

This study found that although FEPAC-accredited graduate forensic science programs exhibited differences (unique characteristics) among their curricula, they did not as a whole exhibit significant inconsistencies (lack of agreement). All the accredited graduate programs covered the natural sciences, particularly the areas related to forensic science, such as forensic chemistry and forensic biology; however, the programs' coverage of the comparative sciences, such as firearms and questioned documents, was limited. Evaluation of the 11 FEPAC self-study reports revealed that, on average, these programs exceeded the required minimum instructional hours of core forensic science topics as specified by the FEPAC guidelines. All programs in this study required students to complete an independent research project as their capstone experience, whether thesis or non-thesis. Additionally, all programs included a requirement for students to attend a graduate seminar where students presented their independent research findings.

The study found the FEPAC-accredited graduate forensic science programs' curricula was consistent with unique characteristics among the graduate programs. The curricula were rigorous, scientific-based, and discipline specific. This study evaluated a snapshot of accredited graduate forensic science programs' curricula. Future research should seek to further flesh out the curricula by gathering documents from the graduate programs, such as course syllabi, conducting interviews with the program director or their designee, and administering surveys.

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Higher Education, Accreditation Standards, Curriculum

F1 Cross Examination and Direct Examination in Criminal Procedure Discussions in Turkey

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After attending this presentation, attendees will better understand the cross-examination criminal procedure in Turkey.

This presentation will impact the forensic science community by explaining the lawyer's role in cross examination and criminal law.

An important innovation brought to the criminal court by the Turkish Criminal Procedure Code (TCPC) No. 5271 is the vested right of parties to ask questions. The right to ask questions, which is prevalent in the Anglo-Saxon legal systems, increases the role of participants or defense attorneys, who are tasked with presenting material facts, and imposes vital responsibilities on the attorneys who are perceived as merely inactive participants at trial. Article 201 in the TCPC clearly states that the attorneys and the public prosecutor can be asked questions on the condition that they keep to the trial discipline. In practice, it is observed that witnesses, perpetrators, and attendants are examined by and address their questions to the judge, merely waiting for his queries; however, TCPC Article 201 decisively ensures that the judge cannot interfere except in the case of an objection and the expediency of the objection.

In the TCPC, the same form of the procedure in Article 2016 (to determine who will take the floor) is pursued for asking questions as in Article 201. This study discusses the notions of cross-examination and direct examination, how practicable these are, and the problems faced in practice, along with their solutions. The similarity of posing a direct question to cross examination, which has taken place in Turkish law with Article 201 of the Code of Criminal Procedure, and its place in the accusation system will be taken into consideration. Article 201 of the Code of Criminal Procedure is not a cross-examination method, but only a questioning procedure. It is an application that provides the possibility for participation of the parties to the judge's examination.

If the goal of the criminal procedure is to reach the material fact, it is necessary to investigate the historical development of the concrete case since it is possible to forget events in the period of time between when the concrete fact that took place during the event, which will be deemed as a crime, and the investigation of the material fact. Additionally, evidence not attained or changed by the parties can complicate reaching the fact. In this context, the statements of the parties and parties' witnesses or public witnesses are very important. In Turkey, which adopted the accusation system, the goal is to have the true facts explained by the subjects, which will be put forward through statement examination.

It can be evidenced in the application, that cross-examination, which is one of the necessities of the right to a fair trial, has no real response in Turkish law. In order to reach the real function of direct questioning, the judges have significant responsibilities. Changing the law for justice depends on the realization of all facts of the right to a fair trial. It is evident that Article 201 of the Code of Criminal Procedure does not comply with the cross-examination system. The most appropriate system to the nature of the institution is the accusation system. Although the criminal procedure in Turkey begins with the accusation, there is also a mixed system. This case is an example that there is no cross-examination in the real sense. There are obstacles before the successful application of direct questioning, such as technical infrastructure deficiencies and an insufficiency of the judgment periods.

Courses are provided to law students so they can develop the necessary questioning skills for the legal profession. Through these courses, the contribution of lawyers, who are important participants of the cross-examination system, can be increased. The application of questioning methods cannot be learned by theories as practical trial and doctrinal education are not provided in law faculties. This situation negatively affects the profession of new graduates who want to practice law.

Cross Examination, Forensic Science, Law

F2 Assessment of the Allegations and Evidence in Criminal Proceedings and the Innocence Project in Turkey

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After attending this presentation, attendees will be familiar with the Innocence Project in Turkey and the assessment of the allegations and evidence in the criminal proceedings.

This presentation will impact the forensic science community by demonstrating that in criminal proceedings, the evidence is important in terms of prevention and impact on the judicial system.

In the legal sense, when a crime is committed, a legal institution subjects the criminal to punishment or security precautions. The history of crime is as old as the history of humanity. Throughout history, states have been subjected to various forms of culpability if they have tried the factions as required by society's dynamics and if the criminal action has been reached by the prosecuted offender. If it is concluded that the person did not commit the crime, he was acquitted.

The crime is not rendered suspect by the issue of proof that comes into play, so the suspect is innocent or must prove himself, which will be hard to prove to the party who claims to have committed a crime. In Roman law and in our legal system, the plaintiff has to prove his claim. In medieval Europe, the plaintiff had to prove that the suspect was innocent. The function of the Law of Criminal Procedure is to show the offender, in return of the actual execution of the application (if it has been exercised) to determine by whom it will be handled. Essentially, the goal is to investigate material facts. The authority that has the authorization to pursue punishment has no right to arbitrarily decide who committed the crime, but must prove it in accordance with the law. Material reality is relative. The parties will naturally go on to influence the court, and the judicial authority will go through the process of finding the truth by examining the evidence.

Article 217/2 of the Criminal Procedure Code in the Turkish Criminal Proceedings, which regulates the judge's discretion, is regulated as follows: "Any crime that has been committed in accordance with law can be proven."

The concept of "evidence," which is the principle of the law of evidence, can be described as each type of trace, effect, document, and record that contributes to revealing whether the acts, having abstract descriptions in the punishment norms, have been realized in the concrete case.

The imputed crime in the Turkish Criminal Procedure can be proven with each type of evidence collected in compliance with the law. As securing justice is achieved by reaching the material fact in the criminal procedure, each type of means can be a proof, which serves to prove the occurring concrete case and the judge has the discretionary power to make a selection between these means.

Although the proof of the material extent of the penal discrepancy in the criminal procedure is limited as to the types of evidences, the principle of evidence freedom does not mean an unlimited freedom.

Evidence, Criminal Procedure, Innocence Project

F3 Evidentiary Neglect: The Failure to Perform an Autopsy

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After attending this presentation, attendees will have a better perspective on when an autopsy should be performed, as well as comprehending the various issues regarding privacy, the public's right to know, and ethical parameters.

The presentation will impact the forensic science community by increasing awareness concerning the various dimensions regarding the role of and need for autopsies.

On February 13, 2016, 79-year-old Supreme Court Justice Antonin Scalia was found dead in bed at a West Texas hunting ranch. His doctor offered that Scalia had suffered from cardiovascular disease and hypertension. Scalia had visited his doctor days before his death complaining of a shoulder injury from tennis. The ranch owner called emergency services and the United States Marshals Service arrived at the ranch. There was no medical examiner in Presido County so the marshals called for the local justices of the peace.[1] When one could not be found, a county judge conducted an investigation "over the phone." The judge declined to order an autopsy after taking into consideration law enforcement's observations of the body and scene, consultation with Scalia's physician, and respecting the wishes of the family. "Our folks never indicated that anything seemed amiss or unusual, but that wasn't our role," declared a spokesman for the Federal Marshals service. "We weren't there to make any determination like that, so I'm not going to be drawn into that."[2]

Almost immediately questions arose from ordinary citizens and conspiracy theorists as to the cause of Scalia's death. The circumstances and rebuttals had a surprising similarity to Teddy Kennedy's Chappaquiddick affair 50 years earlier. Following the tragic death of Secretary Ron Brown in 1996, the federal government had created legislation delegating the responsibilities to invest the deaths of the president, vice president, and members of the cabinet to the Armed Forces Medical Examiner.[3]

A well-performed autopsy is the gold standard for any death investigation. Despite advances in diagnostic technologies, autopsies still detect major findings not identified prior to death in 25%–40% of deaths.[4] Most states have statutes that order mandatory autopsies in deaths in police custody, criminal violence, sudden deaths of infants and children, and others. The National Association of Medical Examiners (NAME) Forensic Autopsy Standards identify 12 types of deaths in which autopsies "shall be performed."[5] On many occasions, medical examiners and coroners have been sued for performing an autopsy against the wishes of the family. There are few, if any, cases in which the medical examiner or coroner was sued for not performing an autopsy.

Performing autopsies protects the public interest and provides necessary information to address legal, public health, and public safety issues in each case. Statutes give medical examiners and coroners wide discretion on the performance of autopsies. In Minnesota, for example, statutes allow for the performance of autopsies when, in the judgment of the coroner or medical examiner, "the public interest would be served by an autopsy."[6] Autopsies should be performed in deaths of prominent persons, recent releases from hospitals, institutional deaths, drug deaths, and, at times, simply at the request of the family in suspicious circumstances.

The failure to perform necessary autopsies creates a gap in medical evidence to explain the cause of a death and can "arouse public interest, raise questions, or engender mistrust of authorities." In the midst of an opioid epidemic and faced with a paucity of trained forensic pathologists, medical examiners and coroners are searching for excuses not to defer autopsies or simply performed external examinations. This practice can have disastrous consequences. In today's media-fueled environment, we cannot be so naïve to believe that "everyone should be treated the same."[7] The deaths of prominent and high-profile government officials need and require careful autopsy examinations. Even a lay person would suggest that the death of Supreme Court Justice would require an in-depth federal investigation. As one family put it, "You are the professional, we are a grieving family, you should have done your job!"[8]

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Autopsies, Ethics, Evidence

F4 The Aaron Hernandez Verdict: Hard Work and Critical Analysis Secure Acquittal

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After attending this presentation, attendees will better understand the significance of the collaborative process in case trial preparation and presentation, including expert consultations, site visits, and demonstrative evidence.

This presentation will impact the forensic science community by reinforcing and demonstrating the importance of the process and the significance of the collaborative process in case trial preparation and presentation, including expert consultations, site visits, and demonstrative evidence. In so doing, the process of pursuing justice will be enhanced.

The critical import of understanding the forensic evidence in a case — from the vantage point of what exactly it *does* and *does not* prove — is critical in the pursuit of justice. There are at least two sides to every story, although sometimes a prosecution moves forward insisting that only one version of events is truthful and that the facts revealed by forensic evidence can lead to only a single version — the state's interpretation. The latter seems obvious, simply by the fact that the case is going to trial. Telling the rest of the story requires knowledge, determination, and time.

The fundamental importance of preparation with all the experts in a case is frequently rewarded with a fuller understanding of strengths and weakness of a case. The case preparation phase should also include not only visiting the crime lab and the medical examiner's office, but also the various crime scenes to evaluate possibilities perhaps not considered or dismissed by primary investigators. Such visits may also include scene re-enactment or reconstruction to facilitate a better understanding of what aspects of a case may actually be fact, as opposed to opinion or interpretation. For example, clearly showing relative body positions when injuries were sustained goes beyond the typical "anatomic position" of the autopsy and leads to the conversation about bodies positioning in the real world. Ultimately, counsel should seek first to understand what happened and how it happened in order to place that information into the larger context. Hand-in-hand with the former is comprehending the different elements of the forensic evidence and trying to ensure that the jurors grasp the nuances of the evidence.

Due to limited resources and overwhelming caseloads, the forensic pathologist actually visiting the scene and incorporating that visit with the autopsy findings is a dying exercise. Fewer and fewer offices are able to send the Medical Examiner (ME) to the scene. A regionalized death investigation system, currently being considered by some jurisdictions, would further limit the forensic pathologist visiting the death scene. In many areas, primary death scene functions are performed by lay investigators — either law enforcement, a coroner, or the ME staff. Sometimes, this may result in little more than the police "telling" the pathologist what happened.

In a death case, prosecutors are commonly urged to bring the decedent to life. The defense is usually admonished to minimize or ignore this strategy. In point of fact, the message of the dead may be more important to the defense than to the prosecution. The old adage that the dead speak to the forensic pathologist has its basis in fact. Counsel should work with the medical experts to translate that conversation to the jury in clear, understandable, and *understood* language.

Utilizing the highly scrutinized and widely publicized double murder trial of former professional football player Aaron Hernandez, which resulted in his acquittal, illustrates the importance of preparation and demonstrations. Taking advantage of the varied strengths of the forensic experts can prove remarkably helpful in facilitating conveyance of the message to the jury. To that end, demonstrative evidence, scene representation, and alternate interpretations can be of critical import in communicating with the jury by visually portraying exactly the points that need to be made.

Collaboration, Demonstrative Evidence, Aaron Hernandez

F5 Care Decisions in Desperate Cases in Infancy: *Parens Patriae* or Birth Parents' Responsibility — Lessons From the Case of Charlie Gard

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The goal of this presentation is to highlight the differing approaches taken in common law jurisdictions where there are differences of opinions between parents and health care professionals as to the care that should be provided to a desperately ill infant.

This presentation will impact the forensic science community by analyzing how care decisions can best be made when there are significant differences between medical professionals and the natural parents as to the appropriate care that should be given to a desperately ill child.

Charlie Gard was born on August 4, 2016. Initially, he developed normally, but his parents took him to their family physician in early October after he failed to gain weight. He was referred to Great Ormond Street Hospital (GOSH), a tertiary pediatrics referral center. Respiratory failure developed and he required ventilatory support. By the end of October, a clinical diagnosis of Mitochondrial DNA Depletion Syndrome (MDDS) was made. This was confirmed as the RRM2B variant. Functional RRM2B is required for the synthesis of mitochondrial DNA. Non-functional RRM2B results in depletion of mitochondria in body tissues, including brain and muscle. The outcome of the disease is early death from respiratory failure

GOSH consulted, nationally and internationally, on options for Charlie, including possible Nucleoside Bypass Therapy (NBT). His condition deteriorated; he began having fits and became unresponsive. By February 2017, the GOSH staff decided that there were no useful treatment options left and that it was in his best interest that active treatment ceased and palliative care be instituted. His parents did not agree, believing that Charlie was not non-responsive and adhered to the view that NBT might have something to offer him. A Crowd Funding appeal for his treatment in the United States raised £1.3 million (US$1.6 million). The GOSH staff felt this would be futile and, if Charlie had any pain perception left, it would be distressing for him.

His parents and GOSH could not agree on Charlie's best interest. Thus, GOSH asked the High Court to exercise its residual *parens patria* function and decide what was best for Charlie. His parents were represented by counsel *pro bono*. Charlie was represented by counsel appointed by the Children and Family Court Advisory and Support Service (CAFCASS), an independent body that represents children in legal proceedings relating to their welfare.

The Judge, dealt with the question that United States commentators raised as to what business it was of the court's rather than the parents, said, "The duty with which I am now charged is to decide, according to well laid down legal principles, what is in Charlie's best interests. Some people may ask why the court has any function in this process, why can the parents not just make the decision for themselves? The answer is that, although the parents have parental responsibility, overriding control is by law vested in the court exercising its independent and objective judgment in the child's best interests. The Great Ormond Street Hospital has made an application and it is my duty to rule on it, given that the parents and the hospital cannot agree on the best way forward."

The Judge adjourned the hearing in February to allow the parents to obtain additional evidence. This included evidence from a United States expert agreeing that as encephalopathy had developed, treatment with nucleosides was very unlikely to lead to improvement. He also said that if Charlie was in the United States, he would nonetheless treat him at the parents' request.

The Court ordered that Charlie should receive palliative care only and not receive nucleoside therapy. The family appealed, first to the Court of Appeal, which held against them, then to the United Kingdom Supreme Court, which affirmed the judgment, and finally to the European Court of Human Rights, which, in effect, declined to take jurisdiction. Subsequently, GOSH received opinions from experts in the United States and Italy suggesting that NBT might be beneficial to Charlie.

The Judge held another hearing, ordering that there should be a meeting of experts following examination of Charlie and his imaging and laboratory findings. It was then agreed in Court that NBT would not now be beneficial to Charlie. On July 24, the Court ordered that he should be taken to a hospice for palliative care with a timetable allowing time for his parents to agree with GOSH on how it should be accomplished. No agreement was reached. Charlie was taken to the hospice on July 28 and died shortly thereafter.

Nowhere in this sad saga has there been mention of "Death Panels," euthanasia, or Charlie being a prisoner of the State or the NHS. There are two main lessons: (1) as every law student knows, always read the full judgment, not just the headlines, before commenting; and, (2) when dealing with a child's welfare, formal mediation is desirable before initiating litigation.

Parental Responsibility, *Parens Patriae,* Care Withdrawal

F6 Bringing Science Back: Strengthening the Foundation of Fingerprint Examination

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After attending this presentation, attendees will have a greater understanding of what it means to demonstrate scientific validity, the issues surrounding the litigation of fingerprint evidence, and a way forward for the forensic fingerprint community to navigate toward a stronger scientific foundation.

This presentation will impact the forensic science community by discussing the current issues and a path forward for the forensic fingerprint discipline to demonstrate scientific validity in the wake of the highly critical Report by the President's Council of Advisors on Science and Technology (PCAST).

Fingerprint evidence has been admissible in legal proceedings for more than a century and has been practiced by nearly every forensic laboratory throughout the world. Once viewed as the gold standard of forensic evidence, the fingerprint discipline, along with nearly every other forensic discipline, is experiencing a great deal of turbulence as it navigates through the criticisms from the National Research Council (NRC), PCAST, and several other professional working groups and academic commentators. Although the forensic fingerprint discipline was determined to be foundationally valid by the PCAST Report in 2016, questions still remain regarding the validity of the methods when applied to a specific case at hand. The primary concern is the legal field's inability to assess the reliability of fingerprint comparison results for a given case at hand without validated statistical data concerning the strength of the findings, thus bringing into question the scientific validity of fingerprint evidence and threatening its admissibility in criminal courts. These concerns have stimulated a great deal of healthy debate within the forensic fingerprint discipline regarding how forensic science laboratories can move forward in light of these criticisms and demonstrate the applied validity of their methods so they may continue to serve the criminal justice community.

This presentation will provide a candid assessment of the current state of the forensic fingerprint discipline through the lenses of scientific validity, discuss existing gaps between the current state of the practice and the ideal future state, and propose a way forward for the forensic fingerprint community to navigate toward a stronger scientific foundation. As a result, forensic science leadership and criminal and civil litigators will have a much better understanding of the issues related to traditional practices of fingerprint examinations, become familiar with recently developed tools and technologies that can be leveraged by forensic science laboratories to promote more objective, transparent, and standardized practices, and become better positioned to advocate for appropriate improvements within their respective jurisdictions.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the United States Department of the Army or United States Department of Defense.

Fingerprints, Validity, Science

F7 Mad (Forensic) Scientist and Murder: A Case of Suspected Innocence After 22 Years

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After attending this presentation, attendees will better understand what constitutes a viable *Brady* Claim; that is, what to look for in the files of police officers and forensic scientists, and how to use that information in a legal framework based on personnel files and forensic psychological analysis of the police officers' and/or forensic scientists' fitness for duty at the time of the criminal investigation.

This presentation will impact the forensic science community by shedding light on a problem that is rarely spoken of or investigated — the mental health of forensic scientists and how it may impact their conclusions, in turn affecting the court and undermining its task of seeking just outcomes in criminal matters.

In 1993, James Parsons was convicted of killing his wife, Barbara Parsons, in the bedroom of their home. It appeared that the assailant hit Mrs. Parsons in the head 15 times with a heavy object. Her husband, James, was investigated and claimed to have been away from home working at his auto repair shop during the time in question. This criminal case had remained unsolved for 12 years, when G. Michelle Yezzo, a forensic scientist on staff at the Ohio Bureau of Criminal Identification and Investigation, claimed that she had solved the murder mystery. Using chemical enhancements to find previously undetected bloodstains on a large mechanic's tool and a blood spatter analysis on a sheet reportedly belonging to James and Barbara Parsons, Yezzo gave testimony in court that proved pivotal to the murder conviction. Yet, at the time of this murder conviction, a close review of Yezzo's personnel records revealed that her colleagues and supervisors repeatedly questioned her investigative methods and conclusions. Specifically, some raised the issue that she tended to present evidence with a prosecutorial bias, and she appeared to make mistakes that may have led to "a substantial miscarriage of justice." Moreover, Yezzo's personnel records were replete with many instances in which she exhibited disturbing behavior toward her colleagues. It got to the point that some questioned her mental health, especially during the time she was investigating and came to conclusions leading to the criminal prosecution of James Parsons. It was Yezzo's findings 12 years after the killing that led to Parson's eventual arrest and prosecution. Only circumstantial evidence had been considered until Yezzo's bloodstain analysis on the suspected murder weapon and bed sheet was offered. Interestingly, at the time Yezzo was getting ready to testify in the Parson's murder case, she was investigated and suspended for threatening a coworker.

Twenty-three years later, Ohio Innocence Project attorney, Donald Caster, studied the James Parsons murder conviction. He found that Parsons consistently maintained his innocence and his children never believed their father had killed their mother. Caster was able to obtain the personnel files of Yezzo, and he was shocked by what he found. He hired a forensic psychologist, Dr. Scott Bresler, to review these personnel files, knowing that Dr. Bresler has performed many fitness-for-duty evaluations on persons in positions of trust and power (e.g., doctors, nurses, police officers, firefighters, air traffic controllers). In so doing, Caster established a viable *Brady* Claim and the criminal conviction of James Parsons was vacated after a judicial hearing with new evidence presented in open court. The legal framework for establishing this *Brady* Claim will be described, and the use of Dr. Bresler's analysis of Yezzo's mental state at the time she came to her weighty conclusions will be discussed.

***Brady* Claim, Innocence Project, Fitness for Duty**

F8 An Interpretation of the 2016 President's Council of Advisors on Science and Technology (PCAST) Document in Terms of Forensic Metrology

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After attending this presentation, attendees will better understand how the scientific validity of forensic science methods can be quantitatively assessed by employing the core methods of metrology. In particular, attendees will gain a better understanding of how this may help in evaluating the probability of wrong decisions, when decisions are based on the results of scientific tests.

This presentation will impact the forensic science community by providing an interesting interpretation of the 2016 PCAST document in terms of forensic metrology. This presentation will broaden understanding of how the fundamental concepts of metrology can help with understanding the scientific validity of forensic science methods, and, most importantly, providing a quantitative assessment of their validity.

Recently, a growing discussion regarding the validity of forensic science methods and the way validity can be ensured has been seen. The most recent and interesting document is the PCAST Report, published in September 2016.[1]

On the other hand, ensuring scientific validity of the results obtained from experimental methods is the task of metrology. It is not by chance that forensic metrology has become a popular keyword of both the measurement science and justice.[2,3]

It can be shown that the PCAST Report can be read in a metrological perspective and that the ways scientific validity has been defined have a clear counterpart in the definitions of the contributions to uncertainty given by the International Vocabulary of Metrology (VIM).[4] In particular, the *foundational validity* can be assessed and quantified in terms of definitional uncertainty, while the *validity as applied* can be assessed and quantified in terms of instrumental uncertainty.

Forensic metrology methods enable the experts to evaluate the different contributions to uncertainty and thus provide a quantitative estimate of the validity of the obtained results. This means that a quantitative estimation of the remaining doubt about how well the measurement result represents the measure and can be provided to the trier of facts.

It is also possible to estimate which is the dominant contribution to uncertainty and focus the efforts to discuss and minimize this contribution. This presentation will consider two widely used forensic science methods: DNA profiling and Breath Alcohol Concentration (BAC) tests. This presentation will illustrate that the forensic practice focuses on the definitional uncertainty (in terms of wrong match probability) for DNA analysis, while the dominant contribution to uncertainty is the instrumental one, and focuses on the instrumental uncertainty in BAC tests, while, in this case, the dominant contribution to uncertainty is the definitional one.

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Forensic Metrology, Scientific Validity, Measurement Uncertainty

F9 The National Institute of Standards and Technology (NIST) Plans and Approaches to Conducting Scientific Foundation Reviews of Forensic Science Disciplines

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The goal of this presentation is to discuss the development of NIST approaches of examining scientific foundations (technical merit reviews) for various forensic science disciplines as requested in the President's Council of Advisors in Science and Technology (PCAST) Report and by the National Commission on Forensic Science.

This presentation will impact the forensic science community by discussing the importance of having documented research and validation studies to support measurement and interpretation claims in forensic science.

Science matters and makes a difference in quality forensic science efforts. Data are necessary to demonstrate that measurement claims are valid and appropriate interpretations can be made. In the past year, the forensic science community has been reminded of the importance of documenting data supporting claims in drawing reliable conclusions on forensic evidence. The PCAST, the National Commission on Forensic Science (NCFS), and the American Association for the Advancement of Science (AAAS) have published recommendations encouraging further research and studies assessing the scientific foundations of forensic disciplines.[1-3]

PCAST and NCFS recommendations request the NIST to examine the scientific literature and conduct technical merit evaluations and validation studies of forensic science methods and practices. For example, the NCFS requested that the results of the technical merit evaluations "be issued by NIST as publicly available resource documents" and that "NIST's evaluation may include but is not limited to: a) research performed by other agencies and laboratories, b) its own intramural research program, or c) research studies documented in already published scientific literature."[2] NCFS also requested that these evaluation documents "should be broadly disseminated in the scientific and criminal justice communities and accompanied by judicial trainings."[2]

The first NIST scientific foundation review underway covers DNA mixture interpretation. In July 2006, the International Society for Forensic Genetics (ISFG) DNA Commission provided a series of recommendations and core principles regarding appropriate interpretation of DNA mixtures.[4] Forensic DNA publications covering the time period of 2007 to 2017 were gathered, analyzed, and summarized as part of understanding how responsive the community has been to the ISFG recommendations. Articles were sorted by topic and relevance to research questions addressed. In addition, interlaboratory study results gathered through NIST studies and other collaborative exercises have been examined as part of this assessment with the hope of designing future interlaboratory studies to explore the capabilities and limitations of probabilistic genotyping systems that aid DNA mixture interpretation.

This presentation will review plans and progress made by NIST and others in these scientific foundation reviews. The important role that interlaboratory studies play in evaluating performance across the forensic science community and the ability to achieve specific levels of measurement accuracy will be emphasized. As part of these scientific foundation reviews, NIST plans to prepare regular reports to help the legal community as well as other scientists understand the capabilities and limitations of specific techniques in forensic science.

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Scientific Foundation, PCAST, Validation

F10 The President's Council of Advisors on Science and Technology (PCAST) Report on Forensic Science: Why It Fails Foundational Validity

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After attending this presentation, attendees will have a clearer understanding of why the PCAST Report on forensic science has not negatively impacted the admissibility of comparative forensic science evidence in state and federal courts, and why this "scientific" Report was foundationally flawed from the outset.

This presentation will impact the forensic science community by allowing it to communicate the validity and reliability of its comparative sciences to the courts without being bound by the unnecessarily narrow standards set by PCAST. This presentation will also encourage the forensic science community to understand that additional research is needed to continuously improve the forensic sciences.

The PCAST Report, *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*, issued in September 2016, endeavored to elucidate the findings of the National Academy of Sciences (NAS) Report.[2] As courts have pointed out, the PCAST Report added little to the realm of judicial analysis of validity and reliability of comparative-type forensic evidence. The Report fails to identify any general acceptance for the proposition that a black-box study is the *only* way to validate the foundation of a subjective discipline and disregards a plethora of studies demonstrating that examiners can reliably and accurately associate forensic evidence samples with known samples.

The PCAST Report also misstates statistical results of studies, miscalculates error rates, misunderstands the nature of foundational studies, and inaccurately claims the outer bounds of the confidence interval as the rate of error. The PCAST Report further inappropriately asserts that general error rates should be applied to the specific examiner and evidential analysis in the case at bar. Further, the Report arbitrarily sets the upper bounds of acceptable error rate for purposes of validity.

While the PCAST was a policy body, the Report purports to make "scientific findings," a role outside of its charter. The Report professes not to tell the judiciary what to do, for it is the courts that interpret the legal rules, but in a not-so-subtle bout of circular reasoning, PCAST boldly tells the courts what it arbitrarily determined was the single test for validity and reliability under Federal Rule of Evidence 702.

Although the Report fails to follow any scientific methodology in its construct, analysis, or publication, its defenders say the Report has value because of the few recommendations that might be helpful to the scientific community (i.e., those that recommend additional funding and research). Unfortunately, the PCAST timed its Report such that it was released just prior to the end of the Obama administration, with no time for the President, who supposedly asked for the Report, to fund the programs necessary for the continuous improvement of forensic science.

Nevertheless, as with the NAS Report, the forensic science community is taking the recommendations to heart and is continuing to conduct foundational research, including black-box studies. In the end, though, the PCAST Report was not peer reviewed, has an unacceptable error rate, is not generally accepted, failed to use standards and protocols for the review of the literature, and did not have the transparency expected of government reports.

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PCAST, NAS, Foundational Validity

F11 Female Murder Victims in Turkey

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After attending this presentation, attendees will understand the ways in which the murders of women in Turkey are handled.

This presentation will impact the forensic science community by providing statistics regarding female victimization and the murders of women in Turkey.

Violence against women is a common problem that many women face, both around the world and in Turkey, consisting of urban dweller and villager, educated and uneducated, rich and poor, young and old, housewife and working woman.

Violence against women is a human rights violation, with femicide at the peak of the violence. In recent years, there has been an increase in the terrifying dimensions of internal physical violence, which has caused severe injuries and deaths in Turkey. This has become one of the main issues for governments in charge of ensuring the safety of individuals and the peace of society.

Among those who are exposed to physical violence are women and children who cannot defend or protect themselves. The most common types of physical violence against women are sexual assault, kicking, slapping, punching, rough beatings, and torture conducted with hurtful instruments and objects seized at that moment. Additionally, there is verbal and psychological violence, such as severe insults, humiliation, underrating, deprivation of fundamental rights, taking precious savings and money, underestimation, mocking, exclusion, intimidation, and threats, all of which make life unacceptable for women. In severe conflicts, the wife is often seen as an opponent and enemy, the consequence of many mismatches and financial difficulties. Some bursts of anger go beyond beating and lead to killing.

Unless violence against women and the problems of violence within the family are solved, it will not be possible to provide equality between men and women and close the gap in private and public life between women and men. Otherwise, the murder of women will not be prevented. Despite legal regulations, according to results of 2015–17 statistics in Turkey, the number of female murders is increasing every year. Violence against women is a multidimensional problem. The matter has social, cultural, familial, and individual dimensions and the factors leading to the violence have different structures; however, in general, these women remain alone in terms of the violence with which they are confronted and do not have sufficient knowledge, support, and awareness to protect their rights. Because of this, it is important to "Strengthen the Women" to fight against this violence.

Today, violence against women is deemed a violation of human rights, and violence prevention laws are in effect; however, the existence of these laws is insufficient as it takes time for society to adopt legal regulations. Forming bureaucratic mechanisms to facilitate the application of effective laws is necessary. Because of this, without waiting for laws to reflect society, a social awareness against femicide must be developed. In addition to the legal regulations preventing discrimination against women and stopping femicide, regulations have to be effected to strengthen the economic power of women in society. Women are subjected to many types of violence before they are murdered, and the subjects of the violence are not only women — children are also included. Additionally, children who have been witness to violence and murder are left with irreversible psychological damage. For these reasons, it is necessary to take strict measures to prevent both this violence and femicide. These protective measures should not derive from a single source; rather, effective solutions can be determined with state and local administrations and non-governmental organizations.

As a result, although Turkey should be evaluated as a country "forming the assurance of human rights, democracy, superiority of the law, and social justice in the region and paying regard to spread them on a large area," it is obvious that Turkey does not sufficiently prevent violence against women and femicide.

Female Murder, Victims, Violence Against Women

F12 Mandatory Vaccination: The Italian Case Between Clinical and Legal Profiles

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After attending this presentation, attendees will understand the importance of improving knowledge regarding mandatory vaccinations.

This presentation will impact the forensic science community by highlighting the importance of the subject of vaccinations. This subject is widely considered one of the greatest medical achievements of modern civilization. Childhood diseases that were commonplace less than a generation ago are now increasingly rare because of vaccines.

To be effective at eliminating communicable diseases, vaccines must be administered to a sufficient number of people in the community. Thus, public health officials have mandated vaccination for certain diseases as a condition of school attendance.

The Italian government has declared ten vaccines mandatory for children up to age 16 attending school in an effort to combat what it characterizes as misinformation about vaccines. The new measures followed an intense public debate regarding vaccines after a measles outbreak. The government approved making ten vaccines, including measles, rubella, and chickenpox, mandatory beginning September 2017 for children attending Italian pre-schools through the second year of high school. Other required vaccines include tetanus, diphtheria, polio, and hepatitis B. The health minister said children will not be accepted into nursery or pre-schools without proof of vaccinations, while parents of children legally obliged to attend school will face hefty fines for non-compliance. The certification will be required every year.

Because of the success and the mandatory nature of vaccinations, most people would probably not consider vaccination an optional method of medical treatment. For most parents, the "decision" to vaccinate is equivalent to the "decision" to feed one's child. Typically, a doctor informs parents of the school vaccination schedule and the parents' consent to having their child vaccinated; however, for some parents, vaccination is no routine matter. From the time the smallpox vaccine was introduced, vaccination has had its critics. In the two centuries since then, many different types of objections have been raised, with some questioning the scientific qualifications of mass immunization. Others have focused on the personal liberty interests at stake and have objected to the paternalistic nature of government imposition of what is viewed as a personal medical choice. Still others have opposed vaccination for personal or religious reasons.

The overwhelming effectiveness of vaccination programs may lead individuals to ignore the benefits of vaccination and focus more on the risk of side effects. Moreover, some have criticized the coercive nature of these programs. These objections may lead to an unacceptably high number of exemptions, which can compromise vaccination programs and leave the population susceptible to outbreaks.

This presentation explores vaccination programs with an eye toward greater public safety without ignoring the reality of a small but committed group of vaccine critics. This presentation concludes by recommending stricter enforcement of mandatory requirements for most vaccines and greater dissemination of information on the continued importance of vaccinations.

Mandatory Vaccination, Mass Immunization, Health of Children

F13 Distorting DNA Evidence: Methods of Math Distraction

Mark W. Perlin, PhD, MD, Cybergenetics, 160 N Craig Street, Ste 210, Pittsburgh, PA 15213*

After attending this presentation, attendees will understand how misleading statements can confuse DNA evidence. Trial lawyers can apply these methods to sow confusion. Opposing lawyers can block these methods to restore evidentiary clarity.

This presentation will impact the forensic science community by providing insight into how scientific testimony can distort DNA match statistics in criminal justice.

DNA is often key physical evidence in a criminal proceeding. Connecting a defendant to a crime through genetic material left at the scene can be highly persuasive to a jury, and the absence of such a reliable connection may weaken a prosecutor's argument.

The DNA expert's ultimate scientific statement is the match statistic. How much more probable is a match between evidence and the defendant than coincidence? With abundant DNA left by a single person, this match number is one over the Random Match Probability (RMP). The numerator is one because of the perfect match between the evidence and defendant genotypes. The denominator expresses match rarity as the RMP of finding the defendant's genotype by chance.

A biological mixture contains DNA from two or more people. Mixture data can be explained by adding together the genotypes of these contributors. Since the data can be explained in multiple ways, a contributor genotype is a list of possibilities with associated probabilities.

Comparing a "probabilistic" genotype with a reference genotype (relative to a random genotype) yields a match statistic. This Likelihood Ratio (LR) measures identification information — the probability of a match relative to coincidence. When comparing with the reference, the numerator becomes an evidence genotype probability at the defendant's genotype, a number usually less than one. The denominator is again the defendant's RMP.

Court is an adversarial process. While match statistic science is clear, legal rhetoric can cloud the findings. How can a defender confuse people about a simple ratio of probabilities? Answer: by distracting the jury with irrelevant arithmetic unrelated to a meaningful LR.

Methods of math distraction infect courtroom testimony. Three common ploys are: (1) The defendant does not have the highest probability genotype; (2) other genotypes have probabilities that add up to more than half; and, (3) the match probability between the evidence and defendant is small. These math statements are often true, but they are irrelevant to the DNA match statistic. Whereas the LR is a ratio of probabilities, these distractions may feature an arbitrary big number, sum of numbers, or partial ratio. How can a prosecutor demonstrate to a jury that this is just bad math distorting good DNA evidence?

Drawing on transcripts of actual DNA expert testimony, this presentation will teach attendees how criminal defenders and their experts have employed these three methods of math distraction to confuse triers of fact. The transcripts will also highlight how prosecutors and their experts were able to rebut these baseless arguments. While honesty is commendable, sometimes rhetoric is a determining factor in an adversarial proceeding.

DNA evidence is only as persuasive as its presentation. Proponent experts communicate DNA results for jurors to understand. Defenders and opponent experts can confuse jurors about DNA match statistics with methods of math distraction. Prosecutors can expose flaws in these misleading digressions.

Ways of confusing and clarifying DNA match statistics for the finder of fact will be presented. This presentation will provide multiple lawyer and scientist perspectives.

Expert Testimony, DNA Mixtures, Trial Tactics

F14 Is Epigenetics Ready for Prime Time? The Potential of Using DNA Methylation Pattern Evidence to Differentiate Between Monozygotic Twins and to Estimate Age in DNA Donors

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After attending this presentation, attendees will understand the potential for using epigenetic science in the form of DNA methylation patterns as forensic evidence.

This presentation will impact the forensic science community by assessing the state of the scientific literature on DNA methylation patterns in light of *Daubert* or *Frye* admissibility standards.

Forensic DNA testing was first used in the 1980s and has since revolutionized forensic science. There have been tremendous advances in DNA technology through the decades, including Polymerase Chain Reaction (PCR) amplification and probabilistic genotyping, yet there remain limits to what traditional DNA testing can accomplish. Among those are: (1) differentiating between monozygotic twins; and, (2) estimating the age of a DNA donor.

The discipline of Epigenetics offers potential answers to these questions that may be on the cusp of qualifying for courtroom admissibility.

The Oxford Dictionary defines Epigenetics as "[t]he study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself." "Epi-" is a Greek prefix meaning "above," "upon," or "in addition to."

The focus of this presentation will be on one area of epigenetics — DNA methylation. DNA methylation means the addition of methyl groups (CH_3) to the DNA molecule. The methyl groups do not change the DNA code or sequence, but they change the way the DNA is expressed. Stated another way, DNA methylation results in gene silencing. This is necessary for normal growth and development but can also affect disease progression.[1,2]

Of the four DNA bases, traditional dogma holds that only Cytosine (C) can be methylated in mammals, but recent research suggests that Adenine (A) can also be methylated; thus, nearly all of the scientific research has involved cytosine.[3,4] When cytosine is methylated it becomes 5-methylcytosine (5-mC). Most 5-mCs are found adjacent to Guanine (G) bases, which are called "CpG sites." Clusters of CpG sites are called "CpG islands." A test called Bisulfite Sequencing can be used to develop a DNA methylation profile. When DNA is processed using sodium bisulfite, methylated cytosine remains cytosine, while unmethylated cytosine changes to uracil, thus making it relatively easy to map DNA methylation patterns via conventional sequencing methods.[1,2,5]

The scientific literature on DNA methylation is extensive and goes back more than a half century. In 1948, scientist Rollin Hotchkiss described his findings on the methylation of cytosine into 5-methylcytosine, which is still the understanding today. In the 1970s, Sir Adrian Bird described the role DNA methylation plays in gene transcription. The Bisulfite Sequencing test was invented in the 1990s by Marianne Frommer and Susan Clark and is considered the gold standard of DNA methylation analysis.[1,5] Over the decades, numerous studies, many of them involving twins, have reported findings based on DNA methylation. Therefore, there exists a mature, extensive body of scientific research involving DNA methylation, and in recent years, some studies have specifically discussed the potential of DNA methylation science as forensic evidence.[6,7]

Several research papers reporting the ability to differentiate monozygotic twins using DNA methylation patterns will be listed, discussed, and summarized.[8-15] The extent and quality of the research will be examined with an eye toward whether they appear sufficient to meet *Daubert* or *Frye* standards.[16,17]

The case of *Commonwealth v. Dwayne McNair* will also be reviewed.[18] In *McNair*, the prosecution attempted to use a different type of DNA test to distinguish monozygotic twins. Specifically, the prosecution attempted use ultra-deep next generation sequencing to search for somatic mutations.[19] In April 2017, the trial judge ruled such testing does not meet *Daubert* standards for admissibility. The case is on appeal.

Finally, several recent research papers report the ability to estimate DNA donor age to a range of approximately 3.5–6.85 years based on DNA methylation patterns.[7,20-27] Such evidence could be used to settle questions of possible lab contamination in certain DNA cold cases. See, for example, *People v. Leiterman*.[28]

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Epigenetics, DNA Methylation, Monozygotic Twins

F15 "Not Suitable for Comparison" Almost Sends the Wrong Man to Prison

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The goal of this presentation is to review a case in which a strict interpretation decision that a DNA mixture was not suitable for comparison ignored the presence of usable data that corroborated the defendant's innocence.

This presentation will impact the forensic science community by informing attendees of the need for full disclosure of the facts, bases of conclusions, and limitations on those conclusions.

This is a case in which the defendant was charged with murder, based on the statements of an eyewitness naming the defendant as the shooter.[1] Relevant physical evidence included a baseball hat that fell off the shooter's head as he fled. The first round of DNA testing at 15 loci plus a gender marker developed results at 14 loci plus gender. The lab concluded there was a mixture of at least two people, the number of contributors could not be determined, and the mixture was potentially incomplete and not suitable for comparison.

Defense investigation found an alternate suspect who had admitted to others that he had committed the murder.

A second testing of the hat was ordered with comparison to the alternate suspect. Testing was again performed at 15 loci and gender and results were developed at all loci. The same conclusion was reported: a mixture of at least two and that the mixture was potentially incomplete and not suitable for comparison.

Subsequent to a request for disclosure of the case file and supporting data, defense review of the data found good, reliable data that could be examined. Based on the data reported, there was evidence to exclude the defendant as a contributor and to include the alternate suspect as a possible contributor to the baseball hat.

The third-party confessions of the alternate suspect were hearsay statements and not ordinarily admissible at trial. The Unites States Supreme Court has recognized that Due Process rights to a fair trial and the defendant's right to present a defense will override the hearsay prohibition at times when the statements can be shown to be reliable.[2] At a pretrial hearing, those statements were presented along with a DNA report tendered by the defense that the defendant was excluded and the alternate suspect was included as a contributor to the hat. After the court had ruled that some of those statements would be admissible and before trial commenced, the State decided not to prosecute (*nolle prose*), effectively dismissing the case and allowing the defendant to be released.

A straightforward reading of the state's lab reports would have left the defendant with no physical corroboration of the alternate suspect's statements. The report wording that the mixture was not suitable for comparison fails to inform the legal practitioner that there may be good, reliable data that may be used for the defense. The recent National Commission on Forensic Science made various recommendations to the Attorney General, including recommendations on pre-trial discovery and documentation, case record, and report contents.[3] These recommendations are a beginning to address potential pitfalls caused by the failure to adequately consider the defense position. Labs are also urged to consider implementing procedures to reconsider the interpretation decisions or to flag the concerns in the report when presented with other viable interpretations.

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Actual Innocence, Due Process, DNA Interpretation

F16 You Be the Judge: An Interactive Session Regarding Admissibility of Scientific Evidence

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After attending this presentation, attendees will be able to: (1) state the elements of admissibility under the Rules of Evidence required of a trial court judge to admit scientific or technical evidence; (2) evaluate the challenges a judge faces in a variety of situations in his/her role as gatekeeper of admissible scientific or technical evidence; and, (3) apply their knowledge of the elements of admissible evidence in specific case situations.

This presentation will impact the forensic science community by increasing attendees' abilities to apply their knowledge of the elements for admitting scientific evidence in several specific case situations. Attendees will have a better comprehension of the trial court's admissibility standards regarding expert testimony through interactive audience participation of the application of these standards in several case studies.

In the tradition-bound adversarial legal process, judges and jurors in the courtrooms must reach definitive decisions for "a particular moment in time, while this scientific process is going on."[1] Within the dynamic intersection of science and law, justice and science must coexist for the good of society. Historically, courts have viewed science as an indispensable ally in their shared project of pursuing truth. Scientists seek truth by working diligently and gradually on their hypotheses, using relevant scientific methodologies to validate core insights. Scientists also recognize their evolving need to revise and refine their hypotheses and methodologies due to peer review and criticism emanating from various scientific communities.

Understanding the role of judges as gatekeepers will assist attendees in comprehending and appreciating the critical admissibility decisions judges have as gatekeepers of scientific evidence in our courtrooms. *Daubert v. Merrell Dow Pharmaceuticals, Inc*. is the leading case regarding this gatekeeper role of admissibility decision-making.[2] The United States Supreme Court in *Daubert* defined the judge's role as a gatekeeper for admitting scientific knowledge to assist the triers of fact in understanding the evidence. Jurors as judges of the facts must determine the facts in issue in a case by applying valid scientific conclusions to the pertinent facts in the case. Judges should admit conclusions that will qualify as scientific knowledge if the proponents of such evidence can demonstrate that such conclusions are the products of sound scientific methodology derived from the scientific method. Understanding the methodology used by an expert is critical to effective, efficient, and proper judicial gatekeeping.

Moreover, Federal Rule of Evidence 702 requires the following for expert testimony: A witness who is qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise if: (1) the expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue; (2) the testimony is based on sufficient facts or data; (3) the testimony is the product of reliable principles and methods; and, (4) the expert has reliably applied the principles and methods to the facts of the case.

This session will focus on teaching attendees to apply these trial court's admissibility standards regarding expert testimony through the vehicle of interactive audience participation.

Reference(s):

1. *Carnegie Comm'n on Sci., Tech. & Gov't, Science, Technology, and Government for a Changing World.* 22 (1993) [hereinafter Carnegie Task Force Report]. http://www.ccstg.org/pdfs/Final-Report0493.pdf.
2. *Daubert v. Merrell Dow Pharmaceuticals, Inc*., 509 U.S. 579 (1993).

***Daubert,* Admissibility, Evidence**

F17 Post-Conviction Relief: The Many Errors Leading to a Miscarriage of Justice

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After attending this presentation, attendees will better understand why discovery and review of DNA testing data pre-trial are critical and how a litany of errors by the prosecutor and expert witnesses led to the inaccurate presentation of DNA test results, resulting in the conviction of an intellectually disabled man in a capital murder case.

This presentation will impact the forensic science community by providing information regarding the significant efforts needed post-conviction to seek justice for a defendant after a "worst-case scenario" of multiple events occurred at trial, including discovery violations, inaccurate demonstratives depicting the DNA test results, and expert witnesses' testimony that misrepresented the significance of the DNA results.

Kenneth Simmons was convicted and sentenced to death in 1999 for the murder and sexual assault of an elderly woman in Summerville, SC. The trial conviction resulted from grossly misleading DNA testimony, lack of adequate scientific review of the DNA testing for the defense, and encouragement of errors by an overly zealous prosecutor.

Simmons's death sentence was commuted to life imprisonment in 2014, based on a judicial finding that he is intellectually disabled. The Supreme Court of the United States ruled in *Atkins v. Virginia* in 2002, after Simmons's trial, that persons with intellectual disability cannot be executed.[1] On June 23, 2017, Simmons was also awarded a new trial, 18 years after his original conviction, based on a judge's finding that multiple errors in the State's presentation of DNA evidence caused him to be "severely deprived of his due process rights."

The evidence offered by the prosecution to secure Simmons's original conviction included only two items: the now-debunked DNA evidence and a confession obtained after multiple non-recorded interrogations during which Simmons (whose intellectual disability is now established) falsely confessed to other crimes before ultimately confessing to the murder. A judge recently ruled that the State's DNA evidence was false, confusing, misleading, and inaccurate because the State presented a DNA chart that contained fabricated results. Moreover, the State withheld material evidence, including the fact that a second round of DNA testing did not incriminate Simmons and was inconsistent with the original DNA results, and gender-typing test results indicated no male DNA was even present in the evidentiary samples, among other things.

The first serious examination of the DNA evidence presented at trial was conducted at the request of post-conviction counsel in 2005. Although the initial defense expert noted a number of problems with the DNA evidence, the post-conviction judge was uninterested and initially denied any further live hearings on the subject. In recorded depositions, the State's trial DNA experts refused to acknowledge many of their errors and instead maintained that their original testimony was largely accurate and any errors they committed were insignificant.

To address this apparent "battle of the experts," two additional experts examined the DNA case records. The primary findings, submitted in a joint affidavit in March 2011, not only agreed with the first defense appraisal of the DNA testing, but found numerous additional issues such as undisclosed gender-typing test results, additional unreported results, and the laboratory's failure to use a reagent blank control. Subsequently, the post-conviction relief judge agreed to hear their testimony, but persisted in ignoring the serious concerns of the DNA experts. Simmons was finally awarded a new trial after the state supreme court remanded the DNA issues back down to the post-conviction judge.

The reversal of the conviction required 18 years, three defense attorneys, three well-qualified DNA experts, five hearings, and a state supreme court ruling to undo the original damage. This presentation will detail the flawed legal and scientific issues that contributed to the original conviction and ultimately produced the order for a new trial for Kenneth Simmons.

Reference(s):

1. *Atkins v. Virginia*, 536 U.S. 304 (2002).

Post-Conviction Relief, Misrepresentation of DNA, Expert Review of Testing

F18 The Need for Scientifically Educated Persons at the Sharp End of Scene Investigations

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After attending this presentation, attendees will have an increased appreciation of the importance of a scientific education to underpin training in both fire scene investigation and crime scene investigation. This presentation will examine the changes in fire investigation techniques and hopefully propose a model for inducing similar changes in the investigation of crime scenes that do not involve fires.

This presentation will impact the forensic science community by promoting an understanding of the need for scientific expertise and of fire and crime scenes. This presentation will examine how fire investigation has changed and will propose a model for changing crime scene investigators as well.

Errors that occur with respect to determinations of cause and origin in fire investigations are far more likely to be the result of problems with the scene investigation than with the analysis fire debris samples in the laboratory. This has long been the case. It is attributable to a lack of the necessary scientific expertise at the many fire scenes. Historically, where such expertise was unavailable, the vacuum was filled by non-scientist investigators utilizing naïve "rules of thumb" to substitute for scientific understanding. In an ongoing repeating pattern, more experienced investigators taught these rules to less experienced personnel. These rules of thumb, based on a fundamental misunderstanding of fire behavior, were first debunked in the early 1990s, and by 2000, the document that debunked them, the National Fire Protection Association (NFPA) 921, *Guide for Fire and Explosion Investigations,* can be said to have become "generally accepted."

Apprentice-style training, described above, is starting to give way to a demand for more scientific training, but we are not there yet. In 2009, NFPA 1033, *Standard for Professional Qualifications for Fire Investigator,* added a list of specific subjects in which a fire investigator was required to have knowledge beyond the high school level. That list was expanded to 16 subjects in 2014.

There is still no requirement for a fire investigator to possess a bachelor's degree in anything, and that is unlikely to change soon due to cost considerations. It is unlikely that fire investigation agencies can attract talented college graduates for the meager salaries they offer; however, some unqualified investigators have been weeded out through the use of NFPA 1033, where adverse counsel has probed the investigator's basic knowledge. A fire investigator who does not know the basic units of energy, the basic units of power, or the difference between energy and power is per se unqualified. Rather than challenging investigators' methodology, these new challenges are of the investigator's qualifications. If an investigator can be shown in a pre-trial proceeding, either a deposition or a preliminary hearing, to lack the requisite knowledge, the case is likely to be dismissed or to be settled.[1]

There is no such standard to which crime scene investigators are held. While it is fairly obvious that fire, a complex phenomenon involving chemistry and physics, requires at least some scientific knowledge, that is often not the perception in ordinary crime scenes, despite the need to understand basic physics, evidence collection and preservation, and documentation. This presentation will suggest ways to improve the quality of personnel on crime scenes, but there may be the same problems that are experienced in fire investigations (i.e., when a scientist can earn twice as much money in a non-law-enforcement position, that is likely to be the career path he or she chooses).

A person can be taught to perform routine or repetitive tasks, but in order to do those tasks well, it is necessary for the person to understand the scientific principles behind that training. This is difficult to accomplish without a scientific education.

Reference(s):

1. Lentini, J.J. What fire litigators need to know in 2017. *The SciTech Lawyer.* 2017 13: 4, Summer 2017, 18.

Scientific Investigation, Fire Scenes, Crime Scenes

F19 How Judges and Juries May Perceive Liability Issues Arising From the Operation of Highly Automated Vehicles

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After attending this presentation, attendees will better understand the dynamic relationship between law and technology regarding highly automated vehicles, as well as related ethical and moral dilemmas.

This presentation will impact the forensic science community by increasing competence in understanding legal and scientific issues as to this technology and will allow attendees to recognize possible solutions in developing and understanding appropriate laws in this area.

This presentation begins with an analysis of human errors associated with 85% of motor vehicle accidents. It is hoped that the Highly Autonomous Vehicle (HAV) will address and diminish most of these error types. The full-scale tests of HAVs in difficult control situations will be presented. The side-by-side comparison of HAVs next to human-controlled vehicles will be demonstrated through modeling and full-scale testing. It's impressive, but many unresolved issues exist.

Federal and state laws regulate HAVs being operated on public roads in the United States. When laws from state to state regarding HAVs are reviewed, one can see a myriad of different laws across the United States. The National Highway Traffic Safety Administration (NHTSA) is a federal agency of the Executive Branch and part of the Federal Department of Transportation. NHTSA's mission is to "Save lives, prevent injuries, reduce vehicle-related crashes. Accordingly, NHTSA has proposed Model State Policy to create consistency and unify the national framework of states to regulate all levels of HAVs. This Model State Policy builds on collective knowledge to date and assists in avoiding inconsistent laws and regulations among the 50 states and other United States jurisdictions. Their goal is to avoid the delay of wide acceptance and promote deployment of lifesaving technologies. NHTSA foresees a system wherein states would treat HAVs as the "drivers" of these vehicles. In the interim, Model State Policy suggests states only allow testing and require application and approval before manufacturers are permitted to begin testing. This policy recommends statutes requiring licensed human drivers possessing special training. Overall, this policy urges states to begin addressing issues arising with increased use but recommends states' current usage be limited to testing only.

The legal landscape involving autonomous vehicles will be complex and can include product liability laws such as negligence, strict liability, misrepresentation, and breach of warranty as well as contract law. Jurisdictions adhering to the Second Restatement of Torts would hold manufacturers liable for the sale of their products containing an "unreasonably dangerous" defect even if manufacturers have "exercised all possible care in the preparation and sale" of their products. Other jurisdictions may be utilizing the Third Restatement of Torts, which would specifically deal with manufacturing defects, design defects, and failure to warn to include liability for design defects and failure to warn to "foreseeable risks." The Third Restatement addresses the failure of manufacturers to identify and mitigate dangerous "foreseeable" risks in a similar vein as negligence concepts rather than as strict liability concepts. Liability insurance is another complicating factor as each state has varying laws for impleading the manufacturers versus initiating separate legal actions against manufacturers after the case concludes.

Another dimension involves ethical concerns regarding autonomous vehicles. Will autonomous vehicles fully replace human drivers? What ethical judgments will manufacturers of autonomous vehicles be called upon to make? What socioeconomic impacts flow from the operation of autonomous vehicles? Will these vehicles disrupt the nature of privacy and security? What dialogue needs to take place with government, industry, academia, and the public?

The Uniform Law Commission (The National Conference of Commissioners on Uniform State Laws) recently established a Study Committee on State Regulation of Driverless Cars. Due to this technology rapidly evolving and ripening, four states and the District of Columbia have already enacted their own autonomous vehicle legislation. Moreover, NHTSA has issued guidelines for states to use to regulate these types of vehicles. This Study Committee of the Uniform Law Commission will study the need for and feasibility of drafting uniform state legislation for autonomous vehicles. Their efforts will be discussed.

Autonomous, Liability, Ethics

F20 Quantification of Scientific Opinions and the American Jury System

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The goal of this presentation is to share concerns with colleagues regarding the conceptual incompatibility of quantification of forensic opinions with the heuristic of the American jury.

This presentation will impact the forensic science community by encouraging thought as to whether quantification is compatible with the current American jury system heuristic. If not, judges, lawyers, and forensic scientists must find ways to adapt the jury system and its present heuristic to a new paradigm that integrates quantification.

Opinions of forensic scientists are generally quantified either in numerical terms or value language that suggest a numerical quantification. Currently, quantification of DNA evidence is typical, but there is an effort to quantify fingerprints, pattern evidence, ballistics, and others. The American jury is not educated in statistics nor in how to integrate quantification into their deliberations. Jurors are generally asked to "weigh" evidence and compare it to a vague "burden of proof" in determining which party wins.

The folk ideal of the American jury is one in which the jurors deliberate impartially, weighing each part of the evidence, considering all the evidence, and then "applying" it to the law; however, the folk ideal of the American jury is seldom, if ever, met in practice. Jurors are human and subject to bias and prejudices, and they are not equipped with the knowledge required to perform statistical analysis. As a result, they employ a folk heuristic.

Lawyers, judges, and forensic scientists must evaluate whether quantification — presenting evidence of Frequentist (F), Random Match Probability (RMP), Likelihood Ratios (LR), Bayesian Analysis (BA), or Bayesian Network analysis (BN) — is compatible with either the folk ideal or the heuristic that characterize the American jury. In other words, if an expert expresses an opinion as to one item of evidence (by F, RMP, or LR) or an opinion about the interrelation of one item of multiple items of evidence (by BA or BN), is this compatible with how the American jury functions ideally or in practice?

It is the position of this presentation that quantification is not compatible with the current American jury system. Juries, in fact, engage in a type of largely non-numerical BN analysis. On a good day (when they do not corrupt the process with, for instance, bigotry), they give some type of folk quantification to the individual pieces of evidence. They then evaluate the evidence as a whole using a heuristic similar to a folk BN analysis, which is then compared to a vague standard of the burden of proof. The jury's enterprise is that of uncertainty, just as is the statistician's. The folk ideal is that the American jury will make an overall network analysis resulting in an LR that favors the unknowable truth within the tolerance stated in the burden of proof.

It is observed anecdotally that expert opinions may carry too much or too little weight with jurors. This intuitive observation is supported conceptually in that quantification by an expert is incompatible with the American jury's heuristic in which one piece of evidence is quantified in a scientific manner and the jury is given no means to integrate that quantification into the heuristic that they are intuitively using to weigh the rest of the evidence. For example, the jury is given the probability of randomly finding a match in a data base (RMP) regarding the DNA profile taken from an object at the scene that corresponds to the defendant's sample. Yet this is only a part of the heuristic by which the jury determines whether the case against the defendant was proven. They weigh this evidence with a network of unquantified evidence before they can weigh whether this quantified evidence even creates a likelihood that the defendant was at the scene (e.g., was the object moved, was there transfer DNA, was the evidence contaminated, was the evidence properly analyzed, was the evidence planted, or was the defendant being framed?). And, if the defendant was at the scene, did the defendant do it? Yet, among these important, unquantified, interrelated, or non-interrelated pieces of evidence, there is a glaring DNA quantification that cannot be statistically multiplied, divided, added, or subtracted with anything else in the jury's analysis.

As we move from quantification of DNA analysis to quantification of fingerprints, pattern evidence, ballistics, and other evidence, thought must be given as to whether quantification is compatible with the current American jury heuristic. If not, judges, lawyers, and forensic scientists need to find ways to adapt the jury system and its present heuristic to a new a paradigm of integrating quantification.

Expert Opinions, Quantification, Jury

F21 What Does "Under the Influence" Mean in Driving Under the Influence (DUI) Drug Cases?

Ronald L. Moore, Esq., JD, Impaired Driving Toxicology, 25422 Trabuco Road, Ste 105-309, Lake Forest, CA 92630*

After attending this presentation, attendees will understand the similarities and differences in the definition of "Under the Influence" in DUI alcohol and DUI drug cases and the additional difficulties in proving "Under the Influence" in DUI drug cases.

This presentation will impact the forensic science community by directing future research in order to overcome present limitations in proving "Under the Influence" in DUI drug cases.

DUI alcohol cases are commonly charged on both per se (over the Blood Alcohol Concentration (BAC) limit) and common law driving while "Under the Influence" theories. Because of the issues involved in establishing science-based equivalent per se limits for drugs, many states only prosecute DUI drug cases under the common law theory. This raises questions regarding to what extent is the alcohol model a basis for understanding what quanta of impairment from drugs is necessary to breach the law, and how the presence or absence of that amount of impairment is proven.

In DUI alcohol cases, there have been several large case-controlled studies that have provided crash-risk-odds ratios for alcohol, which clearly demonstrate the concentration-dependent increase in crash risk from alcohol. This provides an objective basis for setting not only the per se limits based on when the crash-risk-odds ratio increases to a level not tolerated by society, but then setting the quanta of impairment as being equivalent to that which is present at the proscribed BAC. The difficulty with DUI drug cases is that the concentration-dependent crash-risk-odds ratio have not been or cannot be established for the myriad of drugs that may potentially impair driving.

With many drugs or medications, the driver may be less impaired while being treated for his or her medical condition than the person would be without the medication. The mere presence of a medication in the person's blood stream, even when present in therapeutic quantities, is not proof of illegal impairment. Furthermore, there may be some level of effect of the medication which is still consistent with safe driving. The impairing effects of the drug may not occur at the same time or in the same magnitude as the desired effect for which the medication is taken, nor has it been established through research that the presence of physiological side effects of taking medication or drugs (such as pulse rate, blood pressure, pupillary reactions to light, etc.) or performance on field sobriety tests correlate with drug-impaired driving ability. Also, in many DUI drug cases, the traffic stop is predicated on regulatory or equipment violations that do not demonstrate impaired driving. Therefore, with alcohol, crash-risk-odds ratios provide an objective basis for establishing that the requisite amount of driving impairment is present based on an objective measure of BAC and can be extrapolated to the types of behaviors that are present in people intoxicated to that level. Impairment from other substances presents a much more difficult interpretation of how to objectively demonstrate that a driver is too affected by the medication to drive safely.

DUI Drugs, Under the Influence, Driving

F22 Due Process: Unscrambling SCRAM®

Gil Sapir, JD, PO Box 6950, Chicago, IL 60680; and Donald J. Ramsell, JD, 128 S County Farm Road, Wheaton, IL 60187*

After attending this presentation, attendees will appreciate the necessity of unscrambling the Secure Continuous Remote Alcohol Monitor's (SCRAM's®) subjective Transdermal Alcohol Concentration (TAC) test results by the legal community.[1]

This presentation will impact the forensic science community by increasing the realization that the use of convenient biomonitoring devices should not usurp scientific accuracy and reliability in the judicial process.

SCRAM® is designed to measure alcohol content as it diffuses through a person's skin as insensible perspiration.[1] TAC does not directly correlate to Blood Alcohol Concentration (BAC) in a SCRAM®.[2] The Drager fuel cell is contained in an ankle bracelet worn by the offender. The device is manufactured by Alcohol Monitoring Systems (AMS). It is designed for court-ordered alcohol monitoring of TAC readings.[3] The unit's modem transmits continuous periodic measurements every 15, 30, or 60 minutes. Positive TAC readings are sent to a central monitoring location for internal review and confirmation.

The device is commercially available to law enforcement agencies and privately operated correctional institutions. SCRAMs® are not subject to uniform standards and regulations for approval, use, maintenance, and calibration. The units can be purchased without governmental oversight and have lower standards than those promulgated in the Driving Under the Influence (DUI) industry.

SCRAMs® are useful in general population biomonitoring of self-induced alcohol consumption as a passive preliminary testing device; however, SCRAMs® have limitations. This presentation briefly reviews the manufacturer's material and scientific literature for accuracy of results as a basis to suppress the TAC results.

The data is transmitted from the device to a central database for initial screening review by a committee. An analyst subjectively decides if the presumed positive is a confirmed positive, presumed tamper, confirmed tamper, or compliant result. The purported analytical determination is problematic and generates inconsistent reporting. Data from the original SCRAM® device, as compared to the current generation SCRAM® II and SCRAMx® devices, indicate poor performance in identifying alcohol consumption events.[4] SCRAM® results should be considered a presumptive violation followed by a confirmatory test.

Justice Blackmun wrote in *Daubert v. Merrill Dow Pharmaceuticals, Inc.,* "under the Rules the trial judge must ensure that any and all scientific testimony or evidence admitted is not only relevant, but reliable." Accordingly, "evidentiary reliability is based upon scientific validity."[5] Attorneys should question the SCRAM's® results by requiring that the analyst testify in court pursuant to *Melendez-Diaz v. Massachusett*.[6] In *Melendez-Diaz*, the United States Supreme Court held that a laboratory report (evidence affidavit or laboratory certificate) prepared for a criminal prosecution in lieu of court testimony is "testimonial" evidence subject to the Sixth Amendment's Confrontation Clause. It is not a business record.[7] Therefore, the defendant has a right to cross-examine the analyst who conducted the testing — not the probation officer or distributor trained by AMS to validate the results.

Significant issues for a motion to suppress SCRAM® results are: (1) alcohol in the perspiration; (2) alcohol from surficial application of a substance with alcohol or some interfering substance in it; (3) interfering substance in the perspiration; (4) unit being regularly calibrated before and after use; (5) production of separate results for TAC, temperature, and current-times-resistance (IR) voltage; (6) operating procedure not including periodic accuracy checks to validate calibration; (7) calibration log not available to user; (8) valid calibration if within 20% of target value; (9) non-existent independent approved calibration standard to test the transdermal devices; (10) questionable ability to differentiate between methanol, isopropanol, and diethyl ether (huffers); (11) no blank reference prior to sampling; (12) device's inability to make a determination of result; and (13) qualifications and competency of analyst.[8]

The attorney has a constitutional obligation to question scientific evidence. Public policy and convenience should not replace objective, accurate, and reliable monitored TAC results. Flawed evidence should not be admissible. Wrongful convictions must be prevented.

Reference(s):

1. SCRAM® is the registered trademark of Alcohol Monitoring Systems, Inc., Littleton, Colo.
2. *People v. Dorcet*, 29 Misc.3d 1167, 1170 (2010).
3. Marques, P.R., A.S. McKnight, and National Highway Traffic Safety Administration. *Evaluating transdermal alcohol measuring devices.* (Report No. DOT HS 810 875). Washington, DC: US Government (2007).
4. Ibid.; Barnett, Nancy P., E.B. Meade, and Tiffany R. Glynn. Predictors of detection of alcohol use episodes using a transdermal alcohol sensor. *Experimental and clinical psychopharmacology.* 22, no. 1 (2014): 86, 93, fig. 2&3.
5. *Daubert v. Merrill Dow Pharmaceuticals, Inc.* 509 U.S. 579, 589, 590-91 n.9 (1993).
6. *Melendez-Diaz v. Massachusetts.* 557 U.S. 305 (2009); Williams v. Illinois, 567 U.S. 50 (2012).
7. Fed. Rule Evid. 803(6).
8. *SCRAM® Calibration Process, Technical Overview.* Alcohol Monitoring Systems, Inc., Littleton, Colo., p.2, Oct. 10, 2004.

Transdermal Alcohol, SCRAM®, DUI

F23 The Use of Field Sobriety Tests (FST) as Proof of Driving Impairment

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After attending this presentation, attendees will understand the basis for use of Standardized Field Sobriety Tests (SFSTs) as proof of driving impairment in Driving Under the Influence (DUI) alcohol cases and the limitations on extending this rationale to DUI drug cases.

This presentation will impact the forensic science community by educating attendees about the limitations on forensic testimony regarding the use of SFSTs as proof of driving impairment and direct future research to overcome those limitations, if possible.

SFSTs were developed to assist police officers in determining drivers who were likely to be over the legal limit of 0.08% Blood Alcohol Concentration (BAC), as a basis for probable cause to arrest, and in combination with the officer's other observations of driving, post-stop cues, and physical symptoms of alcohol intoxication. Several validation studies (Colorado 1995, Florida 1997, and San Diego 1998) were conducted to illustrate the effectiveness of the SFSTs at predicting whether the officer's arrest decision was correct (subject over 0.08%) based on the number of clues observed.

These tests are then often used by prosecutors as proof that the arrestee's driving was impaired, based on the theory that the SFSTs divided attention skills, which are important in driving, and, therefore, if SFST performance is impaired, then driving would likewise be impaired. This supposition is supported by the relationship between the SFST validation for predicting BACs greater than 0.08% and the demonstrated crash risk increasing at BACs greater than 0.08%.

Crash risk from alcohol is a well-studied objective measure of driving impairment, which increases with increasing blood alcohol level, and forms the basis for laws that make driving at a specific BAC illegal. With drug driving impairment, the association with crash risk at particular drug levels is much less well established. The wide variety of drug effects, and the wide variety of conditions for which drugs are taken, contribute to uncertainty in the meaning of SFST performance when drugs are present.

The mere presence of drugs in the subject's system is not proof of driving impairment. Without a correlation of SFST performance to drug levels, and from drug levels to crash risk, the parallel to alcohol is incomplete. There is no known research which directly links SFST performance to a co-existing crash risk from drugs.

This lack of positive association weakens the assumed association between SFST performance and drugged driving impairment. Due to the immense number of drugs, and their varying type and degree of effects, it would be difficult to establish dose or blood level associations with crash risk for individual drugs. A better approach would be to establish an actual association between the degree of impairment measured, the so-called divided attention skills, and driving crash risk or other objective measures of actual driving impairment, since SFSTs lack face validity for driving impairment.

Field Sobriety Test, DUI, Impaired Driving

F24 WITHDRAWN

F25 The Intersection of Science, Standards, and the Law in Fire Litigation

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After attending this presentation, attendees will understand: (1) how scientific knowledge and methodologies are being embodied in consensus standards in fire investigations; (2) the legal issues that affect the admissibility of or the weight given to standards in court; and, (3) strategies to help lawyers and experts make optimal use of standards in a court case.

This presentation will impact the forensic science community by explaining the rules from the worlds of standards and the law necessary for lawyers, judges, and experts to make science matter in striving for justice in a litigation setting.

How many injustices result from faulty fire science admitted in court? No one knows; however, from what we know and can reasonably infer, this is a huge problem demanding serious attention. This presentation summarizes the nature of this problem, then explains why science-based documentary standards for fire investigations (such as those produced by the National Fire Protection Association and the American Society for Testing and Materials (ASTM)) are an important part of the solution; however, making effective use of such standards can be complicated by legal rules that limit their admissibility in court. The first step in overcoming this hurdle is appreciating the relationships among: (1) fire science and reliable methodologies; (2) consensus standards within the fire investigation field; and, (3) legal rules relating to the admissibility of standards in court. This presentation examines the intersection of science, standards, and the law so the significance of relevant standards are fully appreciated and can be better utilized by all concerned.

First, injustices in fire cases will be discussed. In recent times, scores of people convicted of serious fire crimes, such as murder or arson, have been exonerated because the conviction was based, at least in part, on discredited methods or disproven scientific assumptions. The *National Registry of Exonerations* details such exonerations, described as "cases in which a person was wrongly convicted of a crime and later cleared of all the charges based on new evidence of innocence."[1] This registry documents dozens of cases of erroneous murder or arson convictions based, at least in part, on "false or misleading forensic evidence" from fire investigations.[2] Another factor in several cases was inadequate assistance of counsel, which was caused by counsel's failure to seek or adequately utilize expert assistance or well-known standards, for example.

As horrendous as it is that faulty fire science has played a role in unjustly convicting so many people, these cases represent the mere tip of the iceberg. They do not account for criminal cases in which defendants are charged or tried based on faulty fire science or whose cases are still in the long, arduous processes of post-conviction relief. Nor do these numbers include those who may have been convicted of (or convinced to plead guilty to) fire crimes based on disproven science or unreliable methodologies — cases which do not rise to the seriousness that merit the attention of innocence projects.

Last, but not least, are the injustices that have occurred in civil cases. The same fire investigation science and methodologies are applied in both civil and criminal cases. There is no way of tracking how many bad decisions in civil cases result from disproven fire science or unreliable methodologies.

This presentation describes the development of industry standards relevant to fire investigations that promote the knowledge and methods of science. Next, the legal rules relating to the use of standards in court are summarized, including legal hurdles to admissibility. Attendees are then introduced to the hierarchy of the standards world and the rules governing standards development processes relevant to the admissibility or weight a court will give standards that are proffered. Finally, attendees are shown how to correlate the information regarding the interplay between science and standards to the legal rules of admissibility, so that science-based standards can be more fully employed in the interests of justice.

Reference(s):

1. *The National Registry of Exonerations – Mission.* http://www.law.umich.edu/special/exoneration/Pages/mission.aspx.
2. *The National Registry of Exonerations – Glossary.* http://www.law.umich.edu/special/exoneration/Pages/glossary.aspx#A.

Fire Investigation, Consensus Standards, NFPA Standards

F26 The Case of the Missing Millionairess: How Sante and Kenneth Kimes Did Not Get Away With Murder

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The goal of this presentation is to illustrate how a painstaking investigation, involving several forensic science disciplines, helped to prove a case of murder and theft by two grifters.

This presentation will impact the forensic science community by demonstrating how to build a compelling circumstantial case of motive, opportunity, and intent when there is no dead body, DNA, or blood to be found.

Sante Kimes and her son Kenneth "Kenny" Kimes concocted an elaborate scheme to defraud and murder 82-year-old Irene Silverman, a wealthy Manhattan widow who owned and lived in an eight-million-dollar townhouse on Manhattan's ritzy Upper East Side. Silverman rented apartments within the townhouse to business executives and patrons of the arts on short-term visits to the city. In the spring of 1998, Sante and Kenny Kimes resolved to murder Silverman in order to steal the townhouse from her.

Kenny Kimes secured a first-floor apartment in Silverman's home, posing as a well-to-do designer named Manny Guerrin. Unbeknown to Silverman and her house staff, Sante Kimes moved in with him. The defendants set up eavesdropping devices on Silverman's telephone line and learned as much as they could about Silverman and her household routines. They forged Silverman's name on several real estate documents purporting to transfer the ownership of the townhouse to the defendants' off-shore corporate entity. On July 2, 1998, Sante posed as a bedridden Irene Silverman and had the fraudulent signature on the deed of sale notarized.

Finally, on the morning of July 5, 1998, while Silverman's housekeeper was on one of the upper floors of the townhouse, the defendants grabbed Silverman in the first-floor hallway, dragged her into their apartment, and killed her. They wrapped the body in garbage bags, placed it in the trunk of their car, and drove out of town, where they dumped Silverman's remains. Her staff noticed too late that she was missing. With police assistance, the townhouse was searched from top to bottom, but Silverman was nowhere to be found. With the police in tow, two of Silverman's staff entered "Manny's" locked apartment. His belongings were gone, but he had left behind an opened roll of duct tape, a box of 42-gallon trash bags with four bags missing, and a shower curtain. A large pillow and a comforter were missing from the bed.

Meanwhile, federal agents investigating the Kimes mother/son duo arrested them for fraud and found the car. When they learned that Irene Silverman was missing, they notified the New York Police Department (NYPD) and incriminating evidence was found in the car and in the possession of a defense investigator. Documents evidencing forgery were found, and Silverman's signatures on all of the documents in a folder marked "Final Dynasty," including the deed and the transfer tax form, were not genuine. In several instances, the signature appears to have been traced from the rental receipt, which Silverman signed and gave to Kenny. The handwriting in several of the notebooks matched Sante's. The handwriting on a fax requesting an $8,000 check from the Atlantis account matched Kenny's.

The jury heard about all the instrumentalities of death in the Kimeses' car and the bag they had checked at the Plaza Hotel, all of which were recovered in the days after their arrest on the Utah warrant: two loaded guns, an empty stun gun box, a pink liquid sleeping drug under the car's front seat with nearby syringes, and two sinister "Scream" masks. The few items recovered from Apartment 1B — undoubtedly left behind in the Kimeses' hurried frenzy to vacate the building — told the rest of the story. An open roll of duct tape and balled-up duct tape, both bearing Kenny's prints, four heavy-duty garbage bags missing from the box of ten, the shower curtain that had no place in the apartment and seemed to be missing its plastic liner, all indicated quite clearly that Silverman had met her end inside the Kimeses' apartment and at their hands. The absence of any forensic or trace evidence showing either that Silverman was killed in that apartment or that she was transported afterward in the trunk of the Lincoln® is a testament to the defendants' craftiness.

This is a case study of an intense investigation to amass evidence of a homicide without a *corpus delicti*.

Homicide w/o *Corpus Delicti*, Fraud, Circumstantial Evidence

F27 Expert Witnesses: A View From the Bench

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The goal of this presentation is to provide an overview of the role of expert witnesses in the judicial system from a judge's perspective.

This presentation will impact the forensic science community by providing practical advice to experts who appear in court proceedings.

The testimony of expert witnesses contributes significantly to the United States judicial system. They provide expertise in all types of legal matters, both before, during, and after the proceedings. Experts can be used to enlighten the court or to distill complex information for a jury. They often testify in open court but just as frequently provide expertise behind the scenes in a consultative role. They can be called to impeach an opposing expert or to provide an alternative view of the findings. The training of many experts often excludes any information regarding the judicial system and their role in it. They are not aware of courtroom protocols, the different types of legal proceedings that precede trial, the documents that are prepared in advance of trial, or how to conduct themselves once they are on the stand. This presentation will address those issues from the court's perspective.

The judge has a unique viewpoint on the presentation of expert testimony. There are basic tenets that should be adhered to regardless of the type of legal proceedings at hand. The expert should be impartial and clear about the type of evidence he or she is reporting. The expert should speak in plain language that easily communicates their actions, procedures, thought processes, and conclusions. He or she should be prepared and ready to discuss the pros and the cons of their report. The expert should provide concise answers to questions, should comport himself or herself professionally, and adhere to the rules of the courtroom.

Prior to testimony in court and once the review of evidence is complete and a report of findings has been issued, the expert witness should expect his or her opinion to be made public. There are various stages to the legal process in which the findings will be presented. The expert can expect that those findings will be reviewed by the attorneys and that there will be some form of interview process, which can range from an informal meeting to sworn deposition testimony. Both sides will have the opportunity to question the expert and preparation is critical.

Experts may also be asked to express opinions in the form of a sworn affidavit. Such affidavits often constitute the attorney's interpretation of the expert's findings and may not be fully inclusive or accurate, depending on the level of the attorney's understanding. The expert should carefully review any document of this type to ensure that it accurately reflects his or her findings. If an expert is asked to give a deposition or defense interview, the expert should be aware that the proceeding will be recorded (often by video) and that the transcript can be used to impeach. Experts should maintain their objectivity, speak to the facts, be familiar with the literature that supports their findings, and refrain from expressing conclusions that are not supported by the record. Part of the responsibility of the opposing counsel is to bring out contradictions to the findings when they are helpful to their client(s). This process can be combative, but professional demeanor and honest acceptance of the cons of the findings should be the goal at all times.

Many expert witnesses have not been briefed on courtroom procedure and are unaware of the restrictions on their actions once they are sworn in. They will need to be qualified through preliminary examination as to their education, training, and experience. Providing a résumé or *curriculum vitae* to the attorney ahead of time is a good way to ensure this process goes smoothly. Experts are often the mechanism by which exhibits (reports, photographs, supporting documents, etc.) will be admitted into evidence; however, they cannot discuss them until that process is concluded. Generally speaking, experts may not stand or address the jury directly or review exhibits, photographs, or their report until given approval by the judge. Further, their testimony may be limited by court rulings made in advance of the case or at trial. Preparation for these procedural elements is critical to the effective presentation of evidence.

Experts should be prepared for all encounters they may have with the judicial system. This presentation will provide the practical knowledge necessary to make those encounters successful.

Expert Witnesses, Trial, Testimony

F28 Infrared and Ultraviolet Photography for Individual Identification Using Minor Skin Imperfections

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After attending this presentation, attendees will better understand the use of "smart chips" incorporated into United States passports for rapid and better biometric identification of individuals at the borders. In addition, attendees will learn whether or not digital Ultraviolet (UV) and Infrared (IR) photography can result in enhancing subtle facial details not yet being utilized for identification.

This presentation will impact the forensic science community by suggesting methods of increasing border security measures, as there are presently important decisions being made regarding immigration into this country.

The United States requires biometric passports for visitors, especially those from politically sensitive areas, which require fingerprints, photographs, and pertinent identification information.

This study hypothesized that detailed facial information can be captured by using UV and IR photography and incorporated into digital photographs. Experiments were conducted with a digital and a modified UV/IR camera.

Photographs were taken of subjects under different lighting conditions with the goal of distinguishing facial characteristics. Sunlight, studio lighting, UV penlight, and black light sources were all utilized. In addition, subject characteristics ranged in age, skin type, and use of makeup. In addition to a Digital Single-Lens Reflex (DSLR) camera to take color photographs, five different filters (one UV and four IR) were utilized with the UV/IR camera. Black light IR and UV images were converted into black-and-white photographs. Sunlight experiments were conducted outside on a cloudless June day, at approximately 2:00 p.m. The camera settings were ISO 100, f/13, and shutter speed of 1/350. A white background was used with the UV penlight at a distance of 4ft from the subject's face and the camera manually set for ISO 2000, f/4, and shutter speed of 1/30. Black light photography was conducted in a dark room with the camera settings of f3.5, ISO 400, and shutter speed of 1/25. The studio lighting was conducted with a Calumet® 950-watt light at 1/8 power with an umbrella and white background. Camera settings were f/11, ISO 160, and 1/80 shutter speed. A female subject used mud and silicone-based makeup to see if the UV and IR lighting would show any differences between texture of natural skin and the use of makeup. Black light images revealed skin damage on the subject's face that could have potential identification value. The post-production images were adjusted in Adobe® Photoshop® and brightness compensated for the density of the filter to create the black-and-white photographs. The results were shown to passport photographers, police, and other individuals to see if they detected any differences in skin tone, skin detail, and any special facial characteristics that can be seen in one image compared to another to aid in facial mapping for identification.

Photographers chose the regular color image as showing the best facial details, images taken with a UV filter were second best, and the use of IR filters did not show any improvements in image detail. Laypeople also chose color images as having the most detail and the use of a UV filter, second best. They were also in agreement with the value of using studio lighting. Therefore, photography in conjunction with facial mapping will play a significant role in maintaining passport security and aid in human identification. Using studio lighting for passport photographs will improve their quality and value for screening at points of entry. Incorporating biometric data (fingerprints, iris scans) in a passport chip would further strengthen passport security measures and enhance methods for human identification.

Forensic Photography, Biometrics, Passport Images

F29 Forensic Archaeology: The Legal Aspect for a Practical Application in the Italian Context

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After attending this presentation, attendees will understand how archaeology could be applied in the forensic context, not only in the field, but also in court.

This presentation will impact the forensic science community by demonstrating not only the potential of forensic archaeology as a discipline, but also by proposing a review of the legal tools that can allow the application of archaeological techniques to the forensic context.

According to the etymology (ἀρχαῖος and λόγος), archaeology is an evidence-based science that employs methodologies and procedures to reconstruct past events from the physical traces left under and above the ground; however, past events are not limited to those that occurred hundreds or thousands of years ago. The actions related to a recent crime could be considered an "ended event" frozen in the past. For this reason, it is possible to transfer archaeology's *modus operandi* into the forensic world. Forensic archaeology is, in fact, the discipline that applies the stratigraphic techniques to the search, recovery, and interpretation of evidence to reconstruct events with a forensic relevance.

Unfortunately, even if the contribution of forensic archaeology for both the investigation and the trial is recognized worldwide, in some countries, it is still difficult to observe its extensive use. The reasons are related to the complexity of the legal systems, the prerogative of only a few categories of experts on death investigations, and the general lack of knowledge regarding the discipline and its undoubted potential.

Adopting the Italian perspective as a relevant example, the legal instruments for both forensic archaeologists and all other practitioners involved in the investigation could benefit from the use of stratigraphic techniques. With a comparison between the scientific reliability of archaeological methodologies and the Code of Criminal Procedure, it will be possible to delineate how forensic archaeology can fit into the legal system and can be applied for the benefit of a fair trial.

Stratigraphy and evidence-collecting techniques, if performed according to international protocols and guidelines, provide evidence that can be used in court under the rules that control the scientific evidence and its acquisition. In addition, to perfectly integrate the role of the forensic archaeologist in the trial, the possible scientific tasks that the various parties (judge, prosecutor, and defense) could assign to the practitioner will be addressed. This will help practitioners act properly at the scene and in court; at the same time, it will encourage judicial authorities and lawyers to promote the application of archaeological methodologies in cases in which it is necessary to search and recover concealed pieces of evidence.

In conclusion, useful legal references and tools will be provided, not only for the forensic archaeologist, but also for everyone involved in the crime scene investigation and trial. The practitioner and other parties will be able to cooperate and will be aware of the potential provided by the application of stratigraphic techniques to the forensic investigation, particularly when search, recovery, and documentation of buried evidence is required.

An analysis of the practical and legal aspects of forensic archeology, reviewing the legal principles, and the jurisprudence useful to the application of this discipline is necessary.

Forensic Archaeology, Penal Code, Stratigraphy

F30 The Science and Law of Solitary Confinement

Donald E. Shelton, JD, PhD, University of Michigan-Dearborn, Criminal Justice Program, 4901 Evergreen Road, Dearborn, MI 48128-2406*

After attending this presentation, attendees will better understand the scientific studies of the psychological effects of solitary confinement and whether those effects constitute "cruel and unusual punishment" contrary to the 8th Amendment of the United States Constitution.

This presentation will impact the forensic scientific community by providing attendees with a competency regarding the psychological and legal impacts of solitary confinement.

Solitary confinement in the United States typically means isolating a prisoner in a cell for 22 to 24 hours a day without any meaningful human contact or interaction. As of 2013, there were more than 12,000 federal inmates in solitary, and estimates of the national total exceed 80,000.[1] It was first used in the United States in the early 18th century but was abandoned, only to be reborn in the "law and order" era of the 1980s, when rehabilitation ceased to be an active goal of corrections.

Psychiatrists, psychologists, and criminologists have studied the effect of such isolation on mental health. Criminal justice advocates and the courts are now considering whether such treatment amounts to "cruel and unusual" punishment in violation of the 8th Amendment.

Numerous studies have found that isolation and sensory deprivation causes drastic reactions such as hallucinations, confusion, lethargy, anxiety, panic, time distortions, impaired memory, and psychotic behavior.[2] All of the major studies have found that solitary confinement produces a higher rate of psychiatric and psychological health problems than "normal" imprisonment.[3] There is general agreement among many of those who have studied solitary confinement that this mode of imprisonment can produce severe effects.[4]

The United States Supreme Court first discussed the issue of solitary confinement as early as 1890, in a case concerning solitary confinement of a prisoner under sentence of death in the state of Colorado.[5] The court ruled that solitary confinement "was an additional punishment of the most important and painful character" and described how inmates had reacted to solitary confinement in United States 19th-century prisons: "A considerable number of prisoners fell, after even a short confinement, into a semi fatuous condition, from which it was next to impossible to arouse them, and others became violently insane; others still, committed suicide; while those who stood the ordeal better were not generally reformed, and in most cases did not recover sufficient mental activity to be of any subsequent service to the community."

Notwithstanding this early condemnation, virtually every United States court has found that solitary confinement does not constitute "cruel and unusual punishment."[6] The Supreme Court has held that the 8th Amendment may be violated by the conditions of incarceration if they are imposed with "deliberate indifference" or if the conditions violate "the minimal civilized measure of life's necessities."[7,8] Both elements must be present to establish a constitutional violation but "deliberateness" has been somewhat ameliorated.[9]

The legal question is whether the demonstrated psychological harm of solitary confinement represents the denial of a minimal life necessity. The Court has not directly addressed this question but has indicated that deprivation of adequate mental health care violates a duty to provide "basic sustenance" to prisoners.[10] It has not addressed whether the recklessness associated with the imposition of solitary confinement would amount to deliberate indifference.

Reference(s):

1. U.S. Gov't Accountability Office, GAO-13-429. *Bureau of Prisons: Improvements Needed in Bureau of Prisons' Monitoring and Evaluation of Impact of Segregated Housing*. 14 (2013), available at http://www.bjs.gov/content/pub/pdf/csfcf05.pdf, archived at http://perma.cc/Q9L9-E73B; and see Sadie Dingfelder, Psychologist Testifies on the Risks of Solitary Confinement. *Monitor on Psychol.* Oct. 2012, at 10, available at http://www.apa.org/monitor/2012/10/solitary.aspx (testimony of Professor Craig Haney).
2. Zuckerman et al. 1962; Brownfield 1965; Schultz 1965; Vernon 1965; Rasmussen 1973; Zubek 1973; Andersen 1992; Haney and Lynch 1997.
3. Peter Scharff Smith. The Effects of Solitary Confinement on Prison Inmates: A Brief History and Review of the Literature. 34. *Crime & Just.* 441 (2006).
4. See, e.g., Gray 1847; Hinkle and Wolff 1956; Koch 1982; Grassian 1983; Haney and Lynch 1997; Gamman 2001; Smith 2004.
5. In re Medley, 134 U.S. 160 [1890]; Boston 2000, p. 1.
6. Hafemeister, T. and George, J. The Ninth Circle of Hell: An Eighth Amendment Analysis of Imposing Prolonged Supermax Solitary Confinement on Inmates with a Mental Illness. 90 Denver U. L. R. 1 (2012).
7. *Estelle v. Gamble*. 429 U.S. 97 (1976).
8. *Rhodes v. Chapman*. 452 U.S. 337 (1981).
9. *Wilson v. Seiter*. 501 U.S. 294 (1991); *Helling v. McKinney*. 509 U.S. 25 (1993); *Farmer v. Brennan.* 511 U.S. 825 (1994).
10. *Brown v. Plata.* 131 S. Ct. 1910 (2011).

Solitary Confinement, Cruel and Unusual, 8th Amendment

F31 The Albertani Case: Neuroscience and Criminal Trial in Italy

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After attending this presentation, attendees will better understand the scientific and judicial issues that have characterized the criminal trial held in Italy against Stefania Albertani, accused of the attempted murder of her parents and of murdering of her sister, Mariarosa Albertani. This presentation will explain how neuroscience — the set of scientifically conducted studies on the nervous system — can be used in the Italian trial system, with particular focus on the forensic use of the anatomic-genetic-clinical method and the possibility of cross-referencing heterogeneous clinical data.

This presentation will impact the forensic science community by highlighting the need to improve forensic science performance by using knowledge related to the brain, including behavioral genetics. The uniqueness lies in the utilization and enhancement of the anatomic-clinical method and the possible relationship between genetic alterations and legally relevant behavior — the study of the functioning of the normal and pathological mind through the correlation of data of a biological nature and data of a strictly psychological or behavioral nature. This is particularly relevant in the case as the trial judge, the gatekeeper of the judicial system, is faced with controversial conclusions from the defendant and the prosecution, which may affect his decision.

The Case: Stefania Albertani was accused of several crimes, including the murder of her sister, Mariarosa Albertani, (occurring between May13-14, 2009) and the attempted murder of both of her parents.

From information acquired during the investigation, it emerged that Stefania Albertani, after having caused the financial disruption of the family business, killed her older sister, Mariarosa, after isolating her in the home and forcing her to take drugs for psychosis in doses such as to cause her death. She then set fire to the body.

Soon after the sister's disappearance, Stefania was arrested for an attack on her mother, during which she tried to strangle her mother with a belt. A complex criminal pattern emerged, and the accused was interrogated regarding the kidnapping and murder of her sister as well as suppression and destruction of the corpse and the attempted homicide of her parents.

The Capacity to Understand Right From Wrong: In January 2010, the defense argued that Stefania Albertani had acted under the influence of a psychotic pathological condition that had made her totally incapable of understanding right from wrong. In June 2010, the consultant of the Judge of Preliminary Investigations concluded that histrionic disturbances of personality and dissociative disturbances could not, in any way, affect the state of consciousness and the thoughts of the detainee, who at that time had to be considered a person capable of understanding right from wrong.

In September 2010, the defense obtained permission to complete the psychiatric investigations with additional tests. Newer and more thorough psychological assessments were arranged, including psychodiagnostic tests, neuropsychological examinations, cognitive neuroscientific tests, and behavioral genetics investigations. It was claimed that Stefania Albertani was partially capable of understanding right from wrong. The judge agreed with this appraisal and sentenced Stefania to 20 years of imprisonment, recognizing the presence of "alterations" in an "area of the brain that has the function" of regulating "aggressive actions" and, from a genetic point of view, factors "significantly associated with a higher risk of impulsive, aggressive, and violent behavior." In addition to traditional psychiatric examinations, this decision was supported by more in-depth neuroscientific analyzation that revealed the brain morphology and the inherited genetics of the accused.

This is, therefore, the first recognition in Italy, and among the first in the world, of the validity of neuroscience for the assessment of imputability.

Neuroscience, Criminal Trial, Murder

F32 Improving Forensic Competency Evaluations Through Better Participation by Defense Counsel

Paul R. Spyhalski, JD, 211 2nd Street, NW, Austin, MN 55912*

After attending this presentation, attendees will better understand what information should be provided to forensic competency examiners.

This presentation will impact the forensic science community by encouraging all parties to criminal proceedings, but particularly criminal defense counsel, to follow through with a decision to seek an evaluation of the client to properly inform the examiner of the issues resulting in the referral. This presentation will urge criminal defense counsel to provide referring information, as well as other third-party information, to the examiner to assist in the determination of whether the client may constitutionally be tried in criminal court.

"It has long been accepted that a person whose mental condition is such that he lacks the capacity to understand the nature and object of the proceedings against him, to consult with counsel, and to assist in preparing his defense may not be subjected to trial."[1] The United States Supreme Court has "repeatedly and consistently recognized" that "the criminal trial of an incompetent defendant violates *due process.*"[2]

This presentation explores the critical connection between the fields of psychology and the law in determining who may be constitutionally subjected to the criminal processes of the state. Given this link between psychology and the law, forensic examiners expect information from the referring source, and defense attorneys in particular, concerning the nature, extent, and quality of the attorney-client interactions in helping the examiner determine the contours of the evaluation.

Indications from officials at the Minnesota State Operated System (MNSOS) indicate that defense counsel failed to meet the expectations of the examiner in this regard. In particular, defense counsel failed to respond to even the most basic inquiry from MNSOS asking why the client was being referred for examination in roughly 75% of the cases referred to MNSOS for examination.

This failure to provide even the most basic referring information, let alone the failure to provide other additional important collateral information, such as prior treatment records and/or evaluations, contributes to the potential failure of the evaluator to address the issue that resulted in the referral in the first instance. This failure by defense counsel to respond potentially impacts the due process rights of clients to not be tried for a criminal charge if unable to competently make the following decisions required of a defendant during the course of trial: (1) whether to waive the right to remain silent and testify; (2) whether to waive a jury trial; (3) whether to waive confrontation or cross-examination of witnesses; (4) whether and how to put in a defense; and, (5) whether to raise an affirmative defense.

Defense counsel, in expressing a concern about his or her client's competency to stand trial by seeking a referral for examination, should be prepared, at a minimum, to provide information about the quality of the attorney-client relationship in order for the examiner to frame the issues for their own evaluation, report, and eventual testimony, if necessary. Competent defense counsel should also be prepared to assist in gathering records of prior treatment and evaluations to better inform the examiner for this evaluation of the client.

Reference(s):

1. *Drope v. Missouri.* 420 U.S. 162, 171 (1974).
2. *Cooper v. Arizona.* 517 U.S. 348, 354 (1996).

Competency, Capacity, Psychology

F33 Death in the Line of Duty: A New York Police Department (NYPD) Officer Gunned Down During a Botched Robbery

Kerry J. O'Connell, JD, The NY County District Attorney's Office, One Hogan Place, New York, NY 10013*

The goal of this presentation is to illustrate the anatomy of a major case in which NYPD Officer Anthony Sanchez was murdered while responding to a burglary/robbery in progress.

This presentation will impact the forensic science community by discussing forensic techniques used in 1997 and whether those techniques possess the same scientific validity in 2017.

After midnight on May 19, 1997, Scott Schneiderman shot and killed uniformed Police Officer Anthony Sanchez as Schneiderman fled from a botched attempt to rob his own father. He had entered his father's penthouse apartment that night, wearing a mask and armed with a gun, and had terrorized his father and his father's fiancé in an effort to steal the substantial sums of cash that the defendant knew his father kept in his apartment safe. When police officers flooded into the building in response to the 911 call, Schneiderman abandoned his robbery effort and fled into the building's stairwell.

Officer Sanchez was to wait at the front of the building while his partner went upstairs with two other officers. Instead, when another resident answered the numerous buzzers for initial entry, Officer Sanchez entered the building, went to the fourth floor and, alone, started up the fire stairs toward the roof. Schneiderman ambushed Officer Sanchez, who was climbing the stairs in an effort to gain access to the penthouse. Schneiderman heard Sanchez call out, "police," then waited silently above the tenth-floor landing for the officer to come near him. When Sanchez had passed the ninth-floor landing, Schneiderman fired his gun directly down, six or seven times in rapid succession, striking the officer in the back of the neck with one of those shots. Bleeding and coughing up blood, Sanchez retreated down the steps with Schneiderman following after him. Between the seventh- and eighth-floor landings, the officer paused and fired four shots upward toward Schneiderman, hitting the defendant on his side and arm. The officer then continued down the stairs to the second-floor landing, where he collapsed and died.

Meanwhile, multiple 911 callers reported a robbery in progress, and the shots fired by both Schneiderman and Officer Sanchez were captured on some of the recordings. These would prove critical in proving who shot first. Human error in recounting events occurred, yet forensic analysis of the evidence helped to counteract its effect.

Schneiderman was captured hiding in the elevator when the building was searched. He had left a trail of physical evidence along the way, and crime scene detectives documented bullet trajectory, blood spatter, and other evidence in order to reconstruct the scene. Schneiderman claimed self-defense and said that the slain officer fired at him first. He spent more than four hours on the witness stand trying to convince the jury that he was the victim of over-aggressive policing. The evidence disproved that theory by using a combination of forensic science and eyewitness testimony in one of the most gripping trials in New York City history.

Homicide, Crime Scene Investigation, Police Officer Murder

F34 The Dog Alerts But There's No Body: The Science of Human Remains Detection — K-9 Evidence for the Courtroom

Mary E. Cablk, PhD, Desert Research Institute, 2215 Raggio Parkway, Reno, NV 89512*

The goal of this presentation is to educate attendees concerning the current state of cadaver canine detection science and the elements of a sound, defensible K-9 program.

This presentation will impact the forensic science community by informing attendees regarding the current state of science of human remains detection canines that pertains to the reliability and defensibility of their performance.

Cadaver dogs, referred to by a variety of different names, are trained to locate human remains. Lacking a single, unified standard, the reliability of cadaver dog teams varies widely. Properly trained, they are able to detect human remains that may be verified using physical processing (e.g., blood), but analytical tests for all types of human remains do not exist (e.g., human decomposition). Thus, the validity of an alert by a cadaver dog in which no means of corroboration or validation is possible can and has come into question in a court of law.

Cadaver dogs have been used with success for decades, and their use, particularly in high-profile criminal cases, is increasingly reported in popular media. False positives are rarely reported and on deployment, alerts cannot always be resolved. While the scientific literature on cadaver dog capabilities is sparse, their utility remains robust. Peer-reviewed studies point to a discrete set of compounds detectable from human remains, particularly intact decedents; however, those results represent only what the collection and analysis methods can detect, as interpreted by an analyst. No study has linked machine-detected human remains odor to what a canine recognizes as human remains odor.

Knowing the precise chemical composition of human remains odors is not necessary for a detector dog to be reliable, accurate, and precise. Furthermore, what constitutes "reliable" varies in the legal realm and particularly as applied to narcotics K-9s and probable cause. Cadaver dogs are not typically used for probable cause because their trained target odors may be present for non-nefarious reasons (e.g., a bloody nose). In fact, evidence identified by a cadaver dog and corroborated as correct may not have relevance to a case, although the dog's identification of human remains was correct and independently verified.

When a cadaver dog alerts and that alert is unsubstantiated, for any number of reasons, the validity of the alert can be called into question. Published studies have demonstrated the sensitivity and specificity of dogs to detect residual odors, including human remains. Just as with any other technology, not all dogs are trained to the same level, using appropriate procedures to eliminate bias, or to the same range of types of human remains.

This presentation will include discussion of the types of cadaver dogs and their appropriate uses in criminal cases. The current state of science on cadaver dogs will be presented in addition to more broadly relevant science that applies to any detection canine team and program. Key components challenged in court will include residual odor, bias, and training protocols for teams that deploy for criminal cases.

Canine, Detection, Odor

F35 The Need for Transparency in Forensic Report Writing

Sheila Willis, PhD, Forensic Science Ireland, Garda HQ, Phoenix Park, Dublin, IRELAND*

After attending this presentation, attendees will understand the issues that need to be addressed by the forensic scientist and the areas that need to be explicit to ensure transparency in reporting. Attendees will better understand the difference between source and activity propositions.

This presentation will impact the forensic science community by providing a better understanding of the weight of evidence in forensic reports and how the alternative affects this value.

The reporting of forensic science findings is vital to ensure there is some shared understanding of their significance. This is particularly so when the findings need to be shared with a non-scientific audience. Modern forensic science is being redefined as the results from a testing facility. This is done in the erroneous belief that as long as the frequency of occurrence is known with accuracy and tests are capable of being repeated, all is well. There are some instances in which this is the case. There is no reason why the trigger pressure of a gun, the quantity of the active ingredient in a drug, or the level of alcohol in a sample should have any less or different criteria for reporting than many other analytical results from various testing laboratories.

Even so, the situation changes when two propositions need to be addressed, as is often the case in forensic science. Before beginning the testing, additional information is needed in relation to the discriminating power of the tests to be used and the frequency of occurrence of the characteristics to be measured. When probed further, there is a need to establish what questions need to be addressed and following Cook et al., propositions to be addressed can fall at various places in the hierarchy of propositions — subsource, source, activity, or offense.[1] Clearly, the offense level is the realm of the trier of fact, but the forensic scientist can, on occasion, address the probability of findings in activity level propositions; thus, both add value to the court and increase transparency by being explicit in what is being considered.

A forensic scientist may report matching refractive indices and elemental composition for two glass fragments and be influenced in answering questions in court on their significance based on the number of recovered fragments. Thus, two situations with the same apparent forensic findings could get different significance attached following questions in court. This is caused by the scientist reporting the test results but taking transfer and persistence issues into consideration without making these factors explicit.

The European Network of Forensic Science Institutes (ENFSI) produced guidelines to deal with evaluative reporting that advocate the need for a different approach over factual reporting of test results.[2,3] This presentation promotes this approach, which is based on earlier work by Evett et al. and standards produced by the Association of Forensic Science Providers.[4-6]

The approach calls for the scientist to ascertain what the issues are in the case, decide what propositions can be addressed, and conduct a precise assessment on the likely findings, particularly when addressing activity propositions. This approach recognizes that the findings are context-dependent and these factors also need to be made explicit in reports.

The presentation will highlight the critical role played by the alternative proposition on the significance of the findings and on the need for findings to be evaluated in a context.

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Transparency, Activity, Propositions

F36 A Tale of Two Futures

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This goal of this presentation is to encourage laboratory-based criminalists, laboratory administrators, attorneys, and other stakeholders to reflect on the direction of the future development and utilization of physical traces from crime scenes.

This presentation will impact the forensic science community by having the potential to enhance the contributions and value of physical evidence in the future.

It will be the best of times, it will be the worst of times …. The fallout from the 2009 National Academy of Sciences (NAS) Report, along with the effects of both accurate and exaggerated media reports of laboratory "failures" and increased awareness of wrongful convictions, have all but cast a shadow on criminalistics. Is there hope for transformation — a chance for criminalistics to emerge renewed and reestablished as an essential and integral part of the criminal justice system? While many attempts have been made to address issues raised by the NAS Report and improve criminalistics, many unresolved questions remain. In addition, there are problems that were not recognized or cited in the NAS study. This presentation considers issues with physical evidence driven investigations. Specifically, this presentation seeks to draw attention to the shortcomings and hazards of certain current trends in criminalistics and contrast them with the untapped potential that exists.

Will the recognition of relevant physical traces at a crime scene still be hampered by being dependent on the limited knowledge and insights of non-scientist crime scene investigators or other non-scientist personnel with relatively short-term assignments? Will laboratory scientists still be relegated to reactive technician roles and become further removed from the formulation of scientific questions to be addressed regarding the physical evidence? Will trace evidence capabilities continue to be eliminated in forensic science laboratories? Will highly "intelligent" computerized instruments serve in place of experienced scientific human minds in developing the structure of the physical evidence investigation to follow at the initial stages of the overall investigation?

OR …

Will the typical, large forensic science service finally be seen as something more than an overburdened testing facility contending with backlogs of poorly selected items taken from poorly investigated and triaged crime scenes? Will the entire physical evidence continuum be recognized as the responsibility of scientists? Will the crime scene be conceptualized as a challenging scientific problem as difficult and as important as any work performed on evidence items in the laboratory? Will experienced senior scientists at long last be able to proactively apply their physical evidence expertise at the outset of investigations? Will laboratory scientists be able to expand beyond their constrained *de facto* roles as reactive technicians to work on defining and solving complex physical evidence problems?

These questions raise additional questions, such as: What role does the criminal justice system envisage for forensic science and forensic scientists? How can the criminal justice system help ensure it gets what it needs out of forensic science? How can the criminal justice system help ensure that forensic science develops in a more useful direction?

Can criminalists choose a future? Which future of these two starkly different futures do criminalists want? How many understand the problem? Which future do lawyers and other non-scientist members of the criminal justice system want? Are scientists and lawyers in agreement? What can be done to bring them into agreement? How badly do all relevant parties want change? What forces have served to maintain the status quo? What can be done to increase the level of scientific contributions across the physical evidence continuum? How can it be done? Who can get it done? What positive role can members of the legal profession play in bringing about needed change?

Physical Evidence Recognition, Crime Scene, Laboratory Analysis

G1 Migratory Flows and Unaccompanied Minors: The Age Assessment Protocol of the University of Turin (Italy)

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After attending this presentation, attendees will learn the multidisciplinary age assessment protocol proposed by the Medicolegal Department of the University of Turin (Italy) to allow urgent age assessment in circumstances in which law enforcement officers detain individuals of indeterminate age.

This presentation will impact the forensic science community by confirming the importance of dental age methods in the diagnostic procedures targeted at age estimation of unaccompanied minors.

The Italian legislation is paying special attention to the protection of unaccompanied minors. Once their age as a minor is ascertained, unaccompanied minors cannot be subject to expulsion and acquire the right to a residence permit, which can be converted at the age of 18 into a permit for study, employment or self-employment, or health reasons. The growing phenomenon of migratory flows extends beyond anti-terrorism and border control aspects, resulting in the necessity of ascertaining the age of a large number of unaccompanied minors who do not carry valid documents that certify their age. The Forensic Medicine Unit of the University of Turin (Italy) has signed a memorandum of understanding with the public prosecutor's office at the Juvenile Court of Turin to allow urgent age assessment in circumstances in which law enforcement officers detain individuals whose majority or minority is unknown.

The goal of this research is to compare the forensic odontological methods developed by Olze, AlQahtani, and Cameriere against the overall multidisciplinary evaluation of the alleged minor age of unaccompanied foreign minors to determine the forensic significance of dental methods, including aspects such as digital transmission of panoramic radiographs.

Materials and Methods: The protocol applied for age assessment and majority prediction requires the multidisciplinary adoption of several methods according to the following scheme: preliminary medicolegal and radiological assessment of the wrist; possible dental, pediatric-auxologic, and psychological evaluation, following a diagnostic algorithm based on criteria of progressive invasiveness.

A review was performed on the medical records of age assessment visits conducted over a sample of 68 foreigners without a valid birth certificate or ID documentation. The group was also kept under observation for the period of February to June, 2017, and the full technical protocol of dental examination and panoramic radiographs was performed. The results obtained from the entire multidisciplinary diagnostic process were compared with the age assessment methods performed by applying the Olze, AlQahtani, and Cameriere methods individually through Orthopantomograph (OPG) radiographs transmitted remotely for evaluation by the relevant forensic odontologist consultant.

Results: For ten subjects, the match between the alleged and declared age and the ascertained age could be confirmed. Of the 68 subjects, 45 proved to be of age according to the medical records released by the medical examiner following protocol (physical examination, left wrist radiographs using the FELS and Greulich and Pyle methods, oral cavity inspection, and OPG evaluation according to the AlQahtani and Olze methods), whereas 17 proved to be less than 18 years of age and more than 14 years of age.

The dental age estimation against OPG of the lower third molars according to the Olze, AlQahtani, and Cameriere methods exhibited a match with the estimated age through the multidisciplinary procedure with a P-value lower than 0.05.

Conclusions: This sample highlighted the widespread tendency of immigrants to declare an age lower than their actual age, resulting in the legal requirement of attaining majority estimation through a reliable multidisciplinary protocol from an ethical and technical point of view. It is no longer suitable to entrust this majority/minority estimation only to individual assessment methods. It is also essential to integrate the diagnostic process with the evaluation of the dental maturity and eruption of the entire set of teeth and the systematic execution of OPGs submitted to at least two forensic odontological methods for evaluation, including digital transmission of radiographs to the relevant forensic odontologist consultant. Considering the growing number of self-styled foreign unaccompanied minors, this study believes that the protocol employed could represent a valid model that can be deployed across the country, due to its reliability, cost-effectiveness, and timeliness.

Age Assessment, Unaccompanied Minors, Migrants

G2 Measuring Root Transparency for Age-at-Death Estimation: What About the Light?

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After attending this presentation, attendees will acquire a deeper understanding of how to apply root transparency measurements when implementing age-at-death estimation methods.

This presentation will impact the forensic science community by describing the illumination conditions required for measuring root transparency to achieve an accurate age-at-death estimation.

Age-at-death estimation is one of the main goals in forensic identification since age estimation significantly narrows the search possibilities in cases involving missing persons and unidentified bodies. Moreover, age segregation in cases of mass disaster and multiple victims' situations can be of great help in the process of identification. The study of dental tissues has long been considered a proper tool for age estimation; therefore, several age estimation methods involving dental studies have been developed and widely used. Dental age estimation methods can be divided into three criteria: tooth formation and development, post-formation changes, and histological changes. Tooth formation and growth changes are applied for fetal and infant age estimation; however, at the end of dental and skeletal growth, methods addressing post-formation or biochemical changes should be applied. Lamendin et al. developed an adult age estimation method based on root transparency and periodontal recession.[1] The regression formula demonstrated its accuracy for age estimation on individuals between 40 and 70 years of age. Later, Prince and Ubelaker evaluated the effects of ancestry and sex, and incorporated root height into the equation, developing four new regression formulas for males and females of African ancestry and males and females of Caucasian ancestry.[2] Even though root transparency is a key element in the method, the conditions for measuring this element have not yet been established. The goal of the present study is to describe the illumination conditions measured in lumens that result in increased accuracy when applying the Lamendin et al method as modified by Prince and Ubelaker.[2]

Fifty-five single-rooted teeth clinically extracted from known age and sex individuals (19 upper incisors, 11 males and 9 females; 36 upper and lower canines, 19 males and 17 females) were used for this study. Two observers measured root height, periodontosis, and root transparency by caliper from the labial surface, and recorded the measurement in milimeters. Root transparency was measured applying three different lights: 6,500lux, 3,000lux, and 1,600lux. All measurements were repeated on two different days. After taking the measurements, Lamendin and Prince and Ubelaker formulas (modified from Lamendin's method) were employed to estimate the age of the individual. Comparison with actual age was conducted by statistical analysis. There were no significant differences found between age estimation using 1,600lux illumination and the actual age (p=0.872). In contrast, age estimates using the other illumination levels revealed significant differences with respect to the actual age ($p < 0.05$).

Although previous studies analyzed the use of Lamendin parameters involving creation of new formulas for age-at-death estimation based on population differences (Prince & Ubelaker, 2000; Ubelaker and Parra, 2008; González- Colmenares et al. 2007), none of these studies or other studies focused on setting up the correct conditions for the measurement of root transparency.[1-4] As a result, the present work is the first one recorded in the literature to address this issue. This study depicts how illumination conditions may affect root transparency measurement and, therefore, the age estimation methods that rely on this postformation change. These results pointed out that light conditions must be taken into account for correct determination of this parameter. Therefore, this is a pioneer project in this area, which hopefully promotes further studies regarding correct conditions for age-at-death estimation.

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Age-at-Death, Root Transparency, Human Identification

G3 Implications of Canine Width, Inter-Canine Distance, and Facial Dimensions in Forensic Identification

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After attending this presentation, attendees will understand the significance of canine width and inter-canine distance in forensic examinations, especially with reference to sexual dimorphism and two-dimensional facial reconstructions.

This presentation will impact the forensic science community by presenting the utility of important parameters in forensic odontology (i.e., canine width and inter-canine distance) in sexing of the human remains and in assessment of facial dimensions that are likely to assist in two-dimensional facial reconstructions.

Teeth are the most durable and hardest part of the human body. Consequently, dental evidence is recovered most often from mass disaster sites and crime scenes. Dental evidence is considered as valuable substantiation in the process of identification of human remains. In some terrorists' attacks, high-explosion blasts, and high-impact natural disasters, the dental and facial parts are available to Disaster Victim Identification (DVI) teams for identification purposes. In such situations, the dental evidence acts as sole and, thus, important evidence. A few studies are available on canine width, inter-canine distance, and their relationship with some facial measurements. Canine and inter-canine distance may be helpful in the prediction of the remains' sex. Studying the relationship of the inter-canine distance in addition to certain facial dimensions may be helpful in two-dimensional facial reconstructions in forensic identification. Therefore, the main objectives of the present study were to determine the sex differences with respect to canine width and inter-canine distance and to ascertain whether inter-canine distance may be used to estimate certain facial dimensions.

This study was conducted on 240 adult participants (120 males and 120 females) belonging to Himachal Pradesh State of North India. In addition to the canine width and inter-canine distance, the facial dimensions, such as bizygomatic width, physiognomic facial height, morphological facial height, bigonial distance and intercommissural distance, inter canthal distance, outer canthal distance, and tragus-to-wall distance, were measured with standard anthropometric instruments. The descriptive statistics were performed and the Karl Pearson's correlation coefficient was calculated to study the correlation between the measurements. Sex differences in the measurements were statistically analyzed. The mean canine width (mesio-distal crown width) was found to be greater in males than females on both the right and left sides. The sex differences were statistically significant. Similarly, the inter-canine distance was found to significantly larger in males than females in both maxillary and mandibular arches. Significant correlation was observed between maxillary inter-canine distance and intercommissural distance in both sexes; however, the correlation between mandibular inter-canine distance with physiognomic height and intercommissural distance was found to be significant only in females. In males, the correlation between inter-canine distance and facial measurements was not found to be significant. Prediction equations were calculated between inter-canine distance and facial measurements using regression analysis. The regression models were derived to predict facial measurements.

This study confirms the sex-discriminating potential of the dental measurements and concludes that certain facial dimensions can be estimated from the inter-canine distance, thus making it a useful tool in situations in which facial dimensions are not available for facial reconstruction.

Forensic Odontology, Personal Identification, Dental Evidence

G4 Dental Exclusion: What's Next? The Role of the Odontologist As Part of the Identification Team

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The goal of this presentation is to emphasize that dental identification is one part of the human identification team — even in single fatalities — and to advise attendees that even in cases of exclusion, the odontologist has a role to play in the identification process.

This presentation will impact the forensic science community by explaining that dental exclusions may occur for any number of reasons, including true exclusions, inaccurate antemortem dental information, wrong antemortem chart information provided to the odontologist, or even purposeful deceit. If an exclusion occurs, the role of the odontologist is not over. The odontologist must coordinate with the identification coordinator to facilitate identification by other means, if this is possible.

A man went missing in December of 2016. He borrowed a friend's vehicle for urgent business and was not seen alive again. The vehicle was found in May of 2017 in a farmer's field. The deceased was in the car in the front passenger seat, positioned facedown in the footwell with his legs extending into the backseat. The deceased was partially decomposed and partly mummified. Homicide was suspected. His hands were slightly mummified. His dentition was intact.

The purpose of autopsy in this case was to determine the means, manner, and cause of death — and identification. In the Province of Ontario, most identifications are performed by visual methods by family members or by circumstantial information. Scientific identification methods are preferred and include: dental comparison, fingerprint comparison, DNA, and medical/surgical devices. In all of the scientific methods, antemortem information is compared to postmortem data. In scientific identifications, a single identification method is most often used. In rare cases, multiple scientific comparison methods are performed for the same case.

What happens, then, when one scientific method contradicts another? Is there a mechanism for dispute resolution and how is this handled? This study presents a case in which comparison of antemortem and postmortem records resulted in an exclusion; however, the body was concurrently identified using fingerprint comparison and released to the family After release, the forensic odontologist reported the possibility of exclusion to the identification coordinator.

At the time of dental exclusion, a Friday before a long holiday weekend and after the body had been released, there was still debate as to whether the body was properly identified or not. With that in mind, the odontologist decided to request that a number of stored blood samples that had been gathered for toxicology be set aside for DNA comparison. This was accomplished through communication with the identification coordinator.

Subsequently, DNA identification confirmed that the fingerprint identification was correct. The possible reasons for a dental exclusion will be discussed.

Identification, Exclusion, Identification Coordinator

G5 Multimodality Multidisciplinary Dental Identification in a High Stakes Case of Prior Misidentification

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The goal of this presentation is to illustrate the use of antemortem chart information, conventional radiographic examination, computer-assisted radiographic examination, alternate light photography, and dissecting microscopy used in a single case to finalize identification.

This presentation will impact the forensic science community by demonstrating that most dental identifications are relatively straightforward using comparison of antemortem to postmortem dental records. On some occasions, additional technology needs to be harnessed. This presentation details just such a case.

The identification of all persons is important as part of the autopsy process; however, there are instances in which identification of the deceased is the most important part of the autopsy procedure.

The Syrian conflict began in 2011 and continues to this day. Over one-quarter of a million people have died, and millions have been displaced. The combatants include persons of many nations fighting in numerous factions, including “terrorists” and foreign nationals fighting on various sides.

The body of a young male was traded from ISIS in exchange for an unknown reason. The deceased died at an unknown time, but had gone missing many months before recovery. At the time of recovery, the body was said to have been both autopsied and identified by dental record comparison in Iraq. When the body was returned to Canada, no autopsy had been performed and there was a reasonable concern that the returned body was not the deceased Canadian national.

For that reason, an autopsy was undertaken and a dental identification was requested. The identification was complicated by anatomic issues, continuity of dental evidence issues, and an apparent paucity of congruence between the antemortem information. Considering this, it was decided to return to basic comparison principles and maximize the postmortem information to compare to the records obtained in Canada. This process resulted in postmortem evidence that included Computed Tomography (CT) images, reformatted CT images, conventional dental clinical findings, alternate-light fluorescence photography, and full intraoral radiographs. Additionally, because some of the restorations were so conservative as to be nearly invisible, a dissecting microscope was used and highly refined photographs made to complete the postmortem evidence package. All of the postmortem information was compared to the properly sourced antemortem material, and an identification was finalized. The deceased was returned to his Canadian family properly identified.

Dental Identification, Alternate Light Photography, Computer-Assisted Tomography

G6 The Dental Identification After an Air Disaster 45 Years Ago: The Dubai Accident in 1972 With 112 Victims

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After attending this presentation, attendees will better understand how forensic odontologists worked after a disaster that occurred 45 years ago. In addition, attendees will be familiar with the problems encountered and understand that not all concepts being employed today are new.

This presentation will impact the forensic science community by demonstrating that there is something to learn from old cases that were solved under rather primitive conditions — conditions that may still be encountered in poorly developed countries today.

On March 14, 1972, a Supercarvelle airplane hit a mountainous area in the Emirate of Fujairah, now part of the United Arab Emirates. The plane was from a Danish charter company, Tjæreborg, and carried Scandinavian tourists from Sri Lanka (at that time Ceylon) to Copenhagen. On board were 106 passengers and a crew of 6 persons. All died. The plane struck the upper part of a rugged mountain and went over the top; wreckage and bodies were dispersed over a 300m by 400m area on the back side of the mountaintop. All bodies suffered severe trauma, some after a fall of several hundred meters, and for some, only small fragments were found.

Ad hoc ID teams were sent from Denmark, Sweden, and Norway. A dentist and a police officer from Norway were included, while the Swedish and Danish teams also included forensic pathologists. Only 14 of the victims were Norwegian citizens.

On March 17, the Norwegian and Swedish teams arrived in Dubai, while the Danish team had arrived the day before. As the Danish team had two dentists, there were four dentists in total.

The teams, including the dentists, went to the accident site, registered, and packed up the bodies. The victims were then transported by helicopter to an old British air base at the Emirate of Sharjah. Here, autopsies could be performed outdoors while the bodies were kept in a refrigerated room. Without computers or the internet, the work in Sharjah was completed on March 28 after ten days. The bodies were returned to Denmark, where a few more bodies were identified. The identification work was terminated on April 24, and a total of 96 victims were identified. The remaining 16 victims were buried in a common grave in Denmark. All Norwegian victims were identified, due in part to antemortem information accompanying the forensic team that went to Dubai. The two other teams did not have that advantage and had to establish a home commission to take care of the antemortem material. All information was set up in systematic way. In addition, an enthusiastic police officer's keen interest contributed to this result. Forty-one victims were identified, including a dental comparison, and 28 by dental comparison alone.

With no Interpol form, a newly designed Danish form was used. With no transportable X-ray machine, an iodine-isotope radiation source, which the Swedish dentist brought with him, was used for exposing the radiographs. Searching through this large amount of data was difficult. It was initially decided to look for characteristic restorations; however, often those registered antemortem were lost postmortem, and those found postmortem were not registered antemortem. One full day was lost without a single identification being made by this approach. Then, the postmortem forms were laid out on a long table and teams compared antemortem forms from postmortem form to postmortem form. Separating the men from the women was attempted, but was often impossible due to the severe destruction of the bodies. Considering all these conditions, the teams succeeded well.

Air Disaster, History, Identifications

G7 OdontoSearch: Modifications, Updates, and Proper Usage

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After attending this presentation, attendees will better understand using the OdontoSearch program for dental identification.

This presentation will impact the forensic science community by providing an overview of recent modifications of OdontoSearch and will include case examples highlighting proper usage of the program.

It has been two years since the enhanced web version of OdontoSearch was formally introduced to the forensic community at the 68th Annual AAFS Scientific Meeting in Las Vegas, NV. Since that time, there have been significant changes to the program; this presentation will educate attendees about the recent enhancements to the OdontoSearch program. A brief tutorial will also be given regarding the proper use of the program, and informative case examples will be presented.

OdontoSearch is a computerized program which is available online (www.odontosearch.com). It is a tool used by forensic odontologists to statistically determine the frequency that specific dental patterns would be expected to occur in a population. As such, OdontoSearch provides an objective means for users to recognize whether specific dental patterns formed by combinations of missing, filled, and unrestored teeth are common or unusual. The program utilizes a large database of dental records of known individuals for comparison. Empirical comparison of an "unknown" pattern against the reference data in OdontoSearch will reveal the frequency with which this pattern would be expected in the general population. These results can be used to support dental identification in situations in which the observed matching pattern between an unidentified body and a missing person is found to be extremely rare.

The OdontoSearch reference database is constantly growing as more appropriate samples become available for research. Recently, two new sources of dental data have been obtained for incorporation into OdontoSearch. These include the dental data from the 2013-2014 National Health and Nutrition Examination Survey (NHANES), as well as a portion of the "BigMouth" data repository. OdontoSearch 3.0, released in 2015, had a sample size of 57,980 records. With the availability of the NHANES and BigMouth data, the sample size of the next release of OdontoSearch will increase substantially.

The NHANES dental data are periodically collected as part of an initiative by the Centers for Disease Control and Prevention to study dental health. Participants in the newly released 2013-2014 NHANES study represent a cross-section of the United States civilian population. There are more than 9,400 individuals represented in the latest NHANES release, a subset of which is applicable to the OdontoSearch program. Earlier iterations of the NHANES study have formed the foundation of the OdontoSearch reference data.

BigMouth is an oral health data repository available for research.[1] Currently, the BigMouth dental repository is composed of electronic chartings contributed by six United States dental schools as part of the Consortium for Oral Health Research and Informatics. The total BigMouth database holds dental information on more than one million patients. The participating dental schools have generously agreed to contribute their data to OdontoSearch, and a subset of this massive data repository has been converted for use in the next version of OdontoSearch.

Users of OdontoSearch have the ability to customize searches based on specific demographic parameters (age, gender, and ancestry) and/or specific databases, as desired. This can be useful if, for example, the frequency of a dental pattern was needed for only females between 35 and 50 years of age. In addition, language options have been added to OdontoSearch, so all pages can be accessed in Spanish.

When using OdontoSearch, it is important to perform searches only on teeth with exact matches between the antemortem or postmortem records. Explainable discrepancies (e.g., a filled tooth in an antemortem record corresponding to a missing tooth in a postmortem record) should not be included in an OdontoSearch query. Specific "dos and don'ts" of OdontoSearch usage, such as the points listed above, will be discussed, in addition to illustrative case examples.

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OdontoSearch, Dental Identification, BigMouth

G8 Atypical Dental Identifications: What to Do When Antemortem Radiographs Are Missing?

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After attending this presentation, attendees will have a better understanding of the alternative methods for aiding in dental identifications.

This presentation will impact the forensic science community by presenting cases involving innovative techniques that can be utilized by the forensic odontologist.

When a forensic odontologist is requested to identify a decedent, acceptable antemortem records, especially radiographs, must be available for comparison to the postmortem records. That, of course, is the ideal situation, but quite often circumstances presented to the forensic odontologist require ingenuity to provide the medical examiner's office with help in the identification process. Some interesting examples of cases that required such services are presented in this presentation.

A recent New York City cold case was presented for input in helping with the identification of a military serviceman. The antemortem records, dating back many years, contained only chartings and hand-written treatments; no radiographs were available. OdontoSearch was utilized and provided a statistical likelihood of the records matching, although the software cannot provide a definitive scientific identification; however, the written chart described a unique restorative feature but, unfortunately, it could not be confirmed radiographically. A discussion of why radiographs sometimes failed to image restorative features, as well as the ultimate technique utilized to isolate the hidden features, will be presented.

Following a New York City fire, the forensic odontology team was called in to confirm the identity of several minors. Following standard protocols, the children required postmortem radiographs to verify their identity. Before the process began, the team was informed of religious concerns raised by the community concerning radiographing the decedents. In addition, antemortem radiographs were not immediately accessible, and there was a request by the family for same-day burial. The techniques used to address these concerns, as well as methodology used to confirm the identities, will be discussed.

A fire in New Jersey, the consequence of a homicide/suicide, resulted in the death of a husband, his wife, and their teenage daughter. Reconciliation of the antemortem and postmortem radiographs for the husband and his teenage daughter were straightforward, leading to routine identification for the forensic odontologists. Although the 38-year-old wife had received dental care, no antemortem radiographs existed; however, her dentist could provide high-quality intraoral photographs of the anterior teeth. A technique for modifying the dental autopsy to obtain postmortem photographs of the victim's dentition, the methodology used for the comparison, as well as the pros and cons of the technique, will be discussed.

Forensic odontologists are sometimes asked by the medical examiner offices to confirm an identification despite the fact that antemortem radiographs may not be available to complete the identification process. Routine procedures followed by the forensic odontologist in many instances are not so routine and may require some creativity and innovation, as is described in these cases.

Antemortem, Postmortem, OdontoSearch

G9 The Head in Cement and the Medical Examiner

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The goal of this presentation is to illustrate how multiple forensic disciplines can work in conjunction with each other to solve a case of unidentified remains.

This presentation will impact the forensic science community by re-emphasizing the fact that all forensic departments within a single medical examiner's office can, and must, work together to bring about favorable case results.

This case study involves a skull, found partially encased in cement, on the side of a major highway by an unwitting bicyclist. The mound of cement was brought to the Office of the Suffolk County Medical Examiner (OSCME), NY, where a forensic anthropologist carefully extricated the skull from the concrete. The pathologist and the anthropologist observed evidence of blunt force trauma to the head, as well as fractures of the maxilla, mandible, and facial bones.

After removing the skull from the cement mound, a facial impression was visible on the internal surfaces of the cement mound. Another forensic anthropologist described this phenomenon as a form of a Death Mask. This artifact is created when the fatty acids of the skin form a barrier between the concrete and the head, which then leaves a facial impression when the tissues decompose. Hair was also found imbedded in the concrete and was removed for analysis.

A model of the facial impression was made by pouring a silicone material (Dragon Skin™) into the concrete mound, leaving it to set overnight, then carefully removing the cast the next day.

A forensic odontologist performed a dental examination, and radiographs were taken, but no antemortem dental records were available through the police department or the National Missing and Unidentified Persons System (NAMUS). A forensic artist examined the skull, and created a two-dimensional facial reconstruction of the individual in question. The profile created by the forensic anthropologist described a young-to-middle-aged male, with mixed racial characteristics of European (White) and Asian (including Hispanic) traits. The analysis of the hair samples by the forensic scientist revealed light brown, brown, and dark brown fragments exhibiting Caucasian (European) racial characteristics.

A tooth was used to obtain a DNA profile. The DNA profile was consistent with having originated from a male. The profile was entered into the Combined DNA Index System (CODIS). The profile matched another OSCME case from 2009, in which a torso and some fragments of extremities were found in a landfill. Those body parts were previously identified using DNA collected from the missing individual's toothbrush.

The positive ID through DNA and CODIS was of a man of Pakistani descent. His age at the time of his disappearance was 36 years of age. This case is an ongoing homicide investigation.

This case study will illustrate how multiple forensic disciplines, including pathology, anthropology, trace evidence, odontology, artistry, and DNA, all coordinated their efforts to solve a case of unidentified remains.

Identification, Forensic Collaboration, Unidentified Remains

G10 Evaluating a Selfie Identification App in the Forensic Dental Identification Process

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After attending this presentation, attendees will possess a deeper understanding of how social media and smart phone applications could become a valuable investigative tool in the search for antemortem identifying data.

This presentation will impact the forensic science community by increasing awareness concerning how images of the smile and of anterior teeth could be used in the human identification process.

There are several applications for smart phones that assist in the search for missing persons. Selfie Forensic ID App, developed in 2017 by a forensic odontologist, is the first application developed with the goal of assisting the human identification process using smile photographs archived on the internet, thus increasing the antemortem dental data that can be used in comparison to the postmortem dental data of an unidentified person. Forensic casework portrait photographs are often used in the human identification process using superimposition techniques.

The goal of this presentation is to evaluate the Selfie Forensic ID App by simulating a search of selfie images of potential missing persons taken with the app and that are available on Twitter®, Tumblr®, and Instagram™. The app can be freely downloaded in iOS® and Android™ formats. Selected patients regularly visiting the dentist in Matera and Bari, Italy, were asked to participate voluntarily in this experiment. Fifty patients were selected for their specific and potentially individualizing anterior teeth features that were observable when smiling, such as diastema, rotated or wrongly positioned teeth, fixed prosthetics, or dental crown discolorations. Each of the participants downloaded the app and took a selfie image of the lower third of the face showing the teeth. Registration with name, surname, city, and country is required in order to use the app. Alternatively, users can login via their Facebook® or Google+® profile. Once taken, the selfie images were uploaded to various social networks with the registered names. All patients selected also had a separate portrait photograph taken with a professional camera. After some hours, all selfie pictures taken via the app were available via internet image searches using the names of the persons involved, simulating a missing person search.

The app is able to increase quantity and quality of selfie images of the lower third of the face showing anterior teeth and identifying features combined with the name of the person being searching for. Dental variations and characteristics can become an aid in the comparison process of antemortem and postmortem "matches," thus confirming or excluding the identity of one or more individuals.

It is believed that this new app will promote, to the public, the importance of storing personal identification data in social media in order to avoid bodies remaining unidentified.

Selfie, Identification, Forensic Odontology

G11 Silver-Stain Modification as a Method to Enhance Visualization of Histological Features for Tooth Cementum Analysis (TCA)

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After attending this presentation, attendees will better understand how to modify the standard histology specimen preparation for tooth cementum analysis. This will significantly increase the visualization of histological features in teeth, aiding in a better accuracy of age- and season-at-death estimation, as well as enabling a better understanding of the microscopic features visible in tooth cross sections.

This presentation will impact the forensic science community by providing a detailed description of specimen preparation and staining, examples of tooth cross sections prepared with the silver-stain compared to unstained specimens, and the respective age- and season-at-death estimates for the two methods. Example pictures will enable visual comparison and identification of structures and therefore illustrate the accuracy of TCA obtained on stained and unstained cementum.

Histomorphological techniques used at the Defense POW/MIA Accounting Agency Laboratory are usually applied to distinguish inconclusive osseous remains from non-human and non-osseous remains. To enhance the traditional aging methods for identification purposes, extensive histological research on teeth of active duty military personal has been conducted to test the accuracy of TCA for age- and season-at-death assessment.[1]

It is generally still a challenge to accurately apply cementochronology, and it is even more challenging to use TCA on degraded and taphonomically altered teeth found during recoveries. Both biotic and abiotic processes can alter dental remains after death and thus affect any identification analysis. The role of TCA and its accuracy of approximately ±1-3 years to the chronological age of an individual is still being underestimated and not routinely applied due to multiple factors, such as equipment set up being complex and expensive, and the learning curve of properly preparing the specimen and analyzing the cross sections for age- and season-at-death assessment being difficult since the analyst must be familiar with cementum biology to accurately read and understand the dental histological features utilized in this method.[1-4] Silver-staining is a somewhat new modification of traditional bone histomorphological methods and is providing several benefits for species differentiation on skeletal remains.[5] Silver-staining used for TCA can enhance contrast of dark and light increments, enabling the analyst to more confidently count the cementum lines and increase the accuracy of results. Additionally, this staining method offers the opportunity to work with much thinner specimens in order to minimize optical errors influencing the accuracy of age estimation on dental cementum, such as the "doubling effect."[6-8]

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Cementochronology, Tooth Cementum Analysis, Silver-Staining

G12 Macroscopic and Microscopic Changes of Dental Tissues Exposed to Thermal Radiations: Forensic Interest in Fire Disaster Modeling

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After attending this presentation, attendees will better understand how dental tissues could help investigators in fire disaster modeling involving victims.

This presentation will impact the forensic science community by describing the findings from an experimental study conducted in association with the Section of Engineering Fire in the Central Laboratory of Police of Paris (LCPP), France. The benefit of this study lies in its novelty, as dental tissues were the first organic tools used in fire modeling. These findings could be used in fire disasters involving victims.

Human teeth reveal important features that demonstrate their ability to withstand postmortem shock caused by thermal activities. When bodies are seriously damaged by fire, the role of the forensic odontologist becomes even more essential during the identification process. This study was conducted in association with the Section of Engineering Fire in the LCPP using specific tools: the Calorimetric Cone (CC) and the Thermo Gravimetric Analyzer (TGA), which work in fire disaster investigations by using inorganic burned materials found at the fire scene.

The main goal of this study was to analyze the physico-chemical changes of the tooth when exposed to different thermal radiations. Both macroscopic (using CC) and microscopic (using TGA) changes were analyzed to define the mass-loss rate of dental tissues. This work sought to provide a new tool to help the LCPP investigators in fire disaster scenarios involving victims.

Material and Methods: This study used 33 samples of healthy teeth; 66 samples of dental tissues (enamel, dentine, and cementum) were collected from these 33 teeth.

For the microscopic study (TGA), the first study used three teeth and six samples of dental tissues to determine the repeatability and reproducibility of the TGA settings. Then, the healthy samples of dental tissues were burned and weighed from temperatures ranging from 25° to 1,000°C with a heating rate of 10°C/min.

For the macroscopic study (CC), teeth were divided into six groups of four teeth each and placed into six plates in order to reproduce the physiological environment of human teeth surrounded by alveolar bone. These plates were exposed to different thermal radiations from 5kW/m2 to 95kW/m2, corresponding to internal temperatures of 100° to 600° in the dental tissues. The exposure time for each plate was 30min, which represented the mean time of fire in Paris. Macroscopic changes of the teeth when heated were recorded using four photographies taken at T=0 ; T=10 ; T=20 ; and T=30 minutes. Then, samples of dental tissues from these burned teeth were collected, and another microscopic analysis was performed to compare the difference of the mass-loss rates between virgin and burned dental tissues.

Results: The microscopic study found that enamel does not lose mass when heated due to its strongly mineralized histological structure; however, on virgin samples, the cementum and dentin demonstrated three main reactions of mass loss: 280°C-400°C; 360°C-500°C; and 650°C-800°C, with the highest peak of mass loss found at 370°C.

For the macroscopic study, the results revealed a dislocation of the dental crown between the enamel and the underlying dentin at approximately 350°C. The external dislocation of the enamel crown could be linked to the internal mass loss of the cementum/dentin that appears at approximately 370°C.

Given the good repeatability of the experiments conducted with the Scientific Police Laboratory, the findings are promising in the fire investigation field involving victims. From now, dental tissues have a thermograph available in the LCPP with a mass-loss rate recorded according to the temperature of fire exposure. The tooth can be considered as a comparison point for further investigations in fire disaster modeling.

The human tooth is the first organic indicator of fire modeling used in the scientific police investigation and could be used in mass disaster fire cases involving victims. Moreover, the distance between the fire source and a victim would also be possible to estimate using the modelization system in the LCPP.

Fire Disaster Modeling, Forensic Odontology, Dental Tissues

G13 Mass Loss Reaction of Root Canal Materials Exposed to Thermal Radiations: Forensic Interest in Fire Disaster Modeling

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The goal of this presentation is to provide an understanding of how root canal materials could help investigators in fire disaster modeling that involve victims.

This presentation will impact the forensic science community in fire disaster modeling by submitting the outcomes found following an experimental study conducted in association with the Section of Engineering Fire in the Central Laboratory of Police of Paris (LCPP), France. The benefit of this study lies in the continuity of a previous study on dental tissues used in fire modeling. These findings could be used in fire disasters that involve victims.

In the oral cavity, dental roots are protected by alveolar bone. The endodontic material is the most preserved dental material when bodies are severely burned, such as during fire disasters or airplane crashes involving extreme thermal radiations. This study followed a previous study based on dental tissues. This work was conducted in association with the Section of Engineering Fire in the LCPP using specific tools, such as the Calorimetric Cone (CC) and the Thermo Gravimetric Analyzer (TGA).

The main goal of this study was to analyze the degradation of the gutta-percha with and without cement zinc-eugenol oxide when teeth are exposed to different thermal radiations, both macroscopic (using CC) and microscopic (using TGA), when analyzed to define the mass loss changes and thermogram of these root canal materials. This work is intended to provide further organic tools to assist LCPP investigators in fire scenario modeling involving victims.

Material and Methods: Twenty-seven teeth were divided into three groups of nine teeth each and placed into three plates to reproduce the physiological environment of human teeth surrounded by alveolar bone. For each of the plates of nine teeth, three were filled only with gutta-percha, three others with gutta-percha in combination with cement zinc-eugenol oxide, and the last three were filled only with cement zinc-eugenol oxide.

Two preliminary studies using TGA were conducted on root canal material to determine the repeatability and reproducibility of the settings from 25°C to 800°C with a heating rate of 10°C/min. Then, the three plates of nine teeth were placed under the CC with three different thermal radiations: 20 kW/m2, 35 kW/m2, and 50kW/m2. Another TGA microscopic analysis was performed on these burned samples.

Results: The results of this study demonstrate that, when burned, the gutta-percha leaves an important quantity of residues compared with other polymers. Moreover, the gutta-percha and the cement zinc-eugenol oxide revealed a mass-loss reaction between 540°C-765°C, which is the mean fire temperature in Paris.

Following the previous study on human teeth, these findings are still promising in the fire investigation field and further investigations could also be conducted on alveolar bone. During a fire disaster investigation, the LCPP use inorganic burned materials found at a fire scene to modelize the fire scenario. One milligram of dental tissues and root canal material could also be used and considered as a comparison point for the LCPP in a fire scene involving victim(s).

Fire Disaster Modeling, Forensic Odontology, Endodontic Material

G14 Establishing the Necessity for Ethnic Markers in Forensic Odontology: A Literature Review

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After attending this presentation, attendees will understand the use of radiographic markers, in addition to skeletal markers that are unique to African Americans, as a benchmark to improve the outcome for the identification and resolution of cases in which forensic odontology is applied, as well as broader forensic applications, if appropriate.

This presentation will impact the forensic science community by determining if the need exists for, or improving the efficacy of, an already-existing protocol for ethnic markers for African Americans in forensic odontology.

The subject to be evaluated is the necessity for ethnic standards and/or protocols for skeletal and radiographic markers or the combination thereof. The specific application for African Americans, as well as other ethnic groups, is an effort to systematize and improve accuracy and focus in forensic odontology.

Radiographs, as well as skeletal and odontogenic markers, in forensics have the objective of identifying subjects via dental records for criminal investigations, missing persons, and antemortem and postmortem comparisons for identification. This challenge has been primarily addressed by using radiographs.[1,2] These systems can be applied to a closed and small population, which would be ideal, for example, in a plane crash with known passengers, but not-so-ideal in a missing persons cold case in which only skeletal remains are available at best. Hence, in the case of one study, small experimental databases are encouraged.[1] In multiple forensic scenarios, dental records may or may not be available.

There are other challenges encountered when depending primarily on radiographs, including, but are not limited to, poor-quality radiographs and the length of time between antemortem and postmortem images. This does not account for developmental and/or restorative changes that may have taken place as well.[3] Some non-radiographic considerations exist, including the analysis of morphology, while others evaluate different aspects of third molars with respect to eruption and mineralization.[4,5]

Following a review of the literature on this subject, we should be able to determine if a comprehensive system exists and, if so, the efficacy of that system. The implications of this existence or non-existence have far-reaching implications in the potential for improvement of the delivery of forensic "service," as forensics is a unique combination of law enforcement, health care, and the legal system, which locally, nationally, and internationally has the potential to improve the quality of life for mankind on a multitiered level when applied with academic vigor, logistical efficiency, and social compassion and ecumenicity in service of "we the people."

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Odontology, African American, Markers

G15 Bitemarks in Wrongful Convictions in the United States

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After attending this presentation, attendees will have acquired information regarding the role that bitemarks have played in wrongful convictions in the United States judicial system in the past 40 years.

This presentation will impact the forensic science community by demonstrating the shortcomings of the judicial system, the necessity for basic education, continuing education, certification, recertification, proficiency testing, and the necessity of funding research.

The need for funding research was emphasized in the 2009 National Academy of Sciences (NAS) Report for improving the forensic science disciplines and thus, hypothetically, reducing wrongful convictions.[1] Only one bitemark research project has been funded in the eight-year interval by the National Institute of Justice (NIJ), a branch of the United States Department of Justice, despite numerous applications for funding.[2]

The Texas Forensic Science Commission report investigated a complaint lodged by the Innocence Project.[3] The latter asked the Commission "to investigate and report on 'the integrity and reliability' of bitemark evidence as used in criminal proceedings."

The President's Council of Advisors on Science and Technology (PCAST) Final Report and Addendum was widely criticized by different organizations and boards dealing with forensics (the American Board of Forensic Odontology, the Society of Crime Laboratory Directors, the International Association for Identification, etc.), law enforcement (the Federal Bureau of Investigation (FBI), the National Association of Attorney Generals, etc.), prosecutors (the National District Attorneys Association, etc.), as well as scientists.[4,5] One such scathing criticism from a scientist states that: "… the PCAST Report (1) is **not** scientifically sound, (2) is **not** based on data, (3) is **not** well-documented, (4) misapplies statistics, (5) is full of inconsistencies, and (6) does **not** provide helpful guidance to obtain valid results in forensic analyses."[6] These comments underline the necessity for balance between pure science and the practical application of a discipline.

The system of justice in North America is based on the premise that the accused is innocent until proven guilty and this burden of proof rests upon the prosecution. The prosecution's failure to demonstrate beyond reasonable doubt plays in favor of the accused. Ultimately, a judge or a jury decides on the guilt or the innocence of the accused. The principal adversarial actors on this stage are the prosecutorial and the defense attorneys. They are the ones that ultimately orchestrate the unfolding play in court. Their success or failure largely contributes to the outcome. All other interveners contribute minor roles to the courtroom stage.

This presentation also outlines the role that inadequate legal defense has played in wrongful conviction in bitemark cases, the failure to have or to use an expert witness at trial, and the use of rogue forensic experts.

In conclusion, wrongful convictions are the scourge of a North American judicial system. There is no single factor responsible for this dilemma.

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Bitemark, Expert Witness, Wrongful Conviction

G16 The Defense Expert Witness' Obligation to Silence and Its Consequence of Wrongful Conviction

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After attending this presentation, attendees will be familiar with the homicide of 16-year-old Brigitte Grenier on June 22, 1990, following a rock music festival near Roseisle, Manitoba. The victim was beaten, mutilated, bitten, received head injuries, and was strangled. Timothy Lawrence Houlahan and Kyle Wayne Unger were charged with first-degree murder, found guilty on February 28, 1992, and imprisoned. On appeal in 1993, Unger's conviction was upheld, while a new trial was ordered for Houlahan. The latter committed suicide before the new trial. On December 2, 1993, the Supreme Court of Canada denied Unger's application for leave to appeal the Court of Appeals decision to uphold his conviction.

This presentation will impact the forensic community by demonstrating the role of the defense expert witness. The defense expert witness has an obligation of silence if not called upon to testify by defense. If the defense expert witness informs the contracting attorney that his client is responsible for the bitemarks, the latter may be unwilling to place the expert on the stand for obvious reasons. Can this, on the other hand, lead to or contribute to the wrongful conviction of a co-accused? What are the ethical implications of silence?

Houlahan, a minor at the time, was the last person seen with the victim heading into a wooded area where her body was found the following day. His clothes and face were covered with mud, he had scratches on his hands and face and blood on his chin when he emerged from the woods the evening Brigitte disappeared. The forensic evidence against him was the victim's blood on his shoe, the suspect's hair on the victim, and unknown to the prosecution until now, bitemarks attributed to him. All of this was prior to the advent of DNA technology.

Unger, on the other hand, was unaccounted for between 3:30 a.m. and 4:00 a.m. the day of Brigitte's disappearance. He emerged with intact clothing and appearance, and the only forensic evidence found was a single hair on the victim's body attributed to him microscopically. Mitochondrial DNA analysis in 2004 demonstrated that the single hair did not belong to him. He was granted bail in 2005, pending a Ministerial decision for a new trial, which was granted. In 2009, Manitoba's Deputy Attorney General withdrew the charges against him without financial compensation; however, the story is more complicated than this brief abstract outline.[1-5]

The prosecution consulted three dentists regarding the bitemarks on the body. Unger voluntarily provided samples of his dentition. The opinions of the dentists were contradictory, and the prosecution decided not to use the evidence at trial. Two of the forensic dentists had no practical experience in bitemark analysis, while the third was a highly experienced bitemark expert and an American Board of Forensic Odontology (ABFO) board-certified forensic dentist. The third dentist's opinion was that the three bitemarks could not have been made by Unger.

In Canada at that time, an accused could legally refuse to provide dental models and impressions. Houlahan refused to provide the latter, and these were not supplied to the prosecution's expert witnesses. This study received three sets of marked, but unidentified as to source, dental models and impressions from the defense, as well as those supplied to the prosecution's expert witnesses.

Much has been written regarding discord in bitemark identification and comparison among bitemark experts. Had the prosecution known that two experienced board certified forensic dentists agreed that Unger could not have created the three bitemarks on the body, would he have spent 14 years in jail and 5 years in limbo? Many European systems of justice would have permitted opposing expert witnesses to consult each other, and the agreement between opposing bitemark experts may have avoided prosecution and incarceration in these jurisdictions. This case demonstrates that bitemark evidence could have contributed to freeing a wrongfully accused and the wrongfully convicted as it has in other cases in the United States, such as the Gregory Ralph Wilhoit case in Oklahoma.[6]

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Bitemark, Expert Witness, Wrongful Conviction

G17 Validation of an Algorithm to Mathematically Describe Bitemarks

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After attending this presentation, attendees will better understand the basic principles of defining a dentition mathematically.

This presentation will impact the forensic science community by beginning the process of scientifically validating bitemark analysis.

Recently, bitemark analysis has been criticized for lacking a scientific basis. The individuality of a specific bitemark has at times been questioned. This has led to doubt regarding the accuracy of attributing a bitemark wound to a specific biter's teeth. Research has been undertaken to determine how unique a dental pattern is and whether this uniqueness can be measured.

An algorithm for mathematically describing bitemarks has been detailed in the forensic dental literature.[1] The method involves analyzing an image of a dentition to locate the centroids of the biting surfaces of the teeth of interest. Wikipedia defines a "centroid" as "the geometric center of a plane figure." It is the arithmetic mean, or average, position of all the points in the shape. Once the centroids have been established, the centroids are connected with lines (or vectors). A vector is a quantity having direction as well as magnitude, especially as in determining the position of one point in space relative to another. Finally, the angles between consecutive pairs of vectors are calculated and recorded. An angle in planar geometry is defined as the figure formed by two rays (vectors), called the sides of the angle, sharing a common endpoint, called the vertex of the angle. Angles formed by two vectors lie in a plane. When eight teeth are studied, eight centroids will be found. The centroids will be connected by seven vectors, which will describe six angles.

A computer program, Bite2020, was been written in C# (Microsoft C Sharp). The software is based on a centroids-vectors-angles algorithm. The program does the work of finding the centroids of the teeth of interest, then calculates the vectors and angles. The resulting mathematical description is saved in a Microsoft® Excel® file. It is envisioned that the program will be used by forensic odontologists as a routine part of their bitemark case workup. The program has been developed to be intuitive, self-instructive, and easy to use.

Once a dentition has been mathematically described and recorded, the results can be compared to a database of previously recorded dentitions. This will allow the forensic odontologist to assess how unique the dentition of interest is when compared to the realm of heretofore analyzed dentitions.

It is anticipated that a repository of bitemark metrics will be maintained by either the American Board of Forensic Odontology (ABFO), the American Society of Forensic Odontology (ASFO), or another forensic organization.

The purpose of this presentation is to begin the process of validating the ability of the algorithm to accurately identify, analyze, and record mathematical descriptions of bitemarks. The validation process for the purpose of this presentation has been divided into three parts. First, the ability of the algorithm to correctly find, calculate, and record the centroids of a number of standard geometric shapes will be compared to expected values. Second, the ability of one investigator to reproduce the same values when analyzing the same dentitions at different sessions will be evaluated. This measurement is known as repeatability or test-retest reliability. It is used to assess the consistency of a measure from one time to another. The third part will assess the ability of different investigators to reproduce similar values when analyzing the same dentitions. In statistics, this measurement is known as inter-rater reliability or inter-rater agreement, or concordance, and is the metric or degree of agreement among raters and is used to assess the degree to which different raters/observers provide consistent estimates of the same phenomenon.

This algorithm shows promise in formulating a scientific basis for the art of bitemark analysis.

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Algorithm, Bitemark, Mathematics

G18 Lessons From 30-Year-Old Louisiana Bitemark Cases: Jackson, Keko, and Others

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The goal of this presentation is to assist current odontologists by presenting historical cases in light of today's best practices.

This presentation will impact the forensic science community by highlighting errors that may occur without standards, guidelines, and best practices.

Synopsis: Two 30-year-old bitemark cases that resulted in exoneration will be discussed. The processes and procedures used then will be contrasted to resources that are available today. Additional discussion points will contrast standards, guidelines, and best practices over the decades as well as discuss the ongoing impact from these and other historic cases.

Forensic odontology, specifically bite injury analysis, has progressed and continues to progress since its widespread adoption by practitioners and the United States court system beginning in the mid-1970s. Advances have included educational requirements for certification, increasing educational opportunities in the field, ease and desirability of communication between practitioners, advances in photography to include the use of digital imaging, advances in illumination techniques and equipment, the adoption of and increasing sophistication of digital imaging software, research into the dynamics of bite injuries, and research in computer-aided image analysis, to name just a few.

In Louisiana, several suspects have been convicted of crimes in which bite injury analysis played a role. During a 30-year-period, two convictions have been set aside. In each case, the State of Louisiana, through the office of the local district attorney, declined to retry the suspect. This presentation will discuss those controversial cases, detailing what steps were taken by the odontologist(s), what other steps might have been taken at the time, what additional steps could be taken today, as well as other issues that impacted the cases.

The first case is that of Willie Jackson, convicted of rape — a case that was appealed to the United States Supreme Court, which denied writs. More than a decade after that denial, the Innocence Project was successful in securing a hearing based on DNA evidence not available at the time of the trial. The conviction was set aside and the prosecutor declined to retry the matter. Only a single odontologist was involved in the trial, and a second odontology expert was consulted by the defense during the original appellate process.

In the second case, Anthony Keko, the conviction was reversed on appeal. Six board-certified odontologists were consulted in that case at the trial level, three for the state and three for the defense. Although not all made statements "for the record," there was not complete agreement between a majority of the odontologists on the state side, and likely not for defense, either. In addition to the criminal case, this matter spawned a long-running civil suit that continues to impact the practice of forensic odontology to this day.

This presentation will provide information for the newer odontologist by using these cases to point out errors that may explain, in part, why the field is under such scrutiny today. Hopefully, the experienced odontologists attending this presentation will achieve a greater understanding of the internal conflicts which have contributed to long-standing disagreement over technique and presentation of results in the discipline. In many ways, these two cases are not unlike other cases of that time, as well as other more contemporary cases, some of which have stood the test of time and others that have not. The odontology expert should not ignore the potential of renewed and/or protracted litigation.

Bitemark, Exoneration, Historical Cases

G19 Case Studies of Failure to Diagnose Oral Malignancies: What Is the Standard of Care for Diagnosing an Oral Malignancy?

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After attending this presentation, attendees will be able to: (1) understand what is meant by standard of care for oral health care providers as they encounter suspicious oral lesions; (2) follow a diagnostic algorithm for diagnosing oral malignancies; and, (3) use the concepts of sensitivity and specificity for choosing tests to determine if a lesion is malignant.

This presentation will impact the forensic science community by illustrating the importance of practicing dentists' abilities to competently make decisions in the best interest of their patients when they encounter suspicious hard or soft tissue lesions of the jaws. Additionally, this presentation will assist forensic odontologists when they are called upon to develop opinions regarding standard-of-care issues.

When confronted with potentially confusing diagnostic dilemmas, the competent clinician should, when reasonable, not ignore the diagnostic imperative (the condition that the clinician cannot overlook or dismiss as not possible to the detriment of the patient). If a clinician ignores the diagnostic imperative then that clinician might be considered to have failed to meet the standard of care.

Procedures will be shown that assist in differentiating a possible malignancy from infections and/or inflammatory and reactive lesions. Attendees will also be able to understand the advantages of an early diagnosis over a late diagnosis and recognize benefits associated with the development of a treatment plan that is favorable to patient outcome.

After attending this presentation, attendees will be able to recognize many of the common and less common signs (including those found in radiographs and other imaging studies) and symptoms associated with oral malignancies (carcinomas, sarcomas, and possible combinations of the two). To reduce confusion that may occur when viewing suspicious lesions, different tests are often chosen to diagnose an oral lesion using the principles of sensitivity and specificity. Attendees will be able to understand that a biopsy has high utility and is most often the best way to develop definitive diagnoses leading to effective treatment plans to manage a diagnosed malignancy.

Attendees will further recognize that oral cancer is not common but is certainly not rare. The National Cancer Institute's Surveillance, Epidemiology and End Results Program (SEER) reports that 1.1% of men and women will be diagnosed with an oral cancer at one point during their lifetime. Diagnosis of oral cancers and survival rates following diagnosis have remained stable over the past 20 years in the United States. Increasing immigration from countries where oral/pharyngeal cancers are much more common is expected to increase the number of new cases seen by United States providers.

Several case studies utilizing patient histories, the analysis of physical examinations, and test results leading to a diagnosis of malignancy will be presented. Clinician failures to use a proven diagnostic algorithm resulted in malpractice claims against those clinicians who failed to meet the standard of care.

Attending this presentation will increase the likelihood that health care providers will be able to diagnose oral cancer at the earliest possible time, which will hopefully result in better patient outcomes.

Standard of Care, Diagnosis Utilizing Biopsies, Oral Malignancies

G20 The Good, the Bad, and the Ugly

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The goals of this presentation are to add to the odontologist's awareness of the complexities of this science and to allow the odontologist to observe nature taking its course.

This presentation will impact the forensic science community by illustrating how human bitemark pattern injury analysis is an observational science.

Observational science is a field of science in which controlled observations cannot be conducted in order to study cause and effect. Scientific studies are simply conducted through the observation of nature taking its course and recording the findings over time. An example of an observational science is astronomy, a science in which a person cannot change or control any aspects of the sun, moon, and stars.

The complexities involved with bites inflicted on one human by another involve numerous factors. Just a few of these factors would be position of biter versus the person being bitten, forces involved, bites inflicted antemortem versus postmortem, and the anatomical area being bitten. This is just a short list of the many factors that influence the appearance, evidentiary value, and analysis of human bites. None of these many factors can be controlled or predicted by the observer of the pattern injury. Laboratory studies of human bitemark injuries *in vivo* cannot account for the many variables seen in the real world. Cadaveric studies and *in vivo* animal models lack external validity in that they are not directly relevant to the clinical situation.

In the 1980s and 1990s, the admissibility pendulum swung to a very permissive position relative to bitemark evidence. Recently, the pendulum has swung 180 degrees to a restrictive position, to the point that there are proponents that feel bitemark evidence should never be allowed for prosecution in the judicial system. Bitemark injuries will not go away just because commissions, councils, committees, or panels feel they are not scientific. Indeed, in cases in which bitemark evidence is questioned, the courts still ask for the opinion of the forensic odontologist. There is nothing wrong with pattern injury (i.e., bitemark evidence). It is the decision to go ahead with bitemark analysis with weak evidence and incorrect or even over-interpretation of the pattern injuries that needs to be addressed. The trier of fact has a professional, ethical, and moral obligation to determine the merits of each pattern injury seen in crimes against persons.

It is, therefore, essential for the odontologist to observe as many pattern injuries as possible to be proficient in pattern injury analysis. This study would like to share with attendees three different pattern injury cases. The cases presented will cover a wide range of factors, providing attendees with an opportunity to experience the complexities of pattern injury analysis.

The "Good Case" implies that there are both class and individual characteristics that permitted this study to conclude that the pattern injury was a human bitemark. The reasoning process will be presented to allow attendees to follow the course of events that allowed the determination that the pattern injury was a human bitemark.

The "Bad Case" conveys the idea that although both the victim and the assailant, in a domestic violence situation, admit the pattern injury was a bitemark, this study was unable to arrive at a definitive conclusion.

The "Ugly Case" was a situation in which the authorities investigating the case had determined the pattern injury was a "human bitemark;" but, when an expert was called in for consultation, it was determined from both clinical examination and history that the "human bitemark" was a pattern injury of iatrogenic origin and was not caused by human teeth.

If, in fact, human bitemark pattern injury analysis is an observational science, which this study proposes it is, then the odontologist cannot observe enough pattern injuries. It is hoped that this presentation will add to the odontologist's awareness of the complexities of this science and will allow the odontologist to observe nature taking its course. These cases can then be added to the odontologist's portfolio of pattern injuries.

Observational Science, Pattern Injuries, Bitemarks

G21 Project LifeMeters: A Digital Solution Optimizing Forensic Measurement Tools in Bitemark Analysis

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The goal of this presentation is to introduce a novel digital tool capable of capturing and creating life-size images of items present or physical injuries inflicted on a human body at a crime scene.

This presentation will impact the forensic science community by demonstrating methods to further digitize the processes involved in evidence conservation. By suggesting a concrete idea for such a tool, this presentation seeks to motivate the forensic science community to digitize more of the processes involved in evidence conservation.

It is a known fact that it can be a great challenge to accurately register bitemarks. Obstacles include poor quality photographs, not having a ruler handy, not knowing the real proportions of items, and using complex image processing software to manually improve image quality. Not having the proper tools available at the crime scene makes it impossible to perform a proper and accurate bitemark analysis, as precision must always be the benchmark of any forensic investigation.

Furthermore, time is of the essence when it comes to conserving the structure of a lesion on a human body before the healing process begins and improves the condition of the original wound. Also, in economically challenged countries around the world, forensic measurement tools such as the American Board of Forensic Odontology (ABFO) No. 2 forensic ruler may be difficult to obtain at a moment's notice, whereas a smart phone is nearly always available on demand.

In conclusion, the idea is to develop a mobile application capable of capturing life-size images of evidence at a crime scene with the scale of a digitally implemented forensic ruler calibrated by the user beforehand. Current devices on the market are unable to accomplish this due to their inability to scan object depth without infrared projectors, making 3D object capturing impossible. If there were hardware add-ons available on the market for mobile devices or if smart phone companies were able to integrate a hardware addition to their products, a mobile app would be able to calculate the exact size of an item. The app could also record the data of the captured image in a file format suitable for further processing. The automatized forensic analysis of an image could make a considerable contribution to the quality of the findings.

Project LifeMeters consists of two technical components. The software component includes an artificial intelligence module that tags specific objects or wound types automatically and provides this information in a report. The smart phone camera hardware component is used for 3D object capturing. The concept is plausible because there are already technologies, such as Google's® Project Tango or Occipital's Structure Sensor, that provide the camera features for smart phones performing instant measurements. There are already two smart phones on the market from Lenovo® and ASUS® with an integrated 3D camera. In addition, the scene recognition demo of the Massachusetts Institute of Technology demonstrates how precise current scene recognition systems are in defining objects within a crime scene.

Bitemark, Digitalization, Crime Scene

G22 An Examination of Bitemark Analysis in the Turkish Judiciary and the High Court

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After attending this presentation, attendees will understand how the Turkish judicial system evaluates bitemark analyses in criminal cases.

This presentation will impact the forensic science community by providing information regarding examples of bitemark uses in the Turkish court system.

As societies progress, the justice mechanism needs more reliable and more realistic evidence. This is why forensic dentists should have not only basic dentistry science instruction, but should also be familiar with aspects of medicine and law. The analysis of a human bitemark is one of the most complex and challenging components of forensic dentistry. Courts may use bitemark analysis to increase the certainty with which a perpetrator is convicted or help ensure an innocent suspect is properly exonerated. In this presentation, three cases are demonstrated in which bitemark analyses were used in identifying and convicting suspects. The Supreme Court of Appeals subsequently approved the appeal requests, and the decisions of the Court are examined and evaluated.

Case 1: In Istanbul, in 2002, a 70-year-old man was found dead in the house where he lived alone. The autopsy was conducted by the Forensic Medicine Institute. A bitemark was identified on the right side of the victim. Forensic experts examined this bitemark, which consisted of a seven-tooth impression. At the time, the man's wife was a fugitive and her neighbor was providing the police with contradictory testimonies; thus, the neighbor was taken into custody. As a result of the bitemark comparison, the impressions produced from three teeth in the upper arch and four teeth in the lower arch were found to be consistent with the bitemarks observed on the victim's side.

Case 2: Three separate events within a one-month period left one victim dead and two others injured. The first attack took place in 2002, against a woman who lived alone in Istanbul. There were dozens of deep bitemarks on the victim's face and body and she was admitted to Istanbul University Medical Faculty Hospital Emergency Surgery Service for treatment (evidence of sexual assault was not found). The second attack was in 2003 against an 80-year-old woman found partially dressed in a burning house, also in the district of Istanbul. She had deep bitemarks on her face, arms, legs, and other parts of her body. The killer bit the victim several times, then killed the victim with a piercing tool. Approximately one meter from the body, flesh that was bitten off by the perpetrator was discovered. No signs of sexual assault were found. The third assault also took place in Istanbul in 2003, and involved a United States tourist. The assailant bit the victim over her right eye and on several other places on her face. The killer had two distinct characteristics: the canine teeth were much larger than normal, and the anterior teeth were absent, which created a very specific pattern.

Case 3: In 2010 in Izmir, police discovered a body that had been stabbed in the throat and on various other parts of his body. There was no wallet or cell phone on the body. One of the suspects had bitemarks on his right hand. Examination of three cigarette butts found in the suspect's car revealed that the cigarette butts belonged to the suspect. The suspect was also the owner of the farm where the body was found and was at that particular coffee house on the day of the incident. His son and cousin were with him on that specific day and were also arrested.

In conclusion, in the case of bitemarks, the following protocol is followed: per prosecutor order, the police investigation team collects evidence at the scene from all suspects and victims. The prosecutor's office takes statements from witnesses. Evidence is then sent to the relevant laboratory units to be examined. After obtaining the evidence and laboratory results, a scientific report is prepared by an expert. If there is a contradiction in the reports of the expert panel, the court will send all documents to the relevant specialist department of the Forensic Medicine Institute and request a report. Then, the relevant court will judge the entire contents of the file and issue an independent order. If one of these parties disagrees, the matter is assessed, and a final decision is made by the High Judiciary.

Turkey, Bitemark Analysis, High Court

G23 Recognizing Bitemarks: A Basic Problem

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The goal of this presentation is to raise an alternative to stating in a courtroom or a legal statement that a suspect injury is or is not a bitemark when there is no victim statement to corroborate the diagnosis. Instead, this presentation proposes stating the degree to which an injury matches the class and individual characteristics of a bitemark.

This presentation will impact the forensic science community by illustrating how to reduce the potentially spurious certainty around claims that a particular injury is a bitemark when this is uncorroborated by other evidence. Instead, this presentation takes the conservative option of reporting only on the degree to which the injury meets the class characteristics, enhancing the probity of the evidence, and reducing reliability on "expert experience," which has been shown to correlate poorly with a correct diagnosis.

Several lesions have been reported by Gold et al. simulating bitemark injuries, including fixed drug eruptions, subacute cutaneous lupus erythematosus, pityriasis rosea, tinea corporis, and granuloma annulare.[1] Other injuries may also look suggestively similar to bitemark injuries. Injuries from shoe sole contact, belt buckles, defibrillators, and saws have also been reported as potentially presenting confounding features.[2,3]

A disputed paper purporting to determine the degree to which experienced forensic odontologists could agree on whether or not an injury was caused by teeth raised the issue of whether or not injuries that resemble bitemarks can reliably be differentiated from the real thing.[4] Regrettably, the original project has not been repeated in a rigorous form, but the question is nonetheless an important one. Important elements of a case may turn on the diagnosis.

In the absence of hard research demonstrating that experience in bitemark analysis improves the reliability of the diagnosis, a more conservative approach may be warranted.

Current American Board of Forensic Odontology (ABFO) Bitemark Methodology Standards and Guidelines refer to three categories into which injuries may be classified: (1) Human Bitemark (human teeth created the pattern); (2) Inconclusive (there is insufficient evidence to reach an opinion as to whether or not the pattern is a bitemark); and, (3) Not a Human Bitemark (human teeth did not create the pattern).[5] All of these are subjective judgements.

This presentation discusses the class characteristics of human bitemark injuries and suggests that a more conservative presentation removes the subjective nature of these categorizations. Instead, it is proposed that the class characteristics of a human bitemark be listed and agreed upon (this presentation enumerates a possible list) and that forensic odontologists comment only on the extent to which the injury in question matches these class characteristics. If a threshold is met, then the presence of individual characteristics can be used to determine whether or not the injury is suitable for comparison with a potential suspect dentition.

Reference(s):

1. Gold M.H., Roenigk H.H., Smith E.S., and Pierce L.J. (1989) Human bitemarks: Differential diagnosis. *Clinical Pediatrics.* 28, 329-31.
2. Grey T.C. (1989) Defibrillator injury suggesting bitemark. *The American Journal of Forensic Medicine and Pathology.* 10, 144-5.
3. Goodbody R.A., Turner C.H., and Turner J.L. (1976) The differentiation of toothed marks: Report of a case of special forensic interest. *Medicine, Science, and the Law.* 16, 44-8.
4. Page M., Taylor J., and Blenkin M. (2013) Expert interpretation of bitemark injuries – A contemporary qualitative study. *J Forensic Sci.* 58(3): 664-672.
5. American Board of Forensic Odontology Diplomates Reference Manual. (2017) p102

Forensic Odontology, Bitemarks, Class Characteristics

G24 Dental Age Quicksheets (DAQS): The Use of Rapid Calculation Procedures to Determine "Uncertainty" in Dental Age Estimation (DAE)

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After attending this presentation, attendees will be: (1) aware of the differences in the expression and interpretation of the amount of variation associated with DAEs; (2) able to consider the differences in presenting estimate of "uncertainty;" and, (3) in a position to determine the appropriate method of expressing uncertainty.

This presentation will impact the forensic science community by educating attendees on the differences in error calculation and reporting with DAE cases and by improving the accuracy of DAEs by utilizing the correct method of obtaining the pooled standard deviation.

Introduction: DAEs are widely used in forensic human identification, age estimation of asylum seekers, and in forensic anthropology. When calculating the estimated age, the "error of the method" must also be reported. The most common method of reporting the error in DAE is the Standard Deviation (SD). The usual presentation is to indicate the Dental Age (DA) ±1SD, ±2SD, or even ±3SD. These are helpful ranges as they encompass approximately 68%, 95%, and 99%, respectively, of the data.

Historical DAE studies have utilized Tooth Development Stages (TDS), enabling the dental assessor to use the published mean values of the stages to calculate the DA by taking an average of those values. The associated error of the TDS values are also published and the dental assessor therefore averages the SD values of each TDS value used to obtain the overall SD of the DA. The error of the DA is *usually* reported as a multiple of the SD, and, by convention, this is ±2SD.[1] A more appropriate method of calculating the error associated with the DA is utilizing a pooled SD equation, which takes into account a weighted average of the SD by utilizing n-1.

The purpose of this study is to compare the range of values for the SD calculated using the pooled calculation (n-1) with the range of values using the simple average of SD (n).

Materials and Methods: Dental panoramic tomographs from a study of accuracy of age estimation at the 10-year threshold were re-used. The tooth development stages using the Demirjian description were determined and entered into the DAQS. For each subject, the DAQS calculated the age of the subject and the pooled SD n-1 and simple average SD n separately. The range of uncertainty was then expressed as a range of the SD, namely ±1SD, ±2SD, or ±3SD.

Results: It was found that there was a statistically significant difference between ranges of uncertainty calculated respectively from the pooled SD n-1 and SD n.

Discussion: The DA for a single subject is estimated by averaging the mean values for the TDS present on the subject for whom a DA is required. The variation or level of uncertainty is expressed as the SD or multiples thereof.

The data revealed the pooled SD n-1 to be greater than the averaged SD n for 92 of the 100 subjects and the reverse for 8 subjects. The average difference between SDS and SD n-1 is 0.085. This translates into a difference in the estimated DA range of 0.17 years or 2.04 months for ±1SD, 4.04 months for ±2SD, and 6.06 months for ±3SD.

Conclusion: It is clear that the use of the orthodox method of estimating the pooled SD n-1 provides a wider range for the expression of uncertainty, often referred to as the error of the method, compared to the simplistic method of simply averaging the SD.[2] The differences are small, but in the interests of sound logic and improved accuracy, the correct method of obtaining the pooled SD should be used.

Reference(s):

1. Lewis J.M., Senn D.R. Dental Age Estimation. *A Manual of Forensic Odontology.* Chapter 8. Ed. Senn D.R. and Weems R.A. 5th Edition 2013. CRC Press. Boca Raton. Florida. ISBN: 978-1-4398-5133-3.

2. Vosk E., Emery A.F. Forensic metrology. Scientific measurement and inference for lawyers, judges, and criminalists. *International Forensic Science and Investigation Series.* 2015. CRC Press. Boca Raton, Florida. ISBN 978-1-4398-2619-5. Chapter 16.

Dental Age Estimation, Standard Deviation, Quicksheets

G25 Statistical Tools in Assessing Validity and Reliability of Tools Used in Forensic Odontology

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After attending this presentation, attendees will better understand the importance of using appropriate statistical tools when conducting reliability studies in forensic odontology.

This presentation will impact the forensic science community by explaining in detail the importance of selecting or identifying the best statistical tools available in a broader scientific society that can be applied for forensic purposes.

Background: In 2009, the National Academy of Sciences conducted a review of forensic disciplines and their role as expert evidence in the United States. The review took into consideration cases in which the convicted individual was subsequently exonerated due to DNA testing following judicial reassessment. Together with other latent print identification techniques, the Report was critical of forensic odontology and, in particular, of bitemark analysis and comparison. The National Academy of Sciences reported that the limitations of forensic techniques included inadequate scientific underpinnings, a paucity of research on human observer bias, and lack of technological innovation. The Report indicated that both scientific and systemic changes need to be made to bitemark analyses to ensure their reliability, establish standards, and promote practices that are consistent.

Materials currently used in making dental casts for forensic investigations of bitemarks undergo structural and chemical changes during setting, and the casts may not be completely accurate as a consequence. Intraoral 3D scanning of dentitions has the potential to provide a fast, accurate, and non-invasive method of recording dental information; however, they are yet to be validated for use in forensic investigations.

Goal: The goal of this study was to assess the reliability and validity of a portable intraoral 3D scanner appropriate for recording suspect dentitions in forensic investigations. For the purpose of this presentation, two different approaches of estimating Intraclass Correlation Coefficients (ICC) will be outlined.

Methods: In this study, reliability and validity of the intraoral 3D scanner was quantified by observing the findings of several tests, such as by comparing means of test-retest, rater-rater, and method-method differences, calculation of ICC, and Standard Error of Measurements (SEM) of 110 landmark dental features that were made on 50 sets of human dental casts. To estimate the ICC, along with the conventional method by Fleiss and Shrout, a novel method of concurrent assessment of inter-and intra-rater reliability in a three-factor (subjects, methods, raters) design with replication was conducted by extending the approach of Eliasziw et al. for two factors with replication.[1,2] This presentation will present the findings of ICC analysis using a conventional approach (Fleiss and Shrout) and by using the approach by Eliasziw et al. and the advantages and disadvantages of using the above-mentioned methods in a reliability study with repeated measurements in forensic odontology.

Conclusions: This presentation addresses the paucity of research on using statistical tools to enhance the scientific underpinnings in forensic odontology by understanding their limitations and emphasizing the need for identifying other methodology not previously used in forensic odontology.

Reference(s):

1. Shrout, Patrick E. and Fleiss, Joseph L. Intraclass correlations: Uses in assessing rater reliability. *Psychological Bulletin.* 1979, 86, 420-3428.

2. Eliasziw M., Young S.L., Woodbury M.G., and Fryday K. (1994). Statistical methodology for the concurrent assessment of interrater and intrarater reliability: Using goniometric measurements as an example. *Physical Therapy.* 74, 777-788.

Reliability and Validity, Forensic Odontology, Statistical Analysis

G26 Dental Age Estimation Using the Demirjian Method: A Flawed and Obsolescent System

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After attending this presentation, attendees will be aware of the serious shortcomings of the Demirjian system of Dental Age Estimation (DAE). Attendees will be able to consider the reasons for the difficulty in comprehending and applying this system and will be in a position to use a simpler system of DAE based on the Dental Age Quicksheets (DAQS) approach.

This presentation will impact the forensic science community by providing information that will lead to the adoption of the DAQS system for the accurate estimation of dental age as a surrogate for chronological age.

Introduction: Dental age assessment using the method of Demirjian and colleagues published in 1973 has been widely used over the past 40 years.[1] Despite its widespread use, even a cursory reading of the paper leads to the conclusion that the details of the methodology are elusive. A recent systematic review revealed there were wide discrepancies between the Chronological Age (CA) and the Dental Age (DA) estimated using the Demirjian method.[2] This has led to other criticisms of the reliability of the Demirjian method.[3]

Problems with the Demirjian System: It is convenient to break down the method into two major components: (1) The Demirjian Tooth Development Stages (TDS) — the first of these, the TDS, has made a major contribution to DA studies. The observer agreement when assessing the TDSs has proven to be of the highest level.[4] (2) The System of Mathematical Integration of Ages of Attainment (AoA) — the second issue is the major weakness of the Demirjian system. This is the failure to censor Stage H, which leads to grossly inflated AoA for all the H Stages from LL1 through LL7 (see table below). This results in elevated values for Stage H.

	LL1	LL2	LL3	LL4	LL5	LL6	LL7
Development Stage	H	H	H	H	H	H	H
	Years	Years	Years	Years	Years	Years	Years
AoA UNCENSORED	15.43	15.73	16.69	16.98	14.54	16.29	17.82
AoA CENSORED	12.73	13.05	13.22	13.38	17.59	13.16	15.03
Difference	2.70	2.68	3.47	3.60	3.05	3.13	2.79

The data in this table reveals that the effect of non-censoring is to elevate the AoA by between two and three-fourths and three and one-half years. Although the detailed mathematics are complex, this may explain the underlying cause of the systematic overestimation of DA using the system of constraints.

The original mathematical approach was to use the system of "constraints" to assign to each of the Demirjian TDS a "value" that, when summed, came to 100.[5] This was deemed to be the equivalent of "Full Maturity."

Can It Be Rectified?: The straightforward answer to this is no. Over the past few years, persistent attempts have been made to unravel the mathematical approach to this method, including repeated contacts with the original authors.

Conclusions: The method of constraints used in the original 1973 paper has shortcomings and should no longer be used. The ease of use of modern spread sheets makes elaborate computations straightforward for forensic odontologists. The Demirjian system of TDS remains the choice for DAE. It is the most fully described and most reliable in terms of reproducibility.

Reference(s):

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Demirjian, Obsolescent, DAQS

G27 Root Pulp Visibility (RPV): Validation of Applicability of RPV in Determining Adult Status

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After attending this presentation, attendees will be aware of the applicability of RPV measurements to predict whether or not a subject of unknown date of birth is above the 18-year threshold, will be able to apply RPV to cases in which the lower third molar has completed growth and mineralization, and estimate with a high level of probability if the individual is above 18 years of age.

This presentation will impact the forensic science community by providing a method with great potential to predict the adult status of subjects without birth records.

Introduction: The gradual loss of RPV with increasing age has been reported in the European literature.[1,2] This enables a reliable prediction of the age above the 18-year threshold. To validate the technique, the first stage is collection and publication of reference data.[1,2] Next, Dental Panoramic Tomographs (DPTs) of subjects of known age are assessed (age and gender masked).

The purpose of this study is to assess the reliability of RPV in predicting whether a subject is below or above the 18-year threshold. In the United Kingdom, 18 years is the age of majority (i.e., adult status).

Materials and Methods: DPTs taken early in 2015 were drawn from the archives of Guy's and St. Thomas' hospitals. The inclusion criteria were DPTs of diagnostic quality of both male and female subjects of known ancestry, aged between 16 and 25.9 years. The DPTs were enlarged to X2 to improve visualization. The lower left third molar (LL8) was assessed to predict maturity using the 8 Stage System of development.[3]

For each LL8 Stage H, RPV was recorded using the original criteria, modified to improve interpretation.[1,2] The assessments were made in random order. The age, gender, and ancestry were not known to the assessor. Intra-rater agreement was determined by randomly selecting 10% of the subjects for reassessment one week later. All data was entered into a Microsoft® Excel® spreadsheet, and a filter process was applied to isolate the data for each of the RPV categories by gender.

Results: A total of 396 DPTs were assessed. The proportion of subjects in each RPV category is shown in the table below.

Root Pulp Visibility: Summary Statistics for Stages A, B, C, D						
			$\bar{x}$	**SD**	**p > 18**	**% > 18**
RPV- •f	59 / 238	24.8%	19.96	2.99	0.74	74.0
RPV- Af	42 / 238	17.6%	22.45	2.22	0.98	98.0
RPV- Bf	103 / 238	43.3%	23.52	1.68	1.00	100
RPV- Cf	31 / 238	13.0%	23.83	1.85	1.00	100
RPV- Df	3 / 238	1.3%	23.49	0.15	1.00	100
			$\bar{x}$	**SD**	**p > 18**	**% > 18**
RPV- •m	45 / 157	29.3%	19.04	2.74	0.65	65.0
RPV- Am	19 / 157	12.1%	21.60	2.79	0.14	90.2
RPV- Bm	71 / 157	45.2%	22.53	2.34	0.97	97.0
RPV- Cm	20 / 157	12.7%	24.44	2.02	1.0	100
RPV- Dm	2 / 157	1.3%	24.85	1.25	1.0	100

• signifies unusable data from DPTs. *f = female, m = male*

Discussion: This validation of RPV using the gold standard of chronological age has demonstrated a high level of reliability for predicting the adult status of a subject. Clearly, it is appropriate to apply these findings to individuals without birth records. It appears that the high level of probability of being aged above the 18-year threshold is greater than shown in the original studies.[1,2]

Conclusions: The use of RPV has great potential for predicting adult status for subjects without birth records.

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Root, Pulp, Visibility

G28 Dental Age Assessment From Analysis of Canine Pulp/Tooth Volume Ratios Using Cone Beam Computed Tomography (CBCT)

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After attending this presentation, attendees will better understand the correlation between pulp/tooth ratio volume and age assessment and will appreciate the benefits of using Amira™ radiographic software for *in vivo* volumetric analysis of tooth/pulp volume. Additionally, attendees will better understand the effect of gender and arch location of canine teeth in the accuracy of age assessment.

This presentation will impact the forensic science community by providing a non-invasive and dependable modality for age assessment. This presentation also introduces a segmentation tool that provides the ability to segment both the tooth and the pulp structures without including the surrounding bone.

Background: Dental age assessment presents greater challenges for adult cases than for cases involving adolescents or children with mixed dentitions. Some dental methods are invasive and very technique-sensitive compared to others, and some can be applied only to deceased individual cases. Among different potential parameters for age assessment, tooth and pulp space have gained more interest in recent years. It is well known that pulp space decreases in size with aging. Therefore, several studies have evaluated tooth/pulp size ratio using two-dimensional radiographs. Fewer studies have been published using 3D CBCT. The hypothesis developed for this study is that analyses of the ratios of the volumes of canine teeth and their pulps using CBCT can provide an acceptably accurate estimation of age in adults. The purpose of this study was to develop an accurate and non-invasive age assessment method by performing volumetric measurement of tooth and pulp space using CBCT on living subjects.

Material and Methods: The study population consisted of 135 subjects (67 males and 68 females) aged 18 to 75 years old. Maxillary and mandibular canines were used in this study. All scans had been taken earlier for varying diagnostic purposes, such as implant planning and endodontic evaluations. Teeth with caries, fracture, or a history of orthodontic treatment were excluded. Amira™ software was used to quantify the volume of the tooth and pulp space. Pulp/tooth ratio was calculated. All assessments were performed by a single evaluator (a board-certified oral and maxillofacial radiologist), and 60 of the scans were re-evaluated for intraobserver agreement. Data were analyzed using Pearson correlation and regression analyses and significance was set at 0.05.

Result: There was a strong correlation between pulp/tooth volume ratios and age. The age estimates for maxillary canines correlated with chronological age better than the estimates from mandibular canines. There was good intraobserver agreement. The findings indicate that analysis of pulp/tooth volume ratios of both maxillary and mandibular canine teeth are useful for age assessment.

Age Assessment, CBCT, Pulp/Tooth Volume

G29 The Accuracy of Two Dental Age Estimation Methods on Saudi Children: Cameriere's Measurement of Open Apices and The London Atlas of Tooth Development

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The goal of this presentation is to demonstrate how well two methods of Dental Age Estimation (DAE) perform when used on the Saudi population and how they compare with each other. The two methods are: (1) analysis using The London Atlas of Tooth Development and Eruption; and, (2) Cameriere's Measurements of Open Apices of Mandibular Teeth.

This presentation will impact the forensic science community by providing inter-population evidence to demonstrate the accuracy of two new methods of dental age estimation and highlight the differences in conducting these two methods on Saudi children. This presentation will also enhance understanding of the importance of the assessment of dental development in various dental age estimation methods.

Dental development is frequently used to assess maturity and estimate age. The goal of this study was to test and compare the accuracy of two methods of dental age estimation, Cameriere's formula (measurements of mandibular teeth's open apices) and analysis using The London Atlas of Tooth Development, in Saudi children.

Materials and Methods: The sample consisted of 400 Saudi males and females between the ages of 6 and 15 years. Inclusion criteria were good quality, clear panoramic dental radiographs (Orthopantomographs (OPGs)) of healthy patients with no medical history of systemic diseases/disorders. Exclusion criteria were unclear radiographs, hypodontia (one or more missing teeth), hyperdontia (one or more extra teeth), gross pathology (torodontism, microdontia, amelogenisis imperfecta, dentinogensis imperfecta, tumors, abscesses, fractures, etc.), presence of gross caries, or previous orthodontic treatment. Age estimation was performed using Cameriere's formula (measurements of mandibular teeth's open apices) and The London Atlas of Tooth Development on the left side of the jaw.

Chronological age (real age) was blinded from the researchers until all subjects radiographed were assessed and age estimation was completed. Data were managed and analyzed using the SPSS program (v24). Inter- and intra-examiner reliability tests were calculated on a random 10% sample from the radiographs to determine the kappa statistic.

Results: The intra-examiner reliability test was 0.89 and the inter-examiner reliability test was 0.8, which demonstrates excellent agreement.

Mean difference between Estimated Age (EA) and Real Age (RA) was -0.45 years for The London Atlas with a standard deviation of 1.61 years, and was -0.83 years for Cameriere's formula with a standard deviation of 1.34 years; both methods consistently underestimated actual age. The absolute mean difference was 1.19 years and 1.16 years for The London Atlas and Cameriere's formula, respectively. The mean difference between EA and RA using The London Atlas was -0.61 years for males and 0.23 years for females, with no statistical significant difference between genders. Cameriere's formula gave a mean difference between EA and RA for males of -0.89 years and -0.77 years for females, with no statistically significant difference between genders.

Conclusion: The London Atlas of Tooth Development was easier to use because all teeth in both jaws can be used and the availability of an extra dental development stage resulted in better measures of accuracy on Saudi populations.

Age Estimation, Dental Development, Saudi Arabia

G30 Magnetic Resonance Imaging (MRI) of Third Molars in Forensic Age Estimation: Validation of the Gent and Graz Protocols

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After attending this presentation, attendees will be aware of the strengths and weaknesses of two Siemens Magnetic Resonance Imaging (MRI) protocols for third molars that can be applied for age estimation.

This presentation will impact the forensic science community by providing considerations for the interpretation of two main MRI protocols to examine third molars for age estimation. It will be demonstrated that both approaches have their own characteristics and that research using both should continue. These protocols are a next step toward radiation-free dental age assessments.

Background: Panoramic radiographs are used for age estimation in forensic dentistry; however, they imply an exposure to radiation without a medical indication. Moreover, superposition can lead to misinterpretation of the developmental status of the examined structures. To counter these drawbacks, several research groups are studying the use of MRI in forensic age estimation. Per research, dental age estimation by means of third molar MRI has been studied by three research groups.[1-4] Guo et al. described a protocol for 3 Tesla (3T) MRI with a Philips scanner, while Baumann et al. and De Tobel et al. used a 3T Siemens scanner. MRI sequences of the latter two research groups will respectively be referred to as the Graz protocol and the Gent protocol.

Purpose: To validate the Gent and Graz MRI protocols for third molars by evaluating to which extent staging third molars for age estimation is influenced by: (1) in-plane resolution and slice thickness; and, (2) head fixation using a bite bar.

Materials and Methods: Eleven healthy volunteers (5 females, 6 males, 16-30 years of age) scheduled for surgical removal of third molars were included. *In vivo*, two 3T MRI scan protocols were applied: the Gent protocol with T2 Fast Spin Echo (FSE) and thin slice T2 FSE sequences, and the Graz protocol with T1 3D FSE and 3D Constructive Interference in Steady State (CISS) sequences.[1-3] Although bite bar fixation is inherent to the Gent and not to the Graz protocol, for both protocols scans were obtained with and without bite bar. After surgical removal, 39 third molars were scanned *in vitro* with 7 Tesla µMRI, applying T2 FSE and Zero Echo Time (ZTE) sequences, and scanned with µCT. Three observers evaluated the randomized MRI and µCT images in consensus. Assessability was judged and compared in all applied MRI sequences. Third molar staging was conducted according to the technique by De Tobel et al.[5] Staging outcomes between imaging modalities and between MRI sequences (with and without head fixation) were compared.

Results: The Gent T2 FSE sequence (voxel size 0.33 x 0.33 x 2mm³) with and without bite bar was significantly more assessable (97%=38/39 and 81%=26/32, respectively) than the Graz T1 3D FSE sequence (voxel size 0.59 x 0.59 x 1mm³) with bite bar (80%=28/35; P= 0.02) and without bite bar (59%=19/32; P <0.001). The combination of the voxel size and the inherent high-contrast of µCT rendered the optimal reproduction of the hard tissue specimens. Hence, imaging with µCT was considered as comparison standard to visualize and stage third molars. Allocated stages on MRI were most frequently equal to or higher than those on µCT. The difference between staging based on the Gent and the Graz protocol, using the bite bar, was not statistically significant, and neither was the difference in staging based on MRI with bite bar, compared with the same sequence without bite bar.

Conclusion: Compared with µCT, third molars appeared more developed on MRI. Using a bite bar increased the proportion of assessable third molars. By contrast, it did not influence allocated stages to assessable third molars. The differences in in-plane resolution between Gent and Graz protocols also resulted in differences in assessability rather than differences in allocated stages. Images of the Gent protocol allowed interpretation of the highest proportion of third molars available for age estimation.

Funding: Provided by the department of Radiology and Nuclear Medicine at Ghent University and the American Society of Forensic Odontology (ASFO) Research Grant 2017.

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Age Estimation, Magnetic Resonance Imaging, Third Molars

G31 The Use of Magnetic Resonance Imaging (MRI) in Forensic Age Estimation of Living Children, Adolescents, and Subadults: Protocol for a Systematic Review and Preliminary Results

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After attending this presentation, attendees will understand the need for a systematic review of studies on the use of MRI for age estimation. A protocol for an adequate conduct of the review and its preliminary results will be presented.

This presentation will impact the forensic science community by elaborating on the emerging field of radiation-free age estimation by means of MRI. It will be demonstrated that numerous studies have been conducted, but that an overview is necessary to bring age estimation based on MRI of developing anatomical structures into practice.

Background: Established methods for age estimation mainly use radiographs to register and evaluate teeth, carpal bones, and long bones, which are still developing in children and subadults; however, young individuals especially are highly sensitive to radiation exposure. Moreover, radiographs may be misinterpreted because of superposition. MRI overcomes both of these drawbacks. Therefore, several research groups are studying the use of MRI to register the developmental status of the considered body part. In particular, diverse MRI protocols were established depending on the anatomical structures used for age estimation.

Purpose: To review the use of MRI for forensic age estimation in living children, adolescents, and subadults and to provide data on age distribution of the development of different anatomical structures as registered on MRI. The systematic review aims to comply with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[1,2] The review protocol was drafted according to the Cochrane Guidelines for review protocols (http://training.cochrane.org/), and registered in Prospero, the international prospective register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO).[3]

Research Questions: How does the development of different anatomical structures, as registered on MRI, relate to chronological age? What is the performance of age estimation based on development of different anatomical structures as registered on MRI?

Search Methods: The following databases were searched: MEDLINE, Embase, and Web of Science. Additionally, reference lists of included articles and study registers were searched. No restrictions were made based on the country of publication, language, or publication date.

Selection Criteria: Two reviewers independently selected articles based on titles and abstracts. Study populations including living subjects up to 30 years were considered. MRI of any field strength studying the development of anatomical structures related to age were included. Cross-sectional observational studies, pilot studies, cohort observational studies, and case reports were considered. Review articles and pilot studies of included main studies were excluded. After resolving conflicts by discussion, the full text papers were evaluated independently for eligibility. Again, in case of conflicts, a consensus decision was made.

Data Extraction and Analysis: Study characteristics tables and data extraction tables for the included articles were independently developed. A tool for the risk of bias and paper quality assessment was developed, based on the Effective Practice and Organization of Care (EPOC) overview and Quality Assessment of Diagnostic Accuracy Studies (QUADAS) -2.[4,5] Authors were contacted for further details and data if these were unclear, not reported, or in a format unsuitable for the review analysis.

Preliminary results: The search through the databases produced 468 results: MEDLINE (*n*=99), Embase (*n*=192), and Web of Science (*n*=177). Additional records, identified through other sources, provided two results. After deduplication, 259 records were screened based on title and abstract, rendering 60 records to be assessed for eligibility by reading the full-text articles. Finally, 34 studies were included for qualitative synthesis and data extraction.

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Age Estimation, Magnetic Resonance Imaging, Subadults

G32 A Senegalese Case Study Illustrating a Determination of Age of the Pupils Without Civil Status or With False Declaration of Age by Means of Their Dental Assessment

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After attending this presentation, attendees will better understand how dental age estimation may allow pupils to gain civil status when they are without civil status or when there is a false declaration of age by their parents.

This presentation will impact the forensic science community by illustrating how the dental age prediction may help pupils obtain a civil status. Certain children in the population of regions who have left and then moved back to Senegal often do not make the proper and necessary arrangements with the registry to attend school. Their parents sometimes make a false declaration of their age to allow access to the school. The government may request the forensic odontologist to assess their dental age. This presentation will add to research being conducted in age estimation for children using a dental formula when it concerns a large number of individuals.

Materials and Methods: Among the many methods for estimating age, this case study was based on two complementary methods, Schour and Massler, and Ubelaker, both of which use dental development tables, a viewer, and panoramic radiographs. A clinical examination is first performed in order to detect any pathology, parafunction, or treatment likely to alter the results. The government's five-day deadline for these tasks allows one to work quickly and in a simple manner. The two methods combined offer age-estimate ranges of more-or-less than 6 months, and assessments were adapted to each stage of dental development in age groups of 4 to 35 years. Age estimation was conducted by direct reading on the tables. This sample consisted of 102 children, 58 boys and 44 girls.

Results: By analyzing the age reported by parents on the census list, and the age that was estimated, 83 of 102 age declarations were false. This corresponds to an 81% false declaration of age.

Discussions: By comparing the reported age according to the dental formula, this study found a significant number of false declarations for those 5 and 6 years (86% and 92%) of age. This is due to the fact that these two age groups are highly targeted for the chance of being recruited.

False statements exceeded correct statements for all age groups. Among the false declarations, each age group had a given percentage: 3 years – 16% false declaration of age; 3½ years – 21% false declaration of age; 4 years – 12% false declaration of age; 5 years – 18% false declaration of age; 6 years – 19% false declaration of age; and 7 years – 14% false declaration of age.

Conclusion: In developing countries, particularly in Africa, these cases of unregistered children are recurrent and the estimate of their dental age is of great help in resolving this problem. In addition, the need to benefit from false declarations of age, such as for immigration, is a pressing motive to promote this discipline in forensic odontology.

Dental Age Determination, Pupils, False Declaration of Age

G33 Cephalometric Analysis of Historic Native American Arikara

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The goal of this presentation is to provide attendees with knowledge of a number of dentoskeletal parameters that can help illustrate the relationship of the maxillae, the mandible, and the maxillary and mandibular dentitions to the facial profile.

This presentation will impact the forensic science community by providing the cephalometric analyses on the adult Native American Arikara, which will provide additional information on facial profiles for this historic Great Plains population.

Profile changes over the sample's 232-year time span, if there were any, can be surmised. The proposition being considered is, will a dentoskeletal relationship change enough in approximately 232 years to affect a facial profile change?

This study was conducted on the cephalograms of a historical adult Native American population. The Arikara remains were from five anthropological excavation sites in South Dakota: Rygh (1600-1650), Mobridge (1600-1700), Sully (1650-1700), Larson (1679-1733), and Leavenworth (1802-1832). The time elapsed was approximately 232 years (1600-1832).

Cephalograms of adult Native American Arikara skulls in the sample were made by the University of Tennessee Department of Oral & Maxillofacial Surgery in Knoxville, TN. Fifty-five of the cephalograms were determined to be acceptable for cephalometric analyses. Five cephalograms were from Rygh, 7 from Mobridge, 5 from Sully, 17 from Larson, and 21 from Leavenworth. These cephalograms were digitized and the Dolphin imaging system was used to perform the analyses. Measurements used were taken from the Steiner analysis and the Tweed analysis, both of which are commonly used in orthodontics. From the Steiner analysis, the SNA, SNB, and interincisal angles were used. From the Tweed analysis, the FMA, FMIA, and IMPA were used (Tweed triangle).

SNA measures the angle made by the intersection of lines drawn from the Sella to Nasion and from Nasion to Point A (subspinale). The mean SNA is 82 degrees, so an SNA greater than that suggests a protrusive maxillae while an SNA less than that suggests a retrusive maxillae. SNB measures the angle made by the intersection of lines drawn from the Sella to Nasion and from Nasion to Point B (supramentale). The mean SNB is 80 degrees, so an SNB greater than that suggests a protrusive mandible and an SNB less than that suggests a retrusive mandible. SNA and SNB, therefore, evaluate skeletal protrusion or retrusion relative to the cranial base. The interincisal angle is the angle formed by the intersection of lines drawn along the long axes of the maxillary and mandibular central incisors. The mean interincisal angle is 130 degrees. An interincisal angle less than that reflects protrusive incisors and an angle greater than that reflects retrusive incisors.

The Tweed triangle consists of FMA (the Frankfort-Mandibular plane Angle – the angle formed when the Frankfort horizontal plane intersects with the mandibular plane), FMIA (the Frankfort-Mandibular Incisal Angle – the angle formed when Frankfort horizontal plane intersects the line bisecting the long axis of the mandibular central incisor), and the IMPA (the Incisor-Mandibular Plane Angle – the angle formed by a line that bisects the long axis of the mandibular central incisor and intersects the mandibular plane).

With these measurements, two types of protrusion/retrusion can be determined: skeletal and dental. As noted above, SNA and SNB measure skeletal protrusion/retrusion relative to cranial base. FMA is a skeletal measurement of vertical dimension. Dental protrusion occurs when FMIA is less than normal (60-70 degrees) and/or the interincisal angle is less than 130 degrees. An IMPA greater than normal (85-95 degrees) indicates a protrusion of the mandibular dentition.

Review of the analyses of the 55 cephalograms confirmed that there was no statistically significant change in any of the values during the 232-year period. Therefore, the facial profiles of the sample population quite probably did not change over the time span.

Native American Arikara, Cephalogram, Dentoskeletal Relationship

G34 Correctional Dentistry and Forensic Odontologists: Bridging the Gap

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After attending this presentation, attendees will better understand the careers and challenges of the correctional dental team. There will be an analysis of the demands, need for service, and barriers faced.

This presentation will impact the forensic science community by demonstrating how the United States correctional facilities could better utilize forensic odontologists in the interest of justice.

The United States incarcerates nearly 2.5 million inmates each year. With a substantial rise in substance abuse and criminality, the demands placed on correctional dentists is also increasing exponentially. As a practicing healing branch of medicine, the correctional dentist is faced with the legal and ethical responsibility of providing services to the inmate patient population while maintaining an appropriate standard of care; however, these dental teams are often working in extreme and substandard conditions. Dentists are frequently working with antiquated equipment and many institutions have yet to upgrade to digital radiography and electronic record keeping. There is a lack of consistency in security and medical management at different facilities. The dentists are attempting to work and provide treatment while simultaneously maintaining a sense of safety for themselves and support staff. There are limited, if any, studies available and minimal documentation of the harsh and warlike conditions in which dental care is being rendered. The shortage of dentists and security staff, as well as the marked increase in the level of violence within certain facilities, is creating such an environment. The potential ramifications for the dental provider in regard to the continuous and routine accessibility of the inmate to dangerous shanks and illegal substances will be examined. Institutions that incarcerate mentally ill offenders are faced with an entirely unique subset of challenges. This is setting the stage for risk, both professionally and personally, for the correctional dental providers. There are many medical health organizations bidding on contracts for the correctional health services; however, there is minimal input and/or advocacy from outside government agencies for the correctional dentist (Occupational Safety and Health Administration (OSHA); Board of Dentistry).[1-4]

Forensic odontologists are called upon to assist law enforcement. They are rarely utilized in the correctional system where bitemarks routinely occur. Teeth become the common weapon, both by aggressors and as a line of defense. Officers, health care professionals, and inmates are all affected by this form of violence and victimization. There remains a culture of silence behind the walls. There are documented cases of corruption and abuse within the system, but no expression of the impact and the medicolegal risk that is placed upon the correctional dentist. Case and situational examples will be presented for review and discussion. In the local jail setting, there is generally a much more controlled environment. Yet again, there is increasing criminality and high recidivism. The requests for dental care exceed the funding that many institutions have allocated for dental services. Additionally, the offenders who are housed and released from these facilities are often the future accused, victims, and the missing/unidentified.

Attendees will recognize the expanding need for and challenges faced by correctional dentists. There is a missing link between the correctional dentist/system and forensic odontologist. By bridging the gap between the correctional system and the forensic odontologist, the correctional system can be educated to become integrated as part of the forensic team. This information will challenge the attendees to examine the role they may have in creating professional harmony.

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Corrections, Dentist, Odontologist

G35 An Evaluation of a Multimedia Training Module and Lab for Teaching Radiographic Technique and Safety When Using Hand-Held Portable Dental X-Ray Equipment in Forensic Odontology

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After attending this presentation, attendees will: (1) become familiar with safety protocol when using hand-held portable dental X-ray equipment and radiographic techniques for minimizing errors requiring retake images in the categories of placement, angulation, exposure, and other errors; and, (2) be able to develop teaching strategies to train others for the safe and effective use of hand-held portable dental X-ray equipment in forensic odontology.

This presentation will impact the forensic science community by providing information that can be used for understanding and teaching safe and effective use of hand-held portable dental X-ray equipment in forensic odontology.

Hand-held portable X-ray devices are increasingly used in dental and forensic practice. This development introduces new challenges to radiographers for which original safety and technique evaluations must be made and implemented prior to use.[1] Forensic experts were first introduced to hand-held portable X-ray devices in Thailand following the 2004 Asian Tsunami; these devices proved to be essential to dental identification efforts, particularly in mass fatality events.[2] Currently, there is limited research on teaching safe and effective usage of hand-held portable X-ray devices in forensic odontology. Major issues for safe usage and effective radiographic technique have been outlined in the literature as: difficulties in using aiming devices in conjunction with hand-held portable X-ray equipment, radiographic and angulation techniques for minimizing errors requiring retake images, using infection control and Personal Protection Equipment (PPE), and safeguarding of the operator and persons involved in assisting. These problems could result in safety and manufacturer guideline infractions, as well as deviations from the As Low As Reasonably Achievable (ALARA) principles, when compared with other available intraoral X-ray devices, for example, wall-mount units.[3] Teaching and training with multimedia and using computers to present subject matter with sound, graphics, and video have been used in developing forensic training in dental and dental hygiene curricula.[4-5] Multimedia is specifically recommended in forensic training because it is "interactive" and provides an "active learning" experience.[6-7] Multimedia is used across disciplines because it has been found to support the way the human brain learns.[6-7]

For this study, researchers will use a multimedia module with video demonstrations to teach radiographic technique and safety when using hand-held portable X-ray equipment. This module will be made available via a Blackboard University learning system. Forty junior dental hygiene students (participants) enrolled in an Oral Radiology I course will be recruited for the study; researchers will submit applications for Institutional Review Board (IRB) approval. Participants will be required to view the multimedia demonstration video prior to completion of a radiology lab where in-person demonstrations will occur. Participants will take identical sets of X-rays using both a portable hand-held X-ray unit and a wall-mounted X-ray unit. Real human skulls and a standard image receptor holding device will be used to test radiographic technique between the two types of equipment (portable hand-held X-ray device versus a wall-mounted X-ray unit). Participants (N=40) will expose six X-rays per type of equipment for a total of 480 X-rays to be scored in these four radiographic technique subcategories: placement, angulation, exposure, and miscellaneous or other errors. Analysis of Variance (ANOVA) will be used to compare the sum of the errors for each device (portable hand-held device versus a wall-mounted unit). T-test analysis will be used to determine significant differences between the subcategories of each error category. Safety will be measured by a 12-question post-test related to infection control and PPE, ALARA guidelines, and safeguarding of the operator and persons involved in assisting when using portable hand-held X-ray devices. Scores will be presented as grade percentages.

It is hypothesized that a significant difference will be found at the .05 level between the two types of equipment used for radiation exposure (portable hand-held X-ray unit versus a wall-mounted unit), with the portable hand-held X-ray device unit having the least amount of total errors and higher scores on the safety post-test.

This research could impact the forensic science community by providing data on comparing safety and technique exposure from traditional wall-mounted X-ray units to the use of hand-held X-ray devices and as a way to address common radiographic technique errors specific to forensic odontology.

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Education, Radiation Safety, Radiation Technique

G36 The Integration of Forensic Dentistry/Catastrophe Preparedness Course Into a Dental Hygiene Bachelor of Science Program Using the American Board of Forensic Odontology (ABFO) Curriculum Guidelines: A 12-Year Study

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After attending this presentation, attendees will recognize the value of incorporating forensic classes in pre-licensure dental and dental hygiene curriculum. This presentation will explain the contents for the implementation of the scope of the course, interpret the data as a favorable indication for incorporation in education, and describe the survey results from professionals who have completed the courses.

This presentation will impact the forensic science community by demonstrating the value of the incorporation of the ABFO Guidelines on Forensic Odontology into dental and dental hygiene programs. Attendees will come to value the incorporation of the Catastrophe Preparedness curriculum in course offerings and appreciate the effects these courses have on future interest and participation by dentists and dental hygienists.

A Forensic Odontology/Catastrophe Preparedness course is uncommon in dental and dental hygiene program curricula. The addition of these types of courses has the potential to raise professional awareness and effect future participation in forensic dentistry for new dental professionals and the discipline as a whole. Core Content of Forensic Odontology courses are in place and published by the ABFO. Using these as a guideline, a Forensic Dentistry/Catastrophe Preparedness course is offered at New York University College of Dentistry. This study cumulates 12 years of surveys taken by dental hygiene students upon completion of the Forensic Dentistry/Catastrophe Preparedness from the years 2005 to 2016. Surveys were completed by 85 dental hygiene students who completed the course. It sought to measure students' perceptions of gaining advanced knowledge in core content recommended by the ABFO and courses offered by the National Center for Disaster Preparedness housed at Columbia University in New York for the preparedness modules. The Catastrophe Preparedness Modules are based on the center's competencies and are designed to gain core knowledge for preparedness and response for disasters at work, at home, and in the community.

The survey consisted of 18 questions with multiple-choice answers. A total of 85 students completed the surveys, which represents 100% participation. All of the students willingly participated each year. There were no identifiers nor compensation for participation or consequences for refusal to participate. The survey asked questions concerning gaining knowledge from each of the modules. The survey also sought to relate the reasons for students taking the course and if it affected their future plans to seek further education and participation in forensic dentistry and catastrophe preparedness.

A descriptive analysis was completed and the surveys indicate significant acceptance and satisfaction with the course objectives, content, and experiences. Fifty-nine percent of the students stated they are interested in furthering this type of education. Fifty-two percent stated they planned to join a forensic team and 35 percent stated they plan to join a reserve corps for preparedness and response. Ninety-five percent stated they were prepared in the event of a bioterrorism episode. This study found that students are accepting in expanding their knowledge and experience in this field of study.

From this study, it is clear that the published guidelines for core content for both forensic odontology and catastrophe preparedness in these courses should be a model for the educational experiences and that these educational experiences should be incorporated as essential information for future dental professionals. The results also support the interest and potential for future involvement in the science by students who completed the course. The forensic community will come to value the incorporation of ABFO guidelines on forensic odontology into dental and dental hygiene programs and the effects these courses have on future interest and participation. The incorporation of Forensic Dentistry/Catastrophe Preparedness courses in dental and dental hygiene curricula should become more widespread.

Forensic Dentistry, Dental Hygiene Curriculum, Catastrophe Preparedness

G37 Differential Identification of Three Young House Fire Victims: Methods When Statistics Fail

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After attending this presentation, attendees will be able to recognize how differential analysis of tooth maturity scores in dental age assessment can be used to graphically distinguish between child victims who are statistically the same age. Attendees will also be able to appreciate how radiographic and clinical presentation in childhood identical twins can elicit genetic versus acquired similarities and differences and can be used for exclusion of other victims.

This presentation will impact the forensic science community by discussing how forensic odontology provided a method to aid in the identification of three children, 4.4-year-old identical twins and a third unrelated 4.1-year-old child.

In July 2014, a fire consumed an urban row home in Philadelphia, PA. Four children perished in the fire. One of the victims was an infant and was identified by exclusion.

The medical examiner requested that odontology be employed to compare age estimations and dental uniqueness for the three remaining victims in order to corroborate the correct identity of each. This analysis was aided by the fact that two of the victims were presumed to be female identical twins with a birth date of January 25, 2010 (4 years, 5 months of age at death). For reference, they were designated as "Child A" and "Child B" in this study. The third child was male and had a birth date of May 24, 2010 (4 years, 1 month of age at death) and was designated as "Child C." All three victims presumably had never had professional dental care, so antemortem/postmortem comparisons were not possible.

All three were badly charred, with intact craniofacial structures. Soft tissue resection was accomplished. A full series of radiographs, as well as photographic images, were made on each case.

In comparison of the radiographs obtained to a published graphical standard of dental development, the average maturation of tooth buds, calcification of tooth structure, and eruption pattern indicated an age estimation of 4.5 years (±.58 year) for "Child A" and "Child B".[1] According to the same standard, "Child C" presented with a slightly different estimation of 4.0 years (±.52 year). These estimates via this atlas method were therefore not significantly different from each other.

Staging analysis was then performed on the three sets of radiographs, utilizing 14 permanent teeth, utilizing the unknown sex data set described by Moorees et. al.[2] As expected, the mean average dental ages of the three victims were also not statistically different at 95% confidence; however, a box plot of the 14 individual maturity scores clearly showed the difference between the two identical twins and the third unrelated child. "Child A" and "Child B" shared a strikingly similar growth pattern in their dentition as seen on the radiographs. Both cases exhibit a permanent dentition with the same stage of growth on each tooth, with one exception (tooth #19). Of particular interest is the same delayed calcification of the lower second bicuspids, as compared to the advanced maturation stage of the permanent lower first molars. Based on this analysis, it was determined that "Child C" was, in fact, the non-twin victim and thus positively identified through exclusion.

Mostly due to the lack of antemortem evidence, positive identification between the two twins was not possible; however, it is of academic interest that there existed distinct acquired and developmental differences between them. Both twins exhibited evidence of a finger-sucking or pacifier habit with pre-maxillary protrusion, palatal constriction, and anterior open bite; however, the altered growth was more pronounced in "Child A" with an 8mm anterior open bite versus "Child B" with only a 5mm anterior open bite. Also, "Child A" had an erupted #19 clinically and "Child B" did not. Further, "Child A" presented with slightly advanced eruption of #25 compared to #24, whereas this was reversed in "Child B." Lastly, "Child B" exhibited decay on two primary teeth whereas "Child A' appeared caries-free.

In conclusion, this case demonstrated that individual tooth staging can be useful when the difference in average mean dental age of victims who are close in actual age is not statistically significant. Further, it was shown that even identical twins can have definable differences in dental appearance.

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Maturity Score, Dental Staging, Twins

G38 Dental Forensic Identification Information: How to "Get" What We Require

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After attending this presentation, attendees will have had a thorough review and new information concerning: (1) the challenges and obstacles presented to the forensic odontologist in obtaining requested information from medical examiner investigators, as well as the victim's family dentist; (2) how to recognize and evaluate inaccurate and improper data submissions from the medical examiner investigator and/or the victim's dentist; (3) protocol for improving communications between the victim's dental office and the forensic investigator (this protocol will benefit in circumventing dental office objections and assist those requesting the information to receive the data required, accurate, and in the proper format to achieve the desired identification); and, (4) protocol for improving communications between the medical examiner forensic investigator and the inquiring forensic dentist. A newly designed informational worksheet will assist the medical examiner forensic investigator when interacting with the victim's dental office and handling objections, thus receiving the correct data for presentation to the forensic dental investigator.

This presentation will impact the forensic science community by enabling the forensic dentist, through organized interaction systems with the dental office and medical examiner investigator, to acquire precise and accurate victim data to achieve correct and timely victim identification.

One of the most frustrating things for investigating odontologists is not receiving the requested information that is necessary to perform investigations efficiently and correctly. Information that is received is often inaccurate, incomplete, non-legible, or labeled incorrectly. The many forms of inaccurate submissions making evaluation difficult will be identified and discussed. Revelations with respect to the Health Insurance Portability and Accountability Act (HIPAA) objections will be presented as well.

Victims' dental offices and even many medical examiner investigators are unfamiliar with how to present and format information that the forensic dentist requires. Dental offices and medical investigators do not understand how to digitize radiographs and paper records for presentation. Four to five cases will be presented demonstrating illegible records, mislabeled records, confusion as to what records were submitted vs. what were requested, and HIPAA objections. Specific repeated areas of difficulties will be identified.

This will be followed by: (1) HIPAA objections and solutions; (2) techniques to discuss with the victim's dental office as well as the medical examiner investigator regarding how to digitize records for proper presentation and evaluation; and, (3) a worksheet designed for the medical examiner investigator to standardize what to request and how to request it.

The goal of this presentation is to assist and upgrade communication between the investigating forensic dentist, the medical investigator, and the victim's dentist. Thus, information transfer will be what the forensic dentist requires. This will avoid the difficulty of back-and-forth requests between parties and the waste precious time … time that will delay the goal of the investigating forensic dentist for successful completion of the identification process.

Dental Identification, Medical Examiner, HIPAA

G39 Postmortem Human Identification Challenges Regarding Domestic Disappearance and the Health Insurance Portability and Accountability Act (HIPAA)

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The goals of this presentation are to: (1) identify and discuss the importance of the HIPAA Exception Law; (2) summarize the importance of forensic odontology in the postmortem identification process of partnering in an ongoing police investigation; (3) apply a better understanding of HIPAA Exception Law to specific situations involving domestic disappearance and law enforcement investigations; and, (4) examine the challenges of requesting antemortem dental records from health care providers and public/private insurance companies.

This presentation will impact the forensic science community by exploring how the postmortem identification process is delayed and confounded when requests for dental records go unfulfilled or the dental records are reportedly not available due to failure to understand HIPAA Exception Law.

The identification of missing or unknown persons is the most common role of the forensic odontologist.[1] Postmortem dental remains can be compared with antemortem dental records, including written notes, study casts, and radiographs, to confirm identity.[2] Individuals with numerous and complex dental treatments are often easier to identify than those individuals with little or no restorative treatment.

The HIPAA of 1996 established the privacy rule standards that address the use and disclosure of individuals' health information by organizations. The privacy rule applies to health plans, health care clearinghouses, and to any health care provider who transmits health information in electronic form.[3] Within HIPAA is exception law. It permits, but does not require, the covered entity or health care provider to use and disclose protected health information, without an individual's authorization, for the following purposes or situations: (1) to the individual (unless required for access or accounting of disclosures); (2) treatment, payment, and health care operations; (3) opportunity to agree or object; (4) incident to an otherwise permitted use and disclosure; (5) public interest and benefit activities; and, (6) limited data set for the purposes of research, public health, or health care operations.[3]

A major goal of the privacy rule is to assure that individuals' health information is properly protected while allowing the flow of health information needed to provide and promote high-quality health care and to protect the public's health and well-being. The privacy rule permits use and disclosure of protected health information, without an individual's authorization or permission for law enforcement purposes and decedents.[3] Covered entities may disclose protected health information to law enforcement officials for purposes that include, but are not limited to: (1) as required by law (including court orders, court-ordered warrants, subpoenas) and administrative requests; (2) to identify or locate a missing person; and, (3) in response to a law enforcement official's request for information about a victim or suspected victim of a crime.[3] In addition, covered entities may disclose protected health information to coroners or medical examiners to identify a deceased person, determine the cause of death, and perform other functions authorized by law. The dental identification process must be carefully undertaken and relies on the comparison of information from the antemortem record with findings from the postmortem examination, and the efficiency of this process is dependent on the quality and availability of the dental record.[1]

This presentation will explore how the postmortem identification process is delayed and confounded when requests for dental records go unfulfilled or the dental records are reportedly not available due to failure to understand HIPAA Exception Law.

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HIPAA, Forensic Odontology, Domestic Disappearance

G40 Use of the Radiographic Positioning Device Holder in the Postmortem Dental Examination

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After attending this presentation, attendees will better understand why it is practical and desirable to implement the use of the radiographic positioning device holder in the postmortem forensic dental examination.

This presentation will impact the forensic science community by introducing the radiographic positioning device holder and demonstrating the advantages of using the holder during the postmortem dental examination.

In the past, traditional dental intraoral radiographic positioning devices have not routinely been used in the postmortem dental examination. Typical postmortem dental X-ray positioning devices have included items such as gauze, wet paper towels, putty, wax, surgical towels, and hemostats. Radiographic positioning devices that are currently manufactured for antemortem dental radiography have not transitioned into widespread use in the postmortem dental setting because these devices are designed to be held in place by the patient, who either bites on the device or holds the device by hand.

With the development of the radiographic positioning device holder, X-ray positioning devices that are currently manufactured for antemortem bite-wing and periapical dental radiography can easily be utilized during postmortem dental radiography. The holder is practical because it accommodates both traditional film and digital sensor positioning devices. The holder can also accommodate radiographic positioning devices that are designed for use with the paralleling technique or the bisecting angle technique.

When antemortem radiographs are available during the postmortem dental radiographic examination, it is desirable to take the postmortem radiographs from an angle that most closely replicates the antemortem radiograph. When utilizing the holder during digital radiography, the holder retains the position of the sensor while the image is viewed. From this fixed point of reference, any necessary angular adjustments can be made so the postmortem radiograph most accurately replicates the antemortem radiograph.

Use of the radiographic positioning device is desirable because it potentiates adherence to the As Low As Reasonably Achievable (ALARA) radiation safety principle. While the ALARA principle ceases to be relevant to the decedent, it remains a radiation safety principle that is applicable to dental radiographers in the postmortem setting.

The radiographic positioning device holder can be useful during the postmortem dental examination for purposes other than radiography. The holder can accommodate a scale or an identification label during postmortem photography. The holder can also function as a backdrop stand.

In conclusion, this presentation demonstrates that use of the radiographic positioning device holder is practical and desirable in the postmortem dental examination. The holder facilitates the adaptation of traditional dental positioning devices for use during postmortem dental radiography while facilitating adherence to the ALARA principle. The holder is also useful during postmortem photography.

Postmortem, Dental, Holder

G41 Identification of a Child Using Comparative Overlays of Primary and Permanent Dentition

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The goal of this presentation is to highlight the challenges facing forensic odontologists when making a dental identification of children and young adults. In the events surrounding this case, the Antemortem (AM) radiographs and Postmortem (PM) radiographs displayed very little similarity in that one had mostly primary dentition and the other permanent dentition. This presentation will provide the odontology community with the following objectives: (1) methods of overlaying images to compare developing permanent teeth to erupted teeth radiographically; (2) understanding the importance of collaboration with a team of dentists when a positive identification is not obvious; (3) using age estimation methods as well as comparative analysis to collect several areas of concordance to have a positive identification; and, (4) the protocol for child abuse management within one's community.

This presentation will impact the forensic science community by informing attendees that dental identifications can be made using somewhat non-traditional methods that are quick and effective. This will also bring community awareness regarding the potential shortfalls of an agency such as Child Protective Services and/or school districts to properly report and handle child abuse cases.

Among the various methods used to identify an unknown decedent, identification using dental data is typically quick and definitive; however, in rare instances, traditional methods for dental identification (comparing missing/existing teeth and dental restorations) cannot be utilized easily due to AM radiographs preceding PM radiographs by a long interval of time when a child is undergoing physiological changes of growth.

On April 25, 2017, two men discovered the skeletal remains of an adolescent child in an east valley desert lot in Las Vegas, NV.[1] The body was confirmed by the Clark County Coroner's Office as a young adult, and the coroner called upon the team's forensic odontology section to begin dental charting right away. At the same time, the investigative team acquired the dental records of a boy who went missing from his home in late January 2017. The family and medical examiners were eager to see if this was a match — as always, unknown children's cases are expedited and processed with urgency. The AM radiographs revealed a young child in mixed dentition with only permanent first molars and lower four incisors erupted with all roots still developing. The PM radiographs of the adolescent decedent were all of permanent dentition, and neither set of radiographs nor the clinical evaluation of the remains revealed any evidence of restorations.

This posed a challenge for the odontologists, and initially it was believed that a conclusive finding could not be made; however, upon further investigation and collaboration with one another, they were able to use the Mideo Systems software overlay system to compare several structures of the permanent dentition on the AM radiographs. Also, they overlaid the developing permanent teeth (still in the follicles during development) in the AM radiographs onto the PM radiographs and were able to find consistent anatomical structures in the overlays. By having several areas of concordance, the odontology team was able to make a positive ID quickly. Sadly, the findings of the rest of the investigation were not as celebratory.

It was discovered that this 13-year-old boy's killer was none other than his father. Due to the ability to make a quick ID, law enforcement was able to make the arrest in a timely fashion and bring the suspect to justice. As the story unfolded, it was discovered that this boy was no stranger to Child Protective Services, as he was taken and given back several times in his young life. This case is not only a story of identification using somewhat non-traditional methods, but it is a case of neglect from his family, his school system, and the state agency.

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Abuse, Child, Primary

G42 Back to Nice — July 2016: The Dental Identification Team's Role in the Disaster Victim Identification (DVI) Mission of the Terrorist Truck Attack in France

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After attending this presentation, attendees will better understand how the French Forensic Team identified the victims killed by the terrorist truck attack on July 14, 2016, in Nice, France, and what outcomes were found in terms of forensic odontology.

This presentation will impact the forensic science community by demonstrating the method used and the new organizational procedures conducted over three days to identify the 86 victims of 16 different nationalities on a public holiday in Nice, France.

On the evening of July 14, 2016, a 19-ton truck smashed into a packed crowd of people gathered to celebrate Bastille Day on the Promenade des Anglais, the famous seaside walk in the French Riviera city of Nice, France. This attack was claimed by ISIS. In this case, the terrorist killed 83 people and injured more than 400 people before being shot dead by the police. After a few days, three more victims died of their wounds. In this terrorist attack, a total of 86 victims were killed, including 17 children and 24 foreign victims of 16 different nationalities.

Emergency measures were immediately instituted, employing two separate protocols: the first was dedicated to the identification process with a Disaster Victim Identification (DVI) procedure, while the second was focused on the autopsy. A well-staffed forensic odontology team was immediately set up, including ten French odontologists from all over the country. For three days, the odontologists worked non-stop, with two teams working in parallel.

The dental Antemortem (AM) team worked within the global AM cell, in close collaboration with the French Forensic Police, the National Gendarmerie within the Unité Nationale d'Identification des Victimes de Catastrophe (UNIVC), the International Criminal Police Organization (INTERPOL), and other independent forensic experts. These teams collected antemortem data of missing people in a facility located in the city center of Nice, accommodating an exceptional venue to investigate, interview, and support the victims' families.

The dental postmortem team worked non-stop in the Forensic Institute of Nice in pairs examining decedents to improve the accuracy of the records and reduce the risk of error due to operator fatigue. This team also examined the unconscious and unknown victims' dentition in the intensive care department of Nice hospital.

Both antemortem and postmortem teams used INTERPOL forms to record data. The two teams worked together to compare and establish the identification of victims based on the dental records of missing people and unknown deceased victims.

Every day, one to two identification commissions, including forensic experts, were organized as new matches were found. Among these experts, one dentist was present in each commission to submit the dental findings. A total of six commissions took place, allowing the identification of all dead and unconscious victims.

The excellent outcomes found during the identification of the victims in Nice reflected the efficient collaboration between the different sections of the DVI unit and the forensic odontology team. The dental identification process played an important role in this organization.

The method and outcomes of this identification process will be explained in this presentation. The difficulties and means implemented to counteract these problems will also be highlighted.

Disaster Victim Identification, Terrorist Attack, Forensic Odontology

G43 The Accuracy of Dental Identification of Adults With Unrestored Teeth by Visual Comparison With Radiographs of Mixed Dentition

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After attending this presentation, attendees will better understand the forensic identification process by dental means. Attendees will learn that in a case of unrestored dentition with a long time lapse between Antemortem (AM) and Postmortem (PM) data, visual comparison of dental radiographs is an adequate method.

This presentation will impact the forensic science community by confirming the value of dental radiographs in identification, which should not be restricted to the analysis of the dentition only. On the contrary, all visible cranio-facial structures and bone characteristics surrounding the teeth should be attentively compared.

Nineteen examiners from the United Kingdom, Canada, the United States, Australia, Brazil, Italy, Iceland, Mauritius, and Mexico (15 forensic dentists and 4 forensic anthropologists) participated in a web-based questionnaire to assess identification in 12 cases; each case required the radiographic comparison of one dental PM panoramic radiograph to three dental AM panoramic radiographs belonging to three different individuals, of which only one was the correct match. The examiners were given four options following the American Board of Forensic Odontology (ABFO) guidelines: established identification, possible identification, insufficient data, or exclusion; the observers also explained the reason for each of their conclusions. The radiographic samples were provided by Italian private clinics; the time lapse between the correct AM and PM radiographs varied from 3 to 18 years.

The total of 684 answers were analyzed. The sensitivity of the methodology was 53.5%, the specificity was 86.4%, and the accuracy was 75.4%. This suggested that excluding the incorrect individuals was easier than matching the correct radiographs; however, achieving a positive identification more than 50% of the time should be considered a satisfactory result, considering that only half the panoramic was compared and no background of the subjects was provided. Examiners with sensitivity higher than 80% primarily compared dental anatomy, particularly root and crown morphology, and primarily analyzed incisors and first molars; approximately 41% of the answers also quoted the maxillary sinus morphology or other bone characteristics, such as trabeculation and mental foramen. Anatomy and development of third molars represented an important misleading feature for identification, which was chosen by most examiners with sensitivity lower than 80%; additionally, the comparison of non-dental features dropped significantly.

Dental identification is not an easy task and hasty conclusions may have catastrophic consequences, such as the exclusion of the correct individual and the misidentification of the wrong person. Even in cases with a long interval between AM and PM radiographs and unrestored dentitions, the meticulous attention to anatomical features helped reaching a conclusion. A constant examination of non-dental features would have probably reduced the error rate. This process requires time, knowledge of anatomy, embryology, and development and aging modifications, not only of the dentition, but of all cranio-facial structures; the accuracy of assessment could be improved by specific training.

Radiographs, Identification, Unrestored Dentition

G44 Active Participation of the United Arab Emirates' Disaster Victim Identification Team Using Dental Identification

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After attending this presentation, attendees will be informed regarding the active participation of United Arab Emirates' (UAE) Disaster Victim Identification (DVI) Team in dental identification of the Air Asia QZ8501 airplane crash victims in Indonesia.

This presentation will impact the forensic science community by demonstrating the international contribution of the UAE DVI Team in general, and forensic dentists in particular, under the International Criminal Police Organization (INTERPOL) umbrella.

Human dental identification plays a key role in DVI because it is considered a prime identifier. It is used as a single identification procedure (using WinID™ version 3) or integrated in multidisciplinary procedures (DVI System International version 5); however, Deoxyribonucleic Acid (DNA) and fingerprint analyses are also identifying techniques well practiced in the UAE.

Although a global increase of unexpected large-scale accidents such as transport crashes, war explosions, and terrorist attacks cause an increasing number of unidentified or/and missing persons, only a few situations mandated a forensic odontologist involvement in the UAE.

A major disaster occurred in 1983 in the desert of Abu Dhabi. A Boeing® 737-2P6 from Gulf Air crashed and 112 victims were found. The 26 children among the victims were investigated by dental experts from the United Kingdom. One of the main obstacles that prevented confirmation of the identity of all victims was the lack of antemortem dental data from passengers with an Indian and Pakistani nationality.

In 2004, a Kish Fokker 50 airplane crashed in Sharjah, resulting in 45 victims. Dentists from the Dubai Health Authority were assigned to conduct the dental identification without previous related knowledge, training, or preparedness. It resulted in confusion and delayed identification.

Both of these aforementioned disaster identification interventions highlighted the need to establish a well-prepared UAE human identification team. In 2010, the UAE DVI team was established. The official certificate accession of the UAE as a member of the Steering Group of INTERPOL for DVI was obtained in 2011. The UAE became the first Arabic country member of the International Organization for Forensic Odonto-Stomatology (IOFOS) in 2011. Since 2014, the UAE has had a forensic odontological participant in the Scientific Working Group on Quality Assurance in the INTERPOL Forensic Odontology Section, contributing to updating the INTERPOL dental forms.

In 2015, the first active participation of the UAE DVI with the specific involvement of forensic odontologists was required for the Air Asia QZ8501 airplane crash in Indonesia. Eight forensic odontologists from six countries participated in the identification of 162 victims. Forty-two percent of the victims were identified based on dental methods and 37% with combined techniques. UAE had no countrymen victims in the air crash, but UAE forensic dentists had the ethical obligation to share their scientific approach for identification after the request of the Indonesian DVI team.

Forensic Odontology, Disaster Victim Identification, Human Dental Identification

G45 A Study of Morphological Patterns of Lip Prints in Relation to Gender and Blood Groups Among the Egyptian Population

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After attending this presentation, attendees will understand the study of the distribution of different lip print patterns among large Egyptian samples in Greater Cairo to ascertain whether there was a correlation between lip print characteristics, blood groups, and gender of the studied population.

This presentation will impact the forensic science community by explaining how lip print patterns assisted in the identification of Egyptians.

Background: Dental, fingerprint, and DNA comparisons are considered the most common techniques to obtain fast and secure personal identification. The public awareness of fingerprints is very high so there are usually deliberate attempts to not leave fingerprints at a crime scene. For this reason, among others, there is still an increasing need for reliable alternative methods of establishing identity. The lip-print study (cheiloscopy) is considered a new tool for human identification in both civil and criminal issues.

Objective: The present work was conducted to study the distribution of different lip print patterns among a large Egyptian sample in Greater Cairo and to ascertain whether there was a correlation between lip print characteristics, blood groups, and gender of the studied population.

Methodology: This study included a total of 1,000 subjects, including 640 females and 360 males ranging in age from 12 to 75 years, with 15 sets of identical twins, and 34 families of Egyptian residents in the Greater Cairo area.

Method of Lip Print Recording: Red or brown, non-glossy, non-persistent, non-metallic lipstick was used to obtain clear lip prints. White paper (white A4 ROCO Premium 80g copy paper) and tissue paper (Kleenex®) were used to obtain the impressions as per Aggrawal.[1] Blood groups were recorded from subjects who were aware of their blood groups.

Analysis of the Lip Print: The lip prints were analyzed based on Renaud's classification.[2]

Results: Throughout the entire study, no identically similar lip print pattern appeared in two subjects; however, many subjects displayed the same groove types in the same areas of the lip, but specificity of the groove pattern was evident in either the site or the direction of branching or reticulation of the groove. The groove type A (complete vertical) was the highest recorded pattern in both males and females (47.9% and 49.7%, respectively), followed by groove type C (complete bifurcation), which represented 43.8% of the Upper Left (UL) area in males and 45.4% of the Lower Right (LR) area in females, while groove type G (reticular type) represented 27.0% and 24.4% of the Upper Middle (UM) area in both males and females, respectively. This was followed by groove type E (complete branched), which constituted 25.0% of the Lower Left (LL) area in males and 16.9% of the Lower Middle (LM) area in females. Groove type D (incomplete bifurcated) represented 15.3% of the Upper Right (UR) area in males and 19.5% of the UL area in females, followed by groove type B (incomplete vertical), which constituted 13.0% and 13.6% in the UR areas in males and females, respectively.

As for the distribution of lip print types in relation to blood groups, the current results revealed that the frequency of lip print type F (incomplete branched), B, and E were the highest detected patterns among individuals with blood group A positive. Distribution of types I (horizontal), H (x-shaped), and G (reticular) were the most frequently recorded patterns among individuals with blood group B positive. Moreover, lip print types I and G were the most frequently detected patterns among individuals with blood group AB positive. Lip prints type J (other forms), H, and G revealed increased expression among individuals with blood group O positive.

Conclusion: This study confirms the uniqueness of lip print patterns for every participant, even twins and family relatives, supporting a role for cheiloscopy in the identification process, both in civil and criminal issues, but also disproves any significant statistical correlation of lip print pattern with gender and ABO blood groups. It is recommended that more studies be conducted on different population groups to analyze these variations and to establish a database to be used as a reference in criminal investigations in addition to the provision of technical and financial support to analyze in-depth the correlation between lip prints and ABO blood groups. Also, a newer computer software technique needs to be developed to assess and accurately detect lip print characteristics.

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Lip Print, Blood Groups, Egyptian

G46 Collecting Antemortem Dental Data

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After attending this presentation, attendees will better understand the importance of and polymorphism in antemortem dental data.

This presentation will impact the forensic science community by illustrating the different forms antemortem dental data can take, how to collect and transmit this data, what problems may arise in this process, and possible improvements.

Whether in individual or mass casualty cases, the quality of antemortem dental data is crucial for success in the identification process.

The first problem encountered with an unidentified body is learning the deceased's supposed identity; in the case of a mass disaster, making a list of all the presumed deceased persons is crucial. A missing persons' dental records database may exist in certain states, but there is no real uniformity. The problem of identifying migrants, whose purported identity may be falsified and whose actual dental records may be very difficult to obtain, also arises. Over these past few years, and especially in the case of terrorist attacks, social networks have been used to announce calls for help, and missing persons apps for smart phones have been created. In the case of Disaster Victim Identification (DVI), the International Criminal Police Organization's (INTERPOL's) protocol for collecting antemortem dental data is followed. Several questions about this data collection must be detailed: Where? When? By whom? For whom? How? What? The process of collecting these data must be exhaustive and must try to focus on one sole investigator to contact the family of the deceased. Despite our computerized culture, neither national nor international dental databases exist. Collection must be conducted for each victim, in each country, and attempts must be made to contact the dentist(s) involved.

During data collections, forensic dentists have often had to be very resourceful and imaginative in seeking out dental records. It is sometimes difficult to find the victim's dentist, and these tasks often take on the feel of a true investigation with a race against the clock. This may be the case for those victims who have not been to a dentist, those who have moved residences, and those involved in disasters involving entire families — in this case, DNA problems may also arise — or in disaster scenarios, such as Hurricane Katrina in the United States, where many medical records were destroyed. In case the forensic team is unable to find the victim's dentist, then insurance company, health, mutual, or military records may be consulted.

As opposed to postmortem data, which are systematized (e.g., systematic numbering, photographs, radiographs, odontograms), antemortem data take wide and varying forms. Antemortem data may include family testimony, practitioner's testimony, dental records, odontograms, and radiographs of varying forms, as well as personal or professional photographs, casts, quotes, prostheses, implants, surgical material, letters (professional correspondence), and, more rarely, facial reconstruction.

Once these data have been transmitted, a pair of forensic dentists analyze and transcribe the data into an odontogram that is then completed and verified by analyzing radiographs, which are an essential identification element. Practical cases will help to illustrate this data collection process.

Even if the victim's dentist is identified, other problems may arise. Although rare, physicians may refuse to share data; more often, computer system changes or a lack of data backups may impede access to past data. Files may also have been damaged in a fire, flood, or another natural disaster. Computer problems may also make data transmission challenging.

In addition to the technical elements, the psychological aspect of these investigations must not be overlooked. The investigating forensic dentist contacting the victim's dentist may be the first harbinger of bad news. This work is conducted in a group of two within the odontological team, which is itself incorporated into the antemortem team, where sometimes victims' families are received. Communication and support among team members is fundamental, and third-party psychological support may be provided.

In order to improve the quality of future antemortem data, dentists and future dentists must be informed regarding how important it is to safeguard proper records and radiographs. The creation of a national database of missing persons could facilitate comparisons with the data recovered from non-identified bodies. A standardized procedure and an exhaustive investigation must be conducted to allow exchange of information among different countries, transparency, traceability, and quality control. The quality of our work in collecting antemortem dental data is essential to achieve a positive dental identification.

Antemortem Data, Identification, Dental

H1 A Rare Presentation of Alexander Disease

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After attending this presentation, attendees will better understand Alexander disease, a rare leukodystrophy, and its presentation.

This presentation will impact the forensic science community by increasing attendees' understanding of Alexander disease and the importance of a thorough neuropathologic evaluation in cases of anoxic encephalopathy.

Introduction: Alexander disease is an extremely rare, usually progressive and fatal, neurological disorder.[1] Initially, it was detected most often during infancy or early childhood, but as better diagnostic tools have become available, it has been found to occur with similar frequency at all stages of life.[1] Approximately 95% of Alexander disease cases are caused by mutations in a gene named *GFAP* for a structural protein called glial fibrillary acidic protein that is found exclusively in astrocytes in the central nervous system.[1] Alexander disease has been estimated to occur at a frequency of approximately one in one million births.[1] There are three forms of Alexander disease: infantile, juvenile, and adult. Juvenile Alexander disease is characterized by difficulty in talking and swallowing and the inability to cough.[2] There can also be weakness and spasticity of the extremities, particularly the legs.[2] Unlike the infantile form of the disease, mental ability and head size may be normal.[2] Survival can extend several years following the onset of symptoms, with occasional longer survival into middle age.[2] The course of the disease may involve signs of swallowing or speech difficulty, vomiting, ataxia, and/or spasticity and kyphoscoliosis can occur.[2] The most striking neuropathological feature is the diffuse presence of eosinophilic fibrinoid bodies in the cytoplasm of fibrillary astrocytes.

Material and Methods: The decedent was a 14-year-old girl with a history of narcolepsy, sleep apnea, and scoliosis. Eighteen months prior to her death, the decedent and several others were playing in a backyard pool. When the decedent did not resurface, one of the children notified the supervising adult, who pulled her out of the pool. She was transported to a local hospital and recovered; however, she began to display signs of global developmental delay with onset of impulse control issues, anxiety, depression, self-mutilating behaviors, dysphagia with bouts of aspiration pneumonia, and ataxia. Two weeks prior to her death, she was hospitalized for self-mutilation and her condition progressed to her refusing to eat, talk, or get out of bed. She was also found to be bradycardic and hypothermic. She became unresponsive and was found to have worsening cerebral edema with cerebellar tonsillar herniation.

Results: At autopsy, she was found to have cerebral edema and scoliosis of the thoracic spine. Neuropathologic consultation revealed a non-perfused, respirator-type, macerated brain and spinal cord with diffuse collections of eosinophilic fibrinoid bodies (Rosenthal fibers) in all sections.

Conclusion: The decedent experienced a near-drowning episode and was diagnosed with anoxic encephalopathy when she was 12 years old. Afterward, she experienced a myriad of psychiatric and neurologic issues that were determined to be sequelae of anoxic encephalopathy. A Magnetic Resonance Imaging (MRI) of her brain revealed atrophy of the cervical spinal cord and periventricular white matter changes, which can be seen in both Alexander disease and anoxic encephalopathy. She became unresponsive in the hospital and care was withdrawn after the girl had been on a respirator for multiple days. At autopsy, she had a non-perfused, macerated brain and spinal cord, which is a known complication of being on a respirator. Her brain and spinal cord were submitted for neuropathologic evaluation, which revealed Alexander disease. The decedent experienced onset of the disease at approximately 12 years of age, which unfortunately coincided with a near-drowning event with resultant anoxic encephalopathy, thus confounding her clinical course. This case highlights the importance of performing a thorough neuropathologic evaluation when necessary, even when it is presumed to be of little informative value because of autolytic changes in the brain.

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Alexander, Leukodystrophy, Anoxic

H2 Adrenal Gland Changes in Relation to the Cause of Death

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After attending this presentation, attendees will understand the importance of improving knowledge regarding adrenal gland involvement in response to stress related to the death process and their changes in relation to cause of death.

This presentation will impact the forensic science community by highlighting: (1) whether the adrenal gland is undergoing structural and/or molecular changes in relation to different causes of death; (2) if these changes have significant differences; and, (3) if it is possible to create a timeline of the death process resulting in more or less prolonged pain.

The adrenal response to stress occurs in a syndrome that reflects activation of the sympathoadrenal system and the Hypothalamic-Pituitary-Adrenocortical (HPA) axis, and a "stress syndrome" maintains homeostasis in emergencies, such as "fight or flight" situations. One of the principal tissues involved in the stress response is the adrenal gland; in fact, there is clear evidence of fasciculata activation with the release of cortisol and the release of adrenaline from the medulla.

The literature suggests that the biochemical analyses of catecholamines may be useful markers for investigating various stress responses in the process of death involving bleeding, burns, cold exposure, physical hyperactivity, or drug abuse; this is possible when these markers can be used in combination with other chemical and immunohistochemical markers; however, in postmortem investigation, catecholamines have been considered rather unstable markers for investigating the cause or process of death due to pain, terminal medical care, and postmortem interference.[1]

This study sought an adrenal tissue marker that was involved in the stress response process with special reference to activated/stimulated receptors by the activation of the sympatho-adrenal system and HPA. The β2-AR (adrenergic receptor) was chosen because stress promotes the release of epinephrine, a catecholamine stress hormone that binds to β(2)-adrenergic receptors (β(2)ARs) with high affinity.

Cases with several causes of death were selected in order to conduct an immunohistochemical analysis by the β2-AR antibody. Several causes of death were drowning, sudden cardiac death, sepsis, hanging, strangulation, traffic accident, and fire. This study revealed a different expression of β2-AR immunopositivity in relation to the cause of death; highlighted was the fact that the positive staining varied both for localization (fasciculate and/or glomerulosa and/or reticularis zone and/or medulla) and quantity. The results were then analyzed in relation to factors such as sex, age, and timing of the death process.

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Adrenal Gland, Medicolegal Autopsy, Immunohistochemistry

H3 A Forensic Case of a "Missing Brain" in a Preterm Newborn: A Postmortem Imaging Study Based on Computed Tomagraphy (CT) and Magnetic Resonance Imaging (MRI) With and Without A Contrast Agent

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After attending this presentation, attendees will better understand the advantages and limitations of enhanced and non-enhanced imaging techniques that can be seen in a forensic case of a preterm newborn.

This presentation will impact the forensic science community by revealing the results of a forensic case of hydranencephaly in a male preterm newborn who successively underwent Postmortem Computed Tomography (PMCT), Postmortem Magnetic Resonance imaging (PMMR), Postmortem Computed Tomography Angiography (PMCTA), and Postmortem Magnetic Resonance Imaging Angiography (PMMRA).

In addition to the classic non-enhanced PMCT, a postmortem vascular opacification consists of a PMCTA. Other radiological techniques are the PMMR and the PMMRA. A case is presented of a preterm newborn, forensically managed, who underwent imaging techniques prior to a medicolegal autopsy.

Material and Methods: This case involved a young woman with a denial pregnancy who delivered a premature male newborn with a believed gestational age between the 22nd and 24th week of pregnancy at the hospital. The newborn died four hours after birth. The general attorney requested a medicolegal autopsy. The body first underwent external examination, then radiological explorations. The external examination revealed neither traumatic nor malformative lesions. The umbilical vessels consisted of two arteries and one vein.

The forensic imaging management: PMCT — conducted on a 64-row CT unit using soft and bone tissue kernels. PMMR — the body underwent a total body MRI exploration using a 1.5 Tesla® scanner. Next, 3D T1W (weighted) and T2W axial acquisitions were performed on the brain; the rest of the body (except the members) was explored through 3D T1 coronal and a 2D T2 coronal mDIXON. PMCTA — the umbilical vein was catheterized with a radiological sheath (5 French). The vein was manually filled with a 6% dilution of the oily contrast agent with paraffin oil. A first-enhanced CT acquisition was performed. The umbilical artery was filled in the same manner as the vein. A second, enhanced-CT acquisition was performed. PMMRA — a 3D T1 coronal acquisition was performed on the body. The corpse underwent a medicolegal autopsy. Histological examinations were performed after formalin fixation.

Results: The forensic imaging results: The PMCT revealed the absence of cerebral hemispheres, which were replaced by fluid. A hyperdense central mass was visible in the supra-chiasmatic region. Instead of the posterior part of the left brain parenchyma, an ovoid dense mass was visible, surrounded by a visible hyperdense fluid-fluid level. The brainstem was visible and appeared normal. The cerebellar hemispheres presented cystic lesions. The PMCT revealed no skeletal malformation.

The cerebral PMMR confirmed the absence of the cerebral hemispheres replaced by fluid. The central mass was hyperintense on T1-weighted images and corresponded to a thalamic mass. The posterior fluid-fluid level was hyperintense on T1-weighted images, as was the left posterior mass. Both cerebellar hemispheres revealed cystic changes. MR imaging was typical of hydraencephaly.

The PMCTA revealed the absence of opacification of the intra-cerebral veins. The vertebral arteries and basilar trunk (with both posterior inferior cerebellar arteries) were fully opacified. The posterior cerebral arteries were visible until P3 segments. The internal carotids, in their cavernous portions were visible, opacified through the posterior communicating arteries. The anterior or middle cerebral arteries were not visible. The cervical terminal part of both internal carotids was not opacified.

The PMMRA revealed a correct filling of the venous transverse sinuses and internal jugular veins. The cervical terminal part of both internal carotids was not opacified.

The autopsical and histological results: The autopsy and the neuropathology analysis confirmed the findings in the brain, with parenchymal destruction due to hypoxo-ischemic changes, all corresponding to the diagnosis of a massive necrotico-hemorrhagic hydranencephaly.

Discussion/Conclusion: Hydranencephaly, a pathological change seen in the fetus, is a destruction of the cerebral hemispheres with occasional persistence of occipital and temporal tissue. The destroyed brain is replaced by cerebrospinal fluid and delimited by leptomeninges. Gray nuclei, trunk, and cerebellum may present abnormalities. This massive ischemia of the brain is secondary to a vascular unclear cause that affects the two internal carotids during the second trimester of pregnancy.

Per research, this is the first case describing the forensic management of a preterm newborn with hydranencephaly, including PMCT, PMMR, PMCTA, and PMMRA, which allowed a complete neuro-vascular analysis.

Forensic Imaging, Postmortem Angio CT, Postmortem Angio MR

H4 When the Walls Close In: Chronic Allograft Vasculopathy on Autopsy of an Orthotopic Heart Transplant Patient

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The goal of this presentation is to illustrate a rare form of vascular occlusive changes that can be seen on autopsy and can lead to misdiagnosis.

This presentation will impact the forensic science community by demonstrating the chronic changes of vascular structures in transplant patients and how these changes can lead to ischemic damage of the transplanted organ.

Introduction: Heart transplantation procedures have been steadily increasing in the United States. The Organ Procurement and Transplantation Network reported 3,191 transplants in 2016 in America and 67,301 in total since 1988. Advancements in post-surgical therapies have decreased acute rejections and increased the one-year survival rates. Unfortunately, the long-term survival rates have not followed, as chronic, progressive arteriopathies compromise the blood flow to the transplanted heart and lead to ischemic injuries.

Materials and Methods: This case involved a 46-year-old Caucasian male who had undergone orthotopic heart transplant in 2005 and had multiple episodes of rejection due to medication non-compliance. The patient arrived at the hospital for his scheduled plasmapheresis session for presumed antibody mediated rejection, where he complained of numbness, lightheadedness, and dyspnea. A right heart catheterization revealed hemodynamic signs suggestive of ongoing rejection despite recent aggressive treatment. The patient was admitted and was initially responding favorably, but was found unresponsive on day eight. Despite multiple lifesaving efforts, the patient unfortunately expired.

Results: Postmortem examination revealed acute myocardial infarction involving the septum and the posterior and lateral left ventricular walls. The coronary arteries exhibited varying degrees of chronic allograft vasculopathy with intimal arteritis.[1] The vessel walls revealed concentric tunica intimal thickening, resulting in luminal narrowing of 25%-50%. The epicardial and intramural arterioles showed lymphocytes in an infiltrative and perivascular pattern. Obliterative transplant arteriopathy could be appreciated in the intramural arterioles, with up to 90% luminal narrowing.

Discussion: Chronic allograft vasculopathy is diagnosed in one-third of heart transplant patients five years after the procedure and in half of these patients by ten years.[2] The most widely accepted etiology is immune-based, wherein the recipient's immune system recognizes the foreign tissue, releases a cytokine cascade, resulting in growth factor expression and eventually smooth muscle proliferation in the artery walls.[3] The incidence has remained stable despite advancements in immunosuppressive treatments.[2] With an increase in heart transplant patients surviving beyond five years, recognition of chronic allograft vasculopathy will be needed to avoid misdiagnosis from similar diseases to such as: atherosclerotic coronary artery disease, acute transplant rejection, and autoimmune and/or infectious myocarditis.

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Chronic Allograft Vasculopathy, Intimal Arteritis, Obliterative Arteriopathy

H5 Fatality Following Percutaneous Endoscopic Gastrostomy Tube Insertion

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After attending this presentation, attendees will better understand vascular injury and a fatal hemorrhage as a rare complication of Percutaneous Endoscopic Gastrostomy (PEG) tube insertion. The associated autopsy findings, risk factors for vascular injury and hemorrhage, and the use of therapeutic complication as a manner of death will also be discussed.

This presentation will impact the forensic science community by alerting the forensic pathologist of this complication of percutaneous endoscopic gastrostomy tube insertion, which can be demonstrated at the time of autopsy by careful dissection. This presentation will also impact clinicians by recognizing the potential for vascular injury at the time of tube insertion and patients at increased risk of hemorrhage.

PEG tube placement is a minimally invasive method of providing long-term enteral nutritional support.[1] The procedure was first conceptualized and performed in 1979 in Cleveland, OH, by Drs. Ponsky and Gauderer for use in the pediatric population. Their goal was to avoid a traditional laparotomy and instead provide a minimally invasive technique for gastrostomy tube insertion.[2] Following the initial success of the procedure, an estimated 100,000 to 125,000 PEG tube insertions are now performed every year in the United States.[3] Absolute contraindications to PEG tube insertion include pharyngeal or esophageal obstruction, active coagulopathy, and any general contraindication to endoscopy.[4]

A 54-year-old man with metastatic gastric adenocarcinoma was admitted to the hospital for management of dysphagia and a 30lb weight loss. He was initially receiving total parenteral nutrition via a central line. During his admission, he developed an upper extremity deep vein thrombosis for which he was started on heparin treatment. The heparin was stopped the following day after he developed hematemesis. Subsequently, he underwent a PEG tube placement in the interventional radiology department.

Following his transfer back to the intensive care unit, he was noted to be hypotensive and tachycardic. A massive transfusion protocol was initiated and an attempted coil embolization of the presumed vascular injury was initiated; however, the patient died during the procedure.

At autopsy, the torso was remarkable for diffuse petechiae and ecchymoses. A gastrostomy tube was extending from the epigastrium. There were seven liters of blood in the peritoneal cavity. The gastrostomy tube had perforated the greater omentum immediately adjacent to the transverse colon, with hemorrhage along the serosal surface, but no defect in the colonic wall was identified. The tube then perforated the pylorus of the stomach, but also inadvertently transected the right gastroepiploic artery. The gastric wall was diffusely thickened, up to 1cm. Also of note was advanced hepatic cirrhosis.

Serious or fatal complications following PEG tube insertion are rare. Approximately half of all patients will experience a minor complication, most frequently a wound infection.[5] Mortality rates are reported to be approximately 2% and have resulted from esophageal perforation, peritonitis, and severe respiratory distress.[6] Fatal hemorrhage is very rare with only two prior reported cases.[6,7] In both of these cases, the patient had undergone a prior cholecystectomy resulting in intra-abdominal adhesions, resulting in distorted anatomy. In this particular case, the recent use of heparin as well as the underlying advanced hepatic cirrhosis may have contributed to the profuse hemorrhage. The cause of death was hemorrhagic complications of PEG tube for the treatment of metastatic gastric adenocarcinoma. The manner of death was therapeutic complication. The therapeutic complication manner is used in a few jurisdictions in the United States and is defined as fatalities due to predictable complications of appropriate medical therapy.[8]

Although a rare complication, clinicians should be aware of the possibility of a vascular injury during PEG tube insertion and underlying natural diseases (such as hepatic cirrhosis) or concurrent medical therapy that may confer an additional risk of bleeding. Careful dissection at the time of autopsy with the gastrostomy tube *in situ* will help identify the underlying fatal injury.

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Gastrostomy Tube, Hemorrhage, Therapeutic Complication

H6 Examining the Distribution of Manner and Cause of Deaths at Hotels and Motels

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The goals of this presentation are to compare deaths occurring in hotels/motels to deaths occurring in the decedent's residence with specific attention to: (1) the manner of death; (2) the cause of death; and, (3) the variation in the method of suicides.

This presentation will impact the forensic science community by illustrating the significantly higher incidence of unnatural deaths of people who die in a hotel/motel by comparison to a decedent's residence.

This retrospective analysis is important in order to raise awareness of: (1) the proportion of deaths that occur in a hotel/motel due to unnatural causes; and, (2) the need for a high index of suspicion of unnatural deaths.

Within our counties, 82 deaths occurred at a hotel/motel from January 1, 2011 to June 30, 2017. Twenty-five of the decedents were females from 23 to 85 years old and 57 were male from 21 to 80 years old. Of these 82 deaths, 35 (42%) were classified as natural, 31 (38%) accidents, 12 (15%) suicides, and 4 (5%) undetermined. When compared to deaths that occurred at the decedent's residence in the same period, a far greater percentage of those are due to natural causes. Of 4,970 deaths reported that occurred at the decedent's residence, the manner of death was 3,919 naturals (79%), 567 accidents (11%), 341 suicides (7%), 47 homicides (1%), and 96 undetermined (2%).

Of the 47 unnatural deaths (accident, suicide, indeterminate), 38 (81%) were drug-related. An opioid was present in 27 of the 38 drug-related deaths and the most common opioid present was heroin.

Of the 12 hotel/motel suicides, five drug related fatalities (42%), four hangings (33%), two gunshot wounds (17%), and one plastic bag suffocation (8%) were observed. The modality used in suicides within the hotel/motel deaths differed from suicides at the decedent's residence where a majority of the suicides were due to gunshot wounds. By comparison, at the decedent's residence, suicide modalities were 168 firearms (49%), 93 (27%) hangings, 53 drug-related (16%), 8 (2%) sharp force injuries, 8 (2%) carbon monoxide poisonings, 8 (2%) asphyxiations/suffocations by plastic bag, and 3 (<1%) drownings.

Wasserman and Stack also found a higher number of suicides due to hanging in motels.[1] A study that looked at place compared to method of suicide found hotel or motel suicides were 4.9 times more likely to use drugs in comparison to suicides committed outdoors or on railways.[2] A study from Germany confirmed the increased number of unnatural deaths in hotels: 12 of 22 hotel deaths were natural, 9 unnatural, and 1 undetermined.[3] In Canada, researchers followed 15,100 homeless and marginally housed people from 1991 through 2001 and found mortality rates for those living in shelters, rooming houses, and hotel/motels were higher for drug-related deaths, alcohol-related deaths, and suicides.[4]

A recent study analyzed guest perceptions of hotel/motel rooms where a death had occurred.[5] The researchers found if a participant knew a previous guest had died in a room, they were more likely to see the room as less valuable, opt to stay in a different room, and feel uneasy when imagining an overnight stay. Perception of that room eventually returns to baseline, but many years after the death event.

In conclusion, the number of unnatural deaths exceed natural deaths, and drug-related fatalities were the most common. Investigators should have a high index of suspicion for unnatural deaths, especially drug-related deaths, when investigating deaths occurring in hotels and motels.

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Forensic Pathology, Hotel and Motel Deaths, Suicide

H7 An Unusual Case of Repeat Exertional Rhabdomyolysis With Associated Lymphocytic Thyroiditis and Sickle Cell Trait

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The goals of this presentation are to: (1) highlight clinical findings of a death following a second episode of exertional-rhabdomyolysis in a Sickle Cell Trait (SCT) patient also found to have lymphocytic thyroiditis at autopsy; and, (2) review return to play/duty recommendations for rhabdomyolysis in SCT individuals.

This presentation will impact the forensic science community by detailing a case in which SCT, exertional-rhabdomyolysis, and lymphocytic thyroiditis contributed to the death of an otherwise healthy person.

A 33-year-old male soldier in the National Guard began complaining of difficulty breathing and pain and requested medical assistance during a fitness test. Following emergent transfer to the hospital, he became combative and showed altered mental status. He was diagnosed with rhabdomyolysis. Three days after admission, the patient went into full cardiac arrest and died.

According to the decedent's relatives, he was hospitalized a year earlier for exertional rhabdomyolysis following the same annual fitness test. He was known to be positive for SCT, but was otherwise physically fit and without ongoing medical problems. The patient did not take any prescription medications and did not smoke or drink alcohol. He was not placed on any restrictions following this episode of rhabdomyolysis, other than recommendations to adequately hydrate.

His death resulted from complications of exertional rhabdomyolysis, including acute renal failure, intestinal ischemia, disseminated intravascular coagulation, and multiple organ failure. An unexpected finding at autopsy occurred on microscopic examination of the thyroid gland: significant lymphocytic infiltrates with germinal centers and destruction of thyroid follicles. He had a significantly elevated Thyroid Stimulating Hormone (TSH) and anti-thyroid peroxidase hormone on antemortem and postmortem samples. His toxicology screen of admission blood was negative for all screened substances.

For single-episode cases of rhabdomyolysis, the most common causes are cocaine use, exercise, and immobilization.[1] However, if an individual has recurrent episodes of exertional rhabdomyolysis, that person should undergo investigation for an underlying cause. Repeat episodes may be a result of SCT, hypothyroidism, or acquired myopathies, such as polymyositis, congenital muscle disorders such as muscular dystrophy, or metabolic myopathies impair fat metabolism.[1] Recommendations suggest athletes and military personnel should not return to play or duty if they have SCT since many activities can cause repeat episodes of exertional rhabdomyolysis.[2,3]

SCT and hypothyroidism are both risk factors for developing rhabdomyolysis. A study of 47,944 United States Army Black soldiers on active duty who had undergone testing for SCT had a significantly higher risk of exertional rhabdomyolysis.[4] A few case reports revealed hypothyroidism predisposes individuals to rhabdomyolysis and Acute Kidney Injury (AKI). The pathogenesis is unclear, but the hypothesis is hypothyroidism impairs glycogenolysis and mitochondrial oxidative metabolism, which can lead to rhabdomyolysis under physical stress.[5,6] Hypothyroidism as a cause of rhabdomyolysis and AKI should be considered in patients with decreasing renal function, high creatinine kinase, and no obvious cause.[7]

Though not the most common causes of rhabdomyolysis, underlying etiologies, including SCT and hypothyroidism, should be considered by the pathologist performing the autopsy in deaths due to exertional rhabdomyolysis. Microscopic examination of the thyroid is recommended.

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Rhabdomyolysis, Sickle Cell Trait, Forensic Pathology

H8 Pediatric Death by Macrophage-Activation Syndrome (MAS) Related to Epstein Barr Virus: The Role of Microbiological and Histological Postmortem Investigations

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After attending this presentation, attendees will be able to describe the impact of forensic science in cases of sudden pediatric death.

This presentation will impact the forensic science community by demonstrating the importance of immediate treatment in MAS to minimize the risks of sudden pediatric death.

In the context of immune-mediated diseases, MAS is a severe and potentially life-threatening complication of systemic inflammatory disorders. It may occur in response to an infection (often viral), malignancy, or a rheumatic disease. MAS typically appears in patients with Systemic-onset Juvenile Idiopathic Arthritis (SoJIA) and its adult equivalent, adult-onset Still's disease; it also is reported in other pediatric inflammatory disorders, including juvenile Systemic Lupus Erythematosus (SLE) and Kawasaki disease. It is a rare disorder. MAS expresses a close clinical resemblance to a group of histiocytic cell disorders collectively known as Hemophagocytic Lymphohistiocytosis (HLH). MAS is classified among the secondary, or acquired, forms of HLH. Primary HLH is a genetic disorder of immune regulation caused by mutations in genes encoding proteins required for the cytolytic activity exerted by NK cells and cytotoxic T cells. The clinical presentation of MAS is usually acute, may be dramatic, and is often difficult to distinguish from a severe sepsis. Typically, patients present fever, hepatosplenomegaly, lymphoadenopathy, profound depletion of all cellular blood elements, liver dysfunction, disseminated intravascular coagulation, and central nervous system dysfunction. Blood tests reveal: leukopenia, anemia, and thrombocytopenia; hyperbilirubinemia; high levels of Lactate Dehydrogenase (LDH), ferritin, and triglycerides; an increase in liver enzymes and cholestasis; normal or suddenly decreased Erythrocyte Sedimentation Rate (ESR); and sharply increased SCD25 and CD163. Coagulation is often altered, with elongation of the PT and PTT, hypofibrinogenemia, and the presence of fibrin degradation products (sharply increased D-dimer). In the field of forensic pathology, this postmortem diagnosis it is often difficult to detect; for this reason, cases sometimes remain unsolved. The treatment of MAS is not completely standardized. Steroids are considered the first-line therapy. Cyclosporine has demonstrated efficacy in non-responsive patients. The delay in diagnosis and multiorgan involvement are associated with a worse prognosis, so it is important that treatment is immediately instituted to prevent irreversible damage to the tissues and sudden pediatric death.

Case Report: A 2-year-old child was hospitalized for fever and vomiting. The child was pale and had dry skin. Nothing else was reported from the examination of the remaining systems. The hospital staff gave her antibiotics, antipyretics, and antiemetics. The results of chest and abdominal X-rays during the period of hospitalization were negative. A few hours after her admission, the child began to manifest mottled skin and bilious vomiting. She had kidney failure, absence of peripheral pulses, and loss of consciousness with convulsions and defecation. The child was treated with intubation, diazepam, and cortisone. Laboratory tests revealed lymph monocytosis, hyperglycemia, increased C-Reactive Protein (CRP) and ESR, and marked metabolic acidosis. Approximately six hours later, the first cardiac arrest occurred, but resuscitation was successful. Subsequently, two additional cardiac arrests ocurred, with a fatal outcome. After her death, an autopsy was performed. The microbiological data on postmortem samples from the lungs, pleural fluid, pericardial fluid, and cerebrospinal and ascitic fluid were negative for the detection of bacteria and yeasts. Microbiological investigation of the blood exhibited positivity for Epstein-Barr Virus (EBV) (Cytomegalovirus (CMV) and Mycoplasma pneumonia were negative). The postmortem histological examination revealed: labeled macrophage activation thymic cortical parenchyma; cardio-pulmonary thrombosis; pulmonary atelectasis; foci of interstitial pneumonia; follicular hyperplasia of lymphoid tissue associated with the intestinal and colon mucosa (Mucosa-Associated Lymphoid Tissue (MALT)); chronic hepatitis; and cerebral edema. The comparison of the clinical data with the autopsy data accompanied by microbiological investigations established that the MAS and Disseminated Intravascular Coagulation (DIC) in a child infected with EBV was the cause of death.

Conclusions: Cases of pediatric death with the absence of an antemortem diagnosis require a detailed analysis of antemortem clinical data with multidisciplinary collaboration with a pediatrician; it is necessary to conduct microbiological postmortem surveys on biological fluids in order to detect viruses and bacteria and to conduct histological investigations to explore in detail the thymus and lymphoid organs.

Forensic Science, MAS, Pediatric Death

H9 A Doubtful Case of Suicide by Firearm: The Comparison Between the Forensic Analysis of the Crime Scene and the Computed Tomography (CT) -3D Postmortem Investigation in Reconstructing the Manner of Death

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After attending this presentation, attendees will be able to describe the impact of CT-3D postmortem investigation and forensic analysis of the crime scene in cases in which there were doubts concerning the manner of death.

This presentation will impact the forensic science community by demonstrating the importance of radiological investigations to clarify doubtful cases of suicide by gunshot wounds.

Suicide is a public health problem; generally, it is underestimated. Many different factors may influence personal decisions about the choice of suicide modality. In forensic pathology, some cases of suicide are not clear, particularly when the body is moved, when the dynamics of the crime scene are uncertain, or the method of suicide is unusual. In gunshot suicides, the primary problem is understanding the dynamics of the shot; specifically, after the gunshot, the body may be subjected to passive movements or the shot is not immediately fatal and thus the individual can move away from the exact point where the shot or shots were fired.

The case of a man found dead in the countryside just a few meters from his home is presented. The victim was in a supine position above steep ground near a tree. Next to his right foot was a dark green hat on the ground. A dirty rifle with handcrafted changes was located approximately two meters to the left side of the corpse. There was a gunshot injury to the victim's left chest. Blood spatters were detected between the thumb and the index finger of the left hand. The analysis of the clothes revealed the presence of lacerations on the left thoracic area. A knife was found in his left trouser pocket. Near the corpse, a 96.5cm wooden stick with a V-shaped smooth tip was found, recently honed by a sharp blade. The wife stated the man did not show any psychological problems and that he had gone hunting in the morning. An autopsy was performed that revealed a gunshot wound that had jagged and irregular edges and an oval 3cm x 2.5cm shape with blackish shades as a contact hit. Under the gunshot wound, an ecchymotic-bruised complex of 3cm x 2.5cm was detected on the left intercostal region, at the level of the fifth intercostal space. A CT-3D 64-slice postmortem imaging allowed the reconstruction of the corporeal pathway and the detection of multiple bullets inside the pleural cavity and in the left under-scapula region. At autopsy, 30 bullets were detected, along with a cylindrical plastic element compatible with an explosive cartridge fragment. The directionality of the corporeal pathway determined that the shot was fired from left to right and from bottom to top in an oblique direction; the rifle (leaning on the ground) was activated with the aid of the stick that was used by the victim as a lever. The stick with the V shape was previously honed by the victim himself with the knife found in his trouser pocket.

Conclusions concerning the manner of death were deduced after several simulations that considered as most likely the hypothesis of the barrel being supported under the left armpit and the weapon's being fired by the right-handed and previously prepared stick. This case demonstrates how the analysis of circumstantial data emerging from the inspection, with the support of postmortem radiology, in cases of gunshot wounds is a valuable aid in reconstructing the dynamics of the event. In this case, the question was raised that perhaps the man had fallen and the shot from the rifle was accidental. The accidental theory was excluded due to the circumstantial and radiological elements that made it possible to discover the truth. Therefore, it is suggested that in all cases of firearm injury, a careful inspection is performed of all measurements as well as a pre-autopsy CT-multislice to detect the bullets and perform vector analysis of the shot or shots fired.

Forensic Science, Suicide, CT-3D Postmortem

H10 The Analysis of Pattern Injuries From Blunt Trauma and Sharp Force in a Forensic Case of Homicide: An Experimental Study Using a Pig Head Model

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After attending this presentation, attendees will be able to describe the impact of an experimental study using pig head models in order to identify the compatibility of injuries produced compared to those found on the victim.

This presentation will impact the forensic science community by demonstrating the importance of the correlation between the analysis of pattern injuries and weapon identification.

Blunt trauma can have various patterns. Each injury is different depending on the nature and mass of the weapon and on the force and manner employed. Difficulties arise in the identification of the weapon, in checking the compatibility with the injury, in the determination of the acting force, and in the discrimination of each injury. Investigations are complicated by the use of different weapons at the same time. Therefore, it is necessary to analyze injury patterns by replicating each wound on an experimental model, similar to a human one. In the literature, several studies have used pig bones in order to evaluate various injuries or to reproduce particular conditions, such as for burn injuries.

Often, it is difficult to determine the nature of the injuries, especially when they are multiple, overlapping, and made by blunt objects with an unidentifiable shape. The forensic pathologist must answer questions about the weapon used and is not always able to do so. Detecting the weapon is crucial and investigators search for it at the crime scene or where the attacker may have thrown it away. The pathologist's tools are experimental, especially when the weapon is not found or many weapons have been used. The autopsy is fundamental, as is the analysis of the margins, shape, and morphology of each injury.

A woman was found dead in her apartment with multiple wounds on her chest, head, face, and hands. An inspection was conducted during which all rooms were evaluated to find the crime weapon(s). In a wardrobe, knives were found wrapped in cloths and a cloth of the same color was found on the bed. All of the knives found did not show blood spots or latent traces. At the autopsy, the victim exhibited 11 injuries with clear and infiltrated margins attributable to 11 stab wounds from a cutting weapon. Only three of them were penetrating: one on the chest that lacerated the left lung and two on the right side and abdomen with hepatic lacerations.

Head injuries were present as a result of multiple stab wounds on the same point. There were 20 such injuries; each had indented margins, infiltrated with rounded and convex features, totally different from cutting injuries. These injuries exhibited multiple underlying cranial fractures attributable to the use of a blunt object repeatedly coming into contact with the head of the victim (ascertained by analyzing the victim's position at the inspection), first on the occipital region, then on the right temporo-parietal region. An experimental study was conducted on pig heads obtained from a slaughterhouse in compliance with animal experimentation regulations. Each head was placed on a cloth. Metallic and wood cutting and blunt weapons were chosen. Among the blunt objects classified and measured were: various hammers, metal pipes, screwdrivers, pipe wrenches, sticks, bats, picks, shovels, and hoes. Among the cutting weapons chosen were: single- and double-edge cutting knives, clasp knives, cleavers, and various kitchen knives. All the weapons were tested on pig skin. Head blows were made by a subject of the same weight and height as the suspect, with his right and left hand. Each injury was photographed and measured. From the comparison of the data emerged a compatibility of cutting wounds due to a single-edge cutting knife, with similar morphological characteristics compared to the victim's injuries. The analysis of blunt weapons exhibited a compatibility with metallic pipe injuries with the same rounded and convex morphology of multiple and overlapping wounds found on the head and face of the victim. This study has proven that the injuries were attributable to the use of two distinct weapons with different traumatic mechanical actions and that the aggressor used stronger force on the head with the metal tube, causing multiple cranial fractures. These data allowed for the detection of the inconsistencies declared by the aggressor and the discovery of the weapons that had been discarded in the countryside.

Forensic Science, Blunt Trauma, Pig Head Model

H11 The Role of Crime Scene Investigation and Judicial Inspection in Bath-Related Deaths: A Case Report With Forensic Implications

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After attending this presentation, attendees will understand the role of the forensic pathologist in determining the dynamics of bath-related deaths.

This presentation will impact the forensic science community by describing the procedure performed and the issues encountered in solving a case of bath-related death.

In Italy, more than four million domestic accidents happen every year, of which 8,000 are fatal. Such accidents can occur in the bathtub. In the literature, cases of drowning, sudden deaths, frequently associated with hyperthermia, and electrocution are described. The judicial inspection plays a decisive role in bath-related deaths, providing forensic evidence that could be lost if not immediately collected, especially in cases in which autopsy may not provide sufficient data to clarify the dynamics.

The case of a girl found dead in her home is presented. During the inspection, the girl was found on the couch. From the testimonies of the family, it emerged that the victim was found dead in the bathtub, then the body was moved by her father. An analysis of the rooms of the house, especially the bathroom, was conducted. Every object in the bathroom was cataloged and photographed. The bathtub was placed next to a sink on which an extension cord connected to a charging cell phone was found. An assessment of the electrical installation of the home was then performed. Subsequently, an external examination of the victim was conducted. The corpse had cutis anserine with pilo-erection on the dorsal area and the presence of a large area of skin burns (II and III degree) extended from the right shoulder to the left and caudally to the right gluteus. An impression of a rectangular shape (12cm x 3cm) from contact with an energy source on the dorsal region was found. Two burns on the right arm and a likely electrical mark with the exit point on the third finger of the right foot were collected. Samples of the skin, subcutaneous tissue, and muscle of all injuries were taken. The histological investigation was performed according to a protocol of paraffin-embedded formalin-fixed samples and preparation of slides. The microscopic examination exhibited: (1) the presence of small cavities or air bubbles (alveolus by high temperature) in the stratum corneum of the epidermis, which also presented carbonate residues on the surface; (2) polarization and elongation in clumps of cells of the stratum basale and stratum spinosum; and, (3) coagulative necrosis of the dermal connective.

In bath-related deaths, the judicial inspection and careful evaluation of the circumstantial data are crucial. The forensic pathologist must investigate whether the body has been moved from the bathtub, if it contains water, and measure its temperature. External examination of the victim allows an evaluation of the injuries and determines their nature, shape, dimensions, and microscopy to determine the histopathological features. It is also essential to conduct a toxicological investigation to exclude other causes of death. In this case report, there were difficulties because the victim was found far from the bathtub; however, the correlation of circumstantial data with autopsy data clarified that the burns present were generated by direct contact with an electrical power source, compatible with the extension cord found during the inspection. Plausibly, the girl, during her bath, used a charging cell phone connected to an extension cord near the bathtub. After the accidental fall of the extension cord, the latter was in contact with the water and the skin, causing death by electrocution. Therefore, there was also an increase in temperature on the contact surface of the skin, causing burns by Joule effect. In these cases, the pathologist must examine the electrical system, the current type, and the presence of automatic locking mechanisms. In this case, the electric current had a voltage of 220V and there wasn't any safety device. The comparison of the collected data allowed for the identification of the cause of death and, above all, the clarification of the dynamics of the event as well as the responsibility of other people in the accident. This case also emphasizes the importance of home security systems and using life-saving devices to prevent deaths associated with such accidents.

Forensic Science, Bath-Related Death, Crime Scene

H12 Sudden Death: The Role of Histopathological Investigations in a Case of Eosinophilic Myocarditis (EM)

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After attending this presentation, attendees will be able to describe the impact of histopathological investigation in a postmortem diagnosis of EM.

This presentation will impact the forensic science community by demonstrating the role of timely diagnosis to avoid sudden deaths in the absence of cardiac-specific symptoms.

The heart is a prime target of Polynuclear Eosinophil (PE) toxicity that is due to the release of basic proteins by eosinophils, including major basic proteins, cationic protein, and peroxidase. The most common manifestation of PE toxicity is Chronic Parietal Endocarditis (CPE). The heart damage appears to be a direct result of tissue injury produced by toxic eosinophil granule proteins; however, it is not known what causes eosinophilia in these patients and why the endocardium is especially susceptible to this type of injury. A number of parasitic infections may give rise to EM. Occasionally, drug reactions and rejection of a transplanted heart may produce EM. The incidence of EM is low but probably underestimated. The most common cause of death is the short-term occurrence of cardiogenic shock or dilated hypokinetic cardiomyopathy. Some patients have been successfully treated by early, intensive corticosteroid therapy and/or heart transplantation; however, diffuse myocardial involvement may lead to heart failure, and some of these patients may later develop dilated cardiomyopathy. The causes of sudden deaths are manifold, but, in the forensic field, very few cases of eosinophilic myocarditis are described in the literature. The difficulties of the postmortem diagnosis derive from the need to conduct in-depth histological exams and because the macroscopic data are unspecific.

Case Report: A 32-year-old Black man, a native of Liberia and an immigrant to southern Italy, was found dead in the immigration center. The external inspection of the corpse revealed an athletic constitution. The autopsy showed subarachnoid hemorrhage and fibrotic pleural adhesions of the ribs, and the heart exhibited an increase in volume, with grayish areas on the front surface. The cause of death remained unresolved; thus, the forensic pathologist conducted histological examinations. In particular, the heart was analyzed after fixation in 10% formaldehyde. The slides of the organs were prepared in paraffin and stained with hematoxylin-eosin. Histopathological examination provided the diagnosis of acute necrotizing EM of undetermined origin. Toxicological tests were performed and were negative. From the man's clinical history, it emerged that the day before death, the supervisors of the immigration center had called an ambulance as the man appeared to be in a confused state with pain in the posterior region of the right leg. Despite his recovery at that time, the man then died in the hospital within a few minutes without any possibility of intervention. Histological examination revealed an acute myocarditis associated with cerebral ischemic necrosis and subarachnoid hemorrhage. Therefore, in this case, it is shown that the eosinophilic action carried both cardiac and brain toxicity, generating a detrimental acute necrosis of tissue.

Conclusions: In cases of EM, it is important to make an early clinical diagnosis, and it is especially essential not to underestimate the neurological symptoms manifesting as early symptoms of heart disease, which may more quickly direct a diagnosis. In the forensic field, only histopathological investigation can identify the specific diagnosis.

Forensic Science, Eosinophilic Myocarditis, Sudden Death

H13 The Role of Proteomics for the Forensic Estimation of Postmortem Interval (PMI): A Preliminary Experimental Study

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After attending this presentation, attendees will be able to describe the application of proteomics for the estimation of time of death.

This presentation will impact the forensic science community by describing an operative experimental model used in order to analyze the PMI from the exact moment of death.

Proteomics is a branch of molecular biology that allows the systematic identification of the proteome from a quantitative and qualitative point of view. There are several studies in the literature that have analyzed animal or human biological samples using proteomic methods at different times since death. Regarding humans, no study has analyzed human samples from the exact time of death (time zero). Therefore, not knowing the exact time of death, studies were conducted in which the PMI analyzed was roughly calculated and was not known with certainty. In addition, before discovery, the corpse was exposed to various extrinsic factors, such as temperature or humidity. Therefore, proteomic analysis could be strongly influenced or altered by exposure to thermal variations of the sample.

This presentation introduces the operating model of an experimental study currently underway at the Department of Legal Medicine of the University of Catanzaro. The model is based on taking peripheral blood samples from patients who died at the intensive care unit, following an operation. The study was approved by the Ethics Committee of the University. The informed consent was signed by family members before the death of the patient. Samples were taken according to predefined time intervals, starting from the exact time of death (time zero) and up to two hours after the death. Samples were immediately centrifuged to extract plasma and stored at -80°C and were subjected to proteomic analysis by western blot and mass spectrometry at the Proteomic Laboratories of the University of Catanzaro. Although the experimental study is still ongoing, consistent results both with the time interval examined and the data already known in the literature are expected. In fact, a review of literature on this topic has already revealed that several proteins can undergo not only quantitative changes in terms of increase or reduction directly proportional to the PMI investigated, but also qualitative changes. According to the scientific evidence available in the literature, the expected results of the study are related to the search for quantitative and/or qualitative alterations from the exact moment of death of some markers, already showing time-dependent variations such as: (1) ubiquitous cellular proteins, like High Mobility Group Box 1 (HMGB1), and (2) specific organ proteins (muscle proteins due to progressive degradation, such as Cardiac Troponin I and T (cTn I and cTnT) and proteins related to brain damage, such as Glial Fibrillary Acidic Protein (GFAP) or talin).[1-4]

The proposed model is the first to conduct proteomic investigations on human biological samples from the exact moment of death without exposing the corpse to temperature variations or other extrinsic factors.

Finally, this operating model is intended to: (1) identify the possible role in the estimation of PMIs of new potential protein biomarkers expressed in peripheral blood from the exact moment of death; (2) verify and evaluate in detail the variation of the proteomic profile of markers already known in the literature; and, (3) focus on the analysis of the so-called "early PMI" for forensic purposes.

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Forensic Science, Proteomics, PMI

H14 A Case of Sudden Death From Takayasu Arteritis (TA): The Role of the "Histopathological Autopsy" in the Diagnosis of a Rare Disease

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After attending this presentation, attendees will better understand the role of TA.

This presentation will impact the forensic science community by demonstrating the role of histopathological investigations in cases of sudden death.

The autopsy represents the gold standard to determine cause of death. Sometimes autopsy cannot determine the pathologies that led to death, especially when only microscopic data determines such mechanisms. The pathologist uses histopathological examination even when the cause of death is already known at autopsy, but it becomes crucial when autopsy data are doubtful. In cases of sudden death, a macroscopically visible anatomical anomaly is not always present. Therefore, histopathological autopsy becomes crucial in diagnosis.

Reported here is the case of a 50-year-old woman who suddenly died in the emergency room; she was being seen for dizziness and nausea. An autopsy was performed as the woman enjoyed good health and was a sportswoman. The analysis of the anamnestic data revealed only the presence of a history of dizziness in the absence of other signs or symptoms. The autopsy revealed the presence of sclerosis of the thoracic and abdominal aorta with widespread coronary sclerosis. The lungs and brain appeared to be edematous and congested. There were no other pathological alterations. A histopathological investigation was performed revealing the presence of an ascending left coronary embolization and aortic wall thrombosis. The analyzed emboli, as well as the atheroma on the aorta, detected the presence of an eosinophilic infiltrate compatible with various forms of eosinophilic vasculitis. Histopathologic analysis further demonstrated the presence of non-infectious vasculitis associated with Anti-Neutrophil Cytoplasmic Antibody (ANCA) -associated autoimmune mechanisms. The vessels of all organs without eosinophilic infiltrates were analyzed. The limitation of the vasculitic phenomena to the aorta indicated a diagnosis of TA. Heart sections exhibited disruption areas of intercalated disks, coherent with ventricular fibrillation associated with small areas of necrosis, homogenization of diffuse eosinophilic sarcoplasmas, and wavy fibers. Such aspects were most present at the tip of the heart. These data revealed a terminal cause of myocardial ischemia with left ventricular fibrillation in the genesis of sudden death. This forensic case represents a rare case of autoptsy findings of Takayasu syndrome. This finding, in this case, indicates the cause of death.

TA is a chronic inflammatory large-vessel vasculitis that affects the aorta and its major branches. It can affect the vessel, mainly by stenosis, occlusion, and aneurysm, due to the thickening of the vascular wall. The prognosis of patients with TA is good, and the silent asymptomatic phase of this vasculitis can be long [1-6]. Sometimes the onset of this pathology can be fatal, with complications such as aneurysm rupture and congestive heart failure that may develop as a consequence of hypertension, granulomatous myocarditis, coronary heart disease, and/or aortic regurgitation. Sudden death can also be a rare complication of the disease. In the reported case, a correlation between TA and sudden death caused by aortic involvement with coronary embolization is shown. Such association is not a common finding. The correlation between systemic vasculitis and sudden death even in healthy subjects who do not exhibit comorbidity is emphasizedas is the importance of preventing fatal events, especially through cardiological follow-up of patients presenting with these pathologies. In these cases, from a forensic point of view, especially when the cause of death does not macroscopically emerge at the autopsy, it is fundamental that the histopathological investigation is conducted, which may sometimes be crucial in the diagnosis.

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Forensic Science, Takayasu Arteritis, Sudden Death

H15 Methods for Optimizing Postmortem Fingerprint Recovery From Mummified Fingers

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After attending this presentation, attendees will: (1) understand that traditional methods used to obtain postmortem fingerprints from mummified fingers are not always easy or successful; and, (2) learn two additional methods that can be used to optimize postmortem fingerprint recovery from mummified fingers.

This presentation will impact the forensic science community by introducing two additional techniques for obtaining postmortem fingerprints from mummified fingers.

In many medicolegal death investigation offices, a routine part of the postmortem examination of bodies involves the collection of fingerprints. A variety of challenges exist when attempting to take fingerprints from certain decedents. Decomposition, water immersion with subsequent wrinkling of finger pad skin, and trauma may cause great difficulty in obtaining suitable fingerprints at autopsy. Some of the most challenging cases involve mummified fingers, where shrunken, wrinkled, and hardened finger pads can make it nearly impossible to obtain suitable prints. The forensic literature includes recommendations regarding how to overcome these challenges.[1-4] Commonly employed methods involve attempts at rehydration, followed by various techniques for print acquisition, such as powder and adhesive lifts and Micro-Sil™ Silicone Impression Compound.[4] Despite these recommendations, obtaining useable prints is not always possible. Those responsible for obtaining postmortem fingerprints should use all available means to acquire suitable fingerprints.

This report presents two relatively simple methods using baby powder and transillumination, which may be useful in obtaining suitable fingerprints from mummified fingers. Both can be performed on fingers *in situ* or following finger removal from the body.

The baby powder method is most useful for situations in which the fingers are extremely darkened by mummification. Following attempts at rehydration, as previously reported in the literature, the finger pads are dried. Next, white powder, such as baby powder, is lightly brushed onto the finger pad surfaces, using a fingerprint brush. The powder adheres to the fingerprint ridges and enhances the dark grooves of the fingerprint resulting in what appears to be a "negative image" of how one normally visualizes an inked finger pad. A photograph or 10X to 20X microphotograph of the white-powdered finger pad can then be produced, with subsequent digital inversion for comparison purposes.

The transillumination method is more complex but still relatively simple to perform. Using forceps and a thin, sharp scalpel, the subcutaneous tissues underlying the finger pad are accessed via the skin proximal to the finger pad. The subcutaneous tissues immediately underlying the finger pad are then carefully removed by planed excisioning with the scalpel. The hardened subcutaneous tissues can literally be carved away in thin layers, working outward, toward the skin surface of the finger pad. Once enough tissue is removed, a relatively thin band of epidermis and underlying dermis will remain. Using a fiber-optic light source, the planed surface underlying the finger pad is illuminated. The overlying finger pad will be transilluminated, allowing for photography of the fingerprint ridge detail and subsequent digital inversion of the photograph for comparison purposes.

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Fingerprints, Autopsy, Mummification

H16 Forensic Radiology in Medicolegal Autopsy Practice

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After attending this presentation, attendees will have gained an appreciation of the wide array of case types in which traditional radiography (X-ray (XR)) plays a valuable ancillary role in medicolegal autopsy performance.

This presentation will impact the forensic science community by providing an overview of case types in which traditional XR provides valuable information as a part of a complete medicolegal autopsy examination. This presentation will also provide important examples and reminders of various "pearls" as well as "pitfalls" concerning postmortem forensic XR.

Forensic radiology encompasses the acquisition, interpretation, and reporting of radiologic images for the purpose of medicolegal investigations, including, but not limited to, cases presented in a court of law.[1] In the context of death investigation, forensic radiology is an extremely important component in the evaluation of certain case types. While XR is the oldest and most widely used modality in forensic radiology, Postmortem Computed Tomography (PMCT) and Postmortem Magnetic Resonance Imaging (PMMRI) have been gaining interest in the forensic community.[1] This review will focus on XR, as it is the modality most readily available and widely used by forensic pathologists.

The American Society of Radiologic Technologists (ASRT) formed a task force in 2010 to investigate training discrepancies among personnel performing forensic XR. Focusing on improving image quality and safety of radiologic personnel, the study found large gaps in existing training paradigms.[2] For example, there are still no set training standards for forensic imagers in the United States, and there is wide variability in the forensic XR services available at different forensic autopsy centers.[2] The National Association of Medical Examiners (NAME) Inspection and Accreditation program requires that accredited medical examiners' offices "have access to radiographic equipment or services," but does not specify that such services actually be located within the medical examiner facility.[3] To better address these deficiencies, those who perform and interpret forensic radiologic imaging studies should have a broad understanding of the case types in which postmortem XR is useful.

Not every forensic autopsy requires postmortem radiologic imaging; however, in some case types, forensic XR is essential. The NAME Autopsy Standards state that X-rays must be taken in the following case types: infants, explosion victims, gunshot victims, charred remains, and cases in which decomposition obscures or causes loss of identifying features and/or evidence of trauma.[4] In addition, several other case types may benefit from postmortem XR, including cases of suspected air embolism, sharp force injury, and certain other trauma, including suspected child abuse cases, unidentified remains, elderly or disabled decedents with suspected abuse or neglect, certain cases with implanted medical devices, and others.

This review presents an overview of medicolegal autopsy case types in which postmortem forensic radiology provides valuable, sometimes essential, information. For each general category of case type, specific examples are provided, along with a discussion of important aspects of the radiologic exam, including potential pitfalls. Providing an overview of forensic radiology in medicolegal autopsy practice better enables the forensic pathology community to develop appropriate standards and to address the deficiencies that currently exist within the medical examiner community.

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Autopsy, Radiography, X-Ray

H17 Age Determination of Traumatic Subcutaneous Hematomas Using 3.0T Magnetic Resonance Imaging (MRI): A Feasible Approach

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After attending this presentation, attendees will recognize that the dating of soft tissue hematomas is particularly important for the reconstruction of criminal acts, such as child abuse cases, and thus may have significant medicolegal consequences. Accurate timing of injuries can define or at least set limits on a period of time during which a crime took place and can lead to an inclusion or exclusion of potential suspects.

This presentation will impact the forensic science community by underlining the importance of radiological methods in forensic medicine.

In clinical forensic medicine, it is often important to determine the time of origin of soft tissue injuries. As subcutaneous hematomas are usually not relevant for clinicians, only limited knowledge exists regarding the detection and dating of traumatic lesions in the subcutaneous fatty tissue using Magnetic Resonance Imaging (MRI); however, visual assessment of external hematoma color, the currently used method for estimating hematoma age, is unreliable due to inter-individual differences and its great inter-observer variability.[1] Consequently, dating of hematomas is difficult, due to the lack of an objective and reliable method. The first MRI studies revealed that the contrast behavior between blood and soft tissue could be used to obtain objective information on blood characteristics and temporal changes.[2] Based on initial results regarding artificially created hematomas, the goal of this study was to create an age estimation model and to validate this approach with real hematomas of known age.

In 30 healthy volunteers, without coagulation disorders or medication influencing blood clotting, artificial hematomas were created by injecting 4ml of autologous blood into the subcutaneous fatty tissue of the thigh after a basis MR scan. The artificial hematomas were scanned repetitively at different points in time (directly after the injection and 3h, 24h, 3d, 7d, and 14d after the injection). All measurements (n=180) were performed on a 3T scanner using a multifunctional coil. The MR sequence protocol consisted of a Proton Density-weighted Turbo Spin Echo (PDwTSE) sequence with fat saturation (Spectral Adiabatic Inversion Recovery (SPAIR)) in oblique and axial orientation. Data were analyzed by measuring signal intensities in the hematoma and fatty tissue. Afterward, contrast coefficients were calculated and averaged at single points in time and approximated by a mono-exponential fit.[3] Based on the fitting curve, three contrast cut-off values ($\geq$0.75, <0.75 – $\geq$0.60 and <0.60) for three age categories ($\leq$24h, >24h – $\leq$7d and >7d) were defined, and the suitability of these thresholds was validated with real traumatic hematomas. Therefore, in ten healthy volunteers (exclusion criteria as stated above), hematomas were created using blunt force (paintball shot to the thigh) following a basis MR scan. For all consecutive MR measurements (n=60), the same setup as for the artificial hematomas was used. Subsequently, blinded data evaluation and age categorization according to the predefined thresholds were performed.

Overall, nearly 70% of all investigated hematomas were categorized correctly. For the first age category (hematoma age $\leq$24h), a sensitivity of 73% and a specificity of 93% were found. The Positive Predictive Value (PPV) was 92% and the Negative Predictive Value (NPV) was 78%. In the second age category (hematoma age <24h – $\leq$7d), the sensitivity was 70%, the specificity 68%; PPV was 52%, and NPV was 82%. For the third age category (>7d), a sensitivity of 50%, a specificity of 92%, a PPV of 56%, and a NPV of 90% were calculated.

All real hematomas were detectable at each point of time using MRI. The majority of the tested hematomas was correctly classified, but most notable was the quite accurate age estimation of hematomas within 24h of origin. In forensically relevant cases of living victims, the detection of recent bruises is especially important because it is a well-known fact that not every hematoma is immediately visible. The results of this feasibility study reveal that the presented approach for hematoma dating is a suitable, objective, and examiner-independent method to detect and estimate the age of bruises. The implementation of an objective MR-based age estimation approach may consequently improve the forensic expert's report in court, and thus ensure a higher degree of legal certainty. The applicability of the proposed model will be further validated with a higher number of hematomas of different ages and additional MR sequences.

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Hematoma, Age Determination, MRI

H18 A Comparison of Peak Sound Levels of Non-Contact and Contact Gunshots Into a Gelatin Block

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The goal of this study is to ascertain any difference between peak sound levels of contact and non-contact gunshots.

This presentation will impact the forensic science community by providing insight into whether or not a contact gunshot may reduce the auditory report of the gunfire.

Nearly all firearms create noise that is more than the 140 decibel level.[1] One author reported handgun and rifle sound levels at or near the muzzle ranging from 157 to 165 decibels.[2] A one-year retrospective review of suicidal contact gunshot wounds at the West Tennessee Regional Forensic Center, Memphis, TN, revealed 50 cases examined for either autopsy or external examination. Of those 50 cases, 5 cases included reported circumstances of an on-scene individual, other than the decedent, who reported that they did not hear the lethal gunshot auditory report.

This study will compare the sound levels of contact and non-contact handgun discharges for the calibers .22 long rifle, .38 special, and 9mm North Atlantic Treaty Organization (NATO) firearms fired into ballistic gelatin blocks. Many sound meters have a peak sound level limit of 130 decibels or less, thus testing of gunfire peak sound level at or near the muzzle requires high-quality, laboratory-grade sound meters with much higher decibel limits. The cost and availability of such sound meters could limit future duplication or expansion upon this study. Additionally, this study examines any difference in peak sound level versus highest possible peak levels; therefore, sound levels are obtained at a predetermined distance from the muzzle that does not overload an easily affordable, commercially available meter.

Peak sound level measurements were obtained utilizing a Dayton Audio® iMM-6 calibrated measurement microphone in combination with a sound level meter application from the National Institute for Occupational Safety and Health (NIOSH) installed on an Apple® iPhone® 6. The sound level meter application was programmed for NOISH standard, 80-decibel threshold level, C frequency weighting, fast-time weighting, and 3 decibel exchange rate. The 16" x 6" x 6", 10% clear ballistic gelatin block used meets the Federal Bureau of Investigation (FBI) protocol for testing terminal ballistics of human tissue. The hypothesis is that contact gunshots into a gelatin block will have lower peak decibel levels than non-contact gunshots into a gelatin block. The hypothesis is founded on the proposition that expanding gases will expand inside the gelatin block instead of outside the gelatin block, resulting in a lower peak decibel level. If the hypothesis is supported, it may explain why one-tenth of the suicidal gunshot cases in which an auditory observer was present did not hear the lethal gunshot.

This information may be useful in future death investigations and may be useful for medical examiners, medicolegal death investigators, law enforcement investigators, attorneys, and forensic scientists. The information from this project will better enable death investigators, medical examiners, and law enforcement officials to evaluate the validity of reports of circumstances surrounding and leading up to a death involving a contact gunshot wound.

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Contact, Gunshot, Decibel

H19 The Accuracy of 3D-Printed Models Using Measurements Obtained From Volume-Rendered Computed Tomography (CT) Images

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After attending this presentation, attendees will appreciate the accuracy and usefulness of 3D-printed models created using measurements obtained from volume-rendered CT images.

This presentation will impact the forensic science community by elucidating the precision of volume-rendered models from CT images and raising awareness regarding their potential validity, reproducibility, reliability, and utility in providing visual aids for legal proceedings.

Forensic pathologists are frequently called upon to be expert witnesses in a wide variety of cases. Courtroom testimony often requires pathologists to explain scientific concepts and complicated injuries that can be confusing and difficult to describe to a jury. With the increasing availability of 3D printers, there has been a rising interest in using 3D models to enhance such testimony. A 3D reproduction of a skull with multiple blunt force injuries, for example, can help jurors better understand how, where, and with what force the decedent was struck. In addition to providing a nearly first-hand experience for an audience, 3D models create an opportunity to illuminate physically small but important autopsy findings that can be difficult to appreciate with a 2D photograph or autopsy report.

For a 3D model to be truly impactful in courtroom testimony, it should be a nearly exact representation of the deceased. An effort should thus be made to determine how accurately 3D-printed models reproduce objects that can actually be measured, such as bone. If there is a very small or negligible difference between the measurements from the 3D model and bone itself, a valid argument can be made to use 3D models whenever appropriate.

In practice, though, generating 3D models by means of physical measurements may be difficult. To render a 3D model of a cranium with blunt force trauma, for example, remains must be completely skeletonized to obtain accurate dimensions. A previous study at the Office of the Chief Medical Examiner for the State of Maryland investigated the accuracy of CT-derived dimensions from specific skeletal elements and determined there was a very minimal difference between physical measurements and those obtained from CT imaging. As such, a reasonable step is to create 3D models using data points from CT images of skeletonized remains. To validate the accuracy of these 3D reproductions, their measurements can then be compared to those of the original skeleton.

Seven skeletonized crania from the previous study were analyzed using multiple cranial measurements, such as orbital breadth, orbital height, nasal breadth, and nasal height. They were then scanned with a General Electric® (GE®) Light Speed RT-16 multi-detector scanner at the Office of the Chief Medical Examiner for the State of Maryland with a slice thickness of 1.23mm. The acquired images were then volume rendered using the GE® Advanced CT Workstation (AW-2 version aws-2.0-5.5).

The data from these CT scans will be converted to Stereolithography (STL) format using free software. The STL format will then be converted by free slicing software to generate a G-code file for 3D printing. 3D models will be created via a filament-based 3D printer with a 0.4mm nozzle printing. Models will be printed using Polylactic Acid (PLA) 1.75mm filaments and 0.1mm thick layers.

3D-printed models of a portion of the skulls from the prior study will be made and measurements from these models will be compared to those taken previously. Percent differences between these measurements will determine if there is an acceptable difference between the two values and validate the use of 3D models in legal proceedings as a demonstrative aid. An accurate representation of what was seen at autopsy could be an invaluable resource for all forensic pathologists.

3D Printing, Postmortem CT, Legal Proceedings

H20 Detection of Human Male DNA From Whole *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) Larvae Using the Quantifiler® Trio DNA Quantification Kit

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After attending this presentation, attendees will better understand how the Quantifiler® Trio DNA human quantification kit was used in a novel pilot study to demonstrate that it may be possible to detect human male DNA from multiple whole *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) larvae up to 12 days post-colonization at 24°C under laboratory conditions.

This presentation will impact the forensic science community by revealing that the results from this pilot study indicate that these samples have the potential to extend the window for identification of offenders in cases of rape homicides in which the postmortem interval may exceed the efficiency of swab sampling.

Lucilia sericata (Meigen) (Diptera: Calliphoridae) is a forensically relevant blow fly species and is considered a primary colonizer of remains, both in Australia and around the world. In rape homicide cases, there can be a reduction in the integrity of the anatomical architecture of the cadaver as a consequence of larval feeding. Offender DNA deposited in the cadaver in rape homicides is important evidence that the sexual offense has occurred and the identity of the offender(s). In these cases, it may be that larvae, typically used for the estimation of time since death, now serve as an important reservoir for DNA storage and preservation. The Quantifiler® Trio kit is highly sensitive, capable of quantifying as little as 1pg/µL of human male DNA while also providing a measure of the template degradation.

In this pilot study, *L. sericata* larvae were reared under laboratory conditions at 24°C. The larvae were provided homogenized chicken liver mixed with human semen to mimic a vaginal environment post-coitus, and both larvae and swabs were collected for 12 days. The extracted DNA samples were concentrated with a vacuum desiccator and quantified with the Quantifiler® Trio DNA quantification kit in half-volume reactions using a QuantStudio™ 6 Flex. In this pilot study, it was shown that at 24°C under laboratory conditions, human male DNA could be quantified from multiple whole *L. sericata* larvae up to 12 days post-colonization. Furthermore, the human DNA from larvae typically exhibited lower levels of degradation compared to the swab samples.

Quantifiler® Trio, *Lucilia sericata,* Forensic DNA Analysis

H21 Infant Death Following Home Birth: A Case Report of Fatal Hypoglycemia in a Neonate

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After attending this presentation, attendees will better understand the importance of maternal clinical history, the histologic autopsy findings, and the role of Immunohistochemistry (IHC) in diagnosing islet cell hyperplasia in infant deaths due to hypoglycemia.

This presentation will impact the forensic science community by reviewing a case of fatal neonatal hypoglycemia in an infant born to a diabetic mother and by discussing the autopsy findings that can be used to support that cause of death.

Background: Pancreatic Islet Cell Hyperplasia/Hypertrophy (ICHH) refers to a proliferation and increase in size of the insulin-producing beta cells of the Islets of Langerhans in response to elevated serum glucose levels. ICHH is a common finding in Infants of Diabetic Mothers (IDMs) and a known risk factor for neonatal hypoglycemia. Although most cases are transient, neonatal hypoglycemia can be severe, resulting in infant death. Because hypoglycemia cannot be reliably diagnosed postmortem, ICHH may be the only diagnostic finding in infant deaths related to hypoglycemia, warranting histologic evaluation of the pancreas in all autopsies of IDMs.

Case Report: An infant girl at 37 weeks and 3 days gestation was delivered at home with midwife attendance to a 36-year-old *Gravida* 4 *Para* 3 (G4P3) mother with a history of insulin-dependent gestational diabetes and newly diagnosed, poorly controlled type 2 diabetes mellitus. The mother's previous pregnancy was reportedly complicated by gestational diabetes and transient neonatal hypoglycemia. At delivery, APGAR scores were seven and ten at one and five minutes, respectively; however, several hours after birth, the infant was reportedly not feeding well and was found to be severely hypoglycemic (28mg/dL). Formula and sugar water were administered, bringing the blood sugar to 48. Despite this, the heart rate decreased to 74 and the pulse oximetry went to 68. Emergency Medical Services (EMS) were called and the midwife immediately started Cardiopulmonary Resuscitation (CPR). The infant was transferred to an outside hospital and resuscitated for 70 minutes before being pronounced dead.

Methods: An autopsy was performed in addition to a review of the literature.

Results: External examination revealed the infant to be macrosomic (10 pounds at 11 hours of age) but otherwise without developmental abnormalities. The umbilical stump was desiccated but without purulence or drainage. Autopsy did not reveal evidence of trauma or significant natural disease. The placenta was not submitted for examination. Histologic examination of the pancreas revealed marked hyperplasia/ hypertrophy of the islet of Langerhans and insulin-producing beta cells. The cause of death was determined to be complications of neonatal hypoglycemia.

Discussion: A diagnosis of neonatal hypoglycemia should be considered as a potential cause of death or contributing factor in IDMs who have severe pancreatic islet cell hyperplasia and hypertrophy at autopsy. The finding of ICHH, although not specific, is strongly suggestive of maternal glucose dysregulation and may aid a pathologist in determining the cause of death in infants, particularly in the setting of absent maternal clinical history, prenatal care, and birthing history.

Islet Cell Hyperplasia, Neonatal Hypoglycemia, Infants of Diabetic Mothers

H22 Patterns and Trends of Teenage Homicides in the State of Maryland: A Forensic Autopsy Study (2006-2015)

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After attending this presentation, attendees will better understand the trends and patterns of teenage homicides in Maryland.

This presentation will impact the forensic science community by improving attendees' knowledge regarding the demographic distribution among teenage homicides and the circumstances as well as risk factors of teenage homicides. This presentation will also highlight the role of the forensic pathologist in the surveillance of deadly firearm injuries to teenagers and to our community.

Teenager mortality is an important public health issue because the majority of deaths among teenagers are caused by preventable external causes of injury, such as accidents, homicides, and suicides. Homicide is an extreme outcome of the broader public health problem of interpersonal violence. Homicide in children and youths has received a great deal of deserved publicity in recent years.

This study is a retrospective analysis of teenagers who were the victims of homicide in the State of Maryland from 2006 to 2015. Data analysis included: (1) the trends in teenage homicides from 2006 to 2015; (2) the demographic distribution among teenage homicides; (3) the circumstances of death and risk factors, including ethnicity, gang affiliations, known criminal history, drug and alcohol abuse; and, (4) the autopsy and toxicology findings and cause of death. Over the 10-year period, there were 4,945 homicides in Maryland. Of the 4,945 homicides, 554 (11.2%) were teenagers aged 12-19. The teenage homicide rate ranged from 0.49 to 1.41 per 100,000 population, with highest rate in 2008 and the lowest rate in 2014. The rate of male teenage homicide was more than seven times higher than the rate of females. Race distribution was also significant. African Americans comprised 87% of teenager homicides with a rate of 29.7 per 100,000 population, followed by Hispanic teenagers (5.64 per 100,000 population) and White teenagers (1.24 per 100, 000). The most common known motive/scenario was physical altercation/argument (11.7%, *N*=65), followed by robbery (2.5%, *N*=14), and drug-related (1.8%, *N*=10). The other known motives/scenarios included relationship issues, sexual assaults, and gang-related. There was a positive criminal history for 131 of the decedents. These included traffic violations, such as driving without a license, assault and battery with and without a deadly object, robbery, possession of drugs and narcotics, possession of firearms, attempted murder, and murder. Firearm injuries were the most common cause of teenage homicide (80.1%, *N*=447), followed by sharp force injuries (14.1%, *N*=790) and asphyxia (2.7%, *N*=15). Toxicology analysis revealed that 14.4% (*N*=80) of teenage homicide victims tested positive for alcohol and 18.6% (*N*=103) were positive for illicit drugs, such as cocaine, narcotics, phencyclidine, and methamphetamine.

Homicide is one of the leading causes of death in teenagers in the forensic autopsy population. As presented in this study, firearms and drugs significantly contribute to teenage homicides, primarily due to accessibility and availability. Thus, it is important to educate teenagers regarding the deadly consequences of firearms and drugs in school.

Teenager, Homicide, Forensic Autopsy

H23 An Evaluation of Elderly Deaths: A Retrospective Forensic Autopsy Study in Maryland (2005-2015)

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After attending this presentation, attendees will better understand the epidemiological characteristics of elderly homicides in Maryland.

This presentation will impact the forensic science community by providing insight into the risk factors of elderly homicide, vulnerabilities of the elderly, and characteristics of the suspects. This presentation will also assist the community in developing effective prevention efforts.

Since 1900, life expectancy in the United States has dramatically increased, and the principal causes of death have changed. At the beginning of the 20th century, many Americans died young. Most did not live past the age of 65, their lives often abruptly ended by one of a variety of deadly infectious diseases. But, over time, death rates dropped for all ages, most dramatically for the young. By the dawn of the 21st century, the vast majority of children born in any given year could expect to live through childhood and into their eighth decade or beyond. Elderly care and mortality have become an important public health issue.

According to the definition set by the Center for Disease Control and Prevention (CDC), an elder person is generally regarded as someone aged 60 years or older. The objective of this study was to evaluate the patterns of the causes of death among the elderly whose deaths were investigated by the Maryland Office of the Chief Medical Examiner (OCME). Maryland consists of 23 counties and Baltimore city with a population of 5,773,552, of whom 1,058,253 (18.6%) are aged 60 or older. A total of 4,528 elderly deaths were investigated by the OCME during 2005 to 2015. Of the 4,528 cases, 1,899 (41.9%) individuals died of natural causes, 1,908 (42.1%) were due to accident, 283 (6.3%) committed suicide, and 234 (5.2%) were homicide victims. There were 205 (4.5%) individuals whose manners of death could not be determined after thorough death scene investigations and complete postmortem examinations.

This study focused on the epidemiological profile of elderly homicides in Maryland. The number of elderly homicide deaths ranged from 13 in 2013 to 30 in 2015, with the average homicide rate of 1.63 per 100,000 population per year. More males than females were homicide victims with the ratio *(M: F)*=1.85:1. Of the 234 cases, 48% were White, 48% were African American, 2% were Asian, and 2% were Others, which included Hispanic, Native American, and Unknown. The motive behind the elder homicide was known in 73% (*n*=170) cases. The study revealed that robbery (*n*=45) is the most common motive, followed by altercation (*n*=41) and financial issues (*n*=15). Investigation revealed that of the 234 cases, the perpetrators were family members in 31.2% (*n*=73) cases and roommates, neighbors, or other acquaintance in 18.8% (*n*=44) cases. Therefore, 50% (*n*=117) of the perpetrators were known by the victims. Forty-two (17.9%) victims were killed by strangers, 13 (5.5%) by police, and the suspect was unknown in 62 cases (26.6%). There were 18 deaths involving homicide/suicide. Of the 18 deaths, six victims were killed by their husbands who then committed suicide, one was killed by the wife, one killed by a son, and one by a brother. The main causes of the homicides/suicides were either financial or health issues. The leading cause of death in elderly homicide was firearm injuries, followed by sharp force injuries and blunt force injuries. There were seven victims whose deaths were the result of neglect and/or abuse by their family members.

In conclusion, this study indicates that the elderly have a higher risk of being victims of domestic homicides. Understanding the vulnerabilities of the elderly and the characteristics of suspects and victims is the key to developing effective preventive measures.

Elderly Homicide, Abuse, Forensic Autopsy

H24 WITHDRAWN

H25 The Microbiome of Human Cadavers Can Provide an Estimate of the Postmortem Interval (PMI)

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After attending this presentation, attendees will better understand the human microbiome, how it changes during decomposition, and how it can be used to estimate the PMI.

This presentation will impact the forensic science community by serving as a novel tool for PMI estimation to be used in conjunction with current methods.

Estimating the time since death, or PMI, is critical to criminal investigations for identifying the deceased, validating alibis, and excluding possible witnesses. Estimating the PMI in unintended death scenes can be challenging, particularly when physical evidence is limited. Recently, researchers have developed models using the succession of microorganisms associated with cadavers to estimate the PMI.[1-5] These preliminary models indicate that microbial communities of decomposition can serve as temporal (succession-based) and spatial (origin-based) physical evidence.[2,5] This study proposes that the microbiome on human cadavers is successive and predictable and can be used to calculate the PMI.

Twenty-four human cadavers were placed outdoors to decompose under natural conditions at the Southeast Texas Applied Forensic Science (STAFS) facility. Two bodies were used for each experiment for four experiments per year (corresponding with the four seasons) and repeated each year for a total of three years. Microbial samples were taken from four body locations over the course of decomposition, processed for 16s ribosomal RNA (rRNA) sequencing using the MiSeq® platform and analyzed with QIIME™ 1.9.1.

Results revealed that microbial community structure did change through the course of decomposition of all body sets. While the bacterial community structure of initial samples varied (likely due to the variation of microbiome between individuals), all samples became more similar as decomposition progressed. Bacterial decomposer taxa (bacteria that differentially increased during decomposition) are shared across sample sites within experiments and across environments as well as the skin of host species. Therefore, the community assembly of cadaver microbial decomposers appears to be a generalizable process. A random forest regression model was used to model the PMI as a function of the changes in community structure and found the model to be able to provide accurate estimates of the PMI.

A potential impact of these results is the value of microbial data as physical evidence in medicolegal death investigations. The ability of PM microbes to estimate PMI is therefore a huge opportunity for forensic science to complement and improve upon current methods.

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Microbiome, Decomposition, Postmortem Interval

H26 A Quantitative Assay for Accurate 16S DNA Quantification for High-Throughput Sequencing (HTS) Library Preparation of Microbial Samples

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After attending this presentation, attendees will better understand how to quantify microbial DNA from human samples for improved performance of microbial HTS applications.

This presentation will impact the forensic science community by presenting a new method that will improve performance of microbial sequencing in their laboratories.

Sequencing and classification of microbial taxa within forensically relevant biological fluids has varied applications from individualization to body fluid identification. A distinct advantage as a method for body fluid identification is that it can be easily implemented into a comprehensive HTS panel for human identity. The quantity of bacterial DNA from human samples is currently estimated based on the quantity of total DNA isolated from the sample. This method overestimates the quantity of bacterial DNA due to the mixed nature of the sample and consequently makes library preparation unreliable and variable. The purpose of this project was to develop a reliable assay that can accurately and specifically quantify microbial DNA within a mixed sample for reliable 16S library preparation in advance of high-throughput metagenomics sequencing.

Venous blood, saliva, semen, vaginal and menstrual secretions, urine, and fecal matter were extracted using standard DNA isolation protocols. A quantitative Polymerase Chain Reaction (qPCR) method was developed using universal 16s rDNA primers, and cycling conditions were adapted for qPCR. A commercially available microbial community DNA standard consisting of pooled genomic DNA from eight bacterial and two fungal species was used to develop an accurate, precise standard curve. Five samples of each of the previously mentioned body fluid samples were quantified to determine the dynamic range necessary to detect bacterial communities within all body fluids. Following qPCR optimization, 16S libraries were amplified and evaluated from several samples at various DNA concentrations in order to determine the precise amount of microbial DNA needed for successful HTS library prep. DNA extracts were quantified using a standard human DNA assay to calculate the average ratios of human DNA to bacterial DNA in each biological fluid. Last, the body fluids were subjected to HTS to evaluate the success of the chosen microbial DNA quantity optimal for library preparation.

Bacterial concentrations between body fluids ranged from 5.12ng/µl to 0.001ng/µl. Saliva, vaginal secretions, and menstrual secretions proved to have a higher abundance of bacteria compared to blood and urine, which are considered to be “sterile” in healthy individuals, and beyond dormant bacteria or bacteria collected from skin surfaces upon exit of the respective fluids. A sample was considered negative if quantified below 10pg/µL. Results from gel electrophoresis reveal that successful HTS sequencing can be expected with 20pg of microbial DNA as quantified using this novel method.

This research has shown that quantification of microbial DNA from DNA extracts of forensically relevant body fluids is successful and increases library preparation reliability and success.

Body Fluid Identification, 16S rDNA, Microbiome

H27 Insects Attached to Vehicles Traveling on Roads in Mexico

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The goal of this presentation is to provide information regarding insect data that may be attached to vehicles traveling on different roads in Mexico during various climatic seasons of the year; this information can be used when necessary to establish probable routes taken by a vehicle involved in an investigation.

This presentation will impact the forensic science community by explaining how the information generated will be highly supportive for research that requires establishing probable routes taken by a vehicle involved in an investigation.

Forensic entomology is a discipline that greatly supports the field of legal medicine due to the different manners in which it contributes, such as: the establishment of the postmortem interval or period of insect activity, possible circumstances of death, determination of toxic agents in the body, determination of a possible postmortem move of the body, and assisting in the establishment of possible roads on which a vehicle could have traveled (the latter being the focus of this research).

In Mexico, efforts are being made by researchers to make forensic entomology known and applied in the legal system; thus, it is necessary to generate scientific information that can be available for use when required by experts or researchers, since most of the information available comes from abroad and cannot always be applied to Mexico.

This research was conducted in the south/southeast area of Mexico and covered a large region that stretches from the Gulf of Mexico to the Pacific Ocean. Currently, different insects have been collected from vehicles that travel a 1.130km section of road that begins at the border city of Tapachula, Chiapas, and continues to Mérida, Yucatán. This route consists of different ecosystems, such as wet forest, cloud forest, rivers and lakes, and grasslands, and there is a great diversity of insects that are associated with these ecosystems.

This study will continue on the main roads of the country during different climatic seasons. The information obtained will be shared on the web through a georeferencing database of forensically important insects that researchers have been working on and can be consulted by experts who require this information.

Forensic Entomology, Ecosystems in Mexico, Insect Biodiversity in Mexico

H28 A Checklist of Forensically Important Blow Flies (Diptera: Calliphoridae) Collected From Human Remains in Central Indiana

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After attending this presentation, attendees will better understand the importance of forensic entomology in criminal investigations. Attendees will also be informed about the species of Calliphoridae that colonize human remains in central Indiana.

This presentation will impact the forensic science community by providing case-by-case information pertaining to blow fly colonization of human remains. This will provide insight into blow fly biology and, furthermore, will increase knowledge of how seasonality, temperature, and other variables can play a role in the behavior of these forensically important insects. This presentation will also reveal the most common primary blow fly colonizers of human remains in the study area, which may be applicable to surrounding regions. To date, relatively little information has been published on blow fly species diversity and abundance on actual human remains; consequently, this study will help to fill this deficiency in the literature.

The field of forensic entomology has seen immense growth over the past several decades. Insects can be used to help answer questions pertaining to time since death or movement of remains in homicide investigations as well as providing insight in abuse and neglect cases; however, to improve our use of insects in these types of investigations, we must first know their distribution and biodiversity in a given range.[1] Several areas of the United States have records of surveying forensically important blow flies, using traps baited with beef liver or other decaying remains.[2-4] Many of these studies have shown that these types of traps can provide a sufficient sample of species that would be expected to colonize human remains in that area; however, given the logistical difficulty in sampling insects from human remains, very few studies conducted in the United States have been able to compare blow fly diversity in traps with those found on humans. This study presents the results from a survey of the forensically important blow flies found colonizing human remains in Central Indiana in 2016 and 2017. The findings from this work will provide baseline data that can be compared with future collections of forensically important blow flies on non-humans in Indiana.

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Forensic Entomology, Blow Flies, Human Remains

H29 The Characterization of Louisiana Winter Carrion Decomposition and Its Effects on Accumulated Degree Day (ADD) Estimations

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After attending this presentation, attendees will better understand the potential errors related to postmortem estimations based on ADDs of blow fly development during cooler months of the year. The goal of this presentation is to illustrate differences between observed thermal heat units versus those hypothetically available for growth.

This presentation will impact the forensic science community by demonstrating the importance of understanding the external (ambient) and internal cadaver sources of thermal heat at a crime scene.

Insects are poikilotherms (i.e., cold-blooded animals), thus, their growth and development rates are temperature-dependent. As a result, the summation of degree hours or days (thermal units) at a crime scene can be used to predict the overall energy budgets required to complete specific life stages of necrophilous insects present on human remains.[1] For entomologists to estimate postmortem intervals based on ADDs, three basic requirements must be met: (1) access to species-specific published development data; (2) scientifically based and general acceptance of species-specific development thresholds for growth (i.e., biological minimum and maximum); and, (3) accurate climatological information (ambient temperature, relative humidity, precipitation, etc.) associated with the crime scene or Cadaver Decomposition Island (CDI). Development data are available for various forensically important fly species (i.e., Calliphoridae, Muscidae, Sarcophagidae). In recent years, there has been an increase in forensic entomology studies focusing on improvements for both laboratory-reared blow fly development and thermal summation models (ADDs calculations), particularly associated with the curvilinear portion of insect; however, limited seasonal field experiments have been conducted to document the external and internal sources and fluctuations of thermal heat units associated with blow fly larval masses during cooler seasonal temperatures (i.e., biological minimums for growth).[2,3]

This study analyzed the effects of ambient and carcass temperatures throughout decomposition of adult swine carrion during the winter 2017 season in Hammond, LA. Winter decomposition of large vertebrate carcasses in Louisiana is characterized by prolonged carcass decomposition and periods of reduced insect activity due to fluctuating ambient temperatures.[4] Southeast Louisiana has a humid subtropical climate with long, hot, humid summers and short, mildly warm winters. This region rarely experiences freezing temperatures and regularly records dramatic ambient temperature swings within a 24-hour period (i.e., temperatures dropped from 27.2°C to 1.1°C overnight from January 25 to January 26, 2017). Wide-ranging temperature fluctuations occur sporadically through the cooler months in Louisiana and can cause considerable problems when postmortem estimations are based on traditional ADD formulas. The "typical winter forensically important species" of Calliphoridae in Louisiana varies greatly depending on how warm the winter is. Winter indicator species typically include *Calliphora vicina* (Robineau-Desvoidy), *Phormia regina* (Meigen), and *Lucilia coeruleiviridis* (Macquart), whereas spring and summer species include: *L. coeruleiviridis*, *Cochyliomyia macellaria* (F.), *Chrysomya rufifacies* (Macquart), and *Chrysomya megacephala* (F.). As a result of mild winters and occasional summer-like temperatures, it is not uncommon to recover crime scene evidence in December or January that includes all of the above species at a single site.[5]

This research includes two phases: (1) winter 2017 field study using three adult swine carcasses (36kg-50kg) placed in a hard-bottom flatwoods forest at Southeastern Louisiana University's Outdoor Classroom from January 26, 2017 to May 17, 2017; and, (2) laboratory study using Caron Products® Insect Growth Chamber to simulate observed winter ambient field conditions (thermal units minus carcass affect). Carcasses were sampled daily, every other day, biweekly, and weekly until day 112. Each sampling event included manual sampling of insects, digital photography, and multiple temperature measurements within the CDI, including: (1) FLIR® C2™ compact thermal imaging system (infrared camera) to document carcass surface temperatures and maggot mass activities within the carcass; and, (2) dual-digit temperature probe for internal carcass temperatures (mouth, anus), maggot mass, and ambient temperature. ADD estimations were calculated for *C. vicina* and *P. regina* using observed thermal heat units at the carcasses and climatology data from Hammond Regional Airport.

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4. Watson, E.J. and C.E. Carlton. Insect succession and decomposition of wildlife carcasses during fall and winter in Louisiana. *J. Med. Ent*. 2005; 42(2): 193-203.
5. Personal communication, Erin J. Watson-Horzelski. 2017.

***Calliphora vicina,* Postmortem Estimation, Forensic Entomology**

H30 The Necessity of Glycolic Acid Testing in Suspected Antifreeze Ingestion Deaths

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After attending this presentation, attendees will be more familiar with: (1) Ethylene Glycol (EG) and its toxic metabolites; and, (2) the necessity of EG and glycolic acid testing in forensic toxicology laboratories.

This presentation will impact the forensic science community by discussing a specific case that illustrates the need for glycolic acid testing to be made readily available at all forensic toxicology laboratories.

EG, a colorless, odorless, yet sweet-tasting poisonous diol is found in antifreeze and other industrial products, including hydraulic brake fluids and de-icing solutions.[1,2] The intoxicating effects related to EG ingestion are similar to those of ethanol; however, an ingestion of approximately 100mL is reportedly fatal in most adults.[1] EG poisoning is often intentional and is responsible for approximately 5,000 poisonings each year in the United States.[3] Symptoms of EG poisoning include central nervous system depression, seizures, cardiopulmonary complications, acute renal failure, and delayed neurological sequelae.[1]

EG is metabolized into glycoaldehyde and then into Glycolic Acid (GA), which is metabolized further into oxalic acid and formic acid.[1] Much of the EG is excreted through the kidneys, while its acidic metabolites, especially GA and oxalic acid, account for much of the toxicity of EG.[1] The oxalic acid precipitates as calcium oxalate in the kidneys and into the vascular endothelium of the brain and other organs.[2] Clinical diagnosis of EG intoxication is challenging due to the lack of testing capabilities for EG in anything but large clinical laboratories.[1] Although forensic toxicology laboratories are able to perform testing for EG, most toxicology screening methodologies do not detect the substance; requests for specific EG testing is required. Testing for the acidic metabolites of EG is even more scarcely available, even though the severity of EG intoxication has been directly correlated to the concentration of GA.[1]

This presentation reports a case of a 25-year-old male, found deceased on the floor of a hotel room. A departure note was located in the room, in addition to an empty package of over-the-counter sleeping pills, antifreeze/coolant, toilet bowl cleaner, dietary supplements, an empty bottle of a sleep aid, and an empty bottle of vodka. It was unclear how long the individual had been deceased, as he had not been seen for approximately nine days. Mummification of the distal extremities was present, consistent with early decomposition.

A complete postmortem examination yielded no immediate cause of death. Toxicological testing of postmortem blood was positive for diphenhydramine (87.7ng/mL), nicotine, and cotinine. Toxicological testing of postmortem blood and urine samples for all other substances was negative. Additional specified testing for EG was negative, as well. Histological examination identified crystalline precipitates consistent with calcium oxalate crystals within renal tubules. Additional investigation uncovered screenshots from the decedent's cell phone that involved questions entered into an online search engine such as "how long does it take to die from antifreeze?". In an effort to correlate histological and investigative findings, repeat EG testing was requested, which was again negative. Subsequent GA testing on a postmortem blood sample was obtained at an outside laboratory, with positive qualitative results.

While EG testing was negative, confirmation was made, via histologic identification of renal calcium oxalate crystals and subsequent positive GA toxicology testing, that the death resulted from an intentional antifreeze/ethylene glycol ingestion. The decedent most likely survived for some time, while fully metabolizing the EG into its acidic metabolites, causing organ system malfunction and death. The case serves to emphasize the importance of histologic examination of the kidneys in order to identify EG-related deaths. Additionally, this case illustrates that, in some scenarios, EG testing may be negative, but the identification of GA can be used to confirm that the death resulted from EG intoxication.

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Ethylene Glycol, Glycolic Acid, Antifreeze

H31 When Thromboembolism is Inevitable — A Case Report of a Lung Cancer Patient's Unexpected Death

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The goal of this presentation is to share an uncommon thromboembolism case in a patient suffering from a Large-Cell Lung Carcinoma (LCC).

This presentation will impact the forensic science community by highlighting how pulmonary thromboembolism, a well-known complication of lung cancer, could give access to a medical legal litigation for "unexpected death."

Case: A 49-year-old Caucasian female was taken by ambulance to the hospital after complaining of uneasiness. On this occasion, pulmonary embolism was diagnosed using an angio Computed Tomography (CT) that also detected the presence of solid lesions and parenchymal thickenings, suggestive of an etheroplastic process. The biopsy, performed by bronchoscopy, diagnosed an anaplastic LCC. Moreover, during her hospital stay, venous thrombosis was detected in the lower limbs. Anticoagulant treatment with EBPM followed by warfarin therapy was initiated. After obtaining a good clinical response, the patient was released. Over the next four days, due to leg pain in the lower left side, new tests were conducted and an International Normalized Ratio (INR) of 1.99 was found (range 1.5–2.5). The patient was taken to the emergency room and exhibited blood pressure of 120/60mmHg, a heart rate of 119bpm, and oxygen saturation of 95%; after new hematochemical examinations, an incalculable INR and a positive result of D-dimer (75.2µg/mL) were found. The cardiological and the vascular surgery examinations highlighted the thrombosis of the left poplitial vein. The patient then chose to leave the hospital the same day. After again feeling ill, she arrived at the emergency room and died a few minutes later.

The autopsy findings were a fibrinous saddle thromboembolus present inside the pulmonary trunk; this is a known complication of severe, advanced lung cancer, from which the patient suffered. Cancer thrombosis pathogenesis is complex and involves multiple factors, including general factors, factors related to the inflammatory response of the tumor, and specific properties of cancer cells; the latter, in fact, release substances that induce inappropriate activation of blood coagulation, favoring thrombosis. A patient with an incalculable INR means that the blood was hyperthinned. In cases of deep venous thrombosis/embolism, the therapy is based on anticoagulation and, in this particular case, the drug therapy was over-effective. The presence of very effective anticoagulation (INR incalculable), as in this case, would not have allowed the use of other pharmacological/mechanical therapies.

This clinical scenario, framed as an unexpected death, has given rise to legal litigation. The case presented is not unusual, clinically speaking; in fact, in the absence of a clinical history, the case was treated as an expected death by the public prosecutor.

Unexpected Death, Lung Cancer, Thromboembolism

H32 Fatal Intrahepatic Hemorrhage After Nadroparin Use for Total Hip Arthroplasty

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After attending this presentation, attendees will better understand the physiopathology of spontaneous intra-hepatic hematoma, an exceptional but potentially lethal condition that may occur in patients who receive pre-operative and/or post-operative therapeutic doses of anticoagulants.

This presentation will impact the forensic science community by informing attendees of the possibility of intra-abdominal bleeding from the liver in patients who have received Low-Molecular-Weight Heparins (LMWHs), undergone major surgery, and present post-operative hemodynamic instability, especially in those with a pre-operative diagnosis of hepatic cyst.

LMWHs have become the predominant choice for deep venous thrombosis prophylaxis and treatment; however, their use may cause bleeding complications. Intrahepatic bleeding is exceptional and few cases have been described in the literature thus far.

A unique case of fatal intrahepatic hematoma complicated by nadroparin use in a 65-year-old woman with a hepatic cyst of the anterior part of the right lobe (diameter 2.8cm) admitted to the hospital for a unilateral total hip arthroplasty is presented. The woman did not have a history of any bleeding disorders, coagulopathies, thrombocytopenia, or diabetes and had never received anticoagulant or anti-platelet medication.

Pre-operatively and post-operatively, the patient received nadroparin calcium by subcutaneous injection beginning 12 hours before surgery at 0.4ml (3,800 IU) per day. On post-operative day nine, the patient complained of severe abdominal pain and developed abdominal distention, hypotension, and tachycardia. She was admitted to the intensive care unit and immediately transfused. There was no apparent source of bleeding at the surgical site; however, in view of the abdominal distension, a bedside ultrasound was performed that demonstrated free fluid in the abdomen, primarily around the perihepatic area. Her medical status continued to deteriorate despite supportive care and she went into shock. Attempted manual cardiopulmonary resuscitation was unsuccessful and the patient died before laparotomy could be performed.

A forensic postmortem examination was requested by the inquiring authorities due to suspicion of medical malpractice.

At autopsy, hemoperitoneum (2,000ml of blood and clots) was evident. A ruptured subcapsular hematoma involving the right lobe of the liver was observed, while no other pathological findings were found. The hemorrhage within the simple hepatic cyst (a biliary malformation, which does not have communication with the intrahepatic biliary tree, microscopically lined by a single layer of cuboid or columnar epithelial cells, resembling biliary epithelial cells) induced by the nadroparin use was likely responsible for the subsequent hepatic hematoma, liver rupture, and death.

Visceral injury following cardiopulmonary resuscitation (external cardiac massage), which has frequently been described in the literature (including cardiac, hepatic, splenic, and mesenteric lacerations), was also taken into consideration; however, no fractures of the ribs or sternum were observed at autopsy. Moreover, the epicardium, myocardium, mediastinum, lungs, aorta, and spleen were unremarkable and did not reveal contusions or lacerations. All of these findings tend to exclude the possibility that the hemorrhage within the cyst and the ruptured hematoma of the liver might have been complications of the cardiopulmonary resuscitation and support the hypothesis of a spontaneous intrahepatic hemorrhage.

In conclusion, the need for pathologists to be informed about rare clinical life-threatening conditions is illustrated in order to avoid erroneous evaluation of hypothetic professional liability profiles.

Low-Molecular-Weight Heparin, Intrahepatic Hemorrhage, Sudden Death

H33 A Case Series: A Massive Hepatic Subcapsular Hematoma as an Unexpected Hypoxic Complication — A Preventable Neonatal Death, Not a Latrogenic Rupture of the Liver!

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After attending this presentation, attendees will understand the physiopathology of a massive Subcapsular Hematoma of the Liver (SHL) and its implications for the evaluation of potential medical liability profiles.

This presentation will impact the forensic science community by bringing attention to the possible complication of perinatal hypoxia, awareness of which may help avoid erroneous judgments of the performances of gynecologists and obstetricians. This presentation will also impact society and the clinical community by highlighting the fact that neonatal death due to this complication may be avoided through timely diagnosis and appropriate therapy.

SHL, consisting of a confined collection of a variable amount of blood that raises the hepatic capsule, rarely occurs in neonates and is often undiagnosed or misdiagnosed, even in postmortem examinations. SHL is an unstable condition caused by maternal, placental, or fetal factors that, in cases of pressure surges, can suddenly increase its volume, causing the rupture of the capsule. SHL has a non-specific presentation (characterized by unexplained hypovolemia or anemia) and should be considered, especially in infants with very low birth weight, preterm delivery, and/or suffering from intrauterine hypoxia. The diagnosis is frequently made at autopsy, with the main finding being hemoperitoneum.

As cardio-circulatory collapse is sudden and unexpected, such cases often arouse suspicion of professional negligence or malpractice. The collection of blood under Glisson's capsule sometimes leads forensic pathologists to misinterpret the SHL pattern as iatrogenic rupture of the liver.

The presented cases, which were studied at the Istituto Giannina Gaslini, a highly specialized Italian pediatric hospital, were as follows:

Case	Gestation (weeks)	Birth weight (grams)	Sex	Delivery	APGAR Score (1min and 5min)	Clinical presentation	Diagnosis of hemoperitoneum due to rupture of SHL	Outcome/Age at death (hours)
1	24+2	782	F	Vaginal, breech presentation	5 and 5	IUGR, suspected perinatal asphyxia, leucopenia and low platelet count	Postmortem	72
2	35+4	3065	F	Vaginal	0 and 0	Intrauterine fetal death	Postmortem	0
3	39+4	3410	M	Vaginal, two Kristeller's procedures	9 and 9	Severe respiratory distress with metabolic acidosis	Postmortem	16
4	29+2	960	F	Cesarean section	8 and 9	IUGR, respiratory distress, anemia, pathologic Eco Doppler	Early postnatal	Surviving

The first two cases underwent clinical autopsy and the third case involved a complete forensic approach. In all three cases, after clinical data collection, macro- and microscopic examination led to the conclusion that death had been caused by acute anemia due to hemoperitoneum, following the rupture of the hepatic capsule caused by the presence of a huge amount of sub-capsular blood.

The experience of the three fatal cases led neonatologists to suspect SHL in the fourth case; their early sonographic diagnosis of SHL was promptly followed by the infusion of blood and fresh frozen plasma, by continuous inotropic support with dopamine, and by hematoma drainage.

Hodge was probably the first to report a case of SHL.[1] The recent literature reports a varying incidence of SHL from 1.2% to 9.6% in autopsy series, and the severity of the bleeding is described as potentially life-threatening within minutes, before treatment can be initiated.

In conclusion, it should be stressed that cooperation and the exchange of information among clinical and forensic pathologists, neonatologists, obstetricians, and gynecologists is the only effective method of avoiding long and complex medical malpractice litigation. Achieving this goal requires widespread knowledge of the physiopathology of neonatal conditions predisposing to sudden and unexpected death, which are unrelated to malpractice. Among these, SHL should be considered in newborns affected by very low birth weight, preterm delivery, and intrauterine hypoxia. Additionally, from a clinical point of view, early diagnosis and treatment are essential in order to avoid fatal hemorrhagic shock.

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Subcapsular Hematoma, Unexpected Complication, Neonatal Sudden Death

H34 Video Game-Associated Deaths in the Tidewater District of Virginia (2015-2017)

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The goals of this presentation are to examine the types of fatalities, in a medical examiner's system (forensic deaths), in which video games may have played a role and to consider the significance of video games, including distracted attention, in situations resulting in death.

This presentation will impact the forensic science community by examining and classifying deaths, via a medical examiner's system, that reveal a relationship to playing video games. Video game play may be an unrecognized contributor to forensic deaths; this presentation will provide an analysis of the types of cases encountered in one medical examiner's system and supports tracking whether video game play was present when monitoring forensic deaths.

Video games have been anecdotally reported in association with deaths from exhaustion or natural causes.[1,2] This presentation investigates the types and occurrences of forensic deaths associated with video games in a medical examiner's system in southeastern Virginia.

Nine deaths were identified on initial review. The ages of decedents playing video games when they died ranged from 14 to 48 years, with a mean age of 33.875 years; three of the decedents were teenagers. Two decedents were female; seven were male. Five of the decedents were White, four were Black, and none were Asian or Hispanic. Black race is overrepresented in these video game deaths in comparison to the current population of Virginia, which is 70.0% White, and 19.80% Black. Five of the deaths were witnessed, and four were unwitnessed. Six deaths occurred either while a decedent was playing a video game or the decedent was last seen alive playing a video game and was found dead next to the game. Five of these deaths were eventually determined to have a natural manner; one death was attributed as accidental, having combined natural disease with a methadone and olanzapine overdose. Cardiac factors were significant in five of the six natural deaths, including all of the teenagers (causes of death included dilated cardiomyopathy, anomalous right coronary artery origin, and cardiomegaly). One of the remaining deaths was a witnessed suicide by gunshot that occurred while the decedent was playing a video game; one death was an unwitnessed accidental drowning of a toddler in a backyard swimming pool that occurred while the caretaker was playing a video game.

Representative cases include: a 35-year-old man who developed a myocardial infarction while playing video games; a 14-year-old boy with an unknown history who underwent a witnessed collapse while playing video games and was found at autopsy to have a pituitary stalk/hypothalamic lipoma, as well as cardiomegaly; and a 44-year-old woman with no known history, aside from obesity, who underwent a witnessed collapse while playing video games and was found at autopsy to have unsuspected hypertrophic cardiomyopathy.

Discussion: Video games in relationship to death have not previously been studied in the Virginia medical examiner's system. These cases demonstrate that video games may be associated with sudden collapse from cardiac causes in teenagers and young adults as well as middle-aged people; it is unknown whether the game itself plays a role. Suicide associated with video games has been reported previously in teens; in this study, it occurred only in a 35-year-old man.[3] Questions raised by this case series include whether violent video games show any association with suicide, and whether video game addictive behavior is related to caretaker negligence.[2] These findings suggest that the association with and/or contribution of video games to sudden and unexplained death, particularly in childhood deaths, may need to be tracked for death review.

Reference(s):

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2. Sublette V.A., Mullan B. Consequences of play: A systematic review of the effects of online gaming. *International Journal of Mental Health and Addiction.* 2012 February; 10 (1): 3-23.

3. Messias E., Castro J., Saini A., Usman M., Peeples D. Sadness, suicide, and their association with video game and Internet overuse along teens: Results from the youth risk behavior survey 2007 and 2009. *Suicide and Life-Threatening Behavior.* 2011 June; 41 (3): 307-315.

Video Games, Forensic Deaths, Death Review

H35 Sudden Cardiac Death and Epilepsy-Related Gene Mutations in Sudden Unexpected Death in Epilepsy (SUDEP)

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After attending this presentation, attendees will be able to define SUDEP, understand the application of the definition to individual cases, describe contemporary theories on the mechanism of death in SUDEP, and list several "neurocardiac" genes currently under investigation as biomarkers of SUDEP risk.

This presentation will impact the forensic science community by updating attendees on the theoretical mechanisms of death in SUDEP and how these relate to potential individual genetic differences in "neurocardiac" genes and by reinforcing the definition of SUDEP for uniform cause-of-death certification in these cases.

Background: SUDEP is a sudden, unexpected, witnessed or unwitnessed, non-traumatic and non-drowning death in an individual with epilepsy, with or without evidence of a seizure and excluding documented status epilepticus, in which postmortem examination does not reveal a cause of death.[1] This definition does not predicate that the epilepsy be primary or idiopathic epilepsy. SUDEP is a leading cause of epilepsy-related premature mortality, with an estimated incidence of 1.22/1,000 persons with epilepsy, accounting for more deaths on an annual basis than Sudden Infant Death Syndrome (SIDS).[2] The precise mechanism by which epilepsy results in death remains unknown. Several theories have been proposed, including cardiac arrhythmia, respiratory failure, and "electrocerebral shutdown".[3]

A major difficulty in the clinical treatment of epilepsy is that it is not understood why some individuals die of SUDEP while other patients with epilepsy of a similar "severity" survive.[3,4] One possibility is that those who die of SUDEP have an underlying genetic mutation in a gene related to ion transportation or cardiac function that predisposes them to sudden death.[5] The current work was undertaken to assess the proportion of cases of SUDEP that demonstrate genetic changes in genes associated with sudden cardiac death and/or epilepsy.

Methods: Cases were selected from autopsies performed between January 1, 2014, and December 31, 2016, through the Ontario Forensic Pathology Service. Genetic testing was performed on 36 cases that had been signed out by the pathologist with the cause of death given as SUDEP and that had a sample suitable for genetic testing. Analysis was performed by GeneDx®. Each case was assessed by the Sudden Cardiac Arrest Panel ((SCAP) 120 genes) and Comprehensive Epilepsy Panel ((CEP) 87 genes).

Results: The 36 cases were comprised of 21 males and 15 females, with ages ranging from 3 years to 60 years. Variants of Uncertain Significance (VUS) in SCAP panel genes were detected in 20 cases (proportion with SCAP VUS: 55%) and involved 20 genes. VUS in the following genes was detected in multiple individuals (*n*): *SCN10A* (5), *RYR2* (4), *SCN5A* (2), *LMNA* (2), *TTN* (2). VUS in CEP panel genes were detected in 20 cases (proportion with CEP VUS: 55%) and involved 26 genes. VUS in the following genes was detected in multiple individuals (*n*): *PIGO* (2), *PNKP* (2). Ten cases were called negative on both the SCAP panel and CEP panel (proportion with SCAP or CEP VUS: 72%). No known pathogenic variants were detected. Further in-depth analyses of the VUS are ongoing.

Conclusions: This preliminary interrogation of genes associated with sudden cardiac death and/or epilepsy in a cohort of patients dying of SUDEP reveals a high prevalence of variants currently classified as VUS. Several genes displayed multiple variants across several individuals, which is an intriguing result, and further analyses of the specific variants are ongoing. Forensic pathologists should be aware that the leading mechanistic theories of death in SUDEP involve cardiac arrhythmia, respiratory failure, and electrocerebral shutdown. Genetic variations may account for why some patients with epilepsy die of SUDEP while others survive.

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Epilepsy, SUDEP, Sudden Death

H36 A Novel Approach to Radiographic Identification of Skeletal Remains by the Z-Projection of Cranial Computerized Tomography (CT) Scans

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After attending this presentation, attendees will have a general understanding of how to process a CT image series into a z-axis projected image that can be used in conjunction with or in lieu of conventional antemortem radiographs to establish positive identification.

This presentation will impact the forensic science community by providing a methodology that simplifies a CT image series along the z-axis into one image that can be easily compared to a postmortem radiograph. Additionally, this methodology can be used to provide an antemortem radiograph of a missing person that could be used to compare to unidentified skeletal remains.

A primary objective of the medicolegal death investigation is to establish positive identification of unidentified remains via scientific means. In many instances, identity can be quickly determined via fingerprint comparison when the decedent has prints on record. Unfortunately, establishing positive identification becomes more challenging when the decedent is in advanced stages of decomposition or completely skeletonized, thus requiring the use of other methods, such as DNA or radiographic comparisons. Although DNA provides strong statistical support for identity, it is constrained by the availability of reference samples for comparison and has time and financial drawbacks regarding the processing and analysis of samples. Alternatively, radiographic comparisons are a likely means for positive identification because they obviate the practical and logistic limitations of other methods and are often readily available in many instances.

The use of CT imaging has steadily increased since its introduction into clinical medicine, resulting in the possibility of using antemortem CT data for identification purposes; however, most facilities that are tasked with conducting a medicolegal death investigation do not have access to CT equipment and cannot do slice-by-slice comparisons. Although CT scout films may be taken during a clinical exam, the image quality and/or view may not provide sufficient points of concordance to conclude a positive identification when comparing these antemortem films to postmortem radiographs. One solution to this dilemma is to utilize software that can project the CT image series along the z-axis (a z-projection) to create a 2D image. The resulting z-projection is essentially an inferior-to-superior radiograph, created by "flattening" the CT images, which can then be easily compared to postmortem radiographs taken using conventional equipment. This methodology is particularly useful when dealing with skeletal remains and could provide an additional approach for identification via radiological comparisons from antemortem CT images of missing persons to unidentified skeletal remains within the National Missing and Unidentified Persons (NamUs) database.

Creating a z-projection for identification purposes is, per research, a novel approach that can be easily accomplished using free and readily available software such as ImageJ. This presentation will discuss the following: (1) the steps to import and/or create an image series to generate a z-projection; (2) considerations regarding anatomical planes from a CT series in order to generate appropriate postmortem radiographs for comparison; (3) two examples of z-projection used to identify a decedent; (4) the value of generating z-projections for missing persons in NamUs to possibly aid in the identification of unidentified individuals; and, (5) future research using the projection of antemortem CT series for identification purposes. Implementation of software to create projected images from an antemortem CT series will provide forensic anthropologists, radiologists, and pathologists another avenue for the positive identification of remains.

Forensic Radiology, Positive Identification, CT Scan Project

H37 A Case of Congenital Laryngeal Stenosis Diagnosed at Autopsy

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After attending this presentation, attendees will understand the usual pathophysiology and presentation of congenital laryngeal stenosis and its diagnosis at autopsy.

This presentation will impact the forensic science community by demonstrating the methods necessary for recognizing and diagnosing cases of congenital laryngeal stenosis at autopsy.

Congenital laryngeal stenosis is a rare condition in which the diameter of the upper airway at any point from the epiglottis to the thyroid cartilages is markedly narrowed.[1-7] This condition is usually diagnosed early in pregnancy as a part of Congenital High Airway Obstruction Syndrome (CHAOS) with ultrasound-detected pulmonary abnormalities, abnormalities of the heart and diaphragm, and increases in amniotic fluid levels.[1-7] While most cases are sporadic occurrences, a few cases reported in the literature suggest an autosomal dominant inheritance.[1,4] CHAOS has reportedly occurred in association with other syndromes such as Fraser syndrome, short rib polydactyl syndrome, Vertebral Defects, Anal Atresia, Cardiac Defects, Tracheo-Esophageal Fistula, Renal Anomalies, and Limb Abnormalities (VACTERL) syndrome, or chromosomal abnormalities such as deletion of chromosome 5p and partial trisomy 5.[1-5] Laryngeal stenosis with prenatal care is usually manageable and survival rates are good when emergent treatment is provided; however, if the stenosis goes unrecognized prior to delivery, the mortality rate can range between 80% to 100%.[2,6,7]

This presentation describes a case of congenital laryngeal stenosis diagnosed at forensic autopsy of a 39-week term gestation infant delivered to a 36-year-old *gravida* 5 *para* 3 mother. The maternal gestational history was largely unknown except for reports of a prior pregnancy ending with preterm delivery of a still-birth fetus who had numerous physical deformities. The prenatal history of the current pregnancy was unremarkable except for gestational diabetes; the mother received regular ultrasounds that exhibited appropriate fetal growth and movement. The mother declined an amniocentesis for genetic testing.

After presenting for induction of labor, the infant was delivered in the hospital and was noted to have APGARs of 1, 1, 1, and the following physical abnormalities: "webbed fingers, small ears, abnormal genitalia, and left eye deformity." The infant survived for approximately one hour, during which time she experienced marked respiratory distress and eventually died. The case was referred to the forensic pathologist by the coroner's office for autopsy.

At autopsy, the body measured 49cm long and weighed 3,200 grams. Significant external findings included minor facial abnormalities, including a small left eye with a partially fused lid, a small left nasal opening, syndactyly, and ambiguous genitalia. Internally, there was stenosis of the larynx at the level of the glottis/subglottis and lumen diameter of ~0.2cm; airways distal to the stenosis were dilated with mucus congestion. Additionally, the right and left lungs weighed 38.3 grams and 27.2 grams, respectively, and were congested but otherwise normal in appearance. Ascites was noted with approximately 20ml of serous fluid in the peritoneal cavity. Microscopically, there was vascular congestion of the alveolar capillaries and numerous intra alveolar foamy macrophages. The remaining organs examined were all histologically appropriate for gestational age.

In this case, the clinical presentation and finding of markedly narrowed larynx are consistent with a death due to the obstruction/constriction of the airway; thus, the primary cause of death was determined to be due to congenital laryngeal stenosis. The reported case is an unusual presentation of a very uncommon anomaly.[8] The presentation of congenital laryngeal stenosis at a full-term delivery is rare, especially when the presentation is unexpected.[8]

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Congenital Laryngeal Stenosis, CHAOS, Forensic Pathology

H38 Congenital Hypertrophic Cardiomyopathy in a Neonate: A Rare Etiology for Unexpected Death

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The goal of this presentation is to highlight the necessity for a thorough cardiac examination, prenatal and pediatric medical record review, and discussions with family members when faced with the unusual finding of an enlarged heart in an infant.

This presentation will impact the forensic science community by highlighting the need to consider a broad differential when approaching a hypertrophic heart in the infant population and recognize the presence of posterior rib fractures due to resuscitative efforts.

Full postmortem examinations are critical in sudden unexpected infant deaths. This study presents a rare case of congenital hypertrophic cardiomyopathy with asymmetric hypertrophy, regional myofibrosis with calcifications, and myofiber disarray causing sudden death in a neonate.

A 2-week-old male neonate with no significant past medical history made an unusual crying sound prior to becoming unresponsive while being fed a formula bottle by his biological father. Paramedics responded and found him apneic and asystolic. Resuscitative efforts were continued in route to a local children's hospital with no change in clinical status. His mother had an unremarkable prenatal course with no evidence of diabetes and a normal 19-week anatomy ultrasound. The infant was born at 38 weeks and 0 days gestation via emergent Cesarean section due to fetal decelerations. Birth weight was 3.35kg (25 to 50 percentile) with a head circumference of 35cm (25 to 50 percentile). Initial APGAR scores were 8 and 8 at one and five minutes, respectively. He received supplemental oxygen at birth due to a dusky appearance and responded appropriately. A soft, grade II systolic murmur was heard but resolved prior to hospital discharge. He appeared healthy with no medical issues. Notably, the newborn screen sent at approximately 36 hours of life was within normal limits and he passed the congenital heart disease screen.

Autopsy findings included an enlarged heart with right ventricular enlargement and asymmetric hypertrophy of the interventricular septum (thickness=1.5cm) as compared to the left ventricle (thickness=0.6cm) and an accompanying dilated ductus arteriosus. Multiple regions of white, firm fibrosis were present throughout the myocardium, with the largest measuring up to 1.0cm in the interventricular septum. Microscopically, multifocal hypocellular myofibrosis was present with adjacent calcification and myocyte disarray, seen most prominently in the interventricular septum with extension into adjacent papillary muscles. No additional cardiac anomalies were identified. Other findings included a tetra-lobated right lung. No dysmorphic features were present. Rib fractures were present anterolaterally (right #3-4, left #3-4) and posteriorly (right #3-6, left #2-7) with minimal soft tissue hemorrhage due to cardiopulmonary resuscitation.

This neonate was diagnosed with congenital (infantile) hypertrophic cardiomyopathy. The diagnosis of hypertrophic cardiomyopathy requires left ventricular hypertrophy with a notable absence of other abnormalities to explain the degree of hypertrophy. Infantile hypertrophic cardiomyopathy is extremely rare, with an annual incidence of 3.6 per one million children. Clinical signs are variable and range from presenting with a heart murmur, evidence of heart failure, or sudden death, such as in this case with discovery at the time of autopsy. The etiology for hypertrophic cardiomyopathy is equally heterogenous in the pediatric population and includes glycogen storage diseases, mitochondrial disorders, neuromuscular disorders, certain genetic syndromes (most commonly Noonan syndrome or Beckwith-Wiedemann syndrome), infants born to obese or diabetic mothers, and inherited or *de novo* mutations in the sarcomeric protein genes. No evidence of a storage disorder, syndromic findings with dysmorphic features, or maternal diabetes was ascertained in the clinical history or postmortem examination. Both parents of the infant subsequently have had normal cardiac anatomy on echocardiograms and normal electrocardiograms. Genetic testing has not been performed at this time. This case highlights the need to consider a broad differential when approaching a hypertrophic heart in the infant population and recognize the presence of posterior rib fractures due to resuscitative efforts.

Hypertrophic Cardiomyopathy, Sudden Unexpected Infant Death, Posterior Rib Fractures

H39 Acute Neonatal Appendicitis — An Autopsy Diagnosis

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The goal of this presentation is to present a case of ruptured acute appendicitis in a 19-day-old neonate.

This presentation will impact the forensic science community by illustrating how acute neonatal appendicitis remains a diagnostic challenge. The majority of cases are discovered only during postmortem examination. A high index of clinical suspicion and meticulous analysis of clinical features can lead to an early diagnosis and a more timely surgical intervention to reduce its associated high mortality rate.

Acute neonatal appendicitis is a very rare condition with high mortality. It remains a diagnostic challenge despite the availability of advanced diagnostic imaging. Reported here is a 19-day-old male baby who presented with a clinical picture of a possible small bowel obstruction, and was found at autopsy to have a perforated appendicitis.

Case Report: This case involves a 19-day-old male infant born at full term via spontaneous vaginal delivery following an unremarkable pregnancy. He received a Hepatitis-B vaccine shortly after the delivery and stayed in the newborn nursery for two days prior to discharge. He was diagnosed with thrush at ten days old and was managed with nystatin.

He presented at an emergency department with one-day complaints of increased fussiness and difficulty feeding and was noted to have a slightly distended and tender abdomen. A septic workup was performed and included a Complete Blood count (CBC) (revealed leukocytosis of 28.5k/UL), a negative blood culture, a lumbar puncture (clear fluid with a negative Gram stain), urinalysis (25mg/dL protein with negative nitrite and leukocyte esterase), and elevated C-Reactive Protein (CRP) (194.6mg/L). The patient was subsequently started on antibiotics for neonatal fever. During his three days of hospitalization, the clinical team requested a transfer to the pediatric Intensive Care Unit (ICU) due to worsening of abdominal distension, increasing white count to 43.81k/UL, and suspicions of small bowel obstruction.

The patient further deteriorated during transfer and was significantly obtunded on arrival to the pediatric ICU. He was grossly edematous, with abdominal distension, anisocoria, bruising along the right flank, absence of reflexes, and minimal spontaneous movement. Radiographic studies performed included a Kidneys, Ureter, and Bladder (KUB) (showed absence of air in the rectum, edema of the bowel walls, but no free air on cross-table film), an abdominal X-ray (showed a mild-to-moderate degree of gaseous distention of the bowel), and a chest X-ray (showed bilateral pulmonary opacities). The patient was acutely managed for hypoxia associated with severe metabolic acidosis, hypokalemia, hypotension, and hypoglycemia. He subsequently had several episodes of recurrent wide complex tachycardia and pulseless electrical activity. After multiple resuscitations, he was pronounced dead several hours after the transfer.

An autopsy was performed and the most significant findings were gangrenous appendicitis with evidence of rupture and marked acute serositis identified in the rectal serosa and focally in the mesenteric peritoneum. There was dilation of the transverse colon and proximal descending colon but no obstruction of the bowel was identified.

Discussion: Acute neonatal appendicitis is a very rare clinical entity with 0.04%-0.2% reported incidence.[1] It is more common in males, with up to a half of all reported cases involving premature infants.[2] The different factors attributing to the low incidence include funnel-shaped appendix with a wide opening into the cecum, soft liquid diet, lack of fecalith, recumbent posture, and the presumed infrequent occurrence of viral-induced lymphatic hyperplasia in the periappendiceal region.[3]

The rarity of this disease coupled with the associated diagnostic challenge largely contributes to the reported high mortality rate of up to 28%.[2] Abdominal distension, a non-specific clinical feature, is the most common clinical feature present in up to 89% of the patients and was one of the presenting features of this patient. Other clinical features are non-specific and include vomiting, refusal to feed, irritability, temperature instability, and leukocytosis.[4] Radiological findings also include non-specific findings such as abnormal gas pattern, free peritoneal fluid, obliteration of psoas shadow, right iliac fossa abscess, and a thickened abdominal wall.[5] Other useful diagnostic tools include abdominal ultrasonography and spiral computed tomography.

Perforation plays a significant role in determining the prognosis. Due to the delayed diagnosis and management, the incidence of perforation and subsequent peritonitis is high in neonatal appendicitis. Other factors contributing to the increased susceptibility to perforation in this population include the thin appendiceal wall, a non-distensible cecum, a relatively small omentum insufficient to wall off infection, and a small capacity of the abdominal cavity, resulting in easy dissemination of infection.[6]

In conclusion, similar to this case, acute appendicitis remains a diagnostic challenge and, in the majority of cases, is discovered only on postmortem examination. A high index of clinical suspicion and meticulous analysis of clinical features can lead to early diagnosis and more timely surgical intervention to reduce the associated high mortality rate.

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Neonatal Appendicitis, Rare Clinical Entity, High Mortality

H40 The Sixth Biggest Earthquake in the World: The Working Strategy of a Forensic Identification Team Among Chaos

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After attending this presentation, attendees will understand the organization of the identification team and their importance during the 8.8 earthquake and tsunami that affected the coast of Constitucion City, Chile, on February 27, 2010.

This presentation will impact the forensic science community by indicating that identification processes were successful in this huge mass disaster event.

On February 27, 2010, Chile was affected by the sixth biggest earthquake in human history. The 8.8mww (per the United States Geological Survey (USGS)) had a duration of 3 minutes and 45 seconds and was felt by nearly the whole country. After the earthquake, three tsunamis hit the Chilean coast and created more damage in Constitucion City, Concepcion, and Talcahuano.

The city with the most deceased as a result of this natural disaster was Constitucion. Constitucion is a city in the central south coast of Chile, 359 kilometers south of Santiago (Capital City); they have an urban population of 37,000. At the moment of the mass disaster, Constitucion had a local medical examiner's office (the SML) and a local office of national registration ID office (the RCI). The nearest police forensic laboratory was Laboratorio Carmelo Garcia (LABOCAR) located 111 kilometers away in Talca City.

Immediately after the disaster, all utilities (water, electricity, and gas) and communications were lost with some damage to freeways and highways. The chiefs of the SML, RCI, and LABOCAR created an ad hoc protocol to work collaboratively and create a temporary morgue in the Municipal gymnasium where the SML and LABOCAR received, analyzed, and identified all the victims' bodies.

The main goal for SML and LABOCAR was to examine the bodies and verify if they had injuries related to the event, complete the antemortem International Criminal Police Organization (INTERPOL) form, fingerprint all victims, and attempt preliminary identification with the relatives of the victims. The goal for RCI was to confirm the identity using the preliminary identification and the fingerprints of the victims.

The identification process was divided into three steps: (1) Step 1 — Although a large victim pool, identification was achieved with relative ease using fingerprints and visual identification by next of kin; this step was completed three days after the earthquake; (2) Step 2 — Identification was more difficult due to decomposition, which was observed in nearly all bodies; this step was complete six days after the earthquake; and, (3) Step 3 — Identification using visual appearance or fingerprints was not possible due to decomposition. The only techniques useful for identification were DNA and forensic odontology; this stage was completed 33 days after the earthquake.

During the work on this mass disaster in Constitucion City, the identification team received 94 decedents between February 27 and March 31, 2010. Of the total victims, 51 were female and 43 were male; the ages of the victims were between 0 and 89 years. The cause of death in the victims was crushing, suffocation, drowning, heart attack, polytrauma, and cranial polytrauma.

This collaborative effort resulted in the positive identification of 100% of the victims. The visual and fingerprint identification process was successful in 89 cases. In the other five cases, the identification was confirmed by forensic odontology and reconfirmed with DNA.

This study is significant for several reasons. Understanding the importance of teamwork in the successful victim mass fatality identification process, in difficult/challenging working conditions, along with strategies to work with other agencies is essential. This event is an example of effective interagency collaboration in an emergency situation (without a pre-existing protocol).

Earthquake, Tsunami, Disaster Victim Identification

H41 Differentiating Impact and Heat-Related Skeletal Fractures From a Small Plane Crash

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After attending this presentation, attendees will have a better understanding of skeletal trauma resulting from thermal damage and/or a rapid deceleration event.

This presentation will impact the forensic science community by outlining the differences between skeletal fractures induced by heat and those related to rapid deceleration events. In a medicolegal context, it is important to distinguish the cause of fractures encountered during autopsy. Because heat-related and peri-mortem fractures can be difficult to distinguish, this information will serve to better inform the medicolegal community and increase the accuracy and speed of trauma identification in similar scenarios.

To illustrate differences in skeletal trauma sustained from fire or impact events, this presentation will present a case study of remains recovered from a plane crash. In November 2016, a fixed-wing air ambulance carrying four passengers crashed in Elko, NV. The plane subsequently caught fire, leading to both impact fracturing of the skeletal remains as well as burning and heat-related fractures. The main goal of this investigation was to identify the respective trauma patterns on each decedent and attribute them to the impact or to thermal causes.

Fracture type, location, and cause were documented on each individual and digital photographs were taken in the medical examiner setting, without extensive anthropological preparation. The majority of the fractures were found to result from the impact as evinced by unburned tissue surrounding the trauma sites. Further, these fractures were in the mid-shaft of many long bones, which is also commonly seen in high-velocity impact trauma. Heat-related fractures were seen in areas of charring and burning of the skeletal and soft tissues. The standard pugilistic posture, so-named due to its similarity to a boxer's stance, was observed in two individuals, with associated heat fractures of the bones of the hand and wrist. Pugilistic posture results from protein coagulation and shortening of the muscle fibers in the extremities and torso due to thermal effect; thermal fractures are also commonly present in the extremities, and these fractures are due to heat rather than occurring as a result of any mechanical force of the thermal contracture of the muscles. Several fractures exhibit limited charring (i.e., evidence of thermal damage does not extend across the entirety of the fractured surface) and are likely related to the impact event.

Overall, fractures that exhibit no or incomplete heat damage (i.e., lack charring and/or calcination) can be related to other forces (in this case, a rapid deceleration event). Because heat fractures lack the kinetic energy required to extend into unburned areas, any fracture located in an area unaffected by thermal change can be attributed to the impact event. Fractures related to fire exposure will exhibit fire damage; heat fractures result when heat breaks down the connective tissue, thereby exposing more of the bone to thermal destruction.[1] When fractures exhibit full or incomplete charring/calcination, careful examination is required to determine whether they are due to heat exposure or were sustained from other forces before exposure to the fire. The location and suspected cause of trauma can aid in making this determination, as this case study highlights. Furthermore, such examinations can be made in the typical medical examiner's office setting with some forethought to consider the differential diagnosis for these types of trauma.

This report presents a case study of four individuals from a plane crash and outlines the impact and heat-related skeletal trauma. The means to differentiate the two is also discussed, which includes a careful consideration of the morphology of the fractures and the unique events of the case.

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Thermal Fractures, Blunt Force Injury, Plane Crash

H42 Central Italy Earthquake: A Disaster Victim Identification (DVI) Experience

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After attending this presentation, attendees will better understand the different approaches of DVI, taking into account the challenges and pitfalls that may occur using the same standard protocols in different and complicated cases.

This presentation will impact the forensic science community by sharing the multidisciplinary experiences regarding the earthquake that hit Italy during the night of August 24, 2016. The 6.0-magnitude earthquake caused the death of 297 people, hitting the central part of Italy in the regions of Lazio, Umbria, and Marche. The cities and villages which experienced the worst damage were Amatrice, Arquata del Tronto, Peschiera del Tronto, and Accumuli. Those cities and villages were close to the epicenter of the earthquake.

The DVI forensic team included a biologist, a fingerprint expert, a forensic anthropologist/odontologist, a forensic photographer, and general assistants. The situation was complicated by the fact that, between the ruins, there was not enough space for a mortuary camp, and the examinations had to be performed on the ground, in an extremely hot environment, and during continuous tremors due to the aftershock. The logistic DVI center was set up in Amatrice, one of the most badly hit areas, with very limited road and bridge access, the majority of which collapsed during the earthquake or just after. Because of the limited amount of space available (approximately 3,000 square meters), the family liaison officers had to work very close to the postmortem examination area, rendering their job extremely difficult.

The bodies recovered from the ruins were placed in body bags and sent to the camp with an identification number, precise address of the recovery, and a "possible ID" (i.e., the name or the names of missing people from that address). All the data were collected in a database. The relatives and the next of kin in the immediate area were interviewed in order to collect relevant data for identification, such as photographs, information about clothing, personal belongings, prosthetic implants, scars, tattoos, and distinguishing marks. The names of the family doctor and dentist were also collected to obtain further medical information and, if possible, a dental chart.

During the initial examination, forensic photography of the body, clothing, and personal belongings was performed; fingerprints, DNA samples, and a dental chart were obtained; and a preliminary examination was conducted of the trauma on the bodies. A quick networking system between the forensic anthropologist/odontologist and the victims' doctors and dentists was set up to obtain medical information as quickly and efficiently as possible. This system could not be applied to those medical practitioners whose offices were affected by the earthquake or located in areas that could not be reached.

Since the earthquake occurred at 3:36 a.m., the location that victims were recovered was crucial for identification; in fact, the majority of victims died in their sleep, as a quick escape was nearly impossible.

Despite the many problems encountered, the hot climate, and the limited space, all identification processes were completed within two days by means of anthropological profiles, dental charts, and the examinations of personal belongings. The identification of the remaining bodies (3% of the victims) was conducted by DNA analyses and the further acquisition of dental charts, requiring a few more days. Fingerprint analyses were only performed on immigrants.

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DVI, Identification, Mass Disasters

H43 A Fatal Sex and Drug Party: Understanding the Real Cause of Death

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After attending this presentation, attendees will better understand the death differential diagnosis of the mechanical obstruction of external airways, the depressing effects of heroin on the respiratory centers of the brain, and the anaphylactic disorder caused by the interaction of heroin with mast cell receptors.

This presentation will impact the forensic science community by providing results of a multidisciplinary collaboration, which is fundamental to understanding the cause of death associated with the use of heroin in drug addicts. Moreover, it is hoped that tryptase levels in the blood and in the pericardial fluid will be routinely analyzed in heroin death cases.

In early February 2015, two drug dealers organized a sex and drug party in a flat located in the vicinity of Naples, Italy. The police were wiretapping them. During a telephone conversation, the police heard one of the drug dealers say, "She died by suffocating on by my big penis during the fellatio … we left her on the street." The next morning, a resident found a body on the street near the flat where the party took place. The forensic expert who arrived at the scene said the victim was allegedly 30 years of age, 174cm tall, and had presumably died the previous day. There was a reddish fluid discharge around her mouth and nose. Her leggings were lowered and the gluteal region exhibited signs of dragging via an inflammatory infiltrate. The corpse was identified as the transsexual BC.

The autopsy did not reveal any signs of venipuncture or violence. The victim hadn't been raped prior to death. The medical examiner found pulmonary congestion and general congestion of the parenchymal organs resulting from blood stasis. Petechial hemorrhages and brain swelling were also found. The examiner took oral, vaginal, and anal swabs for genetic analysis, and blood, urine, and bile for toxicological tests. Only the vaginal swabs were positive for the presence of sperm, and the DNA profile obtained was a mixture comprised of BC's epithelial cells and sperm cells of unknown people. The blood tests were positive for morphine (251ng/ml), 6-monoacetylmorphine (15ng/ml), codeine (32ng/ml), cocaine (167ng/ml), and benzoylecgonine (350ng/ml). The urine analysis was positive for morphine (224ng/ml), codeine (11ng/ml), cocaine (39ng/ml), and benzoylecgonine (1,172ng/ml). The bile was also positive for morphine (720ng/ml), 6-monoacetylmorphine (27ng/ml), codeine (28ng/ml), cocaine (1,768ng/ml), and benzoylecgonine (320ng/ml). The ethanol level was 0.91g/l in the blood and 1.90g/l in the urine.

Additionally, the histopathological analysis conducted on fragments of the central lung parenchymal area revealed activated mast cells, lymphocytes, and monocytes. A tryptase quantification analysis was performed on the serum and on the pericardial fluid. The values were, respectively, 15.8µg/l and 11.1µg/l, both higher than normal.[1,2]

In conclusion, the absence of signs of venipuncture on BC's body, the presence in the victim's blood and urine of alcohol, morphine, 6-monoacetylmorphine, codeine, and cocaine was proof that she had inhaled drugs. This scenario would suggest she died of a fatal cocktail of drugs and alcohol. Instead, the presence of active mast cells, together with lymphocytes and monocytes in the lungs, associated with tryptase levels in the serum and in the pericardium, revealed that her death had been caused by an anaphylactic disorder.[3-7] When left by the roadside, she was still alive and, with proper resuscitation efforts, she could have been saved.

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Forensic Science, Heroin, Tryptase

H44 Considerations on Death Caused by Heroin Inhalation: A Literature Review

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After attending this presentation, attendees will better understand how death is caused by the depressing effects of heroin on the respiratory center of the brain after inhalation.

This presentation will impact the forensic science community by illustrating the results of a multidisciplinary collaboration, which is fundamental to understanding the cause of death of individuals who use heroin occasionally and only by inhalation.

The police report containing MP's statements reads that at 8:00 p.m. on a mid-July day in 2010, five men whose average age was 28 went to a secluded park area by the Sabato bridge in Benevento (southern Italy). They first drank two liters of wine and four liters of beer. After approximately one hour, KN took a half-liter plastic bottle, placed a pierced piece of aluminum foil on the mouth of the bottle, made a hole sideways into which he inserted a straw, and lit the dark-colored substance lying on the piece of foil. This phenomenon is known as *chasing the dragon.*[1,2] All the men inhaled the fumes produced by the combustion through the straw. At midnight, the friends went away, leaving KN alone near the bridge. At 9:00 a.m. the next morning, at his mother's request, the police began looking for KN. They found him lifeless just where his friends had left him. The medical examiner arrived at the scene and, based on the thanato-chronological data he had collected, said that the victim had died approximately between 10:00 p.m. and midnight the previous day. He did not have any external skin and/or skeletal lesions except for some reddish fluid leaking from his right ear canal.

During the autopsy, the medical examiner found no signs of venipuncture nor any self- or other-inflicted injuries. The oral cavity was intact and free of contaminations. Instead, the medical examiner found minor signs of cerebral edema, severe pulmonary congestion with a serum-and-blood secretion discharging when pressed, and a general congestion of the internal organs caused by the inhibition of the breathing center. The medical examiner collected samples of blood, urine, and bile for toxicological testing. The blood tests were positive for morphine (252ng/ml) and traces of codeine. The urine was positive for morphine (2,392ng/ml), codeine (9ng/ml), cannabinoids (53ng/ml), and ethyl alcohol (1.09g/l). The bile was positive for morphine (120ng/ml), 6-monoacetylmorphine (17ng/ml), and codeine (11ng/ml).

In conclusion, the absence of signs of venipuncture on KN's body, the presence of morphine, codeine, and alcohol in his blood, and morphine and codeine in the urine were proof that KN had taken drugs by inhalation. This scenario suggests that he died due to an overdose of morphine resulting from heroin inhalation. Through the literature review, the study can affirm that death caused by heroin inhalation, associated with apparent low levels of morphine in the blood, is due to a low drug tolerance and to interactions with other Central Nervous System (CNS) depressants or other systemic factors that have not yet been identified.[3-5]

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Forensic Science, Heroin Inhalation, Overdose Fatality

H45 The Lethal Attack of Cane Corso Dogs: A Multidisciplinary Approach to Solve the Puzzle

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After attending this presentation, attendees will be aware of the importance of a multidisciplinary approach to resolve court cases in which it is difficult to understand the unfolding of events.

This presentation will affect the forensic science community by demonstrating how a multidisciplinary approach was fundamental in establishing the cause of death of a man attacked by Cane Corso dogs. Moreover, this presentation will emphasize the importance of interaction between different forensic professionals, such as pathologists, veterinarians, geneticists, toxicologists, and odontologists.

The Cane Corso is a large and muscular Molosser breed of dog. Males stand 25.0 to 27.5 inches and females 23.5 to 26.0 inches at the withers. Weight is in keeping with the stature, ranging from 90 to 120 pounds. It is believed to be the descendant of a breed of warfare dogs used by the Romans as auxiliaries in the Legions. This ancient Italian breed of dog is usually trained as a guard and hunting dog.

In October 2015 at 8:00 p.m., a 61-year-old man was found dead in a farm orchard located near Sant'Angelo in Formis (Caserta, southern Italy). The corpse was face up (supine position) in a pool of partially dried blood, near an olive tree. He had died 4-6 hours earlier. His face, arms, legs, and abdomen exhibited signs of severe contusions and lacerations from dog bite wounds. He wore socks and shoes, as well as shreds of a sweater, shirt, and undershirt. His trousers were buttoned but pulled down to the ankles. Other shredded clothing was scattered for a range of two meters. All of his clothes exhibited dog bite marks. An overturned basket of olives was found near the tree. A police officer told the forensic expert he had seen a black dog moving away from the corpse and disappearing through a hole in the wire fence. The forensic expert took a sample of blood from the dog's lower lip. Initially, it was assumed that the dog (labeled as dog 1) had attacked the victim while he was picking olives from his trees. Subsequently, during the on-the-spot investigation, five more Cane Corso dogs were located in the area surrounding the crime scene. They were labeled with numbers 2 through 6 and placed in a kennel for further investigation.

The autopsy revealed traumatic wounds caused by several dog bites. The victim's death was due to a hemorrhagic and traumatic shock caused by Molosser dog bite wounds. The lethal bites were discriminated from non-lethal bites and from postmortem lacerations. The dental arches undoubtedly matched those of a dog. With the help of an odontologist, the medical examiner sampled three of the lethal cutaneous arches and fixed them in a formaldehyde/acetic-acid/ethanol solution. A multiple comparison was conducted between the victim's cutaneous arches, the bitemarks on the clothes, and the six dogs' dental impressions[1-4]. Comparisons revealed matches for dog 1 and dog 2.

Several spots on the corpse around the bites were swabbed while cloth samples were taken to perform a genetic analysis.[5,6] The sample taken from the dog 1's lip was the victim's blood. The genetic profiles of dog 2 and of another dog that was not in the kennel were found on the victim's trousers.

The medical examiner sampled only blood specimens for toxicological analysis because the bladder was empty. Gas Chromatography/Mass Spectrometry (GC/MS) analysis was negative.

The histopathologist analyzed fragments of the victim's heart and found that he suffered from coronary artery disease, congestive heart failure, and minor bleeding events of the epicardium. The histological analysis confirmed the vitality of several lesions, suggesting that the man was still alive before the attack.

A multidisciplinary approach was necessary to solve the case. It was determined that the victim's death was due to a hemorrhagic and traumatic shock caused by Molosser dog bite wounds. Moreover, in cases of traumatic death in which animals are directly involved, the collaboration of different forensic professionals is very important. Finally, forensic veterinary medicine has now become an indispensable branch of forensic science in such cases.

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Forensic Science, Dog Bites, Canine DNA Profiling

H46 Patterns of Bruising in Cases With and Without Alcohol Abuse

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The goal of this presentation is to increase the scientific support for conclusions about injury patterns and to better interpret blunt force trauma in cases of alcohol abuse.

This presentation will impact the forensic science community by reviewing the basis for interpreting bruising in cases with and without alcohol abuse.

This presentation will be an example of how the dead teach the living by examining the distribution and extent of bruising in cases of medicolegal autopsies with and without alcoholism and testing the hypothesis that in alcoholism bruising is more extensive. Such information is important when reaching conclusions concerning the distribution of blunt force trauma in both the medicolegal autopsy setting and in the setting of clinical forensic medicine (e.g., examining live victims or suspects of crimes).

In Scandinavian countries, forensic pathologists are concerned with both medicolegal death investigations and clinical forensic medicine. Of importance in both branches is separating injuries inflicted during everyday life and those inflicted by violent assaults, for example.

Since the forensic pathologist gains experience of the normal distribution of injuries in the medicolegal autopsy population, this knowledge can be used to interpret injuries in the living. Generally, it is assumed that chronic alcohol abusers have a heavier burden of blunt force trauma compared to a population without such abuse.

To objectively classify the level of alcohol consumption, blood levels of Phosphatidylethanol (PEth) in whole blood were used to classify the studied population into three groups: low, intermediate, and high consumers of alcohol. PEth is a marker of alcohol consumption registering alcohol intake during approximately two weeks preceding the death and is routinely used at the forensic medicine unit in Lund, Sweden. Cases in which PEth had been analyzed were identified and the autopsy reports were then studied for information regarding bruises. Bruises were counted, their location noted, and, from the original data collected, information about their size and if they were recent was also noted.

In this study, 118 consecutive forensic autopsy cases were identified in which a PEth blood sample analysis had been ordered, beginning in February 2017 and working backward to October 2015. After excluding traffic accidents, homicides, decomposed cases, and cases not finalized, this resulted in a total of 101 cases that were included in the study.

The association between the three categories of PEth (low, moderate, and high alcohol consumption) and having more than three bruises on the body was analyzed using logistic regression. No conclusive associations between the PEth concentration and the numbers of bruises were observed; however, observing only the point estimates indicated a tendency of a U-formed association between the number of bruises and the PEth categories, in which the most number of bruises were observed in the high consumption category and the least number of bruises were observed in the medium consumption category. It appeared as if female sex was associated with an increased number of bruises. No conclusive association between age and number of bruises was observed.

In conclusion, no statistical support for the hypothesis that alcoholics in a medicolegal autopsy setting are subjected to more accidental blunt force trauma reflected in the number of bruises compared to non-alcoholics could be identified in this study. The results may be produced by low statistical power and selection bias, and the study should be extended to include a consecutive and larger population. Nonetheless, this study provides an example of how to increase scientific support for arriving at conclusions regarding patterns of injuries in both the medicolegal autopsy setting and in clinical forensic medicine.

Medicolegal Autopsy, Clinical Forensic Medicine, Bruises

H47 Fatalities Due to the Failure of Continuous Subcutaneous Insulin Infusion Devices: A Report of Six Cases

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The goals of this presentation are to provide: (1) an understanding of how insulin pumps work; and, (2) recognition of potentially fatal malfunctions of insulin pumps.

This presentation will impact the forensic science community by providing the results of a series of cases that have not previously been presented in the literature.

Treatment of diabetes mellitus often requires injection of exogenous insulin to manage the disease. Some patients elect to use Continuous Subcutaneous Insulin Infusion (CSII) devices rather than repeated needle sticks for the administration of insulin. Malfunctions of any component of the CSII device can result in insufficient insulin delivery and, if not recognized in a timely manner, can lead to fatal ketoacidosis.[1] While there are studies describing complications of CSII devices in living patients, the literature is extremely limited in describing CSII failure-associated mortality.

This study presents a series of six cases of fatal diabetic ketoacidosis resulting from insulin infusion set malfunctions in patients undergoing continuous subcutaneous insulin infusion. The six cases were compiled from four different medical examiner offices in the United States. The patients varied in age, ranging from 37 to 79 years old. Both genders are represented as are both types of diabetes mellitus. Autopsy examination, including toxicologic analysis, was performed on all cases. In four of the six autopsies, no anatomical abnormalities were observed. The findings in the other two autopsies were either not significant to cause the patient's death or could be considered secondary to the patient's history of diabetes.

Infusion set malfunctions are among the most common and significant complications of CSII devices.[2,3] In all six cases, the malfunction was identified to be failure of the cannula to puncture or remain in the skin (in five of the six cases, the insulin infusion set failed to puncture the skin, and in one case it appeared that the Insulin Infusion Set (IIS) had initially punctured the skin to some extent, but it was unclear if the cannula had failed to fully insert or had inserted and subsequently dislodged). Also, in five of the six cases, the cannulas were bent or kinked; in one case, the cannula appeared straight but was not inserted through the skin. Four of the six cases involved perpendicularly inserted cannulas, all of which were bent or kinked. Of the two angled cannulas, one was bent and another had failed to insert. In all cases, infusion set malfunction resulted in the failure of insulin infusion, which then resulted in fatal ketoacidosis.

The cause of death in all cases was certified as either diabetic ketoacidosis or complications of diabetes mellitus; however, since it is possible to consider the unexpected natural death of diabetic ketoacidosis which occurred as a result of improper application of their therapeutic device as either natural or accident, there is variation in classification of the manner of death. In the six cases presented in this study, manner of death was classified as accident in three cases and natural in three cases.

Recognition of these potentially fatal CSII-device malfunctions is important in order to prevent future deaths. Though only six cases are presented, given the widespread use of this treatment modality, it is likely there are unrecognized instances in which failure to properly place the IIS cannula during CSII therapy allowed the patient's diabetic condition to progress to a fatal state of ketoacidosis. Examining and understanding these six deaths can lead to future prevention through advancing technological applications and educational practices as well as contribute to proper classification of deaths related to CSII failure.

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Insulin Pump, Insulin Infusion, Mortality

H48 Bathtub-Related Deaths

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After attending this presentation, attendees will better understand the epidemiology and circumstances of bathtub-related deaths. Attendees will also learn about the evaluation of scalp lacerations and how these wounds correlated with deaths due to falls in bathtubs.

This presentation will impact the forensic science community by adding new knowledge regarding injuries sustained in bathtub-related deaths, with emphasis on the correlation with lacerations of the scalp.

This presentation seeks to answer the questions that were of importance in a recent high-profile case in which court testimony centered around the presence and orientation of scalp lacerations as an indication of a fall in a bathtub: Do lacerations of the scalp occur at all in bathtub-related drownings and, if so, is a certain shape of the wounds more frequent?

Determining the cause and manner of death in a bathtub death can be difficult. This study grew out of questions in a high-profile death in which a woman was found dead in a bathtub. This study describes the epidemiology and circumstances of bathtub-related deaths in Sweden. In reference to this case, traumatic head injuries, including the orientation of the scalp laceration, were highlighted. Searches in the database of the National Board of Forensic Medicine in Sweden were conducted for the time period 2007 through 2013. Cases in which the decedent was found in a bathtub, jacuzzi, or hot tub were included. Deaths in showers were also included but were analyzed separately.

A total of 381 cases were identified — 365 bathtub-related deaths and 16 shower deaths. The most common cause of death was, as expected, drowning, followed by intoxication. Suicide was the most common manner of death, but all manners of death were encountered, including two homicides. The prevalence of severe traumatic injuries (AIS>3) was low and was most prevalent among suicides from a violent method, such as gunshot or massive lacerations. A total of six cases had a laceration in the scalp similar to that described in the present case, and four of these cases were bathtub drownings. In three of the four cases, it is plausible that head trauma may have reduced the person's state of consciousness and thus contributed to the drowning and the fatal outcome; however, in neither case was the laceration stated as a (contributing) cause of death by the forensic pathologist. Three of the injuries were located around the bony structures of the right eye (the wounds were transversal, oblique, and vertical), one was on the medial surface of the left ear (vertical), and two were on the back of the head, just right of the inion (oblique and transversal).

In conclusion, all manners of death are possible in a bathtub-related death; however, as expected, suicide was the most common manner of death and drowning the most common cause of death. The prevalence of severe traumatic injuries is low and, when present, it is usually in a suicide from a violent method, such as gunshot or massive lacerations. Lacerations of the scalp in a bathtub drowning *do* occur in various orientations, but the impact of these upon the fatal outcome needs to be explored further.

Bathtub-Related Deaths, Drowning, Scalp Lacerations

H49 Suitcase Concealment: An Interdisciplinary Analysis of the Taphonomic Processes and Their Effect on Postmortem Interval (PMI) Estimation

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After attending this presentation, attendees will better understand the entomological and decompositional changes that are likely to occur when a body is concealed within a suitcase after death.

This presentation will impact the forensic science community by providing results from an experiment that analyzed the taphonomic effects of body concealment within suitcases, a topic that has had only minimal previous research conducted.

In cases of homicide, suitcases provide concealment and may ease the transport of a body with minimal likelihood for detection. In order to create a minimum Postmortem Interval (mPMI) estimate, it is first necessary to understand the unique taphonomic processes that occur when a body is concealed within a suitcase. In this study, the experimental carcasses consisted of pig (*Sus scrofa*) heads, which were concealed within either hard-shell plastic suitcases or fabric suitcases; the control pig heads were left on the surface of the ground to decompose naturally. Starting on day 3 of each study period, and continuing every other day until day 15, three suitcases of each type were removed from the field for analysis of the entomological activity inside the suitcases and the decompositional stage of the pig heads. Additionally, the ambient temperature and the temperature inside each type of suitcase was recorded by temperature data loggers throughout the duration of each study period. The study was conducted at Boston University's Outdoor Research Facility in Holliston, MA. This study was repeated twice, once in May and once in August 2016.

Temperature comparisons revealed that the hard-shell plastic suitcases reached significantly (<0.001) hotter temperatures than both the ambient temperature and the temperature inside the fabric suitcases. Insect activity began immediately on the control samples during both study periods; however, during study one, insect activity was not present inside the fabric suitcases until days 3-5, and did not occur inside the hard-shell suitcases until days 5-7. During study two, insect activity inside both types of suitcases was present by day 3, but not guaranteed to occur until day 4 or later. Some differences in insect species were noted between the controls and the suitcases, as well as between both types of suitcases. Most notable was the presence of a number of fly (Diptera) species inside the suitcases that are generally associated with late decomposition. Additionally, while beetles were present on the control samples, none were found inside the suitcases. All control samples mummified within days, while all of the experimental samples experienced wet decomposition, often resulting in skeletonization by day 15.

In conclusion, this study has shown that not only does concealment within a suitcase change the taphonomic history of the body enclosed, but that the type of suitcase also influences the taphonomic factors that the body will experience. Ultimately, this study will aid in the ability to better predict the mPMI for cases in which a body is concealed within a suitcase.

Suitcase Concealment, Taphonomy, Postmortem Interval

H50 Insects and Bacteria as Forensic Decomposition Markers of Buried Rat Carcasses

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After attending this presentation, attendees will have a detailed understanding of carcasses decomposing in soil and of the necrophagous entomofauna identified during the stages of decomposition. Moreover, attendees will be provided with information regarding the bacterial dynamics of buried carcasses and with the proposal of two bacteria taxa as putative markers for the postmortem interval estimation.

This presentation will impact the forensic science community by adding important data regarding the decomposition process in soil as well as the insects and bacteria associated with carcass decomposition. This type of research, rarely studied to date and the experimental research conducted on exposed remains, will provide much-needed qualitative and quantitative information on necrophagous insects and bacteria associated with buried carcasses.

The decomposition process of surface remains and their associated necrophagous entomofauna has been well studied compared with buried carcasses. The insect diversity and succession pattern for buried and exposed carcasses are different due to the variations of edaphic and environmental conditions. In the case of buried remains, the occurrence of necrophagous insects can be delayed or inhibited by the climatic conditions, soil type, and depth of burial. At the same time, the carcass bacterial community structure is expected to differ between buried and exposed conditions. This becomes an important distinction in cases such as violent criminal actions, when the perpetrator tends to dispose of the cadaver by frequently burying it in a shallow grave.

In this context, the current study focused on monitoring the decomposition process of buried rat carcasses from shallow graves, the diversity and dynamic of insects and bacteria throughout the decomposition stages, and the environmental parameters' influence on these variations at a depth of 40cm. The survey took place in the spring (March) and summer (June) months of 2016 in a green urban area of Bucharest, Romania. For each case, 30 rat (*Rattus norvegicus*) adults were used as study models, and a specimen was destructively sampled every 24 hours, starting immediately after death. Both the necrophagous insect specimens (adults and larvae) and the tissue from the rat small intestine were sampled and taxonomically and genetically identified. Furthermore, the air and soil temperature, relative humidity, precipitation rate, and soil pH were recorded daily.

Necrophagous insect species were absent in March, given the low temperatures that did not exceed 10°C in soil, and thus were observed and sampled solely in July. Two Diptera (Muscidae, Phoridae) and two Coleoptera (Leiodidae, Staphylinidae) families were identified, encompassing five and two species, respectively. All Diptera developmental stages were observed and sampled from the remains, beginning with the active decomposition stage, while Coleptera were present only in the last stage.

From the small intestine and insect tissues, the total genomic DNA was extracted and the bacteria diversity was investigated by Illumina® MiSeq® analysis and taxonomy was assigned via QIIME™ platform and Greengenes database. A preliminary screening of the bacterial community structure of the small intestine during decomposition stages, determined by 16S recombinant DNA (rDNA) Denaturant Gradient Gel Electrophoresis (DGGE), led to the identification of two bacterial taxa belonging to Firmicutes of permanent and sporadic occurrence. The analysis of their relative content during decomposition, quantitated by quantitative Polymerase Chain Reaction (qPCR), led to the identification and proposal of putative bacterial markers for postmortem interval estimation.

The results exhibit correlations between the insect species presence and environmental parameters variation, stages of decomposition and bacterial communities' diversity and dynamics, and modification of soil pH throughout the evolution of the decomposition process. This data represents the first study of bacterial diversity determined by 16S rRNA Illumina® sequencing for buried carcasses in a natural environment, proposing microbial markers for forensic investigations based on quantitative evolution of certain bacterial taxa.

Buried Carcasses, Insects, Bacteria

H51 The Influence of Depth and Mixtures on the Bacterial Profiling of Soil Using Next Generation Sequencing

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After attending this presentation, attendees will understand how different soil depths and mixtures alter the bacterial profiles produced from soil samples and that these factors must be considered when collecting known soil samples in order to produce strong associations between evidentiary soils and the site of a burial.

This presentation will impact the forensic science community by examining how various depths of soil and mixed samples, as may be encountered in burials, affect the bacterial composition of soil, thus expanding the knowledge necessary to successfully individualize forensic soil samples.

Soil is a commonly recovered form of trace evidence found on items such as shovels, tires, or clothing that has the potential to be traced back to its place of origin. Successful identification of soil can be valuable for forensic applications, as it can link a suspect or victim to the scene of a crime. In the past, soil has been analyzed primarily using physical class characteristics. More recently, researchers have sought to individualize soil evidence by analyzing the huge number of bacteria that inhabit it.[1,2] Many factors affecting the bacterial profiling of soil have been studied, including temporal and spatial changes; however, potential vertical changes have yet to be rigorously examined. The bacterial composition of soil may change as depth increases, as was found over horizontal space.[3,4] This could be particularly important in burials, where soil from various depths would be mixed. Understanding how depth and mixtures affect the bacterial profiles of soil is imperative in understanding how to collect and analyze known soil samples.

In the research presented, soil samples were collected from the surface and 5, 10, 20, 40, and 60 inches deep at a central coring location and four nearby corings. Five habitats were examined: two separate agricultural fields, a coniferous forest, an untreated yard, and a deciduous woodlot. Using these depth samples, mixtures were created to simulate a burial by homogenizing equal masses of soil from all six depths for each of the 25 corings. Different combinations of depths were used as training sets for supervised classification to examine the best way to collect depth samples in the case of a mixture or burial.

Bacterial DNA was extracted from soil samples using a DNeasy PowerSoil® kit, and variable regions 3 and 4 of the bacterial 16S recombinant DNA (rRNA) gene were amplified using universal barcoded primers. Bacterial sequences were produced on an Illumina® MiSeq® and visualized via abundance charts and non-metric multidimensional scaling. A bagged trees algorithm was used to objectively associate depth and mixture samples to a habitat and to provide likelihood scores that each of the classifications was correct.

In four of the habitats, there were increases in the bacterial classes Betaproteobacteria and Nitrospira and decreases in Sphingobacteria and Spartobacteria with depth. In contrast, the coniferous forest displayed increases in Deltaproteobacteria and Nitrospira, and decreases in Sphingobacteria and Alphaproteobacteria. The mixture samples for all habitats exhibited a bacterial profile resembling the shallowest soils. Graphically, as depth increased, the soil samples drifted away from the surface samples in multidimensional space, with the 40" and 60" samples being furthest away from the shallow samples while the mixtures grouped closest to the surface, 5", and 10" samples.

The central coring mixtures were then compared to different depths from the four surrounding corings using supervised classification. When compared to surface, 5", or surrounding mixtures, 100% classification accuracy was achieved. This accuracy decreased as depth increased, with the 60" samples producing 20% classification accuracy when used as the training set. This mimics the bacterial composition differences seen between the mixtures and the deep samples, as well as the deep samples being far away from the others in multidimensional space. The results indicate that the deep soil samples contribute very little to the mixture's bacterial profile and, thus, may not need to be collected, even when soils are from a burial.

These results elucidate the effects of vertical spatial changes, along with mixtures, on the bacterial composition of soil via next generation sequencing. Our understanding of these changes aids in the determination of how to best collect and analyze known soil samples when a burial has occurred.

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Soil Bacterial Profiling, Next Generation Sequencing, Bacterial 16S Sequencing

H52 A Survey of Bacterial Diversity Associated With Various Life Stages of *Lucilia sericata* and *Phormia regina* Collected From Central Virginia

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After attending this presentation, attendees will better understand bacteria associated with blow flies that colonize human remains. This information will help forensic scientists in improving accuracy associated with the estimation of the Postmortem Interval (PMI) based on entomological and microbial evidence.

This presentation will impact the forensic science community by helping provide a better understanding of carrion resource utilization, blow fly colonization pattern determination, and in improving precision in PMI estimation using entomological and bacterial evidence.

Reliable and valid methods for the PMI determination are frequently sought. Information obtained from the colonization of insects and the related bacterial succession on carrions may be utilized for PMI estimation, but this can lead to erroneous results due to known variations between individuals and species.[1] Apart from the bacterial communities found with human remains, it is important to note what bacteria may be specifically associated with blow flies colonizing these remains immediately after death. Blow flies often associated with carrions in Virginia are *Lucilia sericata* and *Phormia regina.*

This comparative analysis of the various life stages of *Lucilia sericata* and *Phormia regina* was performed as the initial setup of an ongoing study, including various species of blow flies associated with human remains in central Virginia. Although extensive data exists on the biology of blow flies, a well-replicated study on bacteria associated with different life stages of many blow flies is missing. This study characterized bacteria associated with different life stages of *Lucilia sericata* and *Phormia regina* by using 16S recombinant DNA (rDNA) MiSeq® sequencing. Five *Lucilia sericata* colonies and four *Phormia regina* colonies were established from female flies collected from Richmond, VA, and nearby areas on beef liver bait. First-generation eggs, third-instar larvae, pupae, adults, and second-generation eggs were collected from each colony for DNA extraction using the organic Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method and dual-index 16S rDNA MiSeq® sequencing using the protocol as described by Kozich et al.[2] Sequences were then analyzed using Mothur version 1.39.4, and statistical analysis was performed using R version 3.4.0.[3,4]

The majority of bacteria associated with both *Lucilia sericata* and *Phormia regina* belonged to the phyla Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria and are intragenerationally inherited, varying only in their relative abundance. In *Lucilia sericata,* the class Flavobacteria was present at high relative abundance (12.1 %) in pupal samples, whereas its relative abundance was very low (<1%) in all other samples. In *Phormia regina,* the class Actinobacteria was present at high relative abundance (>7%) in the adult samples, where the relative abundance in the remaining samples was very low (<1%). In *Lucilia sericata*, the genus *Vagococcos* was present at high relative abundance in larva, adult, and second-generation egg samples (>12%). In *Phormia regina*, the genus *Lactococcus* was present at high relative abundance in larva and pupa samples (>20%). In both species, egg samples had high relative abundance of the genus *Yersinia*, whereas its relative abundance was very low (<1%) in all other samples.

In conclusion, this ongoing study provides information on the bacterial communities associated with the various life stages of blow flies and their importance in forensics.

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Blow Fly, Postmortem Interval, Necrobiome

H53 The Utility of Barnacles in Forensic Investigations

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After attending this presentation, attendees will understand the usefulness of barnacles in determining a postmortem submergence time and/or floating time of remains in a marine environment. This, when coupled with sea current knowledge, can impact the provenance of those remains.

This presentation will impact the forensic science community by providing awareness regarding submerged remains as to what to observe and collect and how barnacle growth rate can aid in the total minimum Postmortem Interval (minPMI).

Estimating the minPMI is a necessary part of a forensic investigation. Besides the pathologist's assessment of the typical signs of death, minPMI can be estimated using forensic entomology, the scientific discipline that considers insects and other arthropods that colonize the remains. In an aquatic environment, insects as well as crustaceans have the potential to provide data regarding the time the remains have spent in the water (i.e., Floating Time (FT) and Post Mortem Submersion Interval (PMSI)), and this can also assist in determining the minPMI.

Barnacles (Crustacea: Cirripedia) are common crustaceans that colonize solid and durable substrates in marine environments and can often be found in association with human and animal remains floating in the sea. Scientific literature reports that barnacles are typically found colonizing shoes. Barnacles can colonize both floating remains and submerged remains and their growth rate is dependent on the water temperature. Despite their potential to be indicative of the FT and/or PMSI, at present, research is depleted and only a few case studies have considered it for this purpose.

The present research is focused on the barnacle colonization of different types of shoes (sporty vs. elegant) placed in the sea (Boston Harbor, Boston, MA). The objectives of this study were: (1) the identification of the species of barnacles that colonize shoes; (2) the identification of the settlement preferences of the barnacles associated with the shoes; and, (3) to determine the growth rate of the barnacles associated with the shoes.

For the purpose of this research, in early March 2016, 64 sport shoes and 64 patent leather shoes were placed in the Boston harbor at 8m–10m below the sea level. Four of each shoe type were collected every two weeks from April 2016 to November 2016, inclusive. Each shoe was photographed and the barnacles and other sea life colonization was documented. Individual barnacles from each shoe were sampled and measured to determine species and age as well as the overall colonization density and settlement preference. Data loggers were placed with the shoes to record temperature throughout the course of the study.

Results show that *Amphibalanus improvisus* (Darwin) (Crustacea: Cirripedia: Sessilia) colonized the vast majority of shoes. Colonization occurred quickly and continued throughout the study period. A significant difference in colonization densities was found between the sport and patent leather shoes, with the patent leather seeing higher densities. Barnacles also showed preferential colonization of specific sections on both shoe types. Overall, higher quantities of barnacles were found on the exteriors and bottoms of shoes and low quantities of colonization on the insides, tongues, and laces. Barnacle growth was found to be significantly affected by water temperature. Statistical analysis of the effect of water temperature, time, and shoe type on the size of the largest barnacle revealed a highly significant effect from temperature and shoe type and no significant effect from time. Time and shoe type had a highly significant effect on the total number of barnacles per shoe, whereas water temperature had no significant effect.

Barnacle, PMSI, Growth Rate

H54 The Beaver Dam, Flies, and the Ax: You Can't Hide From Mother Nature

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After attending this presentation, attendees will understand the importance of a cooperative, multidisciplinary approach to investigating and solving a complex homicide.

This presentation will impact the forensic science community by demonstrating the value of incorporating forensic disciplines into complex investigations. Experts in forensic pathology, forensic anthropology, and forensic odontology contributed to law enforcement investigation efforts in the interpretation of homicide injuries and complex postmortem influences occurring within diverse environments (i.e., dismemberment with an edged instrument, mutilation, gunshot wounds from multiple weapons, and evidence of peri-mortem and postmortem influences).

The decedent was taken by two acquaintances to a remote wooded area to inspect a clandestine "below-ground" methamphetamine cooking laboratory. The decedent, who was suspected of being a police informant, was killed and subsequently buried in the same hole as the alleged underground laboratory. Approximately two weeks later, the killers became nervous about the body being discovered and returned to the grave. The decedent was exhumed, dismembered to facilitate removal, packaged in plastic bags, wrapped in tarps, and subsequently submerged in a nearby lake.

The victim remained missing for 22 months and was discovered due to a natural event that changed the landscape. A beaver dam supporting the lake failed, revealing tarps containing the remains. Within the rolled-up tarp were five plastic garbage bags. Initial examination revealed numerous pupal cases associated with the bags. To insure accuracy of trauma interpretation, the contents of each bag were scrutinized and documented.

Radiographs of each bag revealed what appeared to be the fragmented remains of a single decedent, multiple bullets associated with the torso, a severely deformed skull with a "lead snowstorm" appearance, large bone separation that appeared to be straight-edged and occasionally angled, resembling blunt trauma. A cinder block was also observed in the radiographs.

An autopsy on the decomposed remains confirmed a single, incomplete individual. Identification was confirmed through antemortem and postmortem dental radiograph comparisons and DNA analysis. The degree of skeletal representation was highly variable due to the sustained traumatic injuries. The cause of death was diagnosed as multiple gunshot wounds as evidenced by a shotgun wound to the posterior skull and multiple gunshot wounds to the chest and abdomen; gunshot wounds were also observed in the sacrum and lumbar vertebrae.

The sharp trauma was indicative of a heavy edge-beveled blade that cuts soft tissue and bone, but lacks penetration due to a thick blade. The chopping action initially cuts, but does not penetrate far before breaking the bone. Sharp chops associated with blunt trauma occurred on the mandible, posterior C2, and extremities.

The antemortem, peri-mortem, and postmortem analysis of a violent death involved a thorough investigation and the cooperation of personnel representing pathology, anthropology, odontology, forensic autopsy, and local law enforcement. The collective efforts of the above individuals resulted in a positive identification of the decedent, in the determination of the cause and manner of death, and in the conviction of the assailant.

And then there was the ax ….

Dismemberment, Taphonomy, Multidisciplinary Approach

H55 Exogenous Factors Affecting Bacterial Profiling of Soil on Clothing Via Next Generation Sequencing

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After attending this presentation, attendees will understand that bacterial profiles derived from soil and recovered from clothing are a viable source of trace evidence despite exogenous variables affecting the profiles.

This presentation will impact the forensic science community by illustrating that factors affecting soil bacterial profiles do not entirely disrupt the profile, which gives soil identification improved utility in forensic analysis.

Soil is potentially a valuable form of trace evidence, helping associate an object on which it is found with a crime scene. Historically, soil has been analyzed via visual and chemical class characteristics. Today, molecular tools have expanded the scope of forensic soil analysis to include microorganisms (e.g., bacteria, fungi). Most recently, Next Generation Sequencing (NGS) allows the millions of DNA sequence reads to be used to identify soil microbes and determine a microbial profile that distinguishes soil samples.[1] To be useful in forensic applications, stability of these profiles is key, as factors that change an evidential profile could nullify an association with the soil's place of origin.

Soil on clothing has been shown to be a viable source of evidence through bacterial profiling of the 16S ribosomal RNA (rRNA) gene; however, such studies did not involve a person wearing the item.[2] It is possible that the human skin microbiome or body fluids (e.g., blood) might alter a soil bacterial profile on clothing, either by augmenting the bacteria present or through selection for or against certain bacteria when exposed to a new growth environment. Understanding the effect of such variables is key for the use of soil bacterial profiling as a forensic resource.

In this study, 12 participants wore new T-shirts for 24 hours (in accordance with Institutional Review Board (IRB) approval), and cuttings were taken from the back of each worn T-shirt. The T-shirts were then exposed to soil from four different habitats and stored in paper bags. At the same time, pure soil samples from the habitats were aged in weigh boats. Cuttings/samples were taken from each at day 0 and monthly for six months. In a separate experiment, soil from a habitat was mixed with fresh pig blood in three different ratios (1:10, 1:1, and 10:1 soil:blood) and placed on clean T-shirts. Three T-shirt replicates were either stored in plastic bags (wet) or allowed to air dry and stored in paper bags. Soil/blood cuttings were taken from each T-shirt at day 0, week 1, month 1, and month 2.

DNA was isolated from all soil samples using a MO BIO PowerSoil® kit. A 500bp stretch of the 16S rRNA gene containing variable regions 3 and 4 was amplified using universal, barcoded primers. Amplicons were purified and sequenced on an Illumina® MiSeq®. Bacterial profiles from the T-shirts were visualized using abundance charts and associated via non-metric multidimensional scaling. A random forest algorithm was used to objectively assign the soil evidence to habitats, and scores were generated that reflect the confidence of that classification.

Bacterial profiles were obtained from each worn T-shirt and all soiled cuttings. In all instances, the T-shirt soil profiles correctly classified with the habitat of origin, and there was no detectable influence from human microbiomes. For T-shirts exposed to soil and blood mixtures, the effect of blood on the soil bacterial profiles differed depending on storage type, time since exposure, and the soil/blood ratio. All treatments resulted in very similar bacterial profiles on day 0, which was maintained in the dry T-shirts and those with little blood (10:1); however, the wet T-shirts at 1:1 and 1:10 ratios differed markedly from the other samples after week 1 and through month 2. Notably, *Bacilli* and *Gammaproteobacteria* increased substantially in bloody wet T-shirts, bacterial classes that include species that are known blood pathogens.

The results demonstrate that soil on clothing can accurately and reliably associate with soil from a crime scene. The soil dominates the bacterial profile of samples exposed to the human skin microbiome or to small amounts of blood. Even when blood-saturated, dry storage or immediate sampling allows the soil on T-shirts to accurately associate with its place of origin. Despite purposefully introducing potentially confounding variables, bacterial profiling-based soil identification from clothing maintains its viability for forensic analysis.

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Soil Identification, Bacterial Profiling, Next Generation Sequencing

H56 A Comparison of the Geographical Variability of the Thanatomicrobiome of Finnish and American Corpses

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After attending this presentation, attendees will better understand the comparison of the 30S small subunit of the prokaryotic ribosome-based sequencing to characterize the thanatomicrobiome of postmortem liver from corpses derived from different geographical locations, namely the United States and Finland.

This presentation will impact the forensic science community by providing results to support the Human Postmortem Microbiome Project (HPMP) catalog. This presentation will also add to research in regard to distinct geographical locations in which corpses originate.

Human thanatomicrobiome studies have shown that microorganisms inhabit and proliferate externally and internally throughout the body and are the primary mediators of putrefaction after death. Yet little is known about the source and diversity of the thanatomicrobiome in regard to geographical factors in which corpses are found. Prokaryotic 16S ribosomal RNA (rRNA) gene sequences are extensively used in forensic microbiology as reliable biomarkers for the taxonomic classification and phylogenetic analysis of the thanatomicrobiome. Upon death, all immune mechanisms in the human body that were operational during life cease, and commensal bacteria start to degrade the body as a part of the natural calendar of death. Presumably, the postmortem microbial composition dynamics of internal organs (e.g., liver) are distinct due to geographical variabilities. The richness of postmortem bacterial abundances in the liver is due, in part, to several factors: its location juxtaposed to the microbe-rich intestinal tract; its location near pancreatic enzymes, stomach acids, and gallbladder fluids that initiate putrefaction through autolysis that spreads quickly to the liver; and its reservation of nutrient-rich blood from the portal vein and hepatic artery that are excellent growth media for bacteria.

This study investigated the microorganisms obtained from the liver of corpses from Finland and the United States. The thanatos model assessed liver samples from 65 human remains from the two countries (n=130) with postmortem intervals ranging from 3 hours to 264 hours. To distinguish the composition and diversity of thanatomicrobiomic signatures, Polymerase Chain Reaction (PCR) and high-throughput sequencing targeting the V4 region (Class I) of the 16S rRNA gene using bacterial primers 515F-806R was performed. The bioinformatic results revealed that there were significant differences ($p<0.001$) among location (Finland versus the United States) and postmortem interval in unweighted and weighted UniFrac Adonis tests. Also, recent thanatomicrobiome findings for United States cadavers discovered that a majority of the microorganisms in the human body after death were the obligate anaerobes, *Clostridium* spp. On the contrary, in the current study, there was a paucity of *Clostridium* spp. in the Finland corpses.

In conclusion, the influence of different geographic locations in determining the distinct microbial community profiles to provide empirical data that will potentially build predictive thanatos models that can further designate the recovery of bodies from discrete locations was demonstrated.

Thanatomicrobiome, Corpse, 16S rRNA

H57 The Ecology of the Human Postmortem Microbiome: Insights From a Large-Scale Study

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After attending this presentation, attendees will better understand the human postmortem microbiome. The majority of research related to the postmortem microbiome involves the use of surrogate animal models (e.g., swine or rodent) or donated bodies placed in anthropological research facilities.

This presentation will impact the forensic science community and practitioners by providing data from a large-scale (185+ cases) characterization of the human postmortem microbiome.

Studies using these methodological approaches, while scientifically valid, result from financial, legal, and/or resource availability limitations. Few published studies have used real-world cases to investigate microbial community changes during the postmortem interval. This presentation will describe the human microbiome characteristics from samples collected during routine death investigation in Detroit, MI.

Since samples were collected on a daily basis, and not limited to the number of donors to a facility, this presentation will offer a unique perspective to the microbial community constituents, composition, and function across a variety of death circumstances. Further, there is a lack of established, long-term collaborations between basic researchers and forensic pathologists to reliably and consistently collect data during death investigation to study the ecology of microbial communities after death. Therefore, this presentation will provide unique insight into the largest known repository of postmortem microbiome samples and the considerations needed for collecting during death investigations.

Microbial samples were collected from 188 cases seen in the Wayne County Medical Examiner's Office in Detroit, MI. Sterile, DNA-free cotton-tipped swabs were used to collect microbial communities from five individual, external anatomic locations: the eyes, external auditory canal, nose, mouth, and rectum. For this study, a variety of cases were desirable to characterize postmortem microbial community stability and variation; thus, no demographic or manner of death was selected *a priori* to collections to ensure samples were obtained from a diverse set of death circumstances. DNA was extracted under aseptic conditions using a commercially available kit with a modified protocol; DNA was quantified using commercially available kits for a fluorometer and a microchannel-based automated electrophoresis system to ensure adequate sample quality and yield for next generation sequencing. The 16S ribosomal RNA (rRNA) V4 gene amplicon region was sequenced for each sample using a 2 x 250 base pair, paired-end approach using a high-throughput sequencing platform. Samples were processed using bioinformatic pipelines to analyze 16S rRNA gene sequences and to predict metagenome functional content from marker (amplicon) genes.

The dataset consisted of samples collected in 2014–2016. There were a balanced proportion of cases between male (56%) and female (44%), and Black (48%) and White (52%). The average (±Standard Deviation (SD)) age was 44±15 years with a range from 18-88 years. Deaths from homicide had a lower average age (35±13 years), while natural deaths had a higher average age (53±11 years). Accidents and suicides were in between (40±14 years and 49±17 years, respectively). Microbial analysis results revealed distinct postmortem microbiome signatures based on anatomic location and time since death. Microbial taxa characterized in the postmortem communities were consistent with communities previously detected in antemortem studies (e.g., *Alloiococcus otitis* in the external auditory canal samples). There were clear microbiome differences among sampling areas as decomposition time increased. Proteobacteria had a statistically significant increase ($p<0.05$) in abundance two days after death. A statistically significant increase in predicted cellular motility was detected after two days postmortem across sampling locations.

In conclusion, this dataset reveals the spatial and temporal variability of the human postmortem microbiome across the largest-scale survey of death investigation in a major, metropolitan city known to date. While traditional techniques for estimating the postmortem interval are important during death investigations, there is potential for using microbial communities as additional biomarkers to corroborate time-since-death estimates. As a community, we need to build the foundational datasets derived from real-world cases to test the validity of microbial communities as postmortem interval indicators. The collaboration between researchers and practitioners to improve non-traditional datasets, such as this one, will ultimately enhance science with practical application to the broader forensic community.

Forensic Science, Forensic Pathology, Postmortem Microbiome

H58 A Global Partnership to Study Geographic Variation in the Human Postmortem Microbiome (HPMM)

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In recent years, there has been interest in using microbiomes in the forensic sciences. There are also several publications documenting the excellent potential for this new line of evidence collection; however, many of these studies have either used non-human models (e.g., swine) or donated human remains that are evaluated in controlled environments, limiting our understanding of the variability in the HPMM collected during routine death investigation, especially in widely separated geographic areas. After attending this presentation, attendees will have a better appreciation for the variability of the HPMM at two scales: (1) within a large metropolitan city; and, (2) across three European cities. Further, attendees will be introduced to a developing global partnership to study the HPMM. The mission of the global HPMM partnership is to expand studies of postmortem microbial ecology so scientists can better understand the potential broad application of the HPMM in practice, and the challenges and limitations that will be addressed as this new science emerges into forensic use.

This presentation will impact the forensic science community by providing new data from an ongoing, large-scale (>180 cases) study of the HPMM from samples taken during routine death investigation received at the Wayne County Medical Examiner's Office in Detroit, MI, and compared to new collections from France, Italy, and Austria. Additional impact will be to bring awareness of this global effort and perspective to evaluate the HPMM and discuss some of the challenges and limitations with bringing this new technique into practice.

Microbial samples were collected from 188 cases at the Wayne County Medical Examiner's Office from 2014 to 2016 during routine death investigation, and 25-30 cases received as autopsies or during investigation in Lille, France, Napoli, Italy, and Salzburg, Austria. All samples were collected using standardized kits constructed and packaged in the same location at Michigan State University under aseptic conditions. Each kit contained individually packaged, sterile, DNA-free cotton-tipped swabs and 1.7ml microcentrifuge tubes with 200µl molecular-grade 96% ethanol for each case. Kits were shipped to each international location for standardized collections. For each case, individual swabs were used to collect microbial communities from five anatomic locations: the eyes, external auditory canal, nose, mouth, and rectum. After standardized swabbing, each swab head was placed into an individual tube, then the entire kit was placed at -20°C until DNA extraction and sequencing. DNA was extracted and quantified under aseptic conditions using commercially available kits. The 16S ribosomal RNA (rRNA) V4 gene amplicon region was sequenced for each sample using a 2 x 250 base pair, paired-end approach using a high-throughput sequencing platform. Samples were processed using bioinformatic pipelines to analyze 16S rRNA gene sequences.

In the Detroit dataset, cases were a balanced proportion of cases between male (56%) and female (44%), and Black (48%) and White (52%). The average (± Standard Deviation (SD)) age was 44 (±15) years with a range from 18 to 88 years. The cases from France represented 28% male and 72% female with an average age of 82 that ranged from 58 to 95 years; cases from Italy and Austria are being processed. Taken together, the HPMM was most highly influenced by anatomic location and then by the Postmortem Interval (PMI), suggesting that anatomic site should be considered before attempting to estimate PMI. Many of the taxa reflected antemortem communities, but this changed with increasing PMI. There was high variation even within body areas from the large sample size from Detroit; however, Proteobacteria was consistently more abundant at longer PMIs. Not surprisingly, much of the variation in the HPMM, even within body area, is likely attributable to antemortem health and lifestyle that is often associated with geographic area and the sociocultural environment.

The enthusiasm for using the HPMM in the forensic sciences is increasing globally; however, there is an urgent need to obtain a more quantified assessment of the local, regional, and global variability in postmortem microbiome communities. Part of this assessment will also require identifying key microbial taxa that are clear forensic indicators and are consistent among different lifestyles, geographic areas, and sociocultural environments. The new global partnership is intended to provide the collaborative network for achieving such inquiry to move this emerging science into future practice.

Forensic Microbiology, International Partnerships, Proteobacteria

H59 Fluorescent Bacteria in the Gut of Mice Carcasses Provides Insight on Postmortem Microbial Translocation

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After attending this presentation, attendees will better understand how host commensal microorganisms transmigrate and thrive immediately following the death and decomposition of the host. In addition, these microbial communities possess investigative potential during the discovery of remains in determining the postmortem interval.

This presentation will impact the forensic science community by providing original data that investigates how commensal bacterial populations transmigrate, colonize, and proliferate following death and the successional decomposition of the associated host. Data obtained will significantly further knowledge of how genetically marked commensal bacteria in the gut transmigrate through their host as decomposition progresses.

Microorganisms play a vital role in the decomposition of remains. Bacteria, archaea, and eukaryotes begin competing against each other to break down and utilize highly nutritious tissues. Organisms able to withstand the changing environment and equipped with proteins that allow more efficient tissue destruction and cell motility have a competitive advantage. Recent work has shown that these bacterial communities are dynamic during decomposition, leading to questions of their utility for either taxonomic or metabolic markers to the postmortem interval. This presentation describes how two genetically labelled communities of *Staphylococcus aureus*-RFP and *Clostridium perfringens*-pZMB2 were orally introduced to a mouse model for colonization of the gut in a living host and subsequently tracked as they migrated through the carcass during decomposition. Aerobic and anaerobic bacteria were monitored in each organ by plate counts, and genetically labelled organisms were tracked using Real-Time quantitative Polymerase Chain Reaction (RT-qPCR). *S. aureus*-RFP was able to be detected by fluorescent imaging *in vivo* to determine colonization routes associated with different physiological events of host decomposition. Mice were dissected and organs harvested at timepoints starting at 1 hour and up 30 days after death. The organs were swabbed for plating, then preserved for total RNA extraction. The total RNA was used for RT-qPCR to identify the inoculated *C. perfringens* and *S. aureus* loads in each organ at each timepoint. These methods provide original data to uncover how commensal bacterial populations transmigrate, colonize, and proliferate across multiple organs following death and the successional decomposition of the associated host. Data obtained significantly furthers investigations identifying microbial behavior during decomposition that may be unique to the postmortem interval to allow for measurements of the time of death used in forensic investigations.

Microbial Transmigration, Decomposition, Postmortem Interval

H60 Autopsy Sampling to Uncover Human Resistome Diversity

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After attending this presentation, attendees will be informed regarding a novel method for uncovering the human resistome within a given population and will be familiar with an Antibiotic Resistance (AbxR) gene dataset from samples collected during routine death investigation in Detroit, MI.

This presentation will impact the forensic science community by providing novel data characterizing antibiotic-resistance genes identified through samples collected at autopsy of bodies within 24 hours of death. This presentation offers unique methodology demonstrating the utility of autopsy sampling in order to surveil antibiotic resistance within a population to predict potential outbreaks of antibiotic-resistant threatening bacteria. Per research, this is a first-of-its-kind study.

Bacteria have expressed AbxR genes long before the antibiotic era, but the use of antibiotics in agriculture and human medicine has dramatically increased their prevalence and intra- and interspecies transfer (e.g., human-to-livestock and microbe-to-microbe). Most studies have focused on the gut, using fecal samples or other minimally invasive sampling methods in healthy individuals to characterize the human resistome; however, the body contains many unique microbial communities not easily accessible in a living individual that likely also contribute to AbxR. Also, surveillance efforts focus on the clinical cases themselves and seldom focus on the potential cases that can arise in the general population.

This study investigated the ability to use postmortem microbiome sampling during autopsy as a method to detect the AbxR genes present in a human population located in Wayne County, MI. Cases were sampled (as previously described) within 24h after death to assess AbxR gene prevalence, identity, and transfer potential in a human population. Thirty-nine bodies were systematically swabbed during autopsy at eight anatomic sites to collect microbial DNA. The DNA from multiple body locations was combined from 20 individual cases to represent the "overall" body postmortem resistome. The samples were analyzed for the presence of 84 known AbxR genes using quantitative Polymerase Chain Reaction (qPCR) arrays. Samples from the calvarial trabecular space/interhemispheric fissure from the remaining 19 bodies were sequenced using Whole Genome Shotgun (WGS) sequencing and assembled into metagenomes that were aligned to known and putative AbxR genes.

Results from qPCR assays revealed that each of the 20 cases had an average of 7.1 AbxR genes. The most commonly positive AbxR genes detected were *ermB* and *mefA,* which were present in 90% of bodies. Genes resistant to the macrolide antibiotic class predominated, with 46% of all the positive AbxR genes. WGS revealed an average of 13.25 AbxR genes per body with *tetQ* as the most commonly found AbxR gene (42% of all the positive) out of the unique 42 AbxR genes detected. Results of this work expand human resistome research to include samples from locations not easily accessible by using samples taken during autopsy.

These novel surveillance methods will allow investigators to sample multiple anatomic sites in a wide range of individuals in the population to obtain information on the incidence and prevalence of antibiotic resistance genes and their presence in the community. Since death is, to some extent, a process with fewer selection biases and wide geographic distribution, sampling at autopsy can help reduce sampling bias and provide a more robust approach to detect the presence of AbxR genes associated with clinically important drugs, such as vancomycin, methicillin, and polymyxins, in the general population. Continuous sampling will also allow for uninterrupted surveillance of these genes once a baseline for their presence is established and serve as a sentinel when new ones are introduced, potentially even before they become clinically significant.

Antibiotic Resistance, Autopsy, Microbiome

H61 An Affordable Immersion Pump for Postmortem Computerized Tomography Angiography (PMCTA) in Forensic Pathology: The First Ten Cases

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The goal of this presentation is to demonstrate how using an affordable immersion pump (a $16 to $20 device) compares with a roller pump (as part of a Heart-Lung Machine (HLM)) in terms of postmortem vascular filling. Results, particularly for intracranial vessels, are better for the immersion pump.

This presentation will impact the forensic science community by explaining how the replacement of an expensive technology, available since 2005 when PMCTA's were introduced and roller pumps were regarded as de-facto state-of-the-art, with more suitable and massively more affordable technology has the capacity to improve results and expand the user base.

Purpose: Approximately ten years after roller pumps were introduced for forensic postmortem Computed Tomography (CT) angiography, it remains an open question as to why a relatively expensive pump mechanism (costing around $1,000 for a used, old HLM to $80 000 for dedicated top-of-the-line postmortem equipment) is actually necessary for PMCTA. Roller pumps make sense for non-Newtonian fluids like blood, where mechanical hemolysis is also a factor; however, in PMCTA, watery or oily liquid is pumped into the vascular system of a body. After it was established in a feasibility study that a simple immersion pump (priced around $15 to $20) can be calibrated to obtain a linear voltage-flowrate relationship for the contrast agent solution used, and that ideal vascular filling at least as good as a roller pump is achieved, this study presents the results of the first ten cases.

Method and Material: Immersion Pump (IP): a Barwig model 0444 pump (max. 10L/min) was used (required PMCTA flow rate 0.2L/min–0,8L/min) (cost around $16-$20 EUR, power supply from $20 EUR and up). Roller pump/HLM: Stoeckert Shiley HLM (max. 10L/min) was employed. Cases: ten cases from forensic pathology caseload were selected in which PMCTA was seen as relevant and examined with the IP. Ten controls examined with the HLM were used for comparison. Both arterial and venous sides were filled from a femoral access. PMCT/PMCTA: Dual source/energy CT scanner was used (100kVp tube voltage, automatic dose modulation). Reconstructions were obtained on Siemens syngo.via software.

Results: Vascular filling was compared related to cerebral vessels, large vessels, coronary arteries, neck and head arteries, extremity arteries, and, on the same level, veins. Figures show the results. Results were the better with IP. Tube handling was problematic at first but was greatly supported by adding custom 3D-printed support structures.

Discussion: The results may be explained by the fact that the fluid contrast agent admixture is very efficiently pumped by immersion or centrifugal pumps. So, in fact, a physical benefit a roller pump may have for pumping non-Newtonian fluids does not seem to work for this application.

Conclusion: To be able to perform a postmortem CT angiography with very affordable equipment of the same quality as high-priced equipment means that a parametrized method can be validated and employed in far more institutes than when using expensive parts.

PMCTA, Virtopsy, Forensic Imaging

H62 Exsanguination on Postmortem Computed Tomography (CT) — What Remains When Blood Leaves the Body

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After attending this presentation, attendees will understand the typical findings that can be seen or expected on postmortem CT in cases of fatal bleeding.

This presentation will impact the forensic science community by providing a review of the literature on exsanguination on postmortem CT as well as results of a case-control study in order to support the scarce literature on the subject.

Introduction: Exsanguination is a common cause of death and may be differentiated in internal and external hemorrhage. Internal blood loss may occur naturally in gastrointestinal bleedings, but it is also frequent in deceleration trauma and consecutive vascular ruptures. The cause of lethal external exsanguination is nearly always non-natural and most common in cases of sharp force injury, traffic accidents, and any form of dismemberment. In classic forensic pathology, loss of a large volume of blood can be assumed when a body presents with only minor lividity. The spleen, kidneys, and liver usually present with discoloration and pallor at autopsy. For postmortem CT, the low density of the lungs as well as decreased cross-sectional areas of great vessels has been reported.[1,2] This study attempted to compare organ radiodensities organs and measure vascular cross-sectional areas in order to characterize exsanguination on postmortem CT.

Methodology: (Results for the control group are printed in **bold**.) In this case-control study, 60 cases that died from internal or external exsanguination were retrospectively matched with cases of identical age and sex. All controls presented no history or evidence of antemortem or peri-mortem loss of blood at external examination and autopsy. The case group was 66% **(66%)** male with a mean age at time of death of 55.1 (**55**) years. The interval between death and CT examination was 29 (**34**) hours. Causes of death were "blood loss" in all cases, with internal (31), external (27), and both internal and external (3) bleeding. In the control group, causes of death were cardiac (36), central nervous (14), metabolic (4), respiratory (4), due to infection (2), and hypothermic (1). Density of the lungs, spleen, kidneys, and liver and were measured by manually applied regions of interest using syngo.via. Cross-sectional areas of the ascending aorta, the descending aorta, and the superior vena cava were measured in a transversal slide at the height of the tracheal bifurcation.

Results: (Results for the control group are printed in **bold**.) Average cross-sectional areas differed significantly (P<0.001) for the ascending aorta (3.5cm^2; **5cm^2**), the descending aorta (2.36cm^2; **3.36cm^2**), and for the superior vena cava (2.21cm^2; **4.02cm^2**). Radiodensities of kidneys (40.5HU; **39.9HU**) and the spleen (50.9HU; **49.9HU**) did not differ between cases and controls. Radiodensity of the liver was significantly (P<0.001) higher in cases (56.8HU) than in controls (**46.3HU**). Pulmonary density was significantly (P<0.001) lower in cases (-661HU) than in controls (**-529HU**).

Discussion: While kidney and spleen radiodensity exhibited no difference between cases and controls, liver density was higher in cases of lethal exsanguination. This may be explained with the higher-than-blood parenchymal density of the liver antemortem (47.5HU) in comparison to the spleen and kidneys, resulting in an average increase of radiodensity with less blood content.[3] For the lungs, a lesser blood content reliably leads to a reduction in radiodensity. The reduction in cross-sectional areas of vascular structures close to the heart represents a collapse of the vessels in cases of exsanguination. This collapse is more pronounced in the vena cava in comparison to the aorta, most probably due to thinner vascular walls. In conclusion, decreased vascular cross-sectional areas as well as decreased pulmonary density and increased liver density are indicators for fatal exsanguination and may support and grade this diagnosis.

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Exsanguination, Postmortem CT, Radiodensity

H63 Global Illumination in Postmortem Computed Tomography (CT): A Presentation of Its Use in Forensic Medicine

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The goals of this presentation are to: (1) introduce the global illumination technique; (2) explain the application of 3D reconstruction in forensic medicine; and, (3) explain the advantages and disadvantages of 3D reconstruction vs. visual examination.

This presentation will impact the forensic science community by improving the quality and accuracy of 3D reconstruction of postmortem CT. Forensic diagnosis prior to the autopsy will be easier and exposure to a non-medical audience more comprehensible.

Postmortem CT (PMCT) and 3D reconstructions are commonly used in forensic medicine and enable highlighting lesions before any forensic examination, especially the autopsy. They are also a good way to present medicolegal findings to a non-medical audience, such as state attorneys, judges, and juries. For a number of years, the technique frequently used for the modeling of 3D reconstructions was the Volume-Rendering Technique (VRT).

More recently, a novel technique has appeared in medical imaging: Global Illumination (GI) (also referred to as cinematic rendering technique in the literature). This technique simulates the complex illumination of an object and is commonly used in virtual cinematography. Its application in medical imaging enables the production of a more realistic and, therefore, a more accurate and comprehensible 3D reconstruction of the CT images.

The cases presented are from the Institute of Legal Medicine of Nancy, France. In some medicolegal cases (i.e., degraded cadavers, ballistic cases), a PMCT is systematically performed prior to the autopsy. This study treated these images with GI in order to explore the possibilities and interest of this new technique in forensic medicine.

In the case of bone study, three types of approaches were compared: the VRT reconstruction, the GI reconstruction, and the visual examination (during autopsy and/or after anthropologic preparation). Compared to the VRT, GI offers more realistic views of the skeleton: simulation of the shadows helps the observer better understand the reconstruction in 3D (depths and reliefs) and the illumination enables modification of the position of the light source and, therefore, the appearance of the bone lesions.

Compared to visual examination, GI has advantages and disadvantages: it enables the exploration of lesions before any dissection of the cadaver; the application of textures and colors according to the density level permit a reconstruction very similar to visual examination; the segmentation of each anatomical structure or bone fragment enables precise study of bone lesions and the reconstruction of complex fractures. However, the quality and accuracy of the 3D reconstruction depends on the resolution of the PMCT. Thus, in some cases, the finest fracture lines were not visible on VRT and GI but were observed by visual examination. A case is presented in which fine impacts of a blunt or spiky weapon were present on the left temporal bone around a major penetrating wound. All of these impacts were only seen during the autopsy.

In medical imaging, the algorithms of 3D reconstructions are more complex and accurate, permitting the realization of more realistic images. Among them, GI and the software it uses are not routinely employed in France, but the results are promising, both in forensic medicine and for clinical application. In the literature, some papers present clinical applications of GI, with only one in forensic medicine.[1] The examples presented here illustrate that they cannot replace the visual examination at autopsy.

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Postmortem CT, 3D Reconstruction, Global Illumination

H64 Detection of Pulmonary Thrombembolism and Postmortem Clotting on Postmortem Magnetic Resonance Imaging (MRI)

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The goal of this presentation is to learn how to detect and distinguish between postmortem clotting and Pulmonary Thromboembolism (PE) in postmortem MRI.

This presentation will impact the forensic science community by providing another tool to detect natural causes of death by using MRI without performing an autopsy.

The purpose of this study was to develop a feasible imaging protocol superior to Postmortem Computed Tomography (PMCT) and to establish diagnostic parameters for diagnosing PE on Postmortem Magnetic Resonance (PMMR) imaging. The PMCT and PMMR protocol developed here should enable further users to distinguish between postmortem clotting and pulmonary embolism by using postmortem imaging.

The study of 113 subjects consisted of 67 males and 46 females ranging from 17 to 89 years of age (mean 55.8 years in males and 53 years in females). This collective was taken from cases which were brought to the Institute of Forensic Medicine in Zurich. The autopsy of each case was ordered by a district attorney. If the case history provided information which led to the suspected diagnosis of a pulmonary embolism, it was included in the study group and was prospectively investigated by PMCT and PMMR for the presence of PE and/or postmortem clotting (cruor). After the postmortem imaging process, an autopsy was performed to verify the radiological findings.

Pulmonary embolism was detected in 20 cases; the remaining 93 cases were investigated for the morphology of cruor (clotted blood). Age graduation of the pulmonary embolism was performed by PMMR, autopsy, and histology using hematoxylin and eosin and elastic van Gieson staining. The postmortem sedimentation effect in which the cellular components of blood split from the plasma and deposit according to gravity was used for the applied imaging protocol on PMMR (supine and prone position).

Visual distension of the pulmonary arteries in PE was observed in all cases, but not in the cases with postmortem clotting. Repositioning of the corpse from a supine to a prone position proved to be beneficial in 90% of cases; pulmonary embolism did not exhibit any relocation after repositioning of the corpse, except in two cases of hyperacute PE. All cases with cruor showed movement of the clot within the blood vessel. Postmortem motion artifacts are first described in 20.4% of cases.

Hyperacute PE (grade 1) presented with a homogenous and hypointense signal on T2w images; acute PE (grade 2) presented with slightly heterogeneous, but still homogenous hypointense signal; subacute PE (grade 3) presented with heterogeneous and slightly hyperintense signal; and chronic PE (grade 4) presented with predominately homogenous with scarce portions of heterogeneous but hyperintense signal. In contrast, the cruor pattern was homogenous hypointense when the cruor was of a “red currant jelly clot” type and heterogeneous hyperintense in the “chicken fat clot” type.

In conclusion, this study shows that reliable detection of pulmonary embolism is feasible by PMMR and that this method allows for determination of the age and composition of an embolus based on morphology and signal intensity in the PPMR without performing an autopsy.

Postmortem Imaging, MRI, Pulmonary Thromboembolism

H65 A Comparison of Postmortem and Antemortem Computed Tomography (CT) for the Identification of Adults With Unique Anatomical Variations

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After attending this presentation, attendees will recognize the utility of CT comparison in the identification of human remains using antemortem-postmortem CT comparison of unique anatomical variations. Furthermore, attendees will recognize the ability of the forensic pathologist to make these comparison-based identifications.

This presentation will impact the forensic science community by exploring additional skeletal features that would facilitate the identification of human remains using CT, especially fragmented or incomplete human remains from cases of mass fatalities.

Identification of human remains, whether complete or partial, is a critical and challenging task for a forensic pathologist.[1] Although DNA analysis remains a steadfast tool in the identification of fragmented, commingled, or partial human remains, time and cost constraints make it particularly prohibitive. Historically, radiological examination for identification has proven useful in comparing various anatomic structures including, but not limited to, cranial features, osseous structures, soft tissues, and teeth.[2-5] Compared to other modalities, radiological examination, more specifically analog imaging, is more cost-effective and has been widely used in the medical examiner setting as a means of identification.

At the Office of the Chief Medical Examiner (OCME) for the State of Maryland, postmortem CT is routinely used as an adjunct tool for diagnostic or identification purposes. Postmortem CT has been extensively used for its utility in identification based on comparison of anatomical variations, including nasal turbinates and sinuses, cranial sutures, degenerative and idiopathic changes of the spine, and anomalous or unusual development of skeletal structures.

To broaden the spectrum of potential identifying characteristics, a study was conducted by retrospectively and prospectively collecting cases with postmortem CT images obtained in the OCME office from 2015 through 2017. The final cases were then selected based on the availability of antemortem CT images to specifically assist for additional morphological features that could be useful in confirming the identities of incomplete remains. Once all images were obtained, a unique identifier was assigned to each postmortem CT image for the purpose of blinding the designated forensic pathologist, with experience in forensic radiology, who would perform the comparison. The results were qualitatively assessed for accuracy and reliability for identification purposes. CT scanning has proven to be a useful and scientific method of identification, especially in cases of limited radiographic studies or partial anatomic remains available for identification.

This study demonstrates that identification of human remains, even in a fragmented state, could be performed by a forensic pathologist with limited CT experience, in a medical examiner setting.

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Postmortem CT, Identification, Human Remains

H66 Photogrammetry Applied to Forensic Pathology: Low-Cost Support to "Freeze the Body in Time"

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After attending this presentation, attendees will understand the usefulness of the photogrammetry technique during an autopsy.

This presentation will impact the forensic science community by providing a recording 3D technique that would also be user-friendly and a valuable support during an autopsy.

The major challenge in every type of forensic investigation, both during the crime scene and in the autopsy phases, is gathering a sufficient amount of information to reconstruct the conditions of a crime scene or a dead body. This can be achieved by the long and difficult work of gathering data that will, objectively, be insufficient to "fix in time" every aspect of the examined situation. Moreover, traditional acquisition methods risk providing information that is too related to the investigator's point of view. The solution lies in freezing, by virtual means, the scene and/or the body in order to obtain a representation that is free of the investigator's influence.

The technologies that are currently available allow this, but they impose a high monetary cost. The method described in this presentation suggests the possibility of achieving such an objective at a very low cost. The only requirements are a digital camera and appropriate computer software. This technique derives from a full photographic report based on photogrammetry.

Photogrammetry is a highly sensitive technique that allows the gathering of the metrical data of an object (shape and position) by acquiring and analyzing stereometric pictures. Several different types of software are capable of this technique. On average, the different programs allow the acquisition of up to 500 pictures of the object (acquired from several points of view) in order to provide a 3D reconstruction of it. Once the pictures are uploaded in the software, they are processed with a method known as Structure From Motion (SFM), which consists of recognizing a point in common between various images using the Scale Invariant Feature Transform (SIFT) algorithm. This allows the creation of a cluster of points characterized by their special positioning in a system of coordinates and intensity scale values (color, depth, etc.), allowing the re-creation of a 3D version of the object.

The original photogrammetry technique was used in architecture and geology to obtain accurate reproductions of the plans of buildings or natural cavities. In time, it was expanded to include the bio-medical field and became indispensable in the creation of anatomical models and the creation of 3D prosthetic devices.

This study introduces the use of the photogrammetry technique to the field of forensics.

Forensic Science, Photogrammetry, Virtual 3D Rendering

H67 Forensic Photography: Focus on Small Findings Using Digital Consumer Cameras

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After attending this presentation, attendees will understand how a clear, specific instruction for sharp, focused images with consumer cameras can assist trainee doctors in taking better pictures, particularly of small findings.

This presentation will impact the forensic science community by explaining how the importance of having medical personnel able to take sharp, focused pictures of very small findings is not restricted only to doctors; it equally affects forensic nurses, mortuary technicians, and others.

Background: While forensic literature abounds with the mention of petechial hemorrhages or congestive hemorrhages, reliably photographing them using digital consumer cameras has neither been officially attempted nor documented comprehensively in an actual setting. The challenge consists in capturing hemorrhages as small as 0.3mm in diameter. An easy-to-understand instruction set was developed and the success in applying it to five test users was recorded. The impact on the quality of a series of test target photos was scored.

Methods and Materials: Given "feature" size for pinpoint hemorrhages of approximately 0.3mm and given image dimensions of 3,000 pixels with any "feature" requiring a minimum of 40 pixels for adequate digital representation, total metric image size should not exceed approximately 22mm. A step-by-step guide on how to reliably produce focused pictures of small targets with such dimensions was devised. Using a typical compact digital consumer camera (Canon® Powershot® G16, Backside Illuminated-Complementary Metal-Oxide Semiconductor (BSI-CMOS) sensor 1/1.7" with 4,000 x 3,000 pixels equating to 12 megapixels), compared the resulting digital image focus of four targets was compared. These targets contain submillimeter-sized features. Images were taken by five test users (trainee doctors in the same institute, with a self-reported basic to intermediate proficiency level, not advanced or professional). Image quality was compared before and after exposing the test users to a step-by-step guide on how to reliably produce focused images. General recommendations were made available from the outset. In a step-by-step instruction, suggest four criteria were suggested: (1) the use of an aperture priority above 5.6; (2) sensitivity (ISO) of not above 800; (3) an exposure of no longer than 1/60 second; and, (4) an optimal angle perpendicular to the feature being photographed. A score was devised that rated the photos on the basis of these criteria. Thus, an optimal image would yield 40 points.

Targets: This study used a printed array of small dots, a bank note featuring tiny anti-forgery perforations, a textile band featuring fabric structure, and a plastic model of a heart valve that contained small ink spots.

Results: Instructing this visual and easy-to-follow, step-by-step guide for correct setup and image-taking approach resulted in better images. The image series before the instruction across all five participants yielded an average of 25+/-1.8 score points per image; afterward, a mean score of 29.5+/-3.3 score points was achieved (statistically significant, Wilcoxon $p<0.04$); however, these efforts did not achieve perfect 40 score points in all instances.

Discussion: Quality improvement usually requires precise instructions and easy-to-follow approaches. Introducing high-tech to a medical frontline workflow does not succeed without these. General recommendations for forensic photography, typically citing the rule of thirds (usually not even applicable in macro photography) or vague explanations regarding image noise, seemed not overly helpful. The results of this study and where efforts need to be further improved to maximize outcome will be presented and discussed.

Forensic Photography, Consumer Cameras, Forensic Nursing

H68 The Redevelopment of the Mississippi State Medical Examiner's Office

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The goal of this presentation is to reveal how the development of a structured state medical examiner system has improved medicolegal death investigations throughout the state of Mississippi.

This presentation will impact the forensic science community by demonstrating how a structured medical examiner system has improved the quality of the forensic pathology services throughout the state of Mississippi.

For more than 30 years, Mississippi contracted with non-board-certified pathologists to provide autopsy services to coroners, law enforcement, and district attorneys to assist them with medicolegal death investigations. This arrangement resulted in numerous false convictions leading to appeals. Many cases were reopened and overturned due to the efforts of the Mississippi Innocence Project.

The core of the problem was untrained and unsupervised pathologists tailoring their reports and testimony to support the expectations of law enforcement and district attorneys in order to obtain convictions. The majority of the examinations were performed in local funeral homes with substandard facilities. This deficiency compromised documentation of findings, collection of evidence, and chain of custody. Further, two independent pathologists reportedly performed a total of approximately 1,800 autopsies per year under these conditions. This total is unfeasible given the complexity involved in cause- and manner-of-death determinations.

The state of Mississippi finally recognized the need for a structured state medical examiner's system. In an attempt to rectify the issue with non-board-certified forensic pathologists, the state contracted with an adjacent state's medical examiner's office; this situation was terminated following unsatisfactory fulfillment of the contractual agreement.

Since 2011, Mississippi has hired only physicians who are board-certified in forensic pathology by the American Board of Pathology. A new facility was constructed in 2015 to jointly house the Mississippi crime laboratory and the medical examiner's office. The state-of-the-art facility contains four autopsy stations, each with its own 55" monitor for viewing digital radiographs and entering case information into an evidence-tracking database program. The physical continuity between the medical examiner's office and the state crime lab allows immediate access to multidisciplinary services, such as toxicology, firearms and ballistics, trace evidence, fingerprinting, and DNA analysis. Although a satellite office on the Gulf Coast remains unstaffed, the facility is sufficiently equipped to accommodate regional demands.

The medical examiner's office consults with a board-certified forensic odontologist, an arrangement which has facilitated numerous dental identifications. The office also recently hired two forensic anthropologists who are developing a statewide skeletal recovery and identification program.

While the quality and continuity of autopsy services has dramatically improved, there is a continual battle regarding funding and staffing issues.

Mississippi, State Medical Examiner, FP Board Certification

H69 Investigation of the Forensic Pathology Services of the National Institute of Legal Medicine and Forensic Sciences of Colombia

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The goal of this presentation is to show the degree of fulfillment of the facilities requirements for which the forensic pathology services of the National Institute of Legal Medicine and Forensic Sciences (INMLCF) of Colombia conduct medical-legal necropsies, using as a reference the requirements established by the National Association of Medical Examiners.

This presentation will impact the forensic science community by demonstrating the importance of the quality and execution of medicolegal autopsies and how they impact the final judicial investigation.

The INMLCF is the Colombian agency in charge of providing technical and scientific support to the administration of justice. In addition to other forensic services, the INMLCF performs medicolegal necropsies consisting of 114 points of attention to establish the cause and the manner of death.

This report presents the results of a study of the INMLCF forensic pathology services where such necropsies are performed. The goal of this study was to identify certain situations at these facilities, using as the main reference point the requirements established by the National Association of Medical Examiners. From the epistemological point of view, this research is qualitative and essentially corresponds to a descriptive study.

For this purpose, a self-administered survey was designed and applied using the LimeSurvey® platform. The results of this survey reveal that none of the services meet all the requirements; that the degree of fulfillment in the requirements varies widely between the different areas of care; that there are important limitations in the access of resources required to perform certain necropsies, such as access to X-rays. The results of this study will allow the senior management of the institute to determine the scope related to the purpose of accrediting such services. This study will establish actions to intervene at the facilities that require intervention, to make decisions regarding the provision of forensic services, and to suspend services rendered as a result of conditions that are considered to be unworthy (either for their own personnel or for their clients and/or users) or conditions that put the quality of necropsies or the safety of personnel and visitors in jeopardy. This may result in: (1) the relocation of necropsy services, among others; and, (2) submitting the need to incorporate resources to meet the requirements of both the administration of justice and society in general, based on objective facts, to the pertinent authorities.

Accreditation, Forensic Facilities, NAME Requirements

H70 Autopsy By Videoconferencing

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The goal of this presentation is to demonstrate how thanatology by videoconferencing constitutes an easier way to perform investigations, providing many advantages over the current practices.

This presentation will impact the forensic science community by explaining how this innovative project presents many logistical, pedagogical, and economic advantages and is part of a global media-judicial project.

Videoconferencing has existed in nearly all types of large and small business fields since the early 1960s; however, its use for forensic medicine has not been fully utilized to reach experts around the world. The initial objective of this study was to quantify the cost of transporting investigators to the Medico-Legal Institute in France during an investigation versus applying new methods to reduce expenses by finding ways to modernize and innovate forensic medicine.

This study covers a three-year period of evaluating the total expenses for the activities required to investigate crime scenes in the city of Rouen, France. The expenses include distances travelled by investigators, such as the local police and gendarmes (French police force) to be present at the forensic institute of Rouen to participate in an active investigation. Distances were estimated using the mapping features from the website Mappy.fr. The stipends, travel expenses, and material equipment used for the investigators were acquired using the audits from the French courts and tax offices.

Over three years, the investigators traveled more than 75,396km (46,849 miles) for a total of 2,720 hours of travel. This represents a minimum material cost of $33,000, a minimum human expense of $150,000, and vehicle downtime of 1,303 hours. These figures are minimal because they do not take into account the frequent presence of more than two investigators, multiple vehicles, or inherent traffic problems, which would increase the overall cost.

Thanatology by videoconferencing, while recording and encrypting data over secure networks channels, constitutes an easier way to perform investigations, which has many advantages over current practices. In addition to the indisputable cost restraints, the use of videoconferencing allows experts of different specialties to work together on the same case, even when separated by great distances. Delays due to travel are significantly reduced, allowing faster analysis. Judges can to take part from their offices during a live or recorded investigation. The investigator can solicit a forensic pathologist during a corpse discovery from his or her smart phone by an encrypted application dedicated to professional videoconferencing before he or she decides to solicit him or her for the crime scene investigation. Videoconferencing can also be an educational tool for the training and development of new investigators, judges, doctors, and students.

This innovative project presents many logistical, pedagogical, and economic advantages and is part of a global media-judicial project. Videoconferencing will quickly demonstrate its efficiency in view of the current high level of equipment required of police stations, the gendarmerie, and public prosecutor's offices. This system will greatly optimize the collaboration between justice and forensic medicine, and it will constitute a new teaching tool for our services while realizing savings for the Ministries of Justice and the Interior.

Videoconferencing, Thanatology, Forensic Medicine

H71 A Review of In-Custody Deaths in Mississippi

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The goal of this presentation is to review the history and structure of the prison system in Mississippi and to present various cases in which inmates have died while in custody.

This presentation will impact the forensic science community by demonstrating the challenges in determining the cause and manner of deaths occurring while a person is in custody.

The first reported prison to be developed in Mississippi was in 1789 in the city of Natchez. The first centralized prison was not established until April 1840 and was designed to house 200 inmates. Overcrowding soon became an issue. Subsequently, the Governor released 40 inmates in order to increase the manpower of the Confederate Army. The prison was eventually overrun by Sherman's army on his march to Atlanta, GA. The current State Penitentiary in Parchman, MS, was built in 1901.

A federal mandate in 1972 ordered the state to develop a plan to address issues that included racial segregation, possession of weapons, drugs and alcohol, and the punishment of inmates. The Mississippi Department of Corrections (MDOC) was established in July 1976. Under their jurisdiction, there are 3 state-run prisons, 11 regional prisons, 6 private prisons, and 4 restitution centers. In addition, each of the 82 counties has at least one county jail. The MDOC houses approximately 21,000 inmates, 47 of whom are currently on death row. The MDOC inmate population figure is the second-highest in the nation.

The investigation of in-custody deaths is a multidisciplinary team effort and presents challenges at all levels. The most recent data available at the time of this submission shows a total of 65 in-custody deaths reported in 2016. This presentation will provide photographic and video documentation of scene and autopsy evidence that illustrates the variability exhibited by in-custody deaths falling under the jurisdiction of the Mississippi State Medical Examiner's Office.

Establishing the correct cause of death and manner of death is the crux of the issue. Media coverage and reporting of the deaths in conjunction with social media is essentially instantaneous and may cause misrepresentation of the facts and result in premature public bias against law enforcement personnel. As an impartial entity able to perform medicolegal postmortem examinations that elucidate the presence or absence of non-accidental traumatic injury, the State Medical Examiner's Office is uniquely equipped to contribute its expertise in the interpretation of these findings to assist in the education of law enforcement individuals. Identification of in-custody drug deaths is another benefit for the Department of Corrections and the Mississippi Bureau of Narcotics in tracking in-custody drug use.

In-Custody Deaths, Cause of Death, Mississippi

H72 The National Institute of Justice's (NIJ's) National Missing and Unidentified Persons System (NamUs) and the Federal Bureau of Investigation (FBI) Laboratory Collaboration: Using Next Generation Identification (NGI) to Solve Unidentified Persons Cases

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After attending this presentation, attendees will understand new efforts that resulted in the identification of more than 190 Unidentified Persons (UP) cases over the course of six months.

This presentation will impact the forensic science community by explaining how new technology and enhanced methods for fingerprint identification can be used to solve UP cases.

In 2007, NIJ reported on the "Nation's Silent Mass Disaster," describing the more than 100,000 active missing persons cases and more than 40,000 sets of unidentified human remains in the United States.[1] At the time, resources to assist with unidentified remains and missing persons were minimal and disjointed and something needed to be done. Two principal needs were identified. The first was a centralized repository for the nation's unidentified and a way to capture essential information, including biometrics that would allow for comparisons with open missing persons cases. The second was a way to close the communication gap among agencies, jurisdictions, and the public so they could share information, collaborate on cases when needed, and help relatives searching for family members. To help meet these needs, the NIJ created the NamUs. By the end of 2007, NamUs' unidentified decedent database was online, followed by the missing persons' database. NIJ continued to expand the system and added another complimentary database for unclaimed persons that includes deceased people who have been identified, but for whom no next of kin has been located to claim them. As of July 2017, 14,389 UP cases have been entered into NamUs, but 11,483 of these cases remain open. Many of the unidentified human remains residing in NamUs are not readily identifiable. Of the active UP cases in NamUs, only 24% were noted to have recognizable faces. Factors that prevent visual recognition of the decedent include, but are not limited to, burning/charring, insect predation, traumatic injury, decomposition, or skeletonization of the body due to environmental factors.

In February of 2017, the FBI Laboratory and NIJ's NamUs initiated a partnership to begin searching all UP fingerprints through the FBI's NGI system. NGI provides the criminal justice community with the world's largest and most efficient electronic repository of biometric and criminal history information.[2] Using a different type of capability that allowed a more pointed search of each individual finger, a total of 2,184 individual fingerprint images (both 10-print cards and individual recordings) representing 1,465 individual UP cases were submitted to the FBI Laboratory Latent Print Support Unit and, within four months, a total of 193 identifications were effected. Of the 193 UP cases, 12% of the cases were ruled homicides and 31% were undetermined.

Many criminal justice agencies, including medical examiners and coroner's offices, are not aware of this program or have not considered re-examining fingerprints from unidentified decedent cases, especially since the implementation of NGI which came online in 2013. The new searching capabilities and enhanced methodology now available can more effectively identify fingerprints and should be considered by all medical examiners' and coroners' offices, even if previous searches resulted in unsuccessful outcomes.

Reference(s):

1. Ritter, N. (2007, January). Missing Persons and Unidentified Remains: The Nation's Silent Mass Disaster. *NIJ Journal.* (256).

2. Next Generation Identification at https://www.fbi.gov/services/cjis/fingerprints-and-other-biometrics/ngi.

Unidentified Remains, NamUS, Next Generation Identification

H73 Jay Dix Memorial Bonus Day Lecture Series

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After attending these presentations, attendees will understand how medicolegal deaths are evaluated and certified and how and why deaths related to the specified topics occur. Attendees will be aware of a systematic approach to the evaluation of such deaths that can be readily implemented in their daily practices.

These presentations will impact the forensic science community by providing a comprehensive review of what causes and contributes to deaths related to the specified topics. Attendees will better be able to systematically evaluate deaths in their daily practices in which the specified topics may have played a role.

A proper medicolegal death investigation is a multidisciplinary process that often involves non-medical personnel as well as medical professionals. This annual series of lectures is intended to provide the non-forensic pathologist forensic scientist a comprehensive basic review of selected topics in forensic pathology in order to increase familiarity and understanding and enhance inter-discipline communication.

This year's lecturers will discuss the medicolegal investigation of death and its certification -- the investigation of deaths due to firearm injuries, deaths that are temporally related to law enforcement apprehension and custody, deaths involving blunt trauma, and adult deaths involving acute and chronic head trauma.

Blunt force injury is one of the major categories of mechanical injury. Blunt force injuries are among the most common injuries sustained by people. These injuries include abrasions (scrapes), contusions (bruises), and lacerations (tears). Blunt force is also a substantial component of chop wounds, injuries caused by relatively heavy-edged objects, such as a machete or ax. Multiple factors and mechanisms are involved in injuries and deaths involving blunt forces. Understanding and evaluating injuries and deaths in which blunt force injuries may have played a role requires: basic knowledge of injuries caused by blunt forces and how to distinguish them from other types of trauma; recognition of patterned injuries; and recognition of injury patterns (e.g., pattern of falling versus pattern of being struck by an object). This lecture will provide a comprehensive review of these issues.

Firearm injuries constitute a major category of physical injury. Firearm fatalities are a major cause of non-accidental morbidity and mortality in the United States. The appearance of these injuries is affected by the firearm, ammunition, range of fire, victim anatomy, and, in some cases, intervening targets. Multiple factors and mechanisms are involved in injuries and deaths involving firearm injuries. Understanding and evaluating injuries and deaths in which firearm injuries may have played a role requires basic knowledge of injuries caused by firearms, how these injuries are produced, and how to distinguish them from other types of trauma. This lecture will provide a comprehensive review of these issues.

Head injury is a common cause of trauma-related morbidity and mortality among adults. Findings in serious head injury depend on the mechanisms of injury, duration of survival, and, in some cases, co-morbid conditions. Proper evaluation of head injury is important in recognizing how an injury was sustained, excluding various potential or alleged mechanisms, evaluating accuracy and reliability of witness accounts, and aid in identifying the perpetrator in those cases involving inflicted injury. This lecture reviews the features of acute and chronic adult head injury, mechanisms of injury, manifestations of head injury, and the interpretation of anatomic and clinical findings in the context of a medicolegal death investigation and quality of evidence in the literature.

The certified cause and manner of death have far-reaching ramifications, impacting areas such as grief, estate settling, insurance claims, civil litigation, criminal investigation and adjudication, investigations by regulatory agencies, public health policy, and research funding allocation, among others. The medicolegal death investigation is a multidisciplinary process designed to collect information pertaining to the deaths of individuals who die of conditions or under circumstances that place them under the jurisdiction of the medical examiner or coroner so that the medicolegal official is able to accurately and reliably determine cause and manner of death of these decedents. The determination of the cause of death is the practice of medicine and, like other medical practices, is based on integrating and interpreting information about the circumstances (history), physical examination (decedent and scene of incident/death), and ancillary studies (e.g., laboratory analyses, radiographic imaging). Accurate and comprehensive information obtained during a medicolegal death investigation contributes to the ability of the forensic pathologist to reliably and accurately determine the cause of death, identify other factors that contributed to death, and properly classify the manner of death. This lecture explores components of a modern medicolegal death investigation and discusses the fundamentals of the certification of deaths.

There are multiple causes, mechanisms, and contributory factors that can play a role in deaths that are temporally related to custody. The custody process can be divided into several stages — pre-custody, pre-incarceration, and incarceration. Particular diseases and injuries tend to occur and/or become manifest during each of these stages. This lecture will systematically review what diseases and injuries cause/contribute to death in the phases of custody related to apprehension and arrest, how they affect physiology and anatomy, when they are typically operative, and how they are manifest. Recognizing what occurs during the various stages of custody allows a systematic approach to assessing deaths that occur during the custody process. This lecture will review the conceptual and practical aspects of understanding and investigating deaths that are temporally related to the apprehension/arrest phases of custody.

Death Investigation, Forensic Pathology, Trauma

H74 Mind Over Matter: A Death Potentially Related to Non-Epileptic Seizures

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The goals of this presentation are to: (1) review the clinical definitions of pseudoseizures, seizures, and epilepsy; (2) discuss a clinical case in which a young individual with a history of pseudoseizures died suddenly with no other identifiable anatomic or toxicologic cause of death; (3) emphasize the necessity of a thorough neuropathologic examination in cases in which a decedent may have had a history of epilepsy or pseudoseizures; and, (4) review the recommended sections that should be examined histologically at autopsy.

Psychogenic non-epileptic seizures, otherwise known as pseudoseizures, are well known to neurologists and psychiatrists, but are rarely encountered by forensic pathologists. This presentation will impact the forensic science community by reviewing the clinical and psychological aspects of pseudoseizures in comparison to true seizure disorders and discussing how the forensic pathologist should approach an autopsy of a decedent with a history of pseudoseizures. Non-epileptic seizures have not been thought to be a cause of death, but this case will raise the discussion of whether this is entirely true.

Non-epileptic seizures have an incidence of 2 to 22 people per 100,000 people. Many of the people who are diagnosed are worked up for epileptic seizures and have negative results. The seizures are thought to occur from psychological distress, as opposed to epilepsy, in which the seizures are brought on by electrical problems in the brain.

Presented here is the case of a 17-year-old female who was found unresponsive by her mother who then called Emergency Medical Services (EMS). EMS pronounced her dead at the scene. Reportedly, her feet were on the bed and her head was down in an adjoining closet with her face against a plastic bag.

Per medical records, the decedent was being followed for seizure-like events, approximately three to four events per week. An electroencephalogram was performed and reported to be normal and lab work was significant only for a low vitamin D level. The neurologist seeing the decedent diagnosed her with pseudoseizures, otherwise known as Psychogenic Non-Epileptic Seizures (PNES). These seizure-like events began after she had moved to a new place and only occurred when she was awake. There was no family history of epilepsy or neurological problems. Several days prior to her death, she was noted to have had a seizure-like event and knocked out a window with her foot. No major anatomic cause of death was found at autopsy. Postmortem toxicology was positive for therapeutic levels of sertraline and its metabolite.

A literature search was conducted in respect to non-epileptic seizures and death, of which there were no results. There is a link between Sudden Unexpected Death and Epilepsy, commonly known as SUDEP, but there was no known association with non-epileptic seizures. A discussion was had with the decedent's neurologist, who did not believe that non-epileptic seizures could result in death but may result in an inability to control the body. The mother of the decedent reported a plastic bag near the decedent's face, so it is possible there may have been an asphyxial component involved if the decedent had been having a non-epileptic seizure and her head fell near the bag.

Pseudoseizure, Epilepsy, Asphyxia

H75 Subdural Hematoma, Retinal Hemorrhages, and Cerebral Venous Sinus Thrombosis (CVST): Homicidal or Natural Death

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The goals of this presentation are to: (1) identify the spectrum of cerebral changes secondary to CVST; (2) discuss the relationship between subdural hematoma and CVST; (3) discuss the relationship between retinal hemorrhage and CVST; and, (4) better interpret the manner of death in patients presenting with subdural hematoma and CVST.

This presentation will impact the forensic science community by advising attendees that the relationship between sinus thrombosis and subdural hematoma is of critical importance in assessing the manner of death in young children. This case, in addition to the literature review, provides valuable insight into this controversial issue.

Background: In the setting of witnessed, severe traumatic brain injury with subdural hematoma, CVST is not a meaningful differential diagnostic consideration; however, in the pediatric setting, severe traumatic brain injury within the home may be unwitnessed, especially among infants and toddlers. In such cases, the caretaker may not provide an accurate account of the extent, severity, or mechanism of trauma. Moreover, victims of inflicted head trauma often suffer neurological collapse with increased intracranial pressure, resulting in non-perfusion and organizing coagula within venous sinuses, mimicking CVST. The question may then be raised as to whether CVST was a primary process or an event secondary to abusive head trauma.

Methods: This study presents the autopsy findings in a 2-month, 21-day-old male infant who initially presented to hospital with seizures. The infant's father indicated that the infant fell during a diaper change but did not cry or respond. Subsequently, the father placed the child on the living room couch for a nap and within minutes he noted that the infant changed positions with his face toward the back of the couch. The father and paternal grandmother then noticed a diminished level of responsiveness. The father "shook" the infant briefly in an attempt to arouse him. Neither the father nor paternal grandmother report the infant hitting anything while being shaken. A Computed Tomography (CT) scan on presentation demonstrated acute subdural hemorrhage along the high right frontoparietal convexity near the vertex and along the interhemispheric fissure posteriorly with a maximum 3mm thickness. Magnetic Resonance Imaging (MRI) with and without contrast demonstrated restricted diffusion consistent with ischemia, within the right cerebrum and most of the left cerebrum. No venous sinus thrombosis was noted in the initial MRI. Ophthalmological evaluation revealed extensive intraretinal hemorrhages. The infant's neurological status failed to improve and he was eventually placed in hospice care. He expired approximated five weeks after presentation.

Results: Autopsy examination demonstrated bilateral extracerebral fluid collections with neomembranes. The underlying parenchymal tissue showed extensive ischemic brain injury. Ophthalmic pathology examination was remarkable for intraretinal, multilayered hemosiderin deposits and hemosiderin deposits involving the optic nerve sheaths. Noteworthy was organizing CVST with neovascularization and calcification, involving the superior sagittal sinus, transverse sinuses, and torcula.

Conclusion: This case raises the issue of CVST as a potential mimic for abusive head trauma. Literature review reveals that CVST as a cause of subdural hematoma is rare, but not non-existent. Although the MRI findings did not reveal evidence of CVST at presentation, the presence of unequivocal organizing CVST, the paucity of such lesions in longer term survival of abusive head trauma, and the lack of evidence for brain swelling and increased intracranial pressure clinically, suggested that CVST as the primary process could not be excluded. Abusive head trauma with subsequent cerebral venous stasis was also not excluded.

Cerebral Venous Sinus Thrombos, Subdural Hematoma, Retinal Hemorrhage

H76 Postmortem Evaluation of Mild Traumatic Brain Injury (Concussion): Importance and Relevance

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After attending this presentation, attendees will: (1) understand the pathophysiology of mild Traumatic Brain Injury (mTBI); (2) understand the medicolegal implications of mTBI, (3) understand appropriate case selection for postmortem mTBI evaluation; and, (4) understand techniques used to evaluate for mTBI.

This presentation will impact the forensic science community by explaining the importance and relevance of mTBI evaluation in the medicolegal setting, expanding upon the predominantly animal-based research, and increasing the ability of forensic pathologists to appropriately evaluate for mTBI.

MTBI, also referred to as concussion, is caused by mechanical trauma. The clinical features of mTBI include possible transient loss of consciousness and/or amnesia surrounding the event, a Glasgow Coma Scale between 13-15, and only rare imaging abnormalities.[1] The pathophysiology of concussion involves a predominantly excitatory neurotransmitter cascade with variable long-term sequelae.[2,3] Although animal studies have suggested structural changes following mTBI, trauma in humans resulting only in concussion rarely leads to death. Two recent cases elucidate the existence and extent of structural Central Nervous System (CNS) changes in humans following concussion and suggest the importance of postmortem evaluation for diffuse Traumatic Axonal Injury (dTAI) in select cases.

In the first case, a helmeted 72-year-old female bicyclist was struck by a motor vehicle traveling at approximately 30mph. Clinical evaluation revealed anterograde amnesia, a likely transient loss of consciousness, and Glasgow Coma Scale of 14 (of 15) which was 15 (of 15) upon arrival to the emergency department, consistent with mTBI. She sustained clavicle, rib, transverse process, and pelvis fractures and required respiratory support for a "flail chest." Head Computed Tomography (CT) was negative for acute pathology. She refused surgical intervention for her traumatic injuries and died two days later. At autopsy, petechial hemorrhages were extensive within white matter. Histological examination revealed organizing fat emboli. Immunohistochemical evaluation with Amyloid Precursor Protein (APP) unexpectedly revealed distinct axonal swellings in regions characteristic of dTAI.

In the second case, a 77-year-old female pedestrian was struck by a motor vehicle traveling between 20mph and 30mph. She was amnestic toward the event and had a brief loss of consciousness. She suffered head trauma and a scalp laceration, but maintained a Glasgow Coma Scale of 15 (of 15) and was diagnosed with a concussion. She sustained rib, clavicle, and pelvic fractures. Head CT revealed a temporal bone fracture, focal subarachnoid and small subdural hematoma without midline shift. She was anti-coagulated at the time of her injury, so thus was followed with serial head CT scans which unfortunately showed subdural hematoma expansion and midline shift. In spite of medical management, she became globally encephalopathic and died five days after her injury. At autopsy, neuropathological examination confirmed the clinical findings and demonstrated dTAI in addition to changes associated with increased intracranial pressure.

Academic and public interest in concussions is growing. While the sequelae of repetitive mTBI are becoming better understood, research demonstrating structural changes following a single episode of mTBI is predominantly animal-based.[3-8] These two cases show similar findings in humans, supporting the co-existence of both metabolic and structural post-concussive pathology. This is relevant to the medicolegal community for numerous reasons. The finding of structural damage upon neuropathological evaluation may be key to an investigation by elucidating incongruent details. For example, it allows postmortem evaluation for brain injury that may be unexpected given limited gross and/or intracranial pathology but prohibited the decedent from escape or self-defense. Alternatively, it may be relevant when considering if the injury could have impacted decision making. Finally, this microscopic finding enables postmortem correlation with imaging techniques designed to evaluate for mTBI during life.[9]

Appropriate sampling for histology and/or stock tissue is straightforward but must be thorough.[10] Although gross findings are unlikely in mTBI, postmortem evaluation for dTAI following known or suspected concussion samples the same white matter regions as in severe TBI. The posterior corpus callosum just anterior to the splenium is particularly high-yield.[9] Cerebral hemispheric white matter, posterior limb of the internal capsule, and the dorsal brain stem including pons and superior cerebellar peduncle(s) should also be sampled. Both Hematoxylin-Eosin (H&E) and the APP immunostains should be requested on these sections. In addition to highlighting TAI, APP demonstrates Vascular Axonal Injury (VAI) and other trauma sequelae. Experience is needed to differentiate these processes histologically; neuropathology textbooks and colleagues are helpful resources.

Postmortem evaluation for dTAI following concussion can yield information that is highly relevant to the cause and manner of death. Although the demonstration of structural changes of concussion is relatively new, a lower threshold for dTAI sampling is likely warranted. Forensic pathologists and neuropathologists should consider evaluating for dTAI when antemortem mTBI is reported or suspected as part of a complete medicolegal examination.

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Forensic Neuropathology, Traumatic Brain Injury, Concussion

H77 Forensic Neuropathology of Cerebral Palsy (CP): The Implications for Cause-of-Death Determination

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After attending this presentation, attendees will better understand the spectrum of neuropathologic substrates underlying CP.

This presentation will impact the forensic science community by informing attendees of: (1) the differential diagnosis of CP, including perinatal hypoxia-ischemia, developmental anomalies, post-infectious sequelae, and storage/metabolic/genetic disorders; (2) the gross and microscopic presentations of these conditions; and, (3) the approaches for forensic neuropathologic workup at autopsy.

Introduction: CP is the most prevalent physical disability in childhood and includes a heterogeneous constellation of non-progressive movement and posture disorders due to insults to the developing fetal or infant brain. Symptoms are spasticity (hemiplegia, diplegia, quadriplegia), dyskinesia, ataxia, and/or hypotonia, as well as variable cognitive impairment. Colloquially, "cerebral palsy" is applied to many ill-defined neurologic conditions arising in childhood and leading to long-term institutionalization. Thus, medical examiners often investigate their deaths and are responsible for determining the underlying cause and manner of death. This study sought to more accurately categorize the underlying neurolopathologic substrate in cases of "CP" referred to the City of New York Office of Chief Medical Examiner (OCME) over a two-year period.

Methods and Results: During this interval, 20 cases were referred to the OCME with the clinical diagnosis of CP. The investigation report, medical documentation, and clinical history were reviewed, as were gross and microscopic data. Fifteen decedents were male and five female; age range was 2-59 years (median, 28 years). Neuropathology fell into five specific categories: (1) hypoxic-ischemic injury (n=9); (2) genetic/syndromic/metabolic disorders (n= 5); (3) post-infectious sequelae (n=3); (4) traumatic brain injury (n=1); and, (5) other (n=2). For Category 1, examples include perinatal hypoxic-ischemic injuries with periventricular leukomalacia, germinal matrix hemorrhage and periventricular hemorrhagic infarct, status marmoratus, and aqueduct stenosis with hydrocephalus. Category 2 included Coffin-Lowry, Cornelia de Lange, and Klippel-Feil syndromes, and neocerebellar aplasia/hypoplasia.

Neuropathology was directly relevant to cause and manner of death in 15 cases owing to severe disability with general deconditioning, septic and respiratory complications, and malfunctioning of life-support equipment. In occasional cases, the neuropathologic diagnosis had direct impact on an affected family member for whom a specific diagnosis had not been made (e.g., neuronal ceroid lipofuscinosis in a twin).

Conclusion: In virtually all cases labeled as "CP," a specific neuropathologic diagnosis is possible, allowing more accurate categorization of cases and of corresponding cause and manner of death. Such specificity may have important clinical consequences for families and caretakers.

Cerebral Palsy, Hypoxic-Ischemic Injury, Developmental Neuropathology

H78 Preliminary Results of Synaptic Neuroplasticity of Memory Areas: A Comparison Between Violent Deaths and Sudden Deaths

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The goal of this presentation is to understand the differences between cases of violent and sudden deaths in retrieving memories during the antemortem phase by activating dendritic spines. The hypothesis is to demonstrate that in the antemortem phase of violent death, the subject had a dendritic spines modification in areas of memory due to a difficult emotional experience, unlike sudden death.

This presentation will impact the forensic science community by providing valuable assistance to forensic pathologists to determine (for doubtful cases): death by violence; if, during the antemortem period, the subject was a victim of violence by their own killer (psychological violence, physical violence, etc.); and if, during the antemortem period, the subject had a strong emotional experience (suicide, instigation to suicide, etc.). All of these situations produce retrieving memories.

Recent experimental evidence has clarified the important role of some brain areas in the creation and retrieval of memories. In particular, memory mechanisms are linked to the modification of dendritic spines and their plasticity. The scientific literature offers many studies that clarify the framework of synaptic neuroplasticity in chronic mental diseases such as depression or post-traumatic stress disorder, but there are no works that attempt to clarify the neuroplasticity mechanisms that can be realized in a short time, as in violent death cases.

Toward this end, this study compared two groups of subjects. The first group consisted of violent deaths (suicide, homicide) compared to the second group consisting of sudden deaths. All subjects had been autopsied within 24 hours of death. This study includes subjects of a broad range of ages, between 20 and 60 years. During the autopsy, brains were removed and fixed with formalin, then sectioned to separate prefrontal, hippocampus, and amygdala areas in order to obtain samples to analyze.

Samples were treated with the immunofluorescence technique with antibodies against PSD95 and studied with confocal microscopy. PSD95 is one of the proteins involved in the development of dendritic spines from the first week of postnatal life and is part of the protrusions that are contained in the post-synaptic density. Some studies show that this protein is responsible for maintaining the size and binding force of the synapses. During this first step, prefrontal cortexes of six subjects were compared.

The prefrontal cortex is dedicated to the elaboration of current sensory experience in relation to data derived from previous personal experiences. In cases of violent death, a synaptic activation has been demonstrated by the presence of numerous dendritic spines responsive to PSD95 immunofluorescent. This positive response to PSD95 is more represented in violent death, especially when compared with sudden death cases in which the prefrontal cortex is nearly completely negative to the chosen marker.

In conclusion, these preliminary results encourage the continuation of these tests to assess whether there is a change in the synaptic pattern due to the recovery of memories during the antemortem phase and whether memory data retrieval is related to the manner of death (differences were found between a suicide by jumping from a height and a murder by stabbing).

Neuroplasticity, Violent Death, Forensic Science

H79 Cardiac Manifestations of Churg-Strauss Syndrome: A Case Report and Literature Review

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After attending this presentation, attendees will be familiar with the characteristics of the rare disease eosinophilic granulomatosis with polyangiitis, formerly known as Churg-Strauss Syndrome, and the life-threatening cardiomyopathy associated with the syndrome.

This presentation will impact the forensic science community by emphasizing the differential diagnosis of eosinophilic syndromes and their life-threatening sequelae in young populations suspected of substance abuse.

A 22-year-old man with a history of long-standing asthma called 911 with complaints of chest pain and shortness of breath. He had presented to the emergency room on multiple occasions for the past two years, had been admitted eight times, and had undergone nerve biopsies for foot drop without diagnostic findings; he was believed to have mononeuritis multiplex. Earlier in the year, after an episode of eosinophilic pneumonia with leukocytosis, he was newly diagnosed with Crohn's disease. During his longest hospital admission in the last six months, Computed Tomography (CT) scan had diagnosed multiple hepatic abscesses, believed to be due to high-intensity steroid therapy. He was never out of hospital for more than a few days before returning with vague complaints of abdominal or chest pain that did not result in clear diagnoses. Medical records documented growing concern with his narcotics dependence and demands for stronger pain medications.

On the day of his death, the man appeared acutely ill at home. Police identified white powder at the scene. The man displayed combative behavior in the ambulance; with oxygen, he improved enough to transfer himself from the ambulance gurney to the emergency room bed. Within 15 minutes, he suddenly lost pulse and blood pressure and could not be resuscitated. Drug overdose was suspected, versus acute asthma exacerbation.

Autopsy showed "bread and butter" pericarditis and diffuse myocardial lesions without clear etiology that did not correspond to the coronary artery distribution. There were multiple recent wedge-shaped renal infarcts in the absence of any thromboembolic disease. Modest pulmonary hyperinflation was not suggestive of a fatal asthma attack; the lungs showed palpable granularity. The gastrointestinal tract was abnormal, but showed no gross evidence of Crohn's disease; there were no hepatic abscesses.

Histology identified Eosinophilic Granulomatosis with Polyangiitis (EGPA), formerly known as Churg-Strauss disease, in the heart, lungs, kidneys, gastrointestinal tract at multiple levels, and retroperitoneal vessels. There was extensive myocardial scarring, with both acute and resolving lesions. Histology confirmed no Crohn's disease. Toxicology was deemed non-contributory.

Cardiac involvement in EGPA includes carditis (myo-, endo-, and pericarditis), pericardial effusion, rhythm abnormalities, cardiac tamponade, and dilated cardiomyopathy, overall causing half of deaths attributable to EGPA. While only 25% have clinical cardiac symptoms, cardiac evaluations of patients with EGPA yield abnormal results in up to 62% of cases, highlighting the importance of early cardiac evaluation in patients with chronic asthma, gastrointestinal inflammation, and neuropathy.[1] The French Vasculitis Study Group has engendered a five-point system for assessing mortality risk, which includes reduced renal function, proteinuria, gastrointestinal hemorrhage, involvement of the central nervous system, and cardiomyopathy.[2,3] This patient fulfilled five of six diagnostic criteria, and three of five high-mortality criteria, with cardiomyopathy carrying the most significant prognostic weight.

Due to his narcotics dependence and steroid therapy masking his peripheral eosinophilia, his life-threatening EGPA cardiac involvement was not identified prior to autopsy. If cardiac imaging is initiated early in the disease process, and myocardial EGPA is treated aggressively, outcomes improve.[1] Autopsy findings such as those in this case can help guide future diagnoses.

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Churg-Strauss Syndrome, Refractory Asthma, Allergic Eosinophilia

H80 Decomposition Odor Analysis Techniques and Prospective Applications in Postmortem Examinations

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After attending this presentation, attendees will understand the most recent chemical analysis techniques used to profile odor from decomposing mammals, as well as how these techniques are currently being applied to postmortem examination of human remains.

This presentation will impact the forensic science community by demonstrating a new method for obtaining information regarding the postmortem status of the remains by using a minimally invasive postmortem technique.

After death, decomposition processes release numerous Volatile Organic Compounds (VOCs) due to the breakdown of tissues by enzymatic and microbial degradation. These contribute to decomposition odor, which can be a tool used to locate human remains using biological detectors and/or chemical sensors. Since the first study of decomposition odor in 2004, methods for the collection and characterization of decomposition odor have evolved substantially, elucidating numerous new trends in the mechanism of cadaveric VOC production under different circumstances. Simultaneously, whole body Postmortem Computed Tomography (PMCT) has become a more common technique used in medicolegal centers worldwide, providing a means of detecting pockets of gases containing cadaveric VOCs through virtual autopsy. The long-term goal of this research is to use PMCT scanning in combination with new gold standard techniques in decomposition odor analysis to provide quantitative taphonomic information in forensic postmortem investigations.

This presentation will provide an overview of the evolution of odor sampling techniques as well as comprehensive Two-Dimensional Gas Chromatography/Mass Spectrometry (GCxGC/MS) for cadaveric VOC analysis. In addition, new applications of gas analysis for cadaveric VOCs have recently been demonstrated for minimally invasive postmortem examination procedures. It is hypothesized that the combination of whole body PMCT, in combination with Headspace/Solid-Phase Microextraction (HS/SPME) and GCxGC/MS analysis, could provide a valuable tool for postmortem examination procedures in the future.

A first proof-of-concept study was performed using PMCT gas reservoir sampling in combination with HS/SPME of VOCs from gas samples and analysis by GCxGC/MS for gas samples from three bodies associated with forensic casework. This study demonstrated that chemical differences existed between samples collected from different areas of a body, as well as between the same sample collection regions for different bodies. This provided a foundation for a secondary study that focused on full optimization of HS/SPME parameters, introduction of a deuterated internal standard mix for quality control, and stability testing, as well as an expanded study on 20 samples from five bodies associated with forensic casework. This optimized method can now be applied in future work to larger cohort studies as part of an established medicolegal network of laboratories that will contribute samples to a combined database. This will allow temporal trends to be better understood and provide robust data for statistical correlation of VOCs with postmortem interval considering factors such as age, weight, gender, etc.

This research is significant because it provides foundational data for further studies aimed at establishing decomposition odor analysis as a valuable postmortem investigation tool, especially as an alternative to more invasive procedures targeted at taphonomic characterization of bodies.

Taphonomy, Pathology, Volatile Organic Compounds

H81 Time and Temperature Effects on Volatile Organic Compound Generation During Early Decomposition

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The goal of this presentation is to inform attendees of recent advancements in the pattern of volatile organic compound evolution during the decomposition process.

This presentation will impact the forensic science community by providing insight into the relationship of time and temperature in the form of accumulated degree hours with the pattern of evolution of Low Molecular Weight (LMW) alcohols and related compounds in relation to Postmortem Interval (PMI).

Currently, there are few scientific methods based on chemical measurements used to estimate the PMI, highlighting the need for more information regarding this important parameter.

PMI can be an important parameter in many homicide or potential homicide investigations. During the immediate postmortem period, breakdown of organic materials takes place, resulting in the formation of LMW volatile compounds from both autolytic processes and, eventually, microbial activity. It was hypothesized that there is a differential pattern in which LMW alcohols and related compounds are generated from tissue during the process of autolysis and decomposition. Further, it was also hypothesized that the pattern of volatile compounds present in a tissue sample may be used, with consideration of time and temperature in the development of a potentially useful analytical technique of measuring PMI.

This study evaluated the pattern of evolution of VOCs from porcine liver at different time/temperature intervals throughout the decomposition process via Headspace/Gas Chromatography (HS/GC). Identification of specific compounds was accomplished by HS/GC/mass spectrometry. Ethanol was determined to evolve from decomposing pig liver in a time/temperature dependent fashion and to serve as a useful marker against which to evaluate the generation of other low molecular-weight alcohols and amines in both a temporal and quantitative manner. This study, therefore, utilized the ratio of several compounds to ethanol in an effort to relate accumulated degree hours of decomposition to the ratio of the marker compound-to-ethanol ratio.

This research has highlighted the potential for the use of specific chemical measurements and their role in estimating the PMI. The time/temperature-dependent compound ratios have been shown to have potential regarding evaluation of the PMI in death cases, which may augment observational information such as stage of decay and insect activity.

LMW Alcohols, Accumulated Degree Hours, Postmortem Interval

H82 The Big Sleep: Elucidating the Early Sequence of Molecular Events in the First Hours of Death to Determine the Postmortem Interval

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After attending this presentation, attendees will better understand the early sequence of molecular changes triggered immediately after death at the cellular level, ultimately providing a quantitative tool for determination of early postmortem interval.

This presentation will impact the forensic science community by demonstrating the added value of understanding cell death regulatory pathways and the crosstalk between autophagy and apoptosis for the estimation of postmortem interval.

Determination of postmortem interval is analyzed using multidisciplinary approaches. Among them, thanatochemistry methodologies seem to be useful and accurate in respect to classical methods. In this line, some works studied the stability of RNA expression using housekeeping genes, although only a few reports have to date investigated the thanatotranscriptome, thereby analyzing gene expression levels in internal organs of cadavers.

Decomposition begins approximately four minutes after death with a process called autolysis, inducing destructive changes in tissues and cells involving cell death; however, the nucleus remains without alterations until four days after death, thus making feasible the application of molecular and cellular methodologies for time-since-death estimation. In fact, recent trends point to the analysis of the expression of autophagy proteins (thanatophagy) toward this purpose.

Autophagy is often observed in dying cells, trying to mitigate a given stress. If the stress persists, cells can respond by activating processes of apoptosis. Even though autophagy and apoptosis represent distinct cellular processes, the protein networks that control their regulation and execution can be highly interconnected.

Based on these premises, the goal of this research was to study early postmortem interval (between two and eight hours) by analyzing: (1) messenger RNA (mRNA) and protein expression levels of both autophagy and apoptotic genes; and, (2) oxidative stress production and the expression of melatonin receptor, as a regulatory gene implicated in this process.

Four adult male Wistar rats were euthanized at the same time with intra-peritoneal injections of xylazine. The rat bodies were placed in the laboratory at room temperature and 20mg of gastrocnemius muscle were biopsied from each rat at different time points (zero, two, four, six, and eight hours) after death. Each sample was divided in two halves: one for oxidative stress and protein expression analysis and the second half for RNA extraction.

After processing on a standard lysis buffer, supernatants from the first half were divided in two for oxidative stress and protein expression analyses. Proteins were quantified by fluorometric assay, with concentrations ranging between 0.41 mg/ml and 23.18mg/ml. Standard Western blot protocol was used to study protein expression, followed by densitometric quantification.

Oxidative stress production was measured by fluorometric assay and the results expressed as Relative Fluorescent Units (RFUs).

RNA was extracted using Trizol protocol, and quantified by spectrometry, ranging concentrations between 483.5ng/µl and 1,622.1ng/µl. The next step was complementary DNA (cDNA) synthesis by reverse transcription, followed by quantitative Polymerase Chain Reaction (qPCR) to analyze the mRNA expression levels of autophagy (LC3, Beclin-1, ATG7 and ATG12) and apoptotic regulatory genes (FasL and PTEN) as well as the melatonin receptor (MT2). The relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method, and mRNA levels were normalized to Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) levels.

The mRNA expression data for autophagy genes were in good agreement with the sequence of cellular events leading to autophagy. Thus, LC3 expression, implicated in the first phase of autophagy, was found to rapidly increase with a maximum peak at two hours. The other autophagy-related genes, implicated at later stages, showed a time-dependent increase until four hours after death. At this point, it seems that apoptotic signaling was triggered since FasL and PTEN mRNA levels as well as Cytochrome C protein expression consistently increased until six hours after death. In contrast, oxidative stress similar to autophagy expression pattern was induced until four hours, then it decreased at six hours, and finally it further increased at eight hours, which can be correlated with the summit of autolysis process. Furthermore, as expected, induced MT2 expression parallels free radicals production at four and eight hours. Applying a multivariate regression analysis, a strong positive correlation was found between the expression levels of autophagy and apoptotic genes and the time since death.

These findings provide a proof-of-principle for a novel quantitative method to estimate early postmortem interval based on the crosstalk between autophagy and apoptosis. Future research may be directed to search for additional markers extending time-since-death estimates.

Postmortem Interval, Autophagy, Apoptosis

H83 Utilizing Radio Frequency Identification Technology (RFID) to Automate Data Acquisition Between the Baltimore Office of the Chief Medical Examiner (OCME) and the Living Legacy Foundation Organ Procurement Organization (OPO)

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The goals of this presentation are to: (1) accurately and efficiently monitor individual cooling times of decedents at the OCME; (2) assist in the determination of transplantable tissue prior to recovery; and, (3) understand the critical time components that are needed to determine tissue donor suitability.

This presentation will impact the forensic science community by showing a way to automate cooling time data acquisition so that efficiency between OPO and OCME agencies increases and ultimately leads to an increase in tissue donors.

Time of death and cooling times are critical data needed to determine tissue donor suitability. According to the American Association of Tissue Banking (AATB), warm ischemic time shall not exceed 24 hours as long as the decedent was cooled within 12 hours of death. The time limit shall not exceed 15 hours if the decedent was not cooled within 12 hours of death.[1] The current system utilized between the Living Legacy Foundation (LLF) OPO and the OCME in Maryland requires OPO staff to call the OCME to obtain cooling times. Obtaining the crucial and time-sensitive information so as to assist the OPO in determining donor suitability is an additional time-consuming task levied on the forensic investigators and autopsy services personnel.

In 2011, the OCME opened their new facility in Baltimore, MD. In this new construction, the OCME incorporated Radio Frequency Identification Technology (RFID) to track medical case files with a potential expansion to track the location of decedents. In discussions with the OCME to improve current practices, the LLF inquired about the use of existing technologies to obtain accurate and timely access to cooling times. It was determined that the RFID readers located above the autopsy coolers might provide a way to automate this data capture.

In late 2015, the LLF met with the OCME leadership to propose a feasibility study to examine the use of RFID to track the movement of decedents in and out of the autopsy coolers. It was determined at that time that existing chart tracking software could be used to potentially import cooling time data into the OCME electronic intake screen. Over the next several months, the LLF trialed individually barcoded ID tags and performed a preliminary validation to determine the range and accuracy of the RFID antennae.

The next step included the actual tagging of decedents into the existing OCME database upon intake. Cooling time data was captured in "comma separated values" (csv) format, which is not a user-friendly format, and was difficult to interpret by LLF call center staff. With OCME information technology support, an additional table was added to the electronic intake screen that would import in and out cooling times on tagged decedents. A recent revision included a unique barcode field that automatically populates during the intake process to eliminate the possibility of entry error. The LLF created an Installation Qualification and Operation Qualification (IQ/OQ) document co-signed between the OCME and LLF that will be utilized in final validation and for auditing purposes.[2]

Final validation of this project began in August 2017 and will determine accuracy of data reading and import to the intake screen. A secondary goal is to test the system for failure and the ability to recognize and default to the previous process in the event of software outage. It is estimated that this new RFID data capture will have gone "live" by the 4th quarter of 2017. It is anticipated that by automating cooling time data acquisition, it will increase efficiency between both agencies and ultimately lead to an increase in tissue donors. By utilizing RFID, it is predicted that processes will be streamlined, donation will be optimized, and, more importantly, allow OCME staff to continue their daily duties uninterrupted.

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Transplant, Cooling Time, RFID

H84 Nailfold Capillaroscopy Efficacy in Assessing Postmortem Interval (PMI) Compared to Vitreous Potassium Concentration: A Preliminary Study

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The goal of this presentation is to introduce a new method in assessing PMI that seems to be more effective and reliable than vitreous potassium concentration.

This presentation will impact the forensic science community by demonstrating the originality of methods that have never been used in the forensic sciences to assess PMI.

Background: Postmortem vessel changes have been widely investigated to understand the pathophysiology mechanisms entailing capillary leaks. The multiphase Postmortem Computed Tomography Angiography (PMCTA) method allows one to analyze and restore the anatomy of circulation and is of particular interest in investigating the vascular damage-related causes of death.[1] External aspects of the postmortem modifications, such as hypostasis and color changes, are explained by the microvascular rearrangement in which blood clots precipitate within the vessel and the non-corpuscular part of the blood progressively expands throughout circulation.

An unequivocal and reproducible tool to determine the progressive onset of these changes has not yet been identified; even the efforts of forensic science requires several methods to attempt to establish and quantify the PMI.[2] At present, the most reliable seems to be the analysis of the potassium concentration in the vitreous fluid of the eye. By a mathematic model connecting the rise of this ion to the PMI, some authors have proposed a reasonable accuracy of the time-of-death estimation.[3]

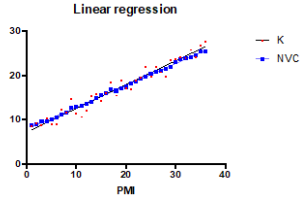
Nailfold Videocapillaroscopy (NVC) is a highly reliable, reproducible, logistically practical, and cost-effective favorable technique, in which the nailfold bed capillaries of the fingers of the hand (excluding the first finger) are directly observed by the operator via a magnification lens (usually 200x). It is extensively used in cardiology, dermatology, and rheumatology settings to assess the capillary shapes, often predictive of clinical outcomes. It has recently been integrated by a software that allows it to perform automatic, instead of ocular, measurements of the number and intensity of capillary abnormalities, thus limiting the inter-observer variability and providing a semiquantitative, validated score.[4] Among the assessable alterations, perivascular edema due to capillary leakage can be easily scored.

Methods: After the approval of the local ethics committee, this study considered one-day consecutive subjects on whom autopsies were performed in the University Hospital. The exact time of death was available for all subjects. The bodies were kept at 20°C and the autopsies were performed in an interval randomly ranging from 12 to 36 hours after death; potassium (mmol/l) vitreous samples were collected, and NVC performed, bilaterally. NVC edema was scored by a dedicated software (VideoCap®) and expressed in a continuous quantitative score, set as *0*=perfectly defined shapes, no flu effect, to 35 (according to the potassium vitreous value range considered in most studies), consisting of completely undistinguishable vessels due to the perivascular "fog."[5-7] Obtained values were plotted versus known PMI by Pearson correlation and multivariate non-linear regression (Analysis of Covariance (ANCOVA)). The PMI was expressed in hours in the decimal system, and the statistical analysis was conducted by SAS/STAT® software Version 9.3. A statistical significance of $p<0.05$ with 95% confidence interval was retained.

Results: A total of 15 non-traumatic and 5 violent deaths were included (13 males and 7 females, average age 60±14 years). Among the 40 collected samples (potassium concentration from both eyes and the average edema score for the eight fingers examined, for both hands), the concentrations of potassium ranged from 8.4mmol/L (PMI of 12 hours) to 35.1mmol/L (PMI of 30 hours), while NVC edema scored, respectively, from 8.8 to 34.9. Both values were positively and significantly correlated with PMI ($p<0.001$) but with a different accuracy: vitreous potassium, R^2=0.83 vs. NVC, R^2=0.99 ($p<0.05$, 95% confidence interval, 34 degrees of freedom). (See Figure 1, below).

Discussion/Conclusion: The PMI assessment has always represented a cardinal objective of forensic science, supported by increasing accuracy due to technology. The obtained data reflects the pathophysiology of the capillary leaks process that begins immediately after death, being conceptually in line with the potassium increasing concentration in the vitreous after metabolic processes stops. Despite the limited number of observations, this work represents a totally original approach in PMI definition, thanks to the contribution of a very simple, reproducible, and standardized technique. Extended data are needed for the validation of the NVC method in capillary assessment to confirm its reliability in determining PMI.

Figure 1:



PMI (hours); K, potassium vitreous (mmol/l); NVC edema score.

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Nailfold Videocapillaroscopy, Vitreous Potassium, Postmortem Interval

H85 Partially Skeletonized Remains Demonstrating Dragging Injuries, Internal Beveling of the Skull, and Tracheal Obstruction: A Team Approach

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After attending this presentation, attendees will recognize how a multidisciplinary team approach to partially skeletonized remains can help assist in determining timing of death and mechanism of trauma.

This presentation will impact the forensic science community by highlighting entomologic and anthropologic findings in partially skeletonized remains that may be found in medical-legal death investigations.

Skeletonized or partially skeletonized remains present challenges in forensic pathology. While identification of the remains has become easier with advances in fingerprinting, DNA, and comparative medical radiography, determining cause of death, manner of death, and approximate time of death can be difficult in this population. This study presents a case of partially skeletonized remains with distinctive bone damage on posterior surfaces and a cigar-like collection of leaves in the trachea.

Adjacent to a field, a partially skeletonized set of remains was discovered with multiple electric cords around the ankles and the neck. At the postmortem examination the next morning, the remains were found to have dry desiccation and mummification of the skin with skeletonization of the posterior aspect of the body, and a noticeable portion of the posterior cranial vault was missing. A forensic anthropologist was consulted to assess the skeleton. In addition to the skull, there were smooth, flat areas of bone disruption on multiple vertebrae, ribs, scapula, humerus, ulna, metacarpals, ilia, sacrum, femur, calcanei, and hand and foot phalanges. Such injury patterns are consistent with vehicular dragging as reported in the literature.[1-3] Furthermore, gross examination of the disrupted bones revealed evidence of gravel embedded in the bone margins. The margins of the residual skull were smooth and flat, with the exception of a single semi-circular defect that appeared to have inward beveling consistent with projectile trauma; however, the sharp, semi-circular defect did not have any associated skull fractures. The forensic anthropology team returned to the recovery site and subsequently recovered a section of skull that matched the area of inward beveling.

Further examination of the remains revealed a tightly rolled collection of green leaves in the trachea. A white larva was identified within the center of the leaf collection. An entomologist was consulted to identify the larva and to collect additional insects on the remains. The larva was determined to be a leafcutter bee (*Megachilidae*). The decedent was subsequently identified by fingerprint analysis. Law enforcement reported that the decedent was last known alive approximately three weeks prior to the discovery of his remains.

This unique case demonstrates the importance of having access to a multidisciplinary team when dealing with skeletonized remains in the forensic setting. The forensic pathologist was not familiar with leafcutter bees and their preference for nesting in open tubular structures, such as a trachea. One should carefully consider the circumstances and the possible mechanism of injury when assessing beveling in skeletonized remains that may have been dragged. While the pattern of inward beveling is closely associated with projectile trauma, it should be considered in cases of dragging. This team approach provided insight into the approximate timing of death and to the mechanism of skeletal injury, even if a discrete cause of death could not be determined.

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Skeletonized Remains, Leafcutter Bee, Drag Injury

H86 Does Black Tar Heroin "Protect" King County, Washington, From Fentanyl-Related Mortality?

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The goals of this presentation are to: (1) describe regional differences in the so-called opioid epidemic; and, (2) explain possible reasons why King County, WA, has experienced a smaller proportion of fentanyl-related deaths.

This presentation will impact the forensic science community by attributing at least part of the high regional variability of drug overdose rates to the supply and demand of various opioids, specifically in the supply of black tar heroin, which appears to reduce the demand for the more potent fentanyl-type drugs.

In contrast to other regions around the country that have experienced rapidly rising opioid overdose death rates, King County, WA, has seen a relatively modest rise in overdose deaths. The present study was conducted to provide possible explanations for the differences.

Methods and Materials: Data on fatal overdoses in King County were obtained from the King County Medical Examiner's Office in Seattle, WA. Similar data were obtained from West Virginia and Erie County, NY. Data on overdose deaths in British Colombia, Canada, were obtained from the British Colombia Coroners Service report of June 30, 2017.

Results: From 1999 to 2016, drug overdose rates in King County increased from 11.9 to 16.9 per 100,000 population. Over the same period, the rates in Erie County increased from 2.6 to 35.5 per 100,000. In West Virginia, the total number of drug overdose deaths for the whole state increased from 212 in 2001 to 879 in 2016. In British Columbia, drug overdose deaths for the province climbed from 269 in 2012 to 967 in 2016. While drug overdoses deaths increased nearly exponentially in Erie County, West Virginia, and British Columbia, the rate of increase in King County was close to linear. The spectrum of drugs responsible for the increasing death toll was also different. In King County in 2016, the most common opioid responsible for fatal overdoses was heroin; only 22 deaths were due to fentanyl and related fentanyl-type drugs. In British Columbia, fentanyl-type opioids accounted for most of the three-fold increase in overdose deaths from 2012 to 2016. In West Virginia in 2001, only 9 deaths were caused by fentanyl type drugs; in 2016, the number was 360. In Erie County, fentanyl-type drugs were present in 229 of the 327 overdose deaths in 2016. Although heroin accounted for a sizeable proportion of opioid-related deaths in all locales, the physical characteristics of the drug varies from place to place. In King County, black tar heroin is the predominant form, while in British Columbia; Erie County, and West Virginia, heroin is predominantly a white or other color powder.

Discussion and Conclusions: Although opioid-related deaths have soared throughout the country in the last several years, the crisis in King County differs from the three places used for comparison in two ways: in King County, the rate of increase of drug overdose deaths is fairly modest rather than explosive; also in King County, fentanyl-type drugs are fairly uncommon in overdose deaths. The most likely explanation for the difference is the physical characteristics of the drug. Black tar heroin, by far the predominant form in King County, is a black gooey substance rather than a powder. This physical characteristic would make it difficult to simply mix black tar heroin with another powdery drug, such as fentanyl, while it would be easy to mix fentanyl into a powdery form of heroin. But this explanation may not be the only one. While it would be relatively simple to adulterate black tar heroin with fentanyl during the "cooking" phase of drug preparation, for unknown reasons, this appears not to happen very often. Very few seizures of black tar heroin have been found to contain fentanyl, and toxicology reports in King County infrequently find heroin and fentanyl together. It is also possible that mixing fentanyl into black tar heroin has no marketing advantage. King County has a strong history of black tar heroin consumption, and it is possible that the current supply of black tar heroin merely suits the local demand. While efforts continue to control both the supply of and the demand for abused drugs, it does appear that, ironically, black tar heroin "protects" King County against more potent fentanyl-type opioids.

Black Tar Heroin, Opioid Epidemic, Toxicology

H87 "Graze Laceration and Graze Fracture": Injuries Which Are Unnamed in Literature

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After attending this presentation, attendees will have a better understanding of the types of injuries found in various traffic accidents not mentioned in the literature. This presentation will also discuss the importance of recognizing such injuries, their mechanism of causation, and their importance in forensic cases.

This presentation will impact the forensic science community by introducing new terminologies for trauma caused by accidents in relation to the mechanism and manner of causation.

Humans have progressed from an early primitive state to the advanced state of the modern era. During this time, humans have been subjected to different types of trauma. Many of the injuries occurring are well classified in the literature. Still, a medical doctor faces different types of injuries that have little-to-no mention in the scientific literature. Naming the injury is very important as the nomenclature of such injuries is very helpful for future identification of the injuries as well as correlating them with the manner or object by which they occurred.

This study highlights some injuries that were found in trauma caused by vehicle mishaps. These injuries occurred due to dragging of a body for some considerable distance by high-speed vehicles. When such injuries occur to a victim and he is brought to the hospital, it becomes imperative for the doctor to name the injury correctly. This will be useful not only for treatment but also for future medicolegal purpose.

Graze laceration: These are injuries that occur when the surface of the body gets dragged over rough surfaces for a considerable period, causing grazing/friction of the body surface by that of the rough surface, resulting in a graze laceration.

Graze/grinding fracture: This fracture is caused by forceful dragging of the body involving bony parts over a rough surface for a considerable period, causing grazing/friction/grinding of the bone coming into contact with the rough surface, resulting in a graze/grinding fracture.

This study presents a case series depicting injuries featuring graze lacerations and graze/grinding fractures from road traffic accidents. This presentation will also discuss the importance of having knowledge of such injuries when dealing with medicolegal cases.

Trauma, Graze Laceration, Graze Grinding Fracture

H88 Fatalities to Children Falling Into Abandoned Borewells: A 10-Year Study

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After attending this presentation, attendees will understand the fatalities caused to children who accidentally fall into abandoned borewells.

This presentation will impact the forensic science community by informing attendees of the danger to children of playing in the vicinity of abandoned borewells regarding manner of causation of death, probability of survival, and the correlation of borewell depth with survival, if any.

There is a growing scarcity of fresh water reservoirs used for drinking, irrigation, and day-to-day washing and other personal usage. India has a varied geography, from snow in the mountains in the north to deserts in the west. The east has large broad rivers like the Ganges and Brahmaputras, while the south faces heavy rainfall. All these regions face the scarcity of potable water. To overcome this difficulty, ground water accessed by constructing open wells and borewells. In drought areas, open wells don't satisfy the need of water for various irrigation and drinking purposes. For this, borewells are a good alternative. The diameter of such borewells should be no more than seven to eight inches. Sometimes these borewells are dug up to 1,000 feet deep. Most often, the borewells are successfully dug to obtain water; however, sometimes the borewell doesn't produce enough water. Such borewells are then abandoned without taking proper measures to seal them.

Such abandoned borewells pose a danger to the public. Due to the small diameter of these borewells, they are not a real threat to a normal adult, but small children are particularly at risk of accidently falling into them. The children can become accidentally stuck and trapped in the tubing or the hole of the borewell and are unable to extricate themselves. If immediate rescue does not occur, chances of survival are diminished. Such a scenario may also happen in other regions of world where such borewells are also dug.

Recently, news of people, particularly children, dying due to a fall in a borewell has received attention from the authorities, the media, and the public at large.[1,2] Such incidents are preventable and valuable lives could be saved.[3,4] A total of 43 incidents of victims falling in borewells was noted during the period from 2006 to 2015. Most of these cases were of children less than 6 years old, with a male preponderance. Most of these victims were not able to survive in the hostile environment inside the borewell.

This presentation will describe the age and sex distribution of fatalities from falls into borewells. The reason for the victims being involved in falls into borewells will be described. The medicolegal death investigator should become more familiar with incidents of victims being trapped in borewells and the manners of death caused by these events.

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Human Fatality, Borewell, Children

H89 A Fatality Caused by Molten Metal Splash During A Field Visit Near A Furnace: A Case Report

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After attending this presentation, attendees will understand the current case of a fatality caused by a molten metal splash during a field visit that occurred near a furnace of a smelter factory.

This presentation will impact the forensic science community by creating an awareness of the possibility that people working on or visiting furnaces containing molten metal should be aware of the possibility that exploding, splashing molten metal could cause a fatality or serious injuries to the operator or bystanders. This presentation highlights the fact that such molten metal explosions do occur and can cause fatal injuries.

Molten metal splash is the most common cause of melt deck injuries and is caused by the addition of wet materials to the molten bath. Wet charge materials are a serious safety hazard in all foundries. Water, moisture, or any liquid-bearing material instantaneously turns to steam when coming into contact with molten metal — expanding to 1,600 times its original volume and producing a violent explosion. This occurs without warning and throws molten metal and possibly high-temperature solids out of the furnace, putting workers, the furnace itself, and nearby plant equipment at risk.

A water/molten metal explosion can occur in any type of furnace; however, for an induction furnace, the aftereffects may be more serious, including the possibility of additional explosions caused by liquid in a ruptured cooling system coming into contact with molten metal in the bath. Molten metal need not be present in the furnace for a water/molten metal explosion to occur. Explosions can also occur if sealed drums or containers containing water are charged into an empty but hot furnace. In this case, the force of the explosion will eject the newly charged material and quite likely damage the refractory lining as well.

This presentation highlights a case in which the victim, while on a field visit to a factory, was fatally injured by molten metal splash. This presentation will also discuss the importance of having knowledge of such injuries when dealing with these medicolegal cases.

Molten Metal, Splash, Explosion

H90 Animal Tusk Injuries: Are They Unique With Specific Animals?

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The goals of this presentation are to discuss: (1) injuries caused by animals that use their tusks when attacking; (2) specific animal tusk patterns; and, (3) the importance of recognizing tusk injuries, their mechanism of causation, and their importance in forensic cases.

This presentation will impact the forensic science community by introducing a better understanding of the manner and pattern of injuries caused by tusks of different animals, particularly wild boars.

Humans have historically always been subject to different types of trauma. There can be increased conflict with humans and animals due to shrinking animal habitat. Some of the animals have various patterns of injury causation. Some attack by using fangs, paws, horns, etc. Other animals have tusks, which they use primarily to search for food. Tusks have a variety of uses, depending on the animal. Social displays of dominance, particularly among males, are common, as is their use in defense against attackers. Elephants use their tusks as digging and boring tools. Walruses use their tusks to grip on ice and to haul out on ice. The presence of a tusk in only the male narwhal suggests that, for these whales, the tusk is a secondary sex characteristic. Though primarily a tool for obtaining food or as a sign of a secondary sexual characteristic, when the animal is in combat, these tusks can become a tool for attacking the encroacher and can cause fatalities.

Many of the injuries occurring to humans have been well reported in the literature; however, a medical doctor encounters different types of injuries that have little or no mention in the available literature. Identifying the injury is important as the method of causation of such injuries is very helpful for future identification as well as correlating the injury with the manner or object of causation. Additionally, the site at which such injuries occur on the human body can, to some extent, determine the possible animal and its method of causing the injuries.

This presentation highlights tusk injuries found in traumas caused by animal tusks. These injuries occurred due to striking, biting, or dragging of the body for some considerable distance. When such injuries occur to a victim and that person is brought to the hospital, it can necessarily be important for the doctor to identify the injuries. This will be useful not only for treatment but also for future medicolegal purposes.

Trauma, Tusk Injury, Animals

H91 The Recovery of Vertebrate DNA From the Gastrointestinal (GI) Tract of Flesh-Eating Insects: A Mass Disaster Simulation Study

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After attending this presentation, attendees will: (1) understand that genetic analyses of flesh-eating insect gut contents found on and near the scene may reveal the identity of missing humans or endangered wildlife species in cases in which the body remains are not visible or available; and, (2) recognize that the value of insects on crime is not limited to Postmortem Interval (PMI).

Mass disasters and terrorist attacks may leave bodies in pieces, burned beyond recognition, or unable to be immediately located. At times, bodies/body parts are moved or scavenged by wild animals in events such as brutal killings, natural deaths, and airplane crashes in remote areas. This presentation will impact the forensic science community by informing attendees that flesh-eating insects are known to colonize decomposing bodies and, when used in forensic cases, may provide crucial evidence that could bring closure to mysterious deaths, determine removal of remains from a suspected crime scene, and establish the credibility of witness statements.

In this investigation, adult *Dermestes maculatus* beetles and larvae were reared in three separate colonies. Each colony fed on fresh flesh meat (*Bos taurus* (beef), *Sus scrofa* (pork), and *Meleagris meleagris* (poultry)). The Quick-DNA™ Tissue/Insect Miniprep Kit was used to extract total DNA. The Polymerase Chain Reaction (PCR) assay utilized species-specific primers that successfully amplified fragments (poultry: 183bp; pork: 212bp; and beef: 271bp) of 12S ribosomal RNA (rRNA), 12S rRNA, and ATPase subunit 6 genes and 8 genes, respectively. The results imply that DNA recovered from the GI-tract reveal what flesh-eating insects fed on.

In a further study, human blood was mixed with beef meat to simulate a mass disaster. The beetles were exposed at room temperature at intervals of 2, 4, 8, 12, and 24 hours. Following extraction of the entire beetle/larvae a Quantifiler® Trio kit was used on a Real-Time PCR 7500 Sequence Detection System and the total amount of amplifiable human DNA and male DNA found ranged from 0.0002ng/µL to 0.184ng/µL. The thermal cycler protocol consisted of holding Stage (1 rep): Step 1, 95° for 2 minutes, cycling Stage (40 cycles): Step 1: 95° for 9 seconds, and Step 2: 60° for 30 seconds.

The GlobalFiler™ amplification kit that contained primers necessary to amplify 21 autosomal loci, Y indel, Y-chromosomal Short Tandem Repeat (Y-STR) locus, and sex determining marker (amelogenin) were used on the genetic analyzer GlobalFiler™ 3500. The protocol was: initial incubation step of 95°C for 1 minute, 29 cycles of denature 94°C 10 seconds, anneal/extend 59°C 90 seconds, final extension 60°C 10 minutes, and final hold was 4°C. A full female DNA profile and partial male DNA profile were developed and matched to the reference profiles made from buccal cheek swabs. The results show that human DNA can be recovered and individualized from flesh-eating insects that could be potential secondary sources of DNA.

Flesh-Eating Insects, Vertebrate DNA, Mass Disaster

H92 Medicolegal Issues in a Death Due to Duchenne's Muscular Dystrophy

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After attending this presentation, attendees will: (1) recognize the importance and appropriateness of performing medicolegal autopsies in certain cases involving chronically disabled persons; (2) recognize the importance of medicolegal death investigation involvement in certain cases involving medical therapy-related complications; and, (3) understand the challenges involved with manner-of-death certification in deaths due to therapy-related complications.

This presentation will impact the forensic science community by focusing on several important issues that can present challenges for those within the death investigation community. These challenges include the investigations of deaths involving chronically debilitated individuals, deaths related to medical intervention/therapy, and the controversies related to certifying the manner of death in such cases.

An important public health function of medicolegal death investigation involves the identification of deaths that are considered preventable. By implementing preventive strategies based on the identification and reporting of such incidents, it is hoped that similar deaths may be avoided. In order to identify such preventable deaths, it is frequently necessary to perform a medicolegal autopsy; however, there are several groups of decedents in which a medicolegal autopsy is rarely performed. Two such groups include persons who have chronic debilitating conditions and those who die in the hospital with severe underlying disease. Although most of the deaths occurring in these two patient populations do not require medicolegal death investigation or autopsy, it is important to note that, in certain circumstances, the investigation of such cases is entirely appropriate. This report presents a case of death in a chronically debilitated, terminal patient, who died as a result of a complication of medical therapy.

Presented is the case of a 28-year-old male with Duchenne's Muscular Dystrophy who died of complications related to gastric tube dislodgment and subsequent sepsis. The patient was hospitalized for an acute-on-chronic episode of respiratory distress and was put on a ventilator and fitted with a gastric tube. The patient later complained of abdominal discomfort and an exploratory surgery revealed peritonitis. The patient died shortly thereafter under general anesthesia during emergency surgery. Autopsy revealed purulent exudate over the serosal surfaces of the intestines that extended into the mesentery and peritoneal lining. There was focal hemorrhagic exudate that was limited to the area immediately surrounding the gastric tube entrance site. Culture of the peritoneal cavity was positive for *Pseudomonas sp.*

Medicolegal autopsies are often not performed on patients with an underlying chronic debilitating condition if they die in a hospital setting. These deaths are common and expected and it is often assumed that, in most cases, the cause of death is either directly or indirectly related to the underlying condition. There is some merit to this assumption. For instance, a recent two-year-long study of the cause of death in an inpatient hospice program found that all 48 patients autopsied died of causes related to their underlying condition.[1] However, assuming that *all* deaths in this subset of patients can ultimately be traced back to the underlying medical condition leads us to overlook preventable causes of mortality such as complications arising from medical interventions.[2] It is thus unfortunate that medicolegal autopsies are not performed when warranted since they can help to identify sources of treatment-related deaths. This would ultimately aid in quality improvement resulting in better outcomes for future patients.

In addition to providing an impetus for discussing deaths in chronically debilitated persons and deaths related to medical intervention, the presented case highlights a classic dilemma faced by those who complete death certificates. Manner-Of-Death (MOD) certification is not an exact science and such determinations can vary. Although general guidelines for MOD determination have been promulgated by the National Association of Medical Examiners, variation remains.[3] The current case raises questions concerning the most appropriate MOD ruling in such cases.

In conclusion, medicolegal autopsies could help improve hospital outcomes in patients with underlying chronic debilitating diseases. The cause of death in these patients is often thought to arise as a natural sequelae of the chronic underlying disease. Performing a medicolegal autopsy on these patients has the possibility of identifying treatment-related deaths that would hopefully improve hospital outcomes.

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Medicolegal Autopsy, Duchenne's Muscular Dystrophy, Forensics

H93 Electrocution Due to Multiple Entry and Exit Wounds — A One-of-a-Kind Case Report

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The goal of this presentation is to introduce a unique case in which the deceased suffered multiple entry and exit wounds at several places on the body, making it difficult to identify the primary site of contact, as well to explain how these could have occurred in the absence of any object that could justify such wounds.

This presentation will impact the forensic science community by describing a situation in which multiple entry wounds may be present without evidence of how the wounds were caused, thereby cautioning attendees that in all cases of multiple entry wounds, the presence of multiple contact points is not a necessity or that there can be multiple contacts occurring in quick succession.

Electrocution injuries are a less-frequently encountered occurence in forensic practice, but they may lead to great morbidity and mortality. Electrical injuries are usually unintentional and occur in household settings. Electrocution is likely to cause injury to the tissues through which it passes. Death is very rapid in cases of electrocution and, in most cases, no gross abnormalities are observed in the internal organs. The only evidence usually found is the presence of an external electrical contact wound.

Passage of current through the human body is usually associated with a single entry and single exit wound. This presentation reports on a rare case of electrocution in which at least 16 electrical contact wounds were found on the body of the deceased male. These were entry wounds distributed over both upper limbs and the left side of the chest and axilla. Histopathology confirmed these were electrical wounds. The exit wounds were present in the form of multiple pinhead-sized lesions, showered over both soles of the feet. According to the eyewitness account, the left side of the body was in contact with an iron gate, thereby suggesting that injury marks on the left side of the body were all entry wounds. The eyewitness account ruled out multiple contacts in short duration. Thus, the only option for the entry wound on the right side of the body was due to the deceased coming in contact with the iron gate in such a way that the gate was in contact with him at multiple places on the body; however, the construction of the gate as well as the position of the deceased was such that this was not possible. One possibility was that the wound on the opposite side was caused when the deceased was being removed from the gate, in which case it would be an exit wound; however, the gross condition suggested it was an entry wound. Thus, it was possible that the right upper limb came into contact with the iron gate because of body movement during the electrical contact. A visit to the scene revealed an iron ceiling that was in contact with the iron gate, and a live wire was in contact with both; due to the rainy season, the surrounding area was wet.

This case highlights the presence of multiple entry wounds in electrocution and emphasizes the importance of the scene visit to correlate the autopsy findings.

Electrocution, Entry Wound, Exit Wound

H94 Is Methamphetamine Use Associated With an Increased Suicide Risk in Adolescents and Young Adults?

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After attending this presentation, attendees will better understand the role methamphetamine abuse plays in leading to mental instability and suicide.

This presentation will impact the forensic science community by drawing attention to the recent increase in suicides associated with methamphetamine abuse occurring in the adolescent and young adult population.

Methamphetamine is a potent central nervous system stimulator elevating mood and leading to a heightened sense of euphoria. Chronic use is known to manifest as mood/anxiety disorders, psychosis, violent behavior, and suicidal ideation. This is achieved by methamphetamine's interaction with the dopaminergic system ultimately damaging nerve terminals leading to psychiatric pathology.

According to the National Institute on Drug Abuse, in 2012, 1.2 million people (0.4% of the United States population) reported having tried methamphetamine. This report has made an observation concerning the association of methamphetamine use with suicide among young adults within the Kansas City, MO, metropolitan area. This study undertook a retrospective record review of cases at the Jackson County Medical Examiner's Office (JCMEO) between the years of 2012 and 2016, reviewing suicide data of individual closed cases. This study defined the age range for "young adult" as less than the age of 40 years. All cases contained associated toxicology and investigative reports.

For the years 2012-2016, the JCMEO had 976 cases of suicide. Of the 976 cases, 416 were cases of individuals ranging in age from 11 to 39 years. In 2016, 12% of reviewed suicide cases involved intoxication with methamphetamine, 75% of which demonstrated recent suicidal ideation and depression per investigative history. Data from 2015 revealed 15% of suicide cases were positive for methamphetamine. Suicidal ideation and depression were noted in 77% of case histories; arguments and pending incarceration were thought to be contributory factors in the remaining cases. In 2014, methamphetamine positivity in suicide cases sharply decreased to 6.6%. Of these cases, 60% of histories reported suicidal ideation, and the remaining 40% were thought to be the result of arguments. Data from 2013 revealed 11% of cases demonstrated methamphetamine positivity, 86% of which revealed suicidal ideation and depression. In 2012, 15% of suicide cases revealed methamphetamine positivity and 62.5% had reported suicidal ideation. The overall average percentage of methamphetamine-positive cases for this total time period was 14.4%.

The suicide data was compared to closed cases ruled as accidents in which methamphetamine was considered the direct cause of death or as a contributing factor. Between the years of 2012 and 2016, 1,983 cases were deemed accidents, 162 of those listed methamphetamine as the cause of death or as a contributing factor, with 54 of these cases within the study's age range. In the adolescent and young adult ages with accidental manners, the overall average of methamphetamine-positive cases for this time period was 3%.

In conclusion, the results of this study reveal that methamphetamine abuse is much higher in younger adults committing suicide than in accidental overdose cases within the Kansas City metropolitan area.

Methamphetamine, Suicide, Young Adult

H95 Acetyl Fentanyl: Trends and Concentrations in Metro Detroit

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After attending this presentation, attendees will be able to recognize acetyl fentanyl on postmortem toxicology as a marker of illicit fentanyl.

This presentation will impact the forensic science community by making forensic pathologists and toxicologists more knowledgeable regarding the possible etiologies of the presence of acetyl fentanyl in postmortem toxicology.

Acetyl fentanyl (N-[1-phenethylpiperidin-4yl]-N-phenylacetamide), a derivative of fentanyl, is a non-prescription synthetic opioid that is 4 to 5 times more potent than heroin and 15 times more potent than morphine, but much less potent than fentanyl and other fentanyl analogs.[1] Acetyl fentanyl is used in the adulteration of fentanyl, heroin, and cocaine and the first outbreak of acetyl fentanyl-related deaths was reported in Rhode Island in 2013.[1]

The trends and concentrations of acetyl fentanyl-related deaths have been followed at the Wayne County Medical Examiner's Office (MEO) in Detroit, MI, from 2015 to the present. Between April 2016 and January 2017, 21 deaths were reported at the Wayne County MEO, where acetyl fentanyl was found in the decedent's peripheral blood; these were compared to the previously published 75 acetyl fentanyl-related deaths between February 2015 and March 2016 from the same MEO.

Of the recent deaths, 62% (13) were male, 38% (8) female, 52% (11) were White, 38% (8) Black, and 1% (2) Hispanic. The mean age was 44.5 years. The mean concentration in the peripheral blood was 0.9ng/mL (range 0.1ng/mL to 2.7ng/mL). All 21 deaths had fentanyl, 52% (11) heroin, 71% (15) other opiates, 24% (5) cocaine, 0.5% (1) both heroin and cocaine, 29% (6) benzodiazepines, and 20% (4) ethanol present in the peripheral blood. Compared to the 75 previously reported acetyl fentanyl-related deaths, in the period between February 2015 and March 2016, the current data exhibits markedly decreased acetyl fentanyl concentrations. The average acetyl fentanyl concentration from the previous data set was 9.25ng/mL with a range of 0.28ng/mL to 37ng/mL; the current data revealed an average acetyl fentanyl concentration of 0.9ng/mL with a range of 0.1ng/mL to 2.7ng/mL. Also in the recent data was a higher percentage of cases with multiple drugs, with other opiates present in 71% of the deaths compared to the 21% reported previously. The other drugs present were found in similar frequencies and with a similar demographic distribution.

All 21 of the recent cases had acetyl fentanyl concentrations below 2.7ng/mL with an associated toxic concentration of fentanyl (>3ng/mL) in the peripheral blood. This means that the primary drug toxicity was due to fentanyl. Since acetyl fentanyl has not been reported in association with the clinical use of fentanyl, the former's consistently observed lower peripheral blood concentrations are most likely an artifact in the manufacture of the consumed illicit fentanyl.

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Acetyl Fentanyl, Concentration, Toxicity

H96 A Lesson Learned From a Case of Unexpected Death of a 73-Year-Old Woman Due to a Congenital Diaphragmatic Hernia

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After attending this presentation, attendees will understand that a diaphragmatic hernia can be a life-threatening condition even in adult patients, as it may remain silent until the onset of serious consequences. These cases might come to the pathologist's attention as unexplained and unexpected deaths.

This presentation will impact the forensic science community by highlighting the need for surgeons to be vigilant regarding congenital internal hernias, as they may become symptomatic later in life. This condition usually presents in the newborn. A few cases may present in older patients, often with non-specific symptoms that make the diagnosis difficult. This study reports a rare presentation of congenital diaphragmatic hernia in a 73-year-old woman.

On a Saturday, a 73-year-old woman was admitted to the emergency department presenting nausea with dark vomiting that had been on-going for several hours. The patient also reported an episode of transient loss of consciousness. The clinical evaluation did not point out anything remarkable, except abdominal tenderness. Laboratory investigations did not reveal any pathological results; no chest radiograph or abdominal echography was performed. Considering the symptoms reported by the woman, a peptic ulcer disease with hematemesis was suspected and the patient was informed about the need to undergo an Esophagogastroduodenoscopy (EGDS); however, the procedure was planned for the following Monday, two days after the symptoms began. In the following hours, the woman, who had been moved to a surgery department, complained of severe abdominal pain, associated with repeated "coffee ground" vomiting. Details concerning the patient's condition were not reported on her medical chart and no medical measures were taken, except for antisecretory drug therapy and parenteral nutrition. Suddenly, on Monday morning, the woman had several episodes of projectile vomiting and became hemodynamically unstable, with a subsequent cardio respiratory arrest. The patient could not be revived despite all resuscitative measures.

A forensic autopsy was performed to determine the cause of death. The gross examination revealed a wide-neck defect of the left diaphragm with herniation of the fundus of the stomach into the left thoracic cavity, covered by a membranous sheet of tissue (hernia sac). Only mild intrathoracic adhesions were present, and the herniated contents could be easily reduced to the abdomen. The size of the defect was 8cm x 6cm. An atelectasis of the left lung's lower lobe was also observed. The stomach presented an altered shape, with no other pathological findings, neither macroscopic nor microscopic. The gastric content consisted of partially digested food.

Therefore, the cause of death could be identified as a cardio-respiratory arrest due to the compression of the left lung and heart by the large diaphragmatic hernia, which was classified as a posterolateral (Bochdalek) congenital hernia. Considering the cause of the death, provided by the evidence from the forensic autopsy, a medical mistake was identified regarding the diagnosis and treatment of this patient. Although it is documented that the clinicians first addressed their suspicions toward a peptic ulcer, by focusing their choices entirely on this hypothesis, they failed to follow the recommended protocol in cases of hematemesis (which specifically suggests an EGDS within 24 hours). Therefore, the doctors' behavior has to be considered negligent, as a large diaphragmatic hernia was missed.

The death of the patient was preventable with an accurate physical examination and further diagnostic exams, which would have allowed an early diagnosis and adequate treatment; however, because of the rarity of the onset of symptoms due to this condition in older patients, and the non-specificity of the symptoms presented, this condition was not considered by clinicians.

Diaphragmatic Hernia, Unexpected Death, Medical Responsibility

H97 Sudden Death of a 3-Year-Old Girl Due to a Rare Thymic Neoplasm

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After attending this presentation, attendees will be familiar with a rare cause of sudden death in children that can be avoided if diagnosed and treated early.

This presentation will impact the forensic science community by highlighting the need for clinicians to consider a mediastinal mass in cases of breathing difficulty in children. The present case outlines one of the many contributions that forensic pathology can make to medical knowledge, providing warnings that can prevent similar deaths in the future.

At home, a previously healthy 3-year-old girl suddenly presented with difficulty breathing associated with cough and stridor. The parents called the emergency services. Doctors gave the baby a corticosteroid and an aerosoltherapy with adrenalin. When the child was taken to the emergency department, the symptoms ceased and she was thus discharged. Five days later, another similar episode occurred. Again, by the time the patient was admitted to the hospital, the symptoms had already stopped. A diagnosis of laryngospasm was rendered and the parents were advised to take the baby to an allergist for a detailed examination. The baby was allowed to go home with a prescription for a corticosteroid and a bronchodilator. No instrumental examinations were performed.

After three days, during which nothing remarkable was noticed by the parents, the little girl had another episode of difficulty breathing, followed by a loss of consciousness. When first aid arrived, the baby was in cardiac arrest. Resuscitation efforts were initiated but were unsuccessful, and death was declared.

A forensic autopsy was ordered by the prosecutor to consider the possibility of a medical mistake.

The external examination revealed a well-nourished, 98cm-long girl. The autopsy disclosed a large mediastinal mass that measured 11cm x 11cm x 6.5cm and weighed 540g. The neoplasm was within the thymus and had already invaded the pericardium and pleura, reaching the diaphragm. The lungs were partially collapsed, due to the large mass occupying the plural space, and showed edema and congestion. Histologically, the neoplastic nature of the tissue of the thymic mass was confirmed, as well as its invasive tendency. Therefore, death was attributed to the mechanical compression by the mass on the airways, the lungs, and the heart. The mass was identified as a malignant thymus neoplasm, classified as a carcinoma.

This type of tumor is extremely rare, especially in childhood, and its prognosis is poor if not diagnosed early, due to its invasiveness and rapid growth. Early diagnosis and complete resection are essential in order to achieve a good outcome.

The evaluation of the medical conduct excluded any medical responsibility in the mismanagement of the patient during the two admissions to the emergency department. This conclusion was reached by considering the lack of specific indications of the instrumental examinations (chest radiography, Computed Tomography (CT) scan), as the hypothesis of a laryngospasm due to an allergic reaction seemed to be the most probable. In fact, the clinical pattern was consistent with this diagnosis and nothing remarkable was observed during the objective examination (especially concerning the thorax). Only by performing the autopsy was it possible to arrive at the diagnosis.

The present forensic case represents a contribution to the improvement of clinical science, as it brings out a very rare cause of sudden death in childhood that, if recognized early, can be successfully treated. This case is worth highlighting as the knowledge it imparts can be helpful in avoiding similar deaths in children.

Sudden Death, Thymic Neoplasm, Medical Responsibility

H98 Sudden Death During Sexual Intercourse: A Fatal Aortic Dissection and Sildenafil Abuse

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The goal of this presentation is to introduce a case of sudden death of a 66-year-old man during sexual intercourse. A complete postmortem examination and toxicological analysis revealed the cause of death.

As there is a rarity of fatal cases in sildenafil users, this presentation will impact the forensic science community by informing attendees of the pathological effects of sildenafil and will emphasize the necessity for an echocardiographic examination before prescribing sildenafil.

Aortic dissection is one of the most dramatic cardiovascular diseases since the first detailed description suggested by Morgagni in 1761. It is defined as blood present between the layers of the aortic wall (false lumen), outside the true lumen, as a result of the decomposition of the media. The separation in the media is in 95% of cases caused by the blood flowing through a tear in the intima (intimal tear or flap), while in 5% of the cases, the cause is bleeding within the media (intramural hematoma). The most frequent etiologic factors reported are chronic hypertension, hereditary connective tissue diseases, and congenital aortic valve diseases (bicuspid and unicuspid aorta). Few fatal cases of acute aortic dissection during sexual intercourse in sildenafil abuse have been reported in literature; it has been supposed that sildenafil use, independent of changes in the aorta pressure, could trigger an aortic dissection. *In vitro* studies reveal that sildenafil has vasorelaxant properties in rat aorta; thus, with a decreasing aortic stiffness, sildenafil can trigger intimal tearing. Other studies performed on pulmonary arteries stated that without changing pulmonary artery or systemic blood pressure, sildenafil increases the pulmonary flow and proliferates pulmonary smooth muscle cells. Based on all of these, sildenafil use can make the aorta wall more sensitive and can trigger the dissection.

Case Report: This study presents the case of a 66-year-old man with no history of cardiac problems who suddenly collapsed during sexual intercourse. His partner stated he used to take sildenafil a few hours before sex. During sexual intercourse, he suddenly complained of the onset of chest pain and fatigue before collapsing. He was not using any other medications regularly. A complete postmortem examination was performed the day after death and type A (DeBakey type I) aortic dissection was detected and recorded as the cause of death. Toxicological examination was performed and confirmed the use of sildenafil. Because dissection occurred in the critical time interval between the peak plasma concentration time and half-life of sildenafil and because the patient had no history of any triggering factor (mechanical stress, trauma, etc.), sildenafil is thought to have triggered the dissection.

Sudden Death, Sildenafil, Aortic Dissection

H99 Aortic Dissection in Cocaine Abuse: A Fatal Case

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The goal of this presentation is to introduce a fatal case of aortic dissection after cocaine abuse. A complete postmortem examination and toxicological analysis revealed the cause of death.

This presentation will impact the forensic community by examining the rarity of fatal cases of aortic dissection in cocaine abusers and by emphasizing the importance for complete postmortem examinations in these cases.

Aortic dissection is generally thought to require a pathogenic co-factor, usually severe arterial hypertension or a connective tissue disorder and a predisposition to weakness in the aortic media and sudden changes in hemodynamic shear stress. The association between cocaine abuse and aortic dissection is well documented in the literature, but few fatal cases are reported. Presumably, the mechanisms relate first to an underlying process that has weakened the elastic media of the aorta and, second, to the severe sheer forces that result from the sudden and profound hypertension and tachycardia that accompany cocaine (particularly crack) use. Cocaine, by inhibiting the reuptake of both epinephrine and norepinephrine at the neural synapses, leads to profound sympathetic stimulation that presumably causes such sheer stress on the aorta's intima that a small "nick" or tear occurs. This physiology is particularly acute with the use of crack cocaine, after which the onset of systemic effects is almost immediate. With the use of cocaine, such tears may occur most often at the ligamentum arteriosum because this region of the aorta is relatively fixed anatomically and is less able to withstand the accelerating aortic pressure wave that speeds down the aorta after ventricular contraction. Once such an intimal tear has occurred, the weakened aortic wall allows entry of luminal blood, followed by propagation of the dissecting hematoma down (and/or up) the aorta. A second possible mechanism is that chronic cocaine use itself may lead to premature atherosclerosis. It has been postulated that recurrent cocaine exposure makes the endothelium more permeable to atherogenic low-density lipoprotein and may accelerate the migration of leukocytes to the aortic wall. Thus, predisposition to aortic dissection could include not only the impact of hypertension, but chronic cocaine's effects on the aorta as well.

Case Report: This study presents the case of a 50-year-old man found lifeless in his car that was parked in front of a disco. Medical history was unremarkable for acute cardiac problems, but he was known as a cocaine abuser. A complete postmortem examination was performed the day after death; type A (DeBakey type II) aortic dissection was detected and cardiac tamponade recorded as the cause of death. Samples of organs were collected for a complete histopathological study. Toxicological examination was performed and confirmed the suspicion of cocaine abuse.

Aortic Dissection, Cocaine, Death

H100 Bilateral Adrenal Hemorrhage Following Arthroplasty: A Case Study

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After attending this presentation, attendees will understand the significant signs, symptoms, and sequelae of bilateral adrenal hemorrhage (BAH) and its correlation with arthroplasty.

This presentation will impact the forensic science community by providing information on the importance of recognizing that BAH can be a fatal consequence to total hip or knee arthroplasty.

Acute BAH, although rare, is a potentially life-threatening condition presenting with non-specific symptoms, such as minor to severe abdominal pain, nausea, vomiting, fever, tachycardia, hypotension, hyponatremia, and hypokalemia. BAH is a common occurrence in post-operative periods, septicemia, and trauma. Total hip and knee arthroplasties are common surgical procedures that are accompanied with anticoagulants to prevent the occurrence of deep venous thrombosis and pulmonary embolism. A rare correlation has potentially been identified between the use of rivaroxaban and other common anticoagulants as possibly causing thrombocytopenia, which may lead to BAH.

The goal of the present work was to present a case of BAH brought on by anticoagulation therapy following total knee arthroplasty and to make physicians aware of this condition in order to prevent unnecessary deaths. Additionally, the information presented could prove vital to forensic pathologists in determining cause of death in these circumstances.

A fatal case of BAH resulting from knee arthroplasty in a 64-year-old male is reported. Arthroplasty was successfully completed with no early post-operative complications. The patient was discharged home on Postoperative Day (POD) one and placed on rivaroxaban to prevent thromboembolic events. On POD seven, he presented in the emergency room complaining of severe abdominal pain, coughing, burping with bright red blood, and labored breathing. He was admitted on POD eight; biochemical exams indicated normal sodium levels (139mmol/L), normal potassium levels (3.7mmol/L), elevated glucose levels (173Hmg/dL), high creatinine (1.25Hmg/dL), normal white blood cell count (7.7 x 10’3/microL), and normal platelets (246 x 10’3/microL). On POD ten, while the decedent was being discharged, he died. Autopsy would ultimately find the cause of death, which was bilateral adrenal hemorrhage due to right knee arthroplasty.

BAH is a rare disease that can follow major joint surgeries and should be suspected in patients presenting with severe abdominal pain, nausea, and coughing who do not respond to standard medical treatment. Computed Tomography (CT) scans and hormone assays should be acquired at earliest suspicion of BAH. Physicians should be aware of admitted patients presenting with the signs and symptoms of BAH, especially following arthroplasty.

Bilateral Adrenal Hemorrhage, Arthroplasty, Anticoagulation

H101 Trends of Cannabis- and Alcohol-Related Single-Vehicle Accident Fatalities at the Jackson County Medical Examiner's Office From 2012 to 2016

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The goal of this presentation is to examine the demographics as well as the toxicology in cannabis- and alcohol-related single-vehicle accident fatalities at the Jackson County Medical Examiner's Office.

This presentation will impact the forensic science community by demonstrating the increasing prevalence of cannabis-related single-vehicle accident fatalities and the increased blood tetrahydrocannabinol levels of the subjects involved at the Jackson County Medical Examiner's Office.

Cannabis use has increased in popularity in recent years; various studies reveal cannabis to be the number one illegal drug used among those reported driving under the influence in several different countries.[1-4] Some of these same studies as well as others demonstrate there is a dose-related association between the increased blood Tetrahydrocannabinol (THC) concentration and the heightened risk of a traffic accident and death.[4,5] Many studies have also shown that when cannabis and alcohol are used in combination, there is a higher incidence of traffic accidents compared to when each drug is used alone.[3,5]

The goal of this study was to examine all of the cannabis- and alcohol-related single-vehicle accident fatalities at the Jackson County Medical Examiner's Office in Kansas City, MO, in the past five years and to discuss the trends of alcohol-only related fatalities versus cannabis-only related fatalities versus combined cannabis- and alcohol-related fatalities. This study also examined the postmortem blood THC concentration trends of the drivers. In addition to toxicology, the race, gender, and age of the subjects were studied. Finally, the time of day the accident occurred was also recorded. All traffic fatalities processed at the Jackson County Medical Examiner's Office from January 1, 2012, to December 31, 2016, were reviewed. Only the drivers of single-vehicle accident fatalities were selected for this study. The postmortem toxicology of the drivers was examined with an emphasis on reviewing alcohol and cannabis levels specifically. During the years studied, the number of traffic fatalities ranged from 93 to 135 per year, with an average of 115. Of those fatalities, 29 to 55 (average 44) individuals were drivers in single-vehicle crashes.

The results of this review revealed that there was an increase in the number of cannabis-only and cannabis-plus-alcohol-related single-vehicle accident fatalities by 57% and 125%, respectively. A 204% increase in the average blood THC concentrations was noted over the five-year period, with the average THC blood concentration being 254.31ng/ml in 2016 compared to 83.6ng/mL in 2012. The demographics of the drivers did not change over the five-year period, with males being more prevalent over females and Caucasians more prevalent than African Americans. Generally, the majority of the drivers were under the age of 40 years. No particular trend was identified in the time of day the single-vehicle fatalities occurred.

Public health concern has grown recently due to the increased numbers of individuals driving under the influence of cannabis as well as the level of intoxication of these drivers. This study provides supporting evidence that there is increasing incidence of cannabis-related traffic fatalities as well as upward-trending blood THC concentrations; this may coincide with increased social acceptance and popularity of cannabis usage as a recreational and possibly medicinal drug.

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Cannabis, Motor Vehicle, Blood Tetrahydrocannabinol

H102 Cranial Abnormalities Seen at Autopsy

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After attending this presentation, attendees will be more familiar with incidental cranial abnormalities that may be present at autopsy. This presentation will explore the etiology, appearance, and what, if any, significance the abnormalities may represent.

This presentation will impact the forensic science community by providing a reference series of select cranial abnormalities that may be encountered at autopsy, with a discussion regarding their significance.

Autopsies are an integral part of the medicolegal paradigm. During an autopsy, a pathologist is typically focused on identifying abnormalities related to the death of the individual. Examination of the brain is an important component of a complete medicolegal autopsy, requiring reflection of the scalp with removal of the upper cranial vault. This is typically followed by dura mater removal. Following brain and dura mater removal, a careful examination of the skull is part of the standard autopsy practice. This includes examination of not only the external aspect of the skull, but also the interior surface adjacent to the brain. A variety of cranial findings may be evident during complete internal and external skull examination at autopsy. Some of these may have significant relation to the cause of death, for example, gunshot wounds and other similar traumatic events; however, many others may be considered incidental findings and likely play no direct role in the death of the patient.

The aforementioned cranial abnormalities can be broadly classified into several categories. One classification scheme includes the following categories: acute and remote trauma, defects related to medical intervention, disease-related entities, genetic anomalies, and developmental or other natural processes. Each category will be discussed, providing specific examples of each, including etiology, appearance, and significance. Examples include: acute and remoted trauma, including cranial defects and healing fractures related to past trauma; defects related to medical intervention, including bone discoloration, healing surgical interventions, and birth-related injuries; disease processes, including Paget's disease and malignancies; and developmental or other natural processes, including arachnoid granulations, prominent parietal foramina, hyperostosis frontalis interna, and intra-sutural bones.

Cranial abnormalities may suggest recent (or remote) trauma, medical therapy and/or intervention, disease processes, or various developmental, genetic, and other natural processes. These abnormalities may represent incidental findings, and it is important for the forensic practitioner to understand the etiology and significance of the findings to determine whether they may be related, either directly or indirectly, to the cause of death. Forensic pathologists should be cognizant of the wide variety of cranial abnormalities they may encounter in order to appreciate their etiology and understand their significance, or lack thereof, in the individual's death.

Cranial Abnormalities, Autopsy, Postmortem Examination

H103 Tied to His Own Apron Strings: A Case of Accidental Strangulation by Power Tool

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The goal of this presentation is to illustrate a rare case of accidental strangulation by a bib apron while using a power tool.

This presentation will impact the forensic science community and the general population by illustrating the unexpected dangers of using power tools while wearing presumed "protective" clothing/attire.

Introduction: Deaths associated with power tool use are rare. Most involve accidental electrocution or, less commonly, penetrating/cutting injuries. Accidental strangulation due to power tools has not been previously reported in the literature. Aprons are considered a type of protective layer, but as with any loose article of clothing, can prove to be a hazard.

Materials and Methods: This case involves a 73-year-old Caucasian man with an interest in restoring and repairing African fertility statues. He was wearing a protective cloth bib apron while using an electric power grinder with a rotating buffer attachment to polish a piece of art. The apron became entangled around the rotating buffer head. Twisting of the apron was transmitted to the neck strap, which then wound quickly and tightly around the decedent's neck, leading to strangulation. The decedent fell backward. During the event, the buffer became unplugged. The victim was found by his wife when she returned home later in the day. Emergency personnel responded to the scene; however, death was obvious and no resuscitation efforts were attempted.

Results: External examination revealed a fully dressed Caucasian male wearing shorts, a T-shirt, clear vinyl gloves, and a blue canvas bib apron. The grinder/buffer was found to be on the "max" setting with the bulk of the apron twisted around the buffer head and approximately 15 turns of the neck strap of the apron wound tightly around the neck. The 1.5-inch-wide apron strap was removed to reveal a circumferential parchment-like ligature abrasion above the level of the thyroid cartilage. Dissection of the strap muscles of the neck was unremarkable and the hyoid bone was intact. Florid petechial hemorrhages were present in the conjunctivae and palpebral fissures. Scleral hemorrhages were also present bilaterally. The upper lip had a midline laceration and contusion.

Conclusion: While personal protective equipment is highly recommended when working with power tools, any loose clothing may prove hazardous. Aprons, neckties, and necklaces may become caught in mechanisms. Power tool users should be especially cautious of tools with rotating heads. Quick disconnects, avoiding distractions, and supervised use are recommended to minimize bodily injury.

Accidental Strangulation, Apron, Power Tools

H104 Troubling Trocars: The Time-Consuming Recovery and Wound Documentation of Fragmenting Bullets

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The goal of this presentation is to illustrate the time-consuming process of wound documentation and recovery of high-performance fragmentation bullets.

This presentation will impact the forensic science community and the general population by illustrating the destructive nature of the copper fragmenting bullets, which have recently entered the public marketplace for self defense. This presentation will also address the complications pathologists face while determining the pathway of the bullet due to its separation into multiple projectiles and the additional time required for projectile recovery.

Introduction: Copper fragmenting bullets entered the public marketplace in 2014, marketed toward self defense. The Liberty Ammunition Civil Defense bullet and G2 Research's Radically Invasive Projectile (R.I.P.®) are designed to inflict maximum trauma by expelling metal trocars that track away from the base of the bullet. These bullets are advertised as able to "defeat all barriers, such as sheet metal, sheet rock, windshields, plywood, and heavy winter clothing." The separation of the trocars cause damage to multiple organs and prove to be problematic when trying to describe the path of the bullet, as they create up to nine separate wound channels. When multiple gunshot wounds are present, the trocars may not be reliably reassimilated with their corresponding base, causing further documentation complications. The process of documenting the wound path and recovering all fragments is tedious and time-consuming, greatly reducing efficiency in the autopsy suite.

Materials and Methods: Two cases involving copper-fragmenting bullets were evaluated. The first involves a 25-year-old African American male who was shot while drinking inside a bar. The decedent was shot in the left chest and forearm. He ran after the assailant before collapsing on a sidewalk outside the establishment. Emergency personnel arrived on scene and found the decedent unresponsive. He expired at a nearby hospital.

The second case involves a 16-year-old White male who was shot while sitting in the back of a friend's car during a marijuana drug deal. He was shot multiple times in the head and neck and was declared dead on arrival of emergency personnel.

Results: Autopsy of the first homicide victim revealed penetrating gunshot wounds to the chest and a perforating gunshot wound to the left forearm. One bullet lacerated the upper left lung, the pericardium, the left atrium, and the root of the aorta. A second bullet lacerated the diaphragm, spleen, liver, left lower lung lobe, pericardium, right ventricle and atrium, and right upper lung lobe. Trocars lacerated the stomach, small intestines, colon, mesentery, the left lung, and the left chest wall. Each were located and retrieved with great effort.

Examination of the second victim revealed multiple perforating gunshot wounds to the head and neck. One bullet lacerated the right neck and fractured the C3 vertebrae, while two others fractured the maxilla. A fourth bullet entered the neck, lacerating the left jugular vein. The fifth bullet lacerated the right parietal lobe. Trocar fragments were recovered from the oral cavity and could not be ascribed to a specific shot.

Conclusion: R.I.P.® and Liberty Civil Defense fragmentation bullets, while marketed for self defense, cause extensive organ damage and significantly increase autopsy time for the forensic pathologist. The extra trocars from the projectile ravage the tissue and can be difficult to locate. Reimaging the body and organs may be necessary for retrieval. The bullets also pose serious complications for the forensic pathologist when describing the wound path. The trocars of these bullets splay outward, causing additional wound paths that may intersect with each other or intersect with the paths from additional bullets. Associating the trocars with their respective bullet base can also be challenging when multiple shots are fired. In addition to the damage done to the body, these bullets do damage to the efficiency of the autopsy suite.

R.I.P.®, Trocars, Fragmentation Rounds

H105 Mixed-Mode Assessment of Reference Lung Weights in a Medicolegal Autopsy Setting — A Bayesian Approach

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After attending this presentation, attendees will understand the relationship between postmortem lung weight and cause of death in a medicolegal autopsy setting.

This presentation will impact the forensic science community by providing new reference lung weights and the association between these reference weights and the cause of death.

Organs are routinely weighed at autopsy and, as such, present immediately accessible objective information that may be of importance for determining disease states and the cause of death.

Lungs are of particular interest as "heavy" lungs have been suggested as an autopsy finding associated with causes of death frequently encountered in a medicolegal autopsy setting, such as drowning and intoxication.[1-9] Problematic is the fact that "heavy lungs" have eluded a definition due to the high variability in lung weight.

Different reference weights have been suggested.[10-16] There have also been attempts at creating linear regression models using individual characteristics to estimate postmortem lung weight.[16] With the exception of one study, neither height, weight, age, nor Body Mass Index (BMI) have yielded R2 values of practical importance.[10] Previous studies were also generally limited by using selected and small populations.

A possible solution to this problem would be to model the lung weight as dependent on the cause of death. It is believed by some that this would be better suited for practical use, as it would appear that lung weight "as is" is far too variable to be of any use. Toward this end, this study attempted to create a varying intercepts regression model using groups based on underlying cause of death as intercepts and individual parameters as case-level predictors.

In Sweden, all medicolegal autopsies are performed at one of six departments of the Swedish National Board of Forensic Medicine. Organ weights and individual characteristics as well as the underlying cause of death are included in the medicolegal autopsy registry.

Using Stan®, interfaced through RStan, this study created multiple mixed-mode Bayesian general linear models using groups based on cause of death as varying intercepts and individual characteristics as case-level predictors.[17] Models were then compared for over- and underfitting, using the Widely Applicable Information Criterion (WAIC), after which a final "meta-model" based on individual model predictions weighted by their relative WAIC weight was created.[18,19]

Data from 2007 through 2013 was analyzed, including decedents 18 years or older but excluding cases with a postmortem interval longer than five days as well as cases with lacking, incorrectly registered, or extreme values. As the International Statistical Classification of Diseases and Related Health Problems (commonly known as the ICD) is ill-suited for a medicolegal autopsy population, groups were created based on the most common case types in our population. As this dataset is also very large, highly granular groupings could be created, allowing for instant subgrouping of different intoxicants in fatal intoxication cases. Results are presented using Highest Probability Density Intervals (HPDI).

This study found that group mean values exhibited a clear difference where expected, for instance between intoxication (mean 1303g (1,053–1,545g 95% HPDI)) and asphyxia (mean 1,029g (788–1,272g 95% HPDI)]; however, when accounting for individual case error rate, predictions were quite wide with significant overlap between case groups (e.g., intoxication (mean 1,303g (684–1,921 95% HPDI)) almost entirely overlaps asphyxia (mean 1,030g (411–1,647g 95% HDPI)).

It is believed this model still yields better, more realistic estimates for what constitutes normal lung weight in the estimated case groups, as these values represent probability distributions and values are less likely closer to the HPDI boundaries.

This reverse approach of assessing lung weight as a function of the cause of death also facilitates differential diagnostics as it provides the forensic pathologist with a better grasp of the context of a given lung weight in relation to one or more possible causes of death. In conclusion, this study submits these estimates are more realistic in forensic practice than previously published raw population means.

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Forensic Pathology, Lung Weight, Bayesian Analysis

H106 Predicting Fatal Intoxications in a Medicolegal Autopsy Population Using the Weight of the Lungs

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After attending this presentation, attendees will understand the issues with defining "heavy lungs" as well as the sensitivity of heavy lungs to identify fatal intoxication cases in a medicolegal autopsy setting.

This presentation will impact the forensic science community by providing clear information on the predictive power of heavy lungs as well as new, easily applicable methods to reduce pre-toxicology uncertainty.

Fatal intoxications are common in a medicolegal autopsy setting, constituting approximately 10% of medicolegal autopsies in Sweden (internal data); however, these cases are associated with sparse findings during autopsy.

Increased lung weight at autopsy has been suggested as a finding suggestive of such deaths.[1-7] Previous literature is generally limited by a descriptive approach, including only opioid deaths and lacking a definition of "heavy lungs". Different reference lung weights have been suggested.[8-14] There have also been attempts to create linear regression models using individual characteristics to estimate postmortem lung weight.[14] With the exception of one study, neither height, weight, age, nor BMI have yielded R2 values of practical importance.[8]

The goal was to: (1) create a model to identify cases with heavy lungs; and, (2) assess the predictive power of heavy lungs in identifying cases of fatal intoxications during autopsy.

This study identified all medicolegal autopsy cases in Sweden from 2000 through 2013 of persons older than 18 years, and the estimated lung weight was calculated in each case using a previously published linear regression model.

Additionally, the mean ratio between lung and heart weight was calculated overall as well as in groups stratified for age and sex. Raw lung weight and regression estimates as well as the ratios were used to identify cases with heavy lungs and evaluated the associations with fatal intoxications.

On average, cases of fatal intoxications had heavier lungs compared to other cases. This difference of 158g (145g–172g 95% Confidence Interval (CI)) was significant ($p<0.001$).

In predicting fatal intoxications, it was observed that raw lung weight had low performance and models based on the lung/heart weight ratio had better predictive power than models based on lung weight point estimates. The ratio may serve as an indication of a fatal intoxication with a sensitivity of 70% to 80% and specificity of approximately 60%.

These findings suggest that using only raw lung weight to estimate the probability of fatal intoxication is of little value in medicolegal autopsy cases. The ratio of lung-to-heart weight may, lacking better methods, be useful as an estimate of the degree to which the forensic pathologist should suspect fatal intoxication.

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Fatal Intoxication, Epidemiology, Lung Weight

H107 How Should Live Entomological Samples Be Stored?

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After attending this presentation, attendees will better understand the current state of the art in collecting and storing entomological samples from a body during autopsy or at the death scene.

This presentation will impact the forensic science community by providing guidelines for sampling and storing living samples of the forensically important blow flies, *Lucilia sericata* and *Calliphora vicina*.

Sampling and storing insect evidence alive is an important task in forensic entomology as different methods can influence survival and growth rates of the living samples. The best practices recommend that living fly larvae should kept under controlled and known conditions (2°C-6°C, preferably) and stored in vials with an air-permeable lid equipped with sawdust or a paper for absorbing excretion liquids. Samples should then be transferred to an expert within 24h. Although this time interval seems to be a realistic approach, cooling the samples appropriately or catering them during storage seems to be a serious logistical problem. Unfortunately, many of the above recommendations are mainly based on expert opinions rather than scientific evidence.

In order to look at the effect of the lack of cooling and/or inappropriate storing of entomological samples before their arrival in the laboratory, the following experiment has been performed. Samples of all larval stages (*L1*=24 hours after hatching at 25°C, *L2*=48h after hatching, and *L3*=72h after hatching) of the forensically relevant blow fly species *Lucilia sericata* and *Calliphora vicina* were divided in two main groups with 33 larvae for each group. The first group was stored at room temperature (~20°C) and the second group in a refrigerator (5°C) for 16h without air, a food supply, or sawdust. Next, they were kept at 2°C-6°C in a Styrofoam™ box for 8h, simulating a transport situation. After 16+8=24h, the storage Mortality Rate (MR) was calculated and 25% of the surviving larvae were killed in hot but not boiling water. Their length was measured and the remaining living specimens were reared (25 C) on their food substrate until adult eclosion. The results were then compared with a control group in which larvae were not sampled but left to feed in the rearing boxes with air-permeable lids on their food substrate at 25°C. All containers were checked for MR every 24h for the effect of 24h of hypoxia on adults; later, hatched flies were frozen and separated according to sex. The length of the left mesothoracic tibia and dc-um vein of the left wing were measured using a dissecting microscope and computer software.

Results revealed a high MR for L3 larvae stored both at Room Temperature (RT) and in a cool environment (100% for both species at RT; for chilled larvae 100% of *L. sericata* and 54.03% of *C. vicina* with an MR in the control 2.53% for *L. sericata* and 8,08% for *C. vicina*). For *L. sericata* L1, the MR was respectively 16.16% at RT, 4.55% for chilled samples, and 2.25% in the control group. For *L. sericata* L2, the MR was 24.73% at RT, 10.61% for chilled samples, and 7.24% in the control group. For *C. vicina* L1, the MR was 35.86% at RT, 10.6% for chilled samples, and 2.94% in the control group. For *C. vicina* L2, the MR was 12.65% at RT, 10.61% for chilled samples, and 9.05% in the control group. Results highlight that the 24h interval time of storage can stop the larval growth in comparison with the control group. The lack of growth was extremely significant in *L. sericata* samples: 0.39cm for L1 at RT and 0.38cm in chilled samples; 0.72cm and 0.71cm for L2 at RT and in chilled samples; 0.17cm and 0.14cm for L3 at RT and in chilled samples. The lack of growth was in *C. vicina* samples of 0.47cm for L1 at RT and 0.45cm in chilled samples, 0.68 cm for L2 at RT, and in chilled samples, 0.25 cm and 0.24 for L3 at RT and in chilled larvae.

The duration of storage needs to be considered when performing the age calculation of larvae to estimate the minimum Postmortem Interval (PMI_{min}). The living larval samples should be stored at least at cool temperatures (e.g., in a refrigerator) instead of room temperature. This is strongly recommended for larvae with a size <1cm (L1-L2 stages) based on the high MR for L3 samples. For larvae >1cm, such as L3 samples, the recommendation is to add paper to the storage vials with air-permeable lids for absorbing the liquids (excrements, enzymes) and to reduce the interval of storage and transport considerably, keeping the temperatures low at a refrigerator level.

Forensic Entomology, Sampling Methods, Storing Methods

H108 Death by Hanging: The High Prevalence of Intervertebral Disc Vacuum Phenomenon in Thoracic and Lumbar Spine in Postmortem Computed Tomography (PMCT)

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The goal of this presentation is to suggest that death by hanging, under various mechanisms, may be associated with the origin of gas collection in the intervertebral discs that is revealed in postmortem imaging, especially by cadavers of a young age.

This presentation will impact the forensic science community by indicating that intervertebral vacuum phenomenon may be an additional finding in hanging cases that have undergone postmortem imaging.

Introduction: Postmortem imaging, in addition to PACT, has become the most frequently applied method in routine investigations.[1] CT provides high sensitivity in gas detection and the assessment of the condition of intervertebral discs.[2,3] Vacuum Phenomenon (VP) describes the gas collection in the intervertebral joint spaces and is associated with various pathologies, including Degenerative (DG) skeletal changes and trauma. Increased volume space of a joint through traction causes intra-articular gas collection.[4] In hanging, gravity pulling the body weight downward will affect the spine.[5] It was hypothesized that the expansion of the spine during hanging may cause gas accumulations in the discs. Also investigated was if Simon's bleedings could be predicted from the presence of VP in PMCT due to a similar origin (i.e., traction of the body and the spine).[6]

Materials and Methods: A group of 36 hanged bodies with PMCT was studied retrospectively. For each hanged case, a control case with another manner of death was chosen of the same sex and age (maximum of differences +/-2 years) and a matching degree of DG conditions. Each group was split into two equal age groups (n=18), one group of ≤43 years of age and one group >43 years of age. Cases with signs of putrefaction, those having undergone resuscitation, and those with trauma were excluded. Gas accumulations of the intervertebral discs and DG skeletal changes were evaluated in PMCT. Then, autopsy reports of the hanging cases were reviewed for the assessment of the presence of Simon's bleedings.

Results: The preliminary results revealed a significant association between hanging and VP in PMCT in the group ≤43 years old in the sample of the hanged group. The proportion of the hanged cases ≤43 years old that exhibited VP in PMCT is statistically different from the proportion of the control cases with other manners of death and VP in PMCT in the same age group. In the sample of the >43 age group, there was no significant association between manner of death and VP. There was no significant association between Simon's bleedings and VP. Simon's bleedings were significantly associated with complete rather than incomplete hanging.

Discussion: In people who died by hanging in the age group under 43 years, VP is to be expected more frequently than in people in the same age group with a different manner of death. This association does not occur in the age group below 43 years. The small number of cases is a limitation. DG skeletal changes and low bone mineral density are already associated with VP.[4,7] Intervertebral VP is also associated with increasing age and the elderly.[7] By hanging, the traction of the intervertebral joint spaces, especially in complete hangings, can play an additional role, among others, for the pathogenesis of vertebral VP. Simon's bleedings cannot be predicted in PMCT, as they are not associated with increasing age and DG skeletal changes because of decreased spinal mobilization.[6]

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5. Mahmoud Rayes et al. Hangman's fracture: A historical and biomechanical perspective. *J Neurosurg Spine.* 14 (2011)**:** 198–208.
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Virtopsy, Hanging, Vacuum Phenomenon

H109 An Autopsy Case of Pulmonary Embolism and Underlying Multiple Myeloma

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After attending this presentation, attendees will better understand how previously undiagnosed plasma cell neoplasm can contribute to fatal pulmonary embolism.

This presentation will impact the forensic science community by providing results from a case report in an area with very little previous research. This presentation will also add to research being conducted in forensic pathology by broadening the understanding of how undiagnosed plasma cell neoplasm can contribute to sudden death, enabling a better appreciation of these processes in death investigations.

Multiple Myeloma (MM) is characterized by the neoplastic proliferation of plasma cells producing a monoclonal immunoglobulin.[1] The diagnosis of MM requires clonal bone marrow plasma cells ≥10% or biopsy-proven bony or soft tissue plasmacytoma plus either the presence of related organ or tissue impairment or the presence of a biomarker associated with near-inevitable progression to end-organ damage.[2] Pulmonary embolus refers to obstruction of the pulmonary artery or one of its branches by thrombus.

A 62-year-old Black male with a past medical history of back pain, obesity, hypertension, and diabetes had a witnessed "falling out" in which he fell backward and became unresponsive. After Emergency Medical Services (EMS) arrival, he was found in pulseless electrical activity. The patient was pronounced dead soon after his arrival at the hospital. The most remarkable gross autopsy findings were bilateral thromboemboli in main, segmental, and subsegmental pulmonary arteries. Microscopically, the histology of vertebral bone showed hypercellular (90%) for the age bone marrow with the majority of the cells being atypical plasma cells. Immunohistochemical stains revealed cells of interest being positive for CD138, light chain lambda, and immunoglobulin G. Protein electrophoresis of postmortem blood showed the presence of M-spike and 1g/dl paraprotein and serum immunofixation exhibited IgG Lambda paraprotein in the gamma region.

In conclusion, this report provides evidence that even when the cause for sudden death is obvious grossly, some routine histology may provide valuable information about the underlying disease. In this case, myeloma significantly contributed to the fatal embolic event by the production of a hypercoagulable state. It was observed that in patients with newly diagnosed and untreated myeloma, increases in Von Willebrand factor and factor VIII and a decrease in protein S levels result in a hypercoagulable state which may promote the development of thrombo-embolic complications.[3]

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Multiple Myeloma, Pulmonary Embolism, Sudden Death

H110 An Analysis of Skeletal Trauma in Suspected Child Abuse Fatalities: A Procedure Involving Radiology, Pathology, Histology, and Anthropology

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After attending this presentation, attendees will have a better understanding of the procedures for the analysis of skeletal fractures associated with child abuse cases.

This presentation will impact the forensic science community by describing a multidisciplinary approach to the interpretation of skeletal trauma in child abuse cases.

Thorough documentation and analysis of fractures is critical for the investigation of suspected child abuse fatalities. This presentation details a multidisciplinary procedure involving radiology, pathology, histology, and anthropology for the documentation, analysis, and interpretation of skeletal fractures.

The forensic pathologist has the ultimate responsibility for evaluating all the different pieces of evidence when making his/her eventual determination of cause and manner of death. In addition to the findings at autopsy, the forensic pathologist will also benefit from findings provided through radiology, histology, and anthropology. These modalities can play critical roles in the final certification of pediatric deaths and will also be useful in criminal proceedings if the case is deemed a homicide.

Radiography is a critical first step in the assessment of skeletal fractures. Antemortem radiographic studies may be available from a hospital and will be of obvious importance to the forensic pathologist. A postmortem series should also be completed prior to autopsy. At a minimum, standard two-dimensional X-ray images should be completed, but high-resolution Computed Tomography (CT) scans can also be extremely useful. Any skeletal specimens removed at autopsy should be re-radiographed as visualization of fractures and associated healing (if present) will be improved. In some instances, it may be helpful to have a radiologist interpret the images, especially regarding fractures in various stages of healing.

During autopsy, the forensic pathologist will document and photograph fractures, sites of hemorrhage, and vital reaction. Areas of fracture (or suspected fracture) are removed during autopsy for additional studies, including histology and anthropology. As noted above, it is advantageous to also take radiographs of the harvested specimens prior to initiating any additional examinations.

In order to allow both histological and anthropological analyses to be completed, a sampling procedure has been developed in which "windows" are cut through the fracture locations. It has been found that a hand-held rotary tool with a diamond blade allows for precise sections to be removed through fracture locations. Cutting a window through the fracture allows for the small section to be decalcified and slide mounted for histological evaluation while the larger section can be submitted for maceration and gross anthropological analysis. Through these studies, it is possible to document whether the fractures are acute, subacute, or show remote stages of healing.

The procedure outlined above is similar to recommendations made by Andrew Baker in the 2013 version of his excellent handout titled *Gross and Microscopic Evaluation of Pediatric Fractures at Autopsy* with only slight modifications.[1] One main difference is the role of anthropology in the process. Maceration of specimens (only to be completed after histology sampling) can provide an additional line of documentation and interpretation of fractures that can be useful to the forensic pathologist and which can be very illustrative in court proceedings. In some instances, additional subtle fractures have been observed after maceration that would have been missed on X-ray and during autopsy. The sampling procedure presented in this report allows both histological and anthropological analyses to be completed.

These recommended procedures for the documentation, analysis, and interpretation of skeletal fractures in suspected cases of child abuse have been applied on numerous cases at the New York City Office of Chief Medical Examiner. This presentation will describe the recommended multidisciplinary procedures in detail. In addition, several case examples will be presented, demonstrating how this approach was applied and how the findings proved to be critical in these challenging cases.

Reference(s):

1. Andrew M. Baker et al. Bones and Children: An Interdisciplinary Approach to Forensic Issues. *Proceedings of the American Academy of Forensic Sciences*, 65th Annual Scientific Meeting, Washington, DC. 2013:18-19.

Child Abuse, Pediatric Fractures, Battered Baby Syndrome

H111 Reducing Misdiagnosis in Child Abuse

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After attending this presentation, attendees will better understand the actions and goals of a Diagnostic Management Team (DMT) and its use in investigating suspected child abuse cases.

This presentation will impact the forensic science community by providing a description of the work of an expert group of coagulation specialists, medical examiners, and pathology residents reviewing suspected child abuse cases to reduce the possibility of misdiagnoses.

A DMT is a group of medical experts, specializing in specific areas, that meet regularly to review cases in real-time to advise on the selection of appropriate diagnostic tests and to interpret complex laboratory test results.

This expert team approach has been successfully implemented in several academic medical centers to provide clinical consultation in coagulation, transfusion medicine, hematopathology, microbiology, and several other areas. At the University of Texas Medical Branch in Galveston, TX, a DMT has been established to specifically evaluate cases of potential child abuse and determine if there is an underlying coagulation or vascular disorder to account for bleeding and bruising.

A 2015 report from the National Academy of Medicine revealed that every adult and many children in the United States have experienced at least one diagnostic error, often with very serious consequences.[1] The report indicates that there are many contributing factors to this problem, some of which are related to cognitive bias toward a specific diagnosis. With published information that 19 out of 20 potential cases of abuse are correctly diagnosed and one is a misdiagnosis, the misdiagnosis frequently occurs because of a significant bias toward a diagnosis of child abuse. There are few true experts in the United States dealing with coagulation disorders, and with 1 out of 20 cases misdiagnosed, approximately 30 percent of which involve a missed coagulation disorder, hundreds of cases are incorrectly diagnosed and treated in the United States annually.[2] Biases involving anchoring, premature closure, context errors, availability bias, and affective bias all influence a diagnosis of child abuse.

This misdiagnosis of child abuse often leads to removal of the child from a loving family and often punishes an innocent and loving parent or caregiver. To avoid a misdiagnosis of child abuse in a bruised or bleeding child, living or deceased, a DMT composed of experts in coagulation, in partnership with a group of pathology residents planning a career in forensic pathology, meets monthly to discuss presumed child abuse cases from all over the United States that have been submitted to a coagulation expert at the University of Texas Medical Branch for review and expert opinion. Each case is reviewed thoroughly by one resident, then presented at the DMT meeting. A set of diagnostic questions, which are relevant to the specific findings in the case, is formulated for further analysis, and each question is researched in the literature to provide published evidence for the conclusions offered in the final report. This expert-driven DMT review of cases has resulted in arguments that support diagnoses for and against child abuse.

In this presentation, three cases analyzed by an expert DMT are shown with the following results: (1) diagnosed as abuse; (2) underlying disease present, which make abuse unlikely; and, (3) no definitive answer because of the high diagnostic complexity.

The concept of introducing true experts in coagulation, bone disease, and dermatology, who review all child abuse questions related to bleeding/bruising, bone fractures, and skin changes, respectively, greatly reduces the risk of diagnostic error presumed in child abuse cases.

Reference(s):

1. National Academies of Sciences, Engineering, Medicine. *Improving Diagnosis in Health Care.* Washington, DC: The National Academies Press, 2015.

2. Metz, Schwartz, Feldman, Lindberg, ExSTRA Investigators. Non-cutaneous Conditions Clinicians Might Mistake for Abuse. *Archives of Disease in Childhood.* 99 (2014): 817-823. doi: 10.1136/archdischild-2013-304701.

Diagnostic Management Teams, Child Abuse, Misdiagnosis

H112 Antemortem Versus Postmortem Bone Fractures: The Usefulness of Morphological Observation Using Scanning Electron Microscope

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The goal of this presentation is to observe the morphology of bone fractures using a scanning electron microscope to ascertain a number of specific characteristics that can be used for distinguishing between peri-mortem and postmortem fractures.

This presentation will impact the forensic science community by providing new tools to distinguish vital fractures from postmortem fractures.

One of the most important evaluations in the forensic assessment of a trauma is the timing of the injuries in respect to the time of death, which is more difficult in bone fractures than skin lesions; however, in many cases, it is crucially important to determine if the person was alive when the fracture occurred.

The evidence of bone remodeling is crucial for determining that a skeletal injury was produced antemortem. When the cortical surface of bone is disrupted by a fracture, a well-known series of events normally ensues. Initially, vascular damage causes a hematoma that forms in the area of injury. Within hours, the clot is invaded by inflammatory cells; after several days, fibrous matrix begins to replace the clot. Later, the callus is remodeled and converted to lamellar bone.

The length of time required for the production of new bone or other signs of healing is very variable and depends on the location of the injury, the health status of the individual, genetic variations, and other factors. The evidence of remodeling associated with the fracture indicates that the injury occurred at least a week before death.

For forensic purposes, it is often necessary to establish the vitality of fractures supposedly produced very shortly before death, when the healing process has no time to progress enough to be observable using the common methods (macroscopic evaluation and light microscopy). Therefore, it was hypothesized that the new morphologic markers, using a scanning electron microscope, are able to distinguish vital fractures from postmortem fractures.

The fracture lines and their relation to bone microstructure were studied on 15 fragments of fractures collected from the skulls of individuals who died from head trauma (i.e., traffic accidents, falls from heights, gunshot injuries). For each case, a forensic autopsy was performed. Each sample was compared to another bone fragment collected from the same subject during the autopsy and then experimentally fractured using a hammer. All samples were fixed in glutaraldehyde solution for 48 hours, then dehydrated and dried. These were coated with gold using a metal ion spattering instrument. The samples were examined with a Zeiss EVO® 40 scanning electron microscope.

The results demonstrated that a relevant, different pattern can be observed in vital fractures compared to postmortem fractures. High-power views indicate that the vital fracture is characterized by a pull-out of elastic and collagen fibers, as well as "bridges" formed by fibers, not seen in postmortem fractures, that have flatter surfaces with characteristic "micro-cracks."

The present study, therefore, suggests the usefulness of the scanning electron microscope in the evaluation of bone fractures, especially in regard to the timing of the injury.

Scanning Electron Microscope, Bone Fractures, Vitality Evaluation

H113 Posterior Rib Fractures in Non-Traumatic Pediatric Deaths

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The goal of this presentation is to present a case series of non-traumatic pediatric deaths in which posterior rib fractures were observed. The learning objective is to understand possible mechanisms causing posterior rib fractures in infants.

This presentation will impact the forensic science community by raising awareness of posterior rib fractures observed in non-traumatic pediatric deaths. Posterior rib fractures are considered suspicious for non-accidental injury, but mechanisms other than non-accidental trauma must be considered.

Rib fractures observed in infants and young children are considered suspicious for Non-Accidental Injury (NAI), especially fractures located in the posterior region of the rib. In fact, Barsness and colleagues found that in children under the age of 3 years, the positive predictive value of rib fractures for NAI was 95%.[1] Dolinak reports that in 11% of consecutive infant autopsies (*n*=70), subtle Cardiopulmonary Resuscitation (CPR) - associated rib fractures were found in the anterolateral region of the ribs.[2] In several of the cases, multiple ribs were fractured bilaterally. No fractures were found in the posterior region of the ribs.

Researchers theorize that the mechanism causing CPR-related rib fractures is different from the mechanism causing posterior rib fractures. CPR rib fractures result from the anterior chest being forced posteriorly while the back is supported, causing the rib to fail in the mid-clavicular region. Posterior rib fractures are caused when the thorax is squeezed in an anterior/posterior direction while the back is unsupported forcing the posterior rib against the transverse process of the vertebra. This action creates a Type I lever at the costrotransverse articulation site.

In 2000, the American Heart Association, in collaboration with the International Liaison Committee on Resuscitation, recommended a new "two-thumb" technique to administer CPR to infants. The method involves placing both thumbs on the sternum of the patient, encircling the chest with the hands, placing the finger tips lateral to the spine, and compressing the sternum. This technique is similar to the mechanism commonly proposed for inflicting NAI rib fractures. Clouse and Lantz reported on four cases of premature infant decedents who each received "two-thumb" CPR.[3] Posterior rib fractures were observed in all decedents and NAI was excluded in each case.

Three infant deaths investigated by the District of Columbia (DC) Office of the Chief Medical Examiner were found to have posterior rib fractures and no other signs of trauma. Each decedent was transported from a private home to the hospital by the DC Fire and Emergency Medical Services (FEMS) with CPR in progress. FEMS standard operating procedure is to perform "two-thumb" CPR on infants. In each case, the posterior rib fractures were observed after the periosteum and intercostal muscles were removed from the pleural surface of the ribs. At each fracture site, little to no hemorrhage was observed. The fractures were both complete and incomplete and were positioned at the rib head or angle. In each case, several ribs were serially fractured, with up to six ribs fractured in one case. In all cases, the fractures were unilateral. Anterior rib fractures were also observed in two of the cases.

The autopsy findings in the presented cases suggest CPR-related posterior rib fractures. In each case, the fractures were subtle and difficult to recognize on radiographs as well as during the autopsy. These three cases should raise caution regarding the diagnostic value of posterior rib fractures for NAI.

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1. Barsness K.A., Cha E.S., Bensard D.D., Calkins C.M., Partrick D.A., Karrer F.M., Strain J.D. The positive predictive value of rib fractures as an indicator of nonaccidental trauma in children. *J Trauma*. 2003;54(6):1107-10.

2. Dolinak D. Rib fractures in infants due to cardiopulmonary resuscitation efforts. *Am J Foresnic Med Pathol*. 2007;28(2):107-10.

3. Clouse J.R., Lantz P.E. Posterior rib fractures in infants associated with cardiopulmonary resuscitation. *Proceedings of the American Academy of Forensic Sciences*, 60th Annual Scientific Meeting, Washington, DC. 2008, 254-255.

Posterior Rib Fractures, Pediatric, Non-Accidental Injury

H114 A Frozen Newborn: A Multidisciplinary Approach in a Case of Infanticide

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The goal of this presentation is to introduce a multidisciplinary approach to a complex case of a newborn infant found deceased in a house freezer. In order to assist the judicial authorities and police inquiries in classifying a crime, radiologists, gynecologists, and forensic pathologists need to be involved. In these cases, the role of the medicolegal team was pivotal in clarifying the newborn's cause and time of death, separate existence, and viability. According to Italian law, infanticide is a crime committed by a mother against her own infant child "immediately after or during delivery," who has suffered "conditions of abandonment connected to childbirth."[1]

Little is written in the literature regarding how to handle this type of forensic case; therefore, this presentation will impact the forensic science community by providing a methodical approach to this medicolegal issue.

A 40-year-old woman, *gravida* 5, *para* 5, was admitted to the emergency department with headache, asthenia, and metrorrhagia. On admission, the patient was conscious and alert, but soon after became confused and disoriented, complaining of loss of sensation in both legs. At the evaluation, she was hemodynamically unstable, with a significant drop in her hemoglobin level (Hb 3.7g/dl); an abdominopelvic Computed Tomography (CT) scan with angiography detected an enlarged uterus (25cm) with thick myometrium and the presence of mixed-density fluid and gas within the endometrial cavity. She was immediately taken to the operating room for an instrumental revision of the uterine cavity. The placenta was spontaneously delivered and macroscopic features revealed that it was full-term. According to hospital records, the previous delivery, which occurred at home in the bathroom, was unexpected, resulting from an unknown pregnancy. Nothing else was reported from her recent gynecological and obstetrical history.

Subsequently, the police, assisted by the medicolegal team, searched the woman's house for the missing newborn. The crime scene investigation revealed the home was full of clutter and garbage, with many household pets and mice. After two days of intensive searching, a newborn infant was found in a hidden freezer. The infant was stacked in the freezer with several frozen foods, wrapped in a wool sweater, and enclosed in two plastic bags. The body was naked and in the fetal position, and sex could not be determined in the frozen state as the genitalia were hidden by the legs.

A multidisciplinary forensic approach included a CT scan and genetic and toxicological analyses; an autopsy was then performed. The histological and immunohistochemical examination of specimens from the newborn, the placenta, and the umbilical cord were helpful for the postmortem investigation.

The CT scan demonstrated the anthropometric parameters were appropriate for the gestational age of a full-term infant. Furthermore, it revealed air in subsegmental regions of the lungs, as well as in the stomach. No fractures were detected.

The body was thawed at the time of the autopsy, seven days after being found. The external examination verified a newborn female infant, full-term at the time of death. The umbilical cord was still anchored to the abdominal wall and lacerated at its distal part. There were no injuries on the skin or signs of suspected asphyxia. The autopsy also revealed a subgaleal hematoma over the right temporal-parietal scalp, and no apparent subdural or subarachnoid hemorrhages. No cardiac malformations were found. When the lungs and the clipped stomach were placed on water, they floated. All findings demonstrate that the newborn infant was born alive. Further investigation revealed characteristics of a dysfunctional family and led to the intervention of protective services.

Reference(s):

1. Italian Law no. 442, art. 2, 5th of August 1981. *Gazzetta Ufficiale.* 10 August 1981; 218:5224-5225.

Infanticide, Frozen Newborn, Histopathology

H115 A Review of Multiple Dog-Mauling Fatalities of Infants Less Than Six Months of Age and Neonates in Travis County, Texas, and Cook County, Chicago, Illinois

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After attending this presentation, attendees will have a greater understanding of the injuries found in neonates and infants mauled by dogs.

This presentation will impact the forensic science community by presenting unique findings regarding neonates mauled by dogs.

There are numerous dog bite fatalities in the United States every year. Most of these fatalities occur in children between 1 and 12 years of age. In contrast, deaths among neonates (less than four weeks of age) and infants less than six months old comprise a small percentage of pediatric deaths. In the 1-to-12-year-old age group, injuries typically involve the head and extremities. The injuries consist of multiple abrasions and lacerations. The lacerations involve the underlying blood vessels and result in death from hemorrhage; however, deaths in infants less than six months of age can also be the result of extensive crushing injuries.

This study reviewed seven dog-mauling fatalities of infants from Travis County, TX, and Cook County, IL. The ages ranged from six days to six months old.

Family members discovered two of the neonates suspended by their heads from the dogs' mouths. The injuries predominately involved the head and neck region and consisted of puncture wounds, lacerations, skull fractures, and brain hemorrhage. In one case, examination of the spinal cord demonstrated hemorrhages along the cord and nerve roots. The third neonate was found on the ground with extensive injuries to the torso, head, and neck. While similar injuries also occurred in the older infant age group, additional injuries to the torso with rib fractures and lacerations of the lungs and liver were identified in this age group as well. The neonates averaged 8 pounds in weight, 20 inches in length, and had an average head circumference of 32.5cm. The older infants averaged 12.4 pounds in weight, 24.5 inches in length, and had an average head circumference of 39.4cm.

Fatal injuries in neonates and infants are related to the physical characteristics of this age group: immobility, low weight, and short stature. Neonates can present with a unique subset of injuries to the neck region. Because they have relatively smaller head circumferences when compared to older infants, larger dog breeds can pick up and shake the neonate by the head, causing cervical spine injuries.

In conclusion, when a neonate is the victim of a dog mauling, the cervical spinal column should be removed and examined for injuries to evaluate shaking as a possible mechanism of death.

Dog Mauling, Neonates, Cervical Spine Injuries

H116 Distinguishing an Accidental Drowning From a Homicide: The Death of a 72-Year-Old Woman in Mississippi

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The goal of this presentation is to stress the significance of a thorough forensic investigation. Attendees will appreciate the nuances of distinguishing accidental from non-accidental trauma, as it relates to forensic pathology.

This presentation will impact the forensic science community by demonstrating how a forensic pathologist can uncover evidence that will prompt an inquiry or change the course of an ongoing investigation. The presence or absence of certain signs can guide the forensic investigation in various directions. This presentation will illustrate the importance of forensic evidence when determining the manner and cause of death.

On June 12, 2017, the Madison County, MS, Sheriff's Office responded to a call concerning a motor vehicle accident. The accident was reported by the driver of a log truck. He stated that he was driving by a lake when he noticed a black car, which was partially submerged. He informed the authorities that he was driving on a two-lane road, so he did not stop until he came across an individual who was working in his front yard. Once he told the homeowner about the vehicle in the lake, he got back into his truck and continued down the road without leaving his name or contact information. Upon arriving at the scene, law enforcement observed a body floating face down in the center of a lake. A purse retrieved close to shore revealed a credit card; the name on the card was recognized by one of the first responders. Authorities contacted the decedent's boyfriend, who explained that the couple had just returned to Mississippi following a brief vacation in Atlanta, GA. He stated that the victim dropped him off at his residence shortly after noon and proceeded to drive in the direction of her own residence. The boyfriend had no further information to provide.

The victim's body and her car, a 2009 black Toyota® Camry®, were retrieved from the lake. The vehicle was submerged in approximately eight feet of water and had sustained damage to the front grill, bumper, and windshield. The passenger window was in a rolled-down position; the glass was intact. The key was in the ignition, but the plastic Frequency Operated Button (FOB) end of the key was missing. The gearshift was in the reverse position, and the hazard lights were turned on and functioning, as the car was removed from the water. The car was towed to a body shop, and the decedent was transported to the State Medical Examiner's Office.

The external examination of the victim was unremarkable except for a laceration noted to the medial aspect of the right first toe and a contusion inferiomedial to the right kneecap. The decedent was cool to the touch, in full rigor, displaying purple fixed lividity posteriorly. On examination of the eyes, bilateral scleral hemorrhage was noted. The autopsy revealed bilateral hyperinflated lungs, hemorrhage of the tongue, bilateral hemorrhage of anterior strap muscles of the neck, petechiae of the scalp, focal hemorrhages of the right scalp, including the right temporalis muscle, and bilateral hemorrhage of the deltoids.

It is important to distinguish the victim's manner of death. The narrative provided by the witness and boyfriend suggest the death was an accidental drowning; however, the damage sustained to the victim's car was inconsistent with the injuries found on the victim's body, as there was an absence of blunt force trauma that should be present in a motor vehicle accident. In addition, the autopsy revealed multiple findings consistent with a struggle. Specifically, the bilateral scleral hemorrhage and scalp petechiae are consistent with hypoxia as a cause of death. Even more significant are the hemorrhages of the anterior strap muscles of the neck, suggesting strangulation as the manner of death.

The investigation is ongoing.

Strangulation, Drowning, Blunt Force Trauma

H117 Diatom Test: Still an Irreplaceable Analysis

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The goals of this presentation are to verify how: (1) the standardization of a diatom test on femoral bone marrow allows for more scientifically correct data to increase the accuracy of the diagnosis of death by drowning and to discriminate it from other causes of death; and, (2) this work seeks to confirm the high specificity of the diatom test.

This presentation will impact the forensic science community by showing how exceptional knowledge of the method, and the ability to apply it rigorously, could be a useful means of confirming a diagnosis of drowning.

Since the early 1900s, the diatom test has been included among tests that allow the diagnosis of drowning. There are many studies on this subject that confirm the diagnostic reliability of this test, while acknowledging its obvious limits (no rigorous implementation by the operator and false positives).

Diatoms are aquatic unicellular algae, widespread in both fresh and salt waters worldwide. They contain a cell wall made of silica (frustule) of various shapes, which reveal gaps for external exchanges (pores). Frustule is highly resistant to chemical and physical treatments that can dissolve human tissues, and for this reason it can be pointed up.

The theory behind the diatom test is that when fluid enters the lung, diatoms enter within the fluid and pass the alveolar-capillary barrier. If the circulatory system is functioning, diatoms are disseminated to peripheral organs; this is an indication of breathing activity at the entrance of the drowning medium in airways. After their diffusion, diatoms can be found in several tissues. The femoral bone marrow is preferred both because of its resistance to external contamination and in cases of a highly decomposed body.

It is important to consider that diatoms decrease their concentration during the transit from outside to the bone marrow, decreasing by 100 to 1,000 times (finding an excess of diatoms means there was a contamination). Moreover, high-dimension diatoms are most likely contaminants (not rigorous implementation by the operator and false positives). It is also important to match the diatoms found in bone marrow and those found in the drowning medium. This comparison could lead to a clear identification of the drowning area.

This study is based on the analysis of 53 corpses recovered from water, of which 36 drowned and 17 died from other causes. In all cases, a complete autopsy, histological investigations, and the diatom test were performed.

In the diatom test, a sample of femoral bone marrow was collected during the autopsy using sterile surgical devices further cleansed with diatom-free alcohol. From this sample, 10ml of bone marrow was processed with nitric acid and successively examined with an optic microscope, using bright field and phase contrast.

Among the drowned subjects examined, 28 tested positive (77.77%) and 8 negative (22.22 %); among the decedents found dead in water, none showed diatoms in the bone marrow. The Fisher test was performed, demonstrating that these two groups were significantly different ($p<0.0001$).

Therefore, a diatom test, executed in a meticulous and standardized manner, could be considered essential support for a drowning diagnosis, in addition to more common practices (analysis of the circumstances, postmortem examination, and histological findings). This method, inexpensive and easy to reproduce, could be an important aid for the pathologist to differentiate drowning cases from other cases of death occurring in water.

Drowning, Diatoms, Forensic Pathology

H118 Armanni-Ebstein Lesions and Hypothermia: A Five-Year Retrospective Study From the Cook County Medical Examiner's Office

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After attending this presentation, attendees will understand the significance of Armanni-Ebstein lesions in the diagnosis of fatal hypothermia.

This presentation will impact the forensic science community by studying the association of these lesions in deaths due to hypothermia in one of the coldest counties of the United States.

Hypothermia is defined as a body core temperature below 95°F (35°C). The final cause of death is due to ventricular fibrillation, asystole, internal asphyxiation or hypoxia due to a left shifting of the oxygen-hemoglobin dissociation curve, or failure of enzymes and electrolyte dysregulation. Usually, the diagnosis of hypothermia is based on circumstantial evidence, but there are some morphological findings that can help the forensic pathologist reach the correct diagnosis: bright red postmortem lividity; hemorrhagic spots of the gastric mucosa (Wischnewsky's spots); several pancreatic changes, such as focal or diffuse pancreatitis, hemorrhagic pancreatitis, patches of fat necrosis; hemorrhages into the core muscles; and fatty changes in the heart, liver, and kidneys. Scene findings of paradoxical undressing or terminal burrowing may assist the pathologist in the diagnosis of hypothermia.

Researchers have found a possible association between the Armanni-Ebstein phenomenon and hypothermia deaths. Armanni-Ebstein changes are subnuclear vacuolization of renal tubular epithelial cells due to glycogen or lipid deposits. These lesions are typically observed in poorly controlled diabetic states.

According to the literature, Armanni-Ebstein changes are constantly observed in subjects with a history of diabetes mellitus. An association between diabetes mellitus and hypothermia has been described. Metabolic complications of diabetes mellitus can cause secondary hypothermia. Conversely, primary hypothermia can worsen a decompensated diabetic state. Hence, Armanni- Ebstein lesions can be found in cases of hypothermia and diabetic ketoacidosis.

Case files from the electronic database of the Cook County Medical Examiner's Office in Chicago, IL, were retrospectively reviewed over a five-year period from July 2011 to July 2016 for cases in which deaths were due to hypothermia. The search was performed using the keyword "cold" in the "Cause," "Due to," and "Injury description" fields. All cases had investigative reports. Cases in which a complete autopsy was performed were selected for this study. Autopsy reports were reviewed, and the age, gender, and pathological findings were summarized. Specific details about diabetic status, if available, were noted. In cases in which histological sections of the kidneys were available, the slides were examined for the presence of Armanni-Ebstein lesions. Periodic Acid-Schiff (PAS) and Oil-red staining were used to demonstrate the material in the lesions.

There were a total of 133 cases of fatal hypothermia, with autopsies performed in 111 cases. Histology slides were available in 44 of the 111 cases. The ages ranged from 0 to 99 years (average 55 years) with a male-to-female ratio of 2.7:1.

This research provides an interesting study targeted at demonstrating the significance of Armanni-Ebstein lesions in cases of fatal hypothermia. The results of this study will be discussed with attendees.

Armanni-Ebstein, Hypothermia, Forensic Pathology

H119 Hypothermia Deaths Due to Environmental Exposure in King County, Washington

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After attending this presentation, attendees will be able to explain the contributing variables in hypothermia deaths in vulnerable populations and describe the paramount importance of scene and circumstances in determining cause of death in the absence of specific anatomic markers.

This presentation will impact the forensic science community by emphasizing the importance of scene and circumstances in determining cause of death in cases of environmental exposure in the absence of specific anatomic findings related to hypothermia. This study has important implications for public health and public policies related to homelessness and protecting vulnerable populations.

The Pacific Northwest has a temperate climate without extremes of temperature, yet hypothermia deaths due to environmental exposure are not uncommon. Data from the King County Medical Examiner's Office (KCMEO) in Seattle, WA, were examined to better understand the contributing factors in hypothermia and the criteria used to determine cause of death.

Methods and Materials: KCMEO records from 2005 through 2016 were selected for all deaths due to hypothermia from environmental exposure. Cases were reviewed in addition to toxicology reports for cause and manner of death, demographics, detailed circumstances, and potential contributing factors. Historical weather data were obtained for each date of death or date of discovery for cases dying in the hospital. Population data were obtained from the 2010 United States Census, and estimates of the homeless population were obtained from local statistics. To assess the specificity of gastric Wischnewski lesions in hypothermia deaths, all autopsy reports for the same time interval containing the term "Wischnewski" were reviewed.

Results: There were a total of 101 cases identified. The annual rate ranged from 5 to 16 per year with a maximum of 16 in 2015. Deaths occurred during all months of the year, with a peak of 32 deaths in December. Daily minimum temperatures associated with each death ranged from 16°F to 56°F with a median of 39°F. The male:female ratio was 69:32. Ages ranged from 24 to 95 years with a median of 57 years. The racial distribution was as follows: 85 White, 6 Black, 4 Asian/Pacific Islander, 5 Native American, and 1 Other. Of all cases, 87 were dead at the scene and 14 were found alive but subsequently died in the hospital. Decedents were found indoors in 15 cases and outdoors in 86 cases. Of those found indoors, all were living alone, and 5 were aged 75 years or older. Altogether, 32 were considered homeless. Based on homeless estimates in King County, this represents an incidence of hypothermia death among the homeless of up to 56 per 100,000 in 2015, compared with 0.76 per 100,000 in the general population. Evidence of intoxication was present in 46 cases. Non-toxic causes of potential incapacitation, ranging from trauma to dementia, were documented in 28 cases. Wischnewski lesions were documented in 25 cases of hypothermia death and in 25 autopsies unrelated to hypothermia. Additional analysis of circumstances found one or more factors that may have contributed to death as follows: 32 were homeless; at least 8 were living indoors in an unheated residence; 65 were incapacitated by intoxicants, injury, and/or natural disease; 7 had underlying dementia or psychiatric illness; and 2 were involved in a motor vehicle collision with a prolonged interval of discovery. Only two cases were engaged in outdoor recreational activities. Paradoxical undressing and/or burrowing were not appreciable features in this study.

Discussion and Conclusions: In this study, the certification of death due to environmental exposure was made by consideration of scene and circumstances. Wischnewski lesions were not found to be a reliable indicator of hypothermia death. Moreover, hypothermia deaths in King County were not necessarily associated with freezing temperatures, indicating that winter in maritime temperate climates may present an unexpected hazard for vulnerable populations, including the homeless and the indigent elderly living alone. Intoxication increases vulnerability as do non-toxic causes of incapacitation, such as underlying dementia or natural disease. Overall, the findings of this study have important implications for public health and welfare services designed for protecting vulnerable populations. As the homeless population in King County continues to rise, it becomes increasingly important to monitor hypothermia deaths as a measure of public policy.

Systemic Hypothermia, Environmental Cold Exposure, Homelessness

H120 Common Cutaneous Injuries Found in Drowning Deaths

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After attending this presentation, attendees will be more familiar with cutaneous injuries that may be seen in drowning deaths. This presentation will explore possible mechanisms underlying these injuries, particularly focusing on cutaneous injuries that occur while the decedent is submerged in water.

This presentation will impact the forensic science community by identifying the frequency of cutaneous injuries in drowning deaths and will provide a reference of cutaneous injuries that may occur to aid forensic pathologists in determining the possible causes of commonly recognized injuries in drowning victims.

Cutaneous injuries are often found in suspected drowning deaths. These injuries are thought to occur as the body drags across the bottom of the body of water or as the body comes into contact with rocks, marine life, underwater objects, and even boats.[1] For example, bodies that have traveled a significant distance in a river may demonstrate grinding down of tarsal and carpal bones due to dragging of the hands and feet along the bottom of the body of water.[2] Classically, these cutaneous injuries are distributed over the face, hands, forearms, knees, and feet as submerged bodies have a tendency to be in a "head down" position.[1] It can become difficult for a forensic pathologist to determine whether these injuries were caused ante- or postmortem, as various types of injuries can occur while submerged in a body of water.[3,4] This also includes cutaneous injuries due to postmortem animal predation.[4]

To date, this study is not aware of previous studies that quantified the frequency and types of cutaneous injuries seen in drowning deaths. In order to accumulate data, this study drew from a web-based database of deaths investigated by medical examiners/coroners from multiple counties in Michigan and Indiana from 2008 to 2017. Cases were identified by use of the term "drown" in the cause-of-death field and further narrowed down by location of death being a lake, pond, or river. A review of the autopsy photographs and the evidence of injury section of the autopsy reports identified cases with cutaneous injuries. All 65 identified cases occurred in freshwater. Approximately 71% of the deceased individuals presented with evidence of cutaneous injuries, including, but not limited to, abrasions, lacerations, and contusions. Injuries on the body were found most frequently on the head (49%) and extremities (43%), but were also found on the trunk (19%) and neck (3%). Some of these injuries appeared to be from postmortem animal activity.

Determining the timeline and cause of cutaneous injuries is an integral part of the medicolegal postmortem examination. This becomes especially difficult in the setting of a drowning, when the body can acquire postmortem injuries from a variety of sources, some of which may mimic antemortem trauma. Members of the forensic community should always remain cognizant of the fact that cutaneous injuries are frequently associated with drowning deaths and, in many cases, may be caused postmortem, with no correlation to the immediate cause of death.

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Drowning, Cutaneous, Injuries

H121 A Probabilistic Analysis of the Cause of a Traffic Death Following Two Crashes Using National Crash Data

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The goal of this presentation is to present the analysis of the most probable cause of a fatal head injury following two traffic crashes occurring in close temporal sequence.

This presentation will impact the forensic science community by illustrating a multidisciplinary, evidence-based assessment of cause of death in a fatal traffic crash investigation.

Medicolegal investigation of the mechanism and cause of death in most fatal traffic crashes is relatively straightforward; there is typically evidence of direct or indirect blunt force trauma to the head, neck, or chest that can be matched with a reconstructed crash injury mechanism; however, in some unique circumstances, there are plausible alternative explanations for observed injuries that require further investigation.

This presentation describes the death of a 19-year-old female driver of a Sports Utility Vehicle (SUV) that was involved in two crashes occurring in close temporal sequence. The collision series began when the decedent's Ford® Explorer® SUV carrying five restrained occupants swerved to avoid a semi-tractor/trailer that had entered its lane. The vehicle began to yaw clockwise, then tipped and rolled 2.5 times, coming to rest on its roof in the same lane in which it had been traveling. The Ford® sustained extensive roof crush. Two of the passengers were able to exit the vehicle, and none of the occupants recalled the driver verbally responding.

Approximately one minute after the vehicle had stopped moving, a southbound semi-tractor/trailer struck the SUV on the driver's side at 33.5mph. The driver, who was found inverted and hanging from her seatbelt with her head and left arm partially ejected, was pronounced dead at the scene. Autopsy revealed a comminuted transverse fracture through the temporal and occipital bones and a pontomedullary transection, as well as extensive abrasions, lacerations, and pressure marks noted about the head, face, neck, torso, and both upper and lower extremities.

There was no reliable evidence from either the autopsy or from an engineering/biomechanical perspective as to which of the crashes caused the fatal head injuries. From a common-sense perspective, it was reasonable to view the second collision as having a very high probability of causing serious head injury, as the decedent's inverted head and neck were at the level of the bumper of the approaching semi. At the same time, the high degree of roof crush sustained by the SUV in the rollover also suggested a high risk of head and neck injury.

An analysis of national crash data was undertaken to quantify the risk of death for each of the two crashes so that a determination of comparative risk could be made. To this end, data were abstracted from the United States National Automotive Sampling System-Crashworthiness Data System (NASS-CDS). The parameters for the analysis included all SUVs that underwent a rollover, and the outcome was risk of death for a belted, non-ejected driver with more than one foot of roof crush at their seating position. The risk of death in such rollover crashes was 1.8% for drivers, or 1 in 56.

The same type of analysis was performed for the second crash, using nearside impacts of a "T-bone" configuration, and adjusted for the severity of the collision, which, at 33.5mph impact speed placed it in the upper 1.5% of all side impacts for severity. The associated risk of death was 16%, or 1 in 6.3. The risk ratio between the two crashes was 16.0/1.8=9.0; thus, a comparison based purely on risk indicated that the second crash was nine times more likely to have caused the fatal injuries.

Further review of the evidence indicated that the inverted driver was likely struck in the head by the bumper of the approaching semi, as there was a small amount of blood found on the bumper. This fact likely increased the risk of death from the second impact substantially, relative to the 16.0% estimated risk for upright occupants. Thus, it was more accurate to consider that the absolute risk of death from the first crash (1.8%) indicated that there was a >98% probability that the decedent survived this initial crash; thus, as the risk of death from the second crash approached 100%, the risk ratio would likewise increase.

Traffic Death, National Crash Data, Forensic Epidemiology

H122 Immune Responses in Opioid Use

Henry J. Carson, MD, Cedar Rapids, IA*

After attending this presentation, attendees will be able to: (1) identify situations in which opioid use is present but not definitely diagnostic of cause or manner of death; (2) collect and store specimens for opioid and immune analysis; (3) apply laboratory and anatomic findings to establish immune response in cases of opioid use; and, (4) apply immune responses in death certificates.

This presentation will impact the forensic science community by demonstrating how these findings can be used to perform forensic, toxicological, and immunological examinations of decedents and to certify the cause and manner of death.

Death from the abuse of prescription or illicit opioids is acutely on the rise in the United States.[1] Death from drug abuse is usually determined by toxicological examination of the blood or other body fluids in conjunction with an autopsy, scene investigation, and detailed decedent history; however, in some cases, the levels of opioids detected do not reach toxic levels, let alone fatal levels.[2,3] It was hypothesized that there is a subset of opioid users in whom immune activiation is a substrate of the mechanism of death, and they can be identified by demonstrating evidence of allergic or anaphylactic reactions.

In this experiment, cases from a ten-year period were reviewed. All cases with toxicological evidence of opioid use were recorded. The cases were further grouped by evidence of immune response in the presence of opioid use. Evidence of immune activation was based on external findings such as rash or scratches from recent pruritis; internal evidence such as overlapping lung apices from asthma; microscopic evidence such as lung changes of asthma; or elevated mast cell tryptase levels. Groups were compared with *t*-tests and chi-square 2x2 contingency tables.

In all, 49 cases of opioid use were identified. Of these, five had evidence of immune response in the presence of opioids: two with scratches, three with evidence of asthma, and one with elevated mast cell tryptase. There was no significant difference between the two groups based on age, race, or sex. There was no significant difference in the types of drugs or opioids identified in the two groups. There was no significant difference in the manner of death. Of a number of clinical histories and causes of death identified, asthma was the major condition that was significantly associated with immune response in the presence of opioid use.

In conclusion, men and women were equally likely to have used opioids in this study group, typically in their fourth and fifth decades. No specific type of opioid was favored. Other prescription drugs were frequently present. The most common cause of death was overdose or drug interaction. The manner of death was typically accident. Immune response was evident in 10% of the cases. A history and evidence of asthma was significantly associated with immune response and as a cause of death.

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Forensic Science, Asthma, Analgesics, Opioid

H123 Preliminary Findings From the Drug Enforcement Administration's (DEA's) National Forensic Laboratory Information System (NFLIS) Medical Examiner/Coroner Office Survey

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After attending this presentation, attendees will understand the DEA's NFLIS expansion and results from their 2017 medical examiner/coroner office survey.

This presentation will impact the forensic science community by providing specific knowledge of medical examiner and coroner office operations, policies, practices, and resource needs of the United States' medicolegal death investigation system.

The NFLIS represents an important DEA resource in monitoring illicit drug abuse and trafficking. Available NFLIS data reflect the results from drug chemistry analyses conducted by federal, state, and local forensic laboratories across the country (NFLIS-Drug). NFLIS-Drug currently includes more than 95% of the nation's crime laboratories that regularly report data from solid-dosage drug analyses from law enforcement seizures. NFLIS-Drug data are used to support drug scheduling decisions and to inform drug policy and drug-enforcement initiatives nationally and in local communities around the country.

Over the next few years, DEA will expand NFLIS to include drug-related mortality data from the nation's Medical Examiner and Coroner offices (NFLIS-MEC) and drug testing results from public and private toxicology laboratories (NFLIS-TOX) across the United States. The new NFLIS-MEC and NFLIS-TOX data collections will extend DEA's radar in identifying new and emerging drugs and to inform drug use patterns. In preparation for this expansion, DEA conducted a 2017 NFLIS-MEC office survey and a Toxicology Laboratory (TL) Survey. Preliminary results from the MEC survey will be discussed in this presentation.

The MEC survey was designed by NFLIS staff with the help of external MEC and toxicology consultants. Mailed surveys were sent to all identified MECs (*N*=2,156) in the United States. NFLIS staff performed several actions to increase survey responses, including verification calls to confirm MEC office contact information and point of contact, prompting call reminders about the mailed survey, and non-response calling to collect two critical questions. In addition, NFLIS staff reached out to all state coroners associations, the National Association of Medical Examiners, and the International Association of Coroners & Medicals Examiners, among others, to encourage participation. Survey responses were collected via a mixed mode data collection (web, mail, and telephone). Data collection began in June 2017 and concluded in October 2017. Survey results highlighting findings related to operation, caseloads, turnaround times, toxicology testing practices, accreditation, and information management systems of MEC offices will be discussed. Where possible, the data will be presented by type of office and size of jurisdiction. The results will provide attendees a broad understanding of the operations, policies, practices, and resource needs of the nation's medicolegal death investigation system. Moreover, DEA will outline its plans for the NFLIS expansion.

NFLIS, DEA, Medical Examiner/Coroner

H124 Developing "Real-Time" Surveillance for Drug Overdose Deaths in King County, Washington

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After attending this presentation, attendees will be able to explain why data from death certificates are not effective for "real-time" surveillance of drug overdose deaths and describe methods by which death investigations can be used more effectively in the face of ever-increasing drug overdose deaths.

This presentation will impact the forensic science community by describing methods by which death investigations can be part of "real-time" surveillance of drug overdose deaths.

Across the country, the opioid epidemic is rapidly evolving. In some regions, there has been a major shift in the type of opioids seen due to fentanyl-type drugs. Unfortunately, overdose data are generally available only after many months and represent a historical analysis rather than a useful surveillance tool. The present study was conducted to increase understanding of the delays in collecting and using overdose data and to develop a method by which information from drug overdose death investigations can be used as part of "real-time" surveillance.

Methods and Materials: Death investigation records and death certificates for drug overdose deaths reported by the King County Medical Examiner's Office (KCMEO) in Seattle, WA, from 2007 through 2016 were examined to evaluate for each case the time delays between when death occurred, when toxicology reports became available, when pathologists cleared the death certificate, and when the death certificates were referred to the Office of Vital Statistics. As a means of developing a "real-time" surveillance model, KCMEO pathologists maintained a daily tally of the overdose deaths, making predictions for each case of the drug(s) that were likely to be detected in the toxicology analyses, based on the scene and circumstances documented by the death investigators. Dialogue has been initiated with local law enforcement agencies to share investigative details regarding all potential overdoses, including predicted drugs. After the toxicology results were reported from the laboratory, the predicted drug(s) were compared with the detected drug(s), and the accuracy of the predictions was evaluated.

Results: Estimates from each of the time periods from death to certification were determined for 2,388 drug overdose cases. The number of days from death to final certification averaged 54 days, with a median of 50 days, ranging 3 to 217 days. Toxicology reports accounted for the greatest delay from death to certification, averaging 40 days, with a median of 44 days, ranging 5 to 519 days. In some cases, the pathologist called the laboratory for results and certified deaths before the reports were available, and in other cases, analyses continued long after the deaths were certified. Administrative functions comprised a minor proportion of the delay — usually less than one week. Comparing the accuracy of predicting what drug(s) might be identified in each drug overdose case, made when the case was initially examined, with the drug(s) subsequently reported by the toxicology laboratory found predictions to be at least partially correct in approximately 93% of cases. Cases in which predications were incorrect often involved deaths associated with white powders.

Discussion and Conclusions: Death certificates are useless for "real-time" surveillance of drug overdose deaths. In the present study, half of the death certificates were not completed until more than 50 days after the death. Not surprisingly, the major portion of the delay was the time required for toxicology results to be reported. This delay is not unique to one office; it is a universal barrier to using drug overdose data as a timely surveillance tool. In the extended period between death and final determination of cause of death, the investigation is essentially stalled. Information regarding drug source is frequently difficult to discover, and witnesses, often protected by Good Samaritan laws, are unlikely to provide valuable information; however, as the results of this study show, experienced death investigators and forensic pathologists, when presented with information gathered from the scene, are fairly accurate in their ability to predict the drugs responsible for death. Hopefully, in the future, rapid reporting of details regarding suspected overdose deaths to local law enforcement agencies will make "real-time" surveillance worthwhile for many different agencies.

Drug Overdose Investigation, Real-Time Surveillance, Toxicology

H125 New Psychoactive Substances (NPS) -Related Deaths in Sweden — An Alarming Development

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After attending this presentation, attendees will better understand the prevalence and other data of NPS in Swedish medicolegal autopsy cases.

This presentation will impact the forensic science community by informing attendees of abuse trends in general for NPS in Sweden and of the increased toxicity of new synthetic opioids in particular.

Introduction: This study was conducted to follow up on a previous investigation of NPS in medicolegal autopsy cases in Sweden with analyses of, for example, time trends, demography of the decedents, and the formation of clusters of cases caused by the same NPS.

Method: Data from medicolegal autopsies positive for NPS in blood and/or urine were reviewed regarding age, sex, cause of death, place of death, and type of drug. It was also noted whether the decedent had a documented substance abuse at the time of death. The cases were divided into three groups: true lethal intoxications (A), possible intoxications (B) in which NPS was a possible contributing factor, and cause of death not directly related to drug intoxication (C). The study period was 2016 and the results were compared to earlier findings from the time period between 2007 and 2015. A cluster was defined as more than five true lethal intoxication (A) cases in Sweden in the same year due to the intake of one specific NPS.

Results: In 2016, 129 NPS-positive cases were found. Of the decedents, 112 were male (87%), the average age was 34 years, and the median age was 32 years. Seventy-nine of the cases (61%) were determined as true lethal intoxications (A), and in 42 cases (33%), NPS was determined as a possible contributing factor (B). Thus, NPS was the sole or contributing cause of death in 94% of all NPS-positive cases. A majority of the decedents (72%) were found in their own home, the fatalities were scattered across Sweden, and 63% of the decedents had a substance and/or alcohol abuse mentioned on the death certificate. Three clusters due to intoxication of one specific NPS were identified within the time period studied, namely by the synthetic opioids acrylfentanyl, tetrahydrofuranfentanyl, and 4-fluoro-isobutyrfentanyl. Variants of "spice" caused a fourth cluster. In all, 48 different NPS were detected in 2016.

Discussion: Unlike previous years, the number of NPS-positive cases did not increase in 2016, but the true lethal intoxications (A) nearly doubled and remarkably few of the NPS-positive cases were entirely caused by something other than NPS. This study found seven different fentanyl analogues, and these caused nearly all of the true lethal intoxications (A). The emergence of new potent opioid analogues is challenging for forensic pathologists as well as toxicologists, and awareness of these particular NPS is important to all professionals working in connection to substance abuse.

Conclusion: As previously shown, there is a high risk of death due to NPS intoxication if the user is male, approximately 30 years of age with a known substance abuse, and uses the NPS in the own home environment. The emergence of new fentanyl analogues in 2015 and 2016 has further elevated the risk of lethal intoxication compared to previous years.

NPS, Postmortem, Increased Toxicity

H126 Deaths in Denver With the Detection of Cannabinoid Metabolites: 2010-2016

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After attending this presentation, attendees will understand the timeline of legalization of marijuana in Colorado and the impact this has had on deaths in Denver, CO.

This presentation will impact the forensic science community by reporting on the incidence of detection of cannabinoid metabolites in forensic cases and discussing trends for deaths in Denver, CO, in relation to the legalization of marijuana in the state of Colorado, a previously unreported topic.

Federal law prohibits cannabis for medicinal and recreational purposes by its classification as a Schedule I Controlled Substance. Amendment 64 to the Colorado State Constitution legalized the personal possession of cannabis for medicinal and recreational consumption in November 2012; state-regulated commercial sales of cannabis products to the general public commenced on January 1, 2014.[1] This study explores the effects that cannabis legalization has had on mortality statistics in Denver, with examination of pre/post legalization trends regarding manner of death.

The Denver Office of the Medical Examiner (DOME) is responsible for medicolegal death investigation in the City and County of Denver, where three Board-certified forensic pathologists and one forensic pathology fellow oversee investigation of sudden or unexplained deaths. In this jurisdiction, deaths are categorized by manner, which consist of natural, accident, suicide, homicide, and undetermined. Samples of postmortem peripheral blood or antemortem specimens (if available) are retained and submitted for testing of commonly abused drugs, including cannabinoids (Tetrahydrocannabinol (THC), and metabolites of 11-hydroxy delta-9 THC, delta-9-THC, and delta-9 carboxy THC).[2] Toxicological testing was performed according to internal laboratory protocols with appropriate controls at National Medical Services, Inc, Willow Grove, PA, by means of High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS).

DOME employs a searchable database regarding cases within its jurisdiction, which includes a coding system that allows documentation of substances detected by toxicological testing. Query of the DOME database revealed a total of 3,075 postmortem examinations in which toxicology studies were ordered during 2010–2016. Of these postmortem examinations with toxicology, 688 (22%) were positive for cannabinoids. Given that the state legalization for commercial sales of marijuana commenced on January 1, 2014, it is determined that 1,682 cases occurred "pre-legalization" and 1,393 cases were "post-legalization." Those cases that resulted positive for the detection of cannabinoids during these time periods, as well as the trends within various manners of death, will be discussed.

Of the total cases with toxicology, 1,185 were ruled with a manner of non-traffic accident (39%), natural 989 (32%), suicide 344 (11%), homicide 252 (8%), traffic-related accident 163 (5%), and undetermined (5%). Pre-legalization, there were 653 non-traffic accidental deaths, and of these, 82 (13%) resulted positive for cannabinoids. Post-legalization, there were 543 non-traffic accidental deaths, and of these, 88 (16%) resulted positive for cannabinoids. Pre-legalization, there were 615 natural deaths, and of these, 127 (21%) resulted positive for cannabinoids. Post-legalization, there were 374 natural deaths, and of these, 98 (26%) resulted positive for cannabinoids. Pre-legalization, there were 126 suicides, and of these, 16 (13%) resulted positive for cannabinoids. Post-legalization, there were 218 suicides, and of these, 75 (34%) resulted positive for cannabinoids. Pre-legalization, there were 128 homicides, and of these, 67 (52%) resulted positive for cannabinoids. Post-legalization, there were 124 homicides, and of these, 56 (45%) resulted positive for cannabinoids. Pre-legalization, there were 73 traffic-related accidental deaths, and of these, 23 (32%) resulted positive for cannabinoids. Post-legalization, there were 90 traffic-related accidental deaths, and of these, 40 (44%) resulted positive for cannabinoids. Pre-legalization, there were 98 deaths with an undetermined manner, and of these, 11 (11%) resulted positive for cannabinoids. Post-legalization, there were 44 deaths with an undetermined manner, and of these, 5 (11%) resulted positive for cannabinoids.

Frequent detection of cannabis metabolites was identified in all manners of death in Denver for the years 2010–2016, and the results of this study reveal an overall increase in the presence of cannabinoid metabolites in fatalities during the three years following the legalization of marijuana sales in Colorado. Furthermore, cannabis metabolite detection was increased post-legalization in manners of non-traffic accidents by 3%, suicide by 17%, traffic-related accidents by 12%, and natural by 5%. Homicides demonstrated a decrease in detection of cannabinoids by 7%. Deaths which remained undetermined in manner revealed no significant change.

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Marijuana, THC, Legalization

H127 Levamisole: A High-Performance Cutting Agent

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After attending this presentation, attendees will better understand the mechanisms of action of cocaine and amphetamines and will learn about the physical, chemical, and pharmacologic properties that have made the anthelminthic agent levamisole one of the most unlikely and widely encountered cocaine adulterants. Attendees will also be acquainted with the possible role of levamisole in cocaine-related fatalities.

This presentation will impact the forensic science community by reviewing the rise of levamisole as a cocaine adulterant and explain the properties of the chemical that almost make it the perfect cocaine cutting agent. An argument will be made for screening of the compound in cocaine-related fatalities to more accurately determine cause of death in these cases. Cocaine cut with levamisole has been associated with a number of deaths; the role levamisole plays in these deaths should be considered.

Drugs like cocaine and amphetamines are abused because of their stimulatory effects. Illicit drugs are frequently diluted (cut) with a variety of materials prior to sale/resale to bulk up the product and increase profit.[1,2] Adulterants are generally selected due to their physical resemblance to cocaine, low cost, or added physiological effects.[2,3] One such adulterant is the imidazothiazole derivative levamisole, an amphetamine and anthelminthic agent acting as a ganglion stimulant in mammals and a depolarizing muscular blocker in nematodes (roundworms).[4,5] Although marketed for numerous uses in humans, including an appetite suppressant, immunomodulatory agent, and antineoplastic agent, it was withdrawn from the United States market in 2000 due to side effects; it is currently marketed only for veterinary purposes as an anthelminthic.[1,4,6-8] Recent estimates suggest that the proportion of cocaine laced with levamisole may now be higher than 80%.[9,10]

In vivo, levamisole is metabolized to aminorex, which is bioactive.[1] The potency of aminorex has been found to be comparable to cocaine, and aminorex's amphetamine-like properties may be responsible for levamisole's psychostimulant effects.[1] Levamisole also readily crosses the Blood Brain Barrier (BBB) and has a longer half-life than cocaine.[1] Through various biochemical mechanisms, levamisole prolongs the action and potentiates the effects of cocaine and may continue to provide amphetamine-like stimulation after the direct effects of cocaine have worn off.[1,4,7,11] Additionally, levamisole is inexpensive relative to other potential adulterants, increasing cocaine profits.[4,9,12] These features make levamisole (and aminorex) an attractive choice as a cocaine additive.[1]

Levamisole use can present with a number of adverse effects, include nausea and vomiting, headache, fatigue, fever, diarrhea, myalgia, dizziness, confusion, and rash.[13] Serious complications include agranulocytosis, leukopenia, thrombocytopenia, vasculopathy and vasculitis, dermal necrosis, leukoencephalopathy, psychosis, pulmonary hypertension and hemorrhage, glomerulonephritis, emboli, arthritis, Coronary Artery Disease (CAD), and circulatory collapse.[1,4,13,14] Some of these complications appear to be associated with levamisole's propensity to provoke hypersensitivity reactions in people with certain genetic predispositions.[7,11] Such reactions have been reported previously, with reoccurrence of symptoms after re-exposure.[11] Reviews on immunologic associations note the presence of Anti-Neutrophil Cytoplasmic Antibodies (ANCA), including perinuclear ANCA (pANCA), cytoplasmic ANCA (cANCA), and type III cryoglobulinemia.[11,14] Given its association with so many potentially deadly side effects, it is not surprising that numerous fatalities, typically attributed to pathology of the heart, brain, or lungs, have been linked to levamisole exposure through tainted cocaine.[7,8,15-18]

Easy to obtain, inexpensive, physically similar in presentation, and with the inherent ability to potentiate and perpetuate the effects of the primary agent, levamisole seems the perfect choice for maximizing profit while leaving consumers none the wiser, except in those cases with deadly consequences. Cause of death in patients with cocaine and levamisole intoxication may be due to any number of mechanisms, ultimately severely impacting the heart, lungs, and brain. Given its prominence as a cocaine additive, its presence in a majority of cocaine specimens evaluated in numerous centers around the world, its known side-effect profile and its role in autoimmune reactions, and its potential role in facilitating or exacerbating pathological processes leading to sudden death, toxicological screening for levamisole is an important element in the analysis of suspected cocaine-related fatalities.

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Cocaine, Levamisole, Cutting Agent

H128 Fire Marshal and Medical Examiner Collaboration in the Investigation of a Complex Homicide

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After attending this presentation, attendees will better appreciate the advantages of collaboration between law enforcement and other agencies in the investigation of complex death scenes, especially those in which the presence of trained fire scene investigators are involved.

This presentation will impact the forensic science community by exploring the ability of trained investigators from disparate disciplines to interact synergistically to enhance the scientific analysis of death scenes.

When fire department Emergency Medical Services (EMS) personnel were called to the scene of a fire, they discovered an unconscious but living 15-year-old Black female. The victim exhibited evidence of a gunshot wound to the head and superficial thermal damage to the face, trunk, and extremities.

She was transported to a local hospital where she was found to have a carbon monoxide level of 40%. A tangential gunshot wound to the right side of the neck and face was also present.[1] This wound fractured the right mandible but exited the head in the right temporal scalp without damage to deeper vital structures in the head or neck. Attempted resuscitation efforts, which began at the scene and continued in the emergency department, were unsuccessful.

Investigation by the fire marshal revealed that two separate fires had been started in separate rooms in the house using material found at the scene, but resulted in only minimal damage to the structure. Unsuccessful attempts to burn the victim could be identified by burn patterns on the body, and the patterns of the thermal trauma could be positively correlated with objects used to start the fire.

Scene investigation utilized blood-spatter patterns and ballistic trajectory analysis to reconstruct the scene of the shooting. Autopsy findings were of a superficial gunshot wound of the head and dense, soot-laden blood in the upper airways.

Death was certified as being a combination of acute fume inhalation and gunshot wound of the head.[2]

A suspect was quickly identified and confessed to the shooting, but related a scenario that was at variance with the scene and autopsy evidence. He was subsequently found guilty of first-degree murder, arson, recklessly endangering, illegal possession of a firearm, and theft.

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Fire Death, Gunshot Trauma, Homicide

H129 An Atypical Suicide by Submachine Gun: A Case Report

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The goal of this presentation is to disclose the forensic and imaging evidence that led to the hypothesis of suicide by submachine gun, namely, the characteristics of entrance and exit wounds and the trajectories and specifications of the weapon used.

This presentation will impact the forensic science community by exposing an atypical suicide method rarely described in the literature.

After a shooting in the neighborhood, Mr. K entrenched himself in his house, waiting for the police. The Groupe d'Intervention de la Gendarmerie Nationale (GIGN), an elite unit of the French police, laid siege to the house for 24 hours before hearing gunshots from inside. During their intervention, they found Mr. K dead, cross-legged on the floor, backing onto a bed frame, collapsed on his right side, with several gunshots wounds on his body. Next to the cadaver, they found a submachine gun whose safety lever, located on the back of the pistol grip, was neutralized with tape.

The weapon, a 1949 French submachine gun from the weapons manufacturer of Chatellerault, was loaded with 9×19mm Parabellum bullets, full metal jacket. Four cartridges seem to have been loaded into the weapon, three of which seemed to have entered the victim. The cadaver was then transported to the forensic institute of Nancy, France, for further examination.

Postmortem Computed Tomography (CT) revealed a wound on the left section of the occipital bone, a fracture of the seventh cervical vertebra, a fracture of the back of the left sixth rib, a wound in the manubrium, and a defect in the third costovertebral junction. The 3D reconstruction was rendered using the global illumination technique and revealed the presence of a metallic object in the neck and of metallic fragments near the two sternal lesions.

The autopsy revealed three ballistic wounds. All entrance wounds were located on the front of the thorax and neck and the trajectories were nearly in the sagittal plane, oblique from bottom to top. The first projectile penetrated on the left side of the sternum and crossed the proximal aorta and the back part of the left sixth rib. The entrance wound presented a zone of soot easily swiped away and revealing blackened seared margins, typical of a loose-contact wound. The exit wound was unusual due to a probable fragmentation of the projectile and the presence of the bed against the skin. It consisted of two shored exits, with elongated orifices and abrasion edges. The second projectile penetrated next to the jugular notch of the sternum and transfixed the trachea and the sixth cervical vertebra. The entrance wound was very unusual; it consisted of a large round opening (4x3cm) with irregular abraded edges. The wound was only penetrating, and the projectile was found in the subcutaneous tissue of the back of the neck. The third projectile penetrated under the chin, on the median line, fractured the hyoid bone, penetrated the base of the skull, and exited through the occipital bone. The entrance wound presented a blackened, seared margin on the upper side, suggesting a near-contact angled wound. The exit wound was typical with a beveled-out hole in the skull and a stellate skin lesion.

The first and third projectiles were the cause of lethal lesions (perforation of the aorta and the brain). The characteristics of the entrance wounds suggested short-range shots. Finally, the first trajectory was nearly horizontal and the two others were more obliquely directed upward. One can therefore imagine a first loose-contact shot in the sternum and the next two shots were due to the neutralization of the safety lever, keeping the weapon in automatic mode. With a firing rate of 600 rounds per minute, five milliseconds were sufficient to fire the four loaded bullets, and recoil can explain the changes in trajectories. In conclusion, the forensic evidence was consistent with the hypothesis of suicide, given the conditions of cadaver discovery and the observations reported by the investigators.

Atypical Suicide, Submachine Gun, Global Illumination

H130 Ballistic Analysis of an Attempted Murder Using a Porcine Model

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The goal of this presentation is to describe an experimental analysis of two competing theories for the circumstances of a gunshot wound, in which a porcine model was used as a proxy for a human thigh.

This presentation will impact the forensic science community by describing a porcine model experiment for the purpose of assessing the plausibility of two competing scenarios in the investigation of an attempted murder.

This presentation will provide the description of the investigation of competing explanations for the circumstances of a close-range gunshot wound from a hunting rifle and the associated prosecution of an attempted murder charge, including an ad hoc experiment to test the victim's explanation for the circumstances of a gunshot wound to the proximal thigh.

The case involves a gunshot wound to the proximal thigh from a jacketed .243 Winchester® round. The victim, a 21-year-old male, had described the injury as resulting from a point-blank (<2 feet) shot into the thigh from a hunting rifle. He claimed to have been held at gunpoint, with the rifle leveled at his chest while standing, and to have slapped the muzzle of the rifle downward, at which point the weapon was fired. The bullet entered at the upper thigh, resulting in a comminuted fracture of the proximal femoral metaphysis, with the bullet fragment remaining largely intact and retained in the soft tissues near the fracture. The injuries included a transected femoral artery and lacerated femoral vein. The round that was fired was a 100-grain (6.5gm), copper-jacketed, tapered, solid lead-core bullet, with a 2,960fps (902m/s) muzzle velocity, and 1,945 ft-lbs (2,637Nm) of energy. The defendant claimed that the rifle discharged while pointed at a concrete floor, and that the injury resulted from a ricochet from the floor. The defendant was charged based on the victim's account of how the events transpired.

An initial review indicated there was no physical evidence supporting either scenario. In the plain radiograph of the femur fracture, the bullet and fragments were in a superior position to the fracture. The entry wound was described as 2cm in diameter. An initial review of the evidence cast doubt on the victim's account, as (1) the size of the entry wound was inconsistently large for a direct shot; and, (2) the fragments from the bullet were above the fracture, indicating an inferior-to-superior travel path rather than the opposite. As there were no reference materials to consult regarding the specific circumstances of the case, it was determined that an experiment would be conducted to test the plausibility of the victim's account of the shooting.

Pig models have been previously described as a relatively close proxy for a human anatomical/pathological response to gunshot; thus, a fresh pig hind leg was selected as the closest proxy for a human thigh.[1] A jig was constructed to hold the pig leg, which was first radiographed, then marked to identify the location of the femoral metaphysis. Damp newspaper was used as a backstop to retain and retrieve any bullet fragments that might penetrate the specimen. High-speed video was used to capture the bullet entering and exiting the specimen. Once the pig leg was secured, a sharp probe was used to further ascertain the underlying bony anatomy of the femur. Using ammunition from the same box used in the shooting, a shot was fired from a distance of 17 inches (43cm) from muzzle to specimen and recorded at 10,000 frames per second. The shot resulted in an entry wound that was approximately 0.6cm in diameter, which struck the femur and exited the rear aspect of the specimen, and subsequently penetrated ~2cm of the damp newspapers. The exit wound was approximately 8cm in diameter, and palpation and radiograph of the femur indicated it was shattered, and fragments of bone were missing (projected into the backstop).

Based on the results of the experiment, it was determined that the scenario of the shooting claimed by the victim was implausible. The explanation for the shooting provided by the defendant was deemed much more probable (i.e., that the bullet struck the defendant as the result of a ricochet).

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Gunshot Wound, Porcine Model, Ballistic

H131 The Effect of Public Awareness and Legislation Against Strangulation on the Occurrence of Gender-Based Violence in King County, Washington

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After attending this presentation, attendees will be able to: (1) list various national and local efforts directed against intimate partner and domestic violence; (2) explain the legal definition of strangulation; (3) trace the rates of all female homicides and those due to strangulation from 1980 to 2016; and, (4) evaluate the evidence that national and local policies and efforts are important in controlling gender-based violence.

This presentation will impact the forensic science community by tracing the declining occurrence of female homicides in King County, WA, from 1980 to 2016 and by illustrating that female homicides due to strangulation constitute a substantial proportion of deaths due to intimate partner and domestic violence. The results of this study provide evidence that local and national efforts directed against gender-based violence are important for controlling these crimes.

Since the Violence Against Women Act (VAWA) was passed in 1994, a number of national and local initiatives have increased public awareness, criminal investigations, and prosecutions directed against Intimate Partner Violence and Domestic Violence (IPV/DV). More recently, in 2007, Washington State enacted legislation promoted by the King County Prosecuting Attorney's Office making strangulation an aggravated assault (Assault 2 felony offense) if prosecution proves that the defendant interfered with the victim's ability to breathe or restricted blood flow to the brain. Because IPV/DV continues to be a major concern in gender-based violence, the present study was conducted to analyze the occurrence of female homicides over the past four decades with special attention to the proportion of female homicides by strangulation.

Methods and Materials: Records of the King County Medical Examiner's office from 1978 to present were reviewed to identify all female homicides occurring in King County according to demographics and mechanism of injury. Data from the Washington Attorney General's Office Homicide Investigation Tracking System were used to identify motives for assault. Data from the King County Prosecuting Attorney's Office and Washington State Administrative Office of the Courts were used to estimate the number of non-fatal domestic violence assaults charged with strangulation.

Results: There were a total of 848 homicides resulting in the death of females in King County over the nearly four-decade period of this study. Despite considerable year-to-year variation, there was a progressive decrease in the number of female homicides. From 1980 to 1989, there were an average of 28.6 female homicides per year; from 1990 to 1999, 25.2 per year; from 2000 to 2009, 15.7 per year; and from 2010 to 2016, 14.4 per year. From 1980 to 2016, strangulation was involved in 20% of cases in which there was a known cause of death and in 14% of female homicides perpetrated by a current or former intimate partner. The proportion of female homicides by strangulation declined as follows: from 1980 to 1989, 23%; from 1990 to 1999, 17%; from 2000 to 2009, 15%; and from 2010 to 2016, 12%. After the King County Prosecuting Attorney's Office Domestic Violence Unit was established in 2000, the number of domestic violence felony filings averaged approximately 1,200 per year, and after the strangulation law went into effect in 2007, strangulation became one of the most frequently filed charges. Correspondingly, from 2007 through 2016, 13% of female homicides were by strangulation, compared to 19% of female homicides by strangulation from 1980 to 2006.

Discussion and Conclusions: The results of this study reveal that strangulation accounts for a considerable proportion of female homicides, especially in IPV/DV. Over the past four decades, the annual number of female homicides has declined by 50%. The proportion of deaths due to strangulation has declined by nearly the same extent. While it is difficult to attribute any single policy or initiative to the declining rates, it is reasonable to conclude that public campaigns to enhance awareness, as well as criminal investigation and prosecution of gender-based violence, are important deterrents of intimate partner and domestic violence.

Female Homicides, Domestic Violence, Strangulation

H132 Two Dead Bodies in a Cemetery: An Unexpected Lightning Strike

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The goal of this presentation is to educate police, scene investigators, medical examiners, and coroners on classic scene and autopsy findings in a lightning strike.

This presentation will impact the forensic science community in regard to the scene and autopsy findings of a death due to a lightning strike, as well as the performance of a scene investigation and examination of these deaths.

In early August 2016, the bodies of a man and a woman were found in a cemetery. They were 34 and 32 years old, respectively, and had a history of intravenous as well as other illicit drug abuse. Drug paraphernalia was identified at the scene, which included syringes and small bags of white powder. Both decedents were also noted to have recent injection sites of their anterior forearms; however, examination of the scene revealed damage to a tree adjacent to where the decedents were discovered that appeared recent and possibly electrical in nature. Additionally, there were thunderstorms in the region overnight, with multiple lightning strikes to the ground reported. The bodies were transported to the medical examiner's office for an examination.

The following day, an examination of both decedents was performed at the medical examiner's office. Examination of the woman revealed a thermal injury of the upper right side of her back associated with a burn of her overlying shirt and bra. She also had "blow-out" damage to the toes of both of her sneakers and socks. Examination of the man revealed classic Lichtenberg figures (ferning) of the anterior torso. Toxicology testing was performed and revealed the presence of fentanyl, as well as other illicit drugs including methamphetamine and heroin at concentrations which could be consistent with a drug overdose in the blood of both decedents. The cause of death for both decedents was certified as: (1) electrocution due to lightning strike; and, (2) acute mixed drug intoxication.

Lightning strikes are an uncommon cause of death with 39 fatalities reported in the United States in 2016 and 312 deaths reported since 2007 according to data collected by the National Oceanic and Atmospheric Administration. The vast majority of fatal lightning strikes occur in the summer (July and August) with men outnumbering women nearly 9 to 1. Classic findings at autopsy include Lichtenberg figures, thermal burns, and associated damage to clothing where present. Examination of the scene and knowledge of the weather at the time of death is crucial to making the diagnosis of an electrocution due to a lightning strike. Additionally, a full internal examination and toxicology testing are a necessary components of a thorough investigation of a possible lightning strike. Electrocution due to a lightning strike will have classic scene and physical findings that should be documented to make the appropriate diagnosis. Other potential causes of death should be considered and excluded where necessary.

Lightning Strike, Electrocution, Fentanyl

H133 An Unusual Case of Suicide in a Young Skydiver

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The goal of this presentation is to report a well-documented case of suicide in a young, experienced civilian parachutist.

This presentation will impact the forensic science community by raising awareness that, as in all medicolegal investigations, every skydiving fatality should require a thorough forensic investigation involving a multidisciplinary approach to allow the forensic pathologist to ascertain the exact cause and manner of death in light of all available relevant information.

Deaths associated with parachuting are very uncommon and the vast majority of these are accidental.[1] Very few suicides have been reported in survey reports on skydiving fatalities worldwide, and the scientific literature contains very limited data about suicidal deaths of skydivers.[2]

This study reports an unusual case of suicide in a 26-year-old civilian skydiver with no significant past medical history, whose body was found dead on an airfield after a 4,000-meter freefall jump. According to witnesses, the victim's main and reserve parachutes did not open and his body remained in a stable freefall arched position until ground impact, then bouncing once on the ground and falling back a few meters away. The backpack the victim was still carrying contained the main and reserve parachutes still packed and properly attached to the harness. The victim's altimeter, helmet, and shoes were found nearby the weed-free area adjacent to the impact site.

A postmortem examination performed two days later revealed a severe multiple blunt trauma with a significant antero-posterior flattening of the body, consistent with a high-energy impact with the ground. Extensive abrasions were arranged symmetrically on the front of the body, whereas no injury was observed on the back, consistent with a ground impact in a stable "belly-down" position. Upon internal examination, there were multiple skull fractures, as well as spinal, rib, pelvic, and limb fractures, with open and symmetrical shoulder and knee fractures. These skeletal injuries were associated with widespread soft tissue hemorrhages and severe injuries to the internal organs, including the brain, heart, and lungs, that exhibited extensive lacerations. No additional injuries that could have been inflicted to the victim prior to the jump nor any evidence of previous natural disease that could have precipitated or contributed to the death were noted. Toxicological analyses performed on the organs, the gastric content, and the vitreous were negative.

Police investigation revealed that the victim, who had considerable skydiving experience and had performed more than 130 jumps, had died during his third consecutive jump of the day, as weather conditions were good. The investigation also discovered that he had recently experienced marital problems and had expressed suicidal thoughts in text messages sent to his wife just before jumping out of the plane.

An expert examination of the parachuting equipment ruled out any evidence of gear malfunction, such as incorrect packing or failure of the chute. In addition, the examination found the Automatic Activation Device (ADD) that allows the reserve parachute to be automatically deployed at low altitude if the skydiver has not released his main chute had been disabled prior to the third jump, with no evidence of criminal intent found.

On the basis of the autopsy findings, testimony of witnesses, and police investigation data, manner of death was considered to be suicide.

Per research, this is the first well-documented case of suicide in a parachutist that has been reported in the scientific literature to date. This case stresses the need to conduct a thorough forensic investigation to determine the cause and manner of death in skydiving fatalities.

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Suicide, Skydiving, Forensic Pathology

H134 An Exceptional Case of Acute Respiratory Failure Caused by Intra-Thoracic Gastric Perforation Secondary to Overeating

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After attending this presentation, attendees will better understand a very rare and devastating mechanism of death, including autopsy findings and related patient history, allowing them to completely and efficiently assess similar cases.

This presentation will impact the forensic science community by reporting a very well-documented case of gastric perforation due to overeating in a psychiatric patient that resulted in death. This study provides information regarding a rare occurrence reported through the clinical history and the results of the forensic investigation, providing an in-depth and complete point of view.

An 18-year-old female patient arrived at the emergency department complaining of feeling abdominal pain and fullness after a heavy meal. She reported a history of *anorexia nervosa* without previous abdominal surgery. At the physical examination, she was reported to be filthy with feces and had a severe abdominal distension with dull percussion and mild abdominal tenderness in the epigastric region. Intestinal sounds were absent. She also reported intermittent nausea without vomiting. A total body computed tomography revealed massive stomach dilatation. The stomach occupied the abdominal cavity and the left hemi-thorax. Physicians began an intravenous fluid replacement and attempted to place a nasogastric tube; however, during the maneuver, a generalized seizure occurred, followed by cardio-respiratory arrest. A diagnostic autopsy was requested by the hospital. During the autopsy, the pathologist observed a significant dilation of the anal sphincter; suspecting a sexual assault, the judicial authorities were alerted, as required by Italian law. The case was assigned to the Legal Medical Department for a forensic autopsy. During the second autopsy, an anal orifice expansion (3.5cm x 25cm), with no signs of violence, was observed; food was found in the epiploon retrocavity. The stomach was hyper-distended and perforated at three different points; the diaphragm was also perforated. The left lung was completely collapsed and was covered with traces of food.

The history of *anorexia nervosa*, clinical objectivity, and autopsy evidence led to the suspicion of an intra-thoracic gastric perforation secondary to acute massive dilation of the stomach due to overeating. Diaphragmatic perforation caused by the stomach is a less-common complication than gastric perforation in patients with eating disorders. Physicians must have knowledge of this complication in order to reach a quicker diagnosis, perform a timely intervention, and provide patients with all necessary information regarding possible risks. Medical-legal aspects of this event though a comparison with scientific literary evidence will be discussed.

Respiratory Failure, Gastric Perforation, Overeating

H135 Determining the Difference Between Blunt and Sharp Force Traumas in Human Head Hair

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After attending this presentation, attendees will understand how hair can be utilized as a reconstructive tool at crime scenes to assist in narrowing the cause of death when the victim's body cannot be found.

This presentation will impact the forensic science community by creating standards for microscopic hair analysis, thus allowing cases to be solved more efficiently with the use of hair as evidence.

Hair has been proven to be a useful specimen for evaluating past drug use and in distinguishing between animals and humans via the measurement of the medulla.[1] In addition, numerous studies have been published on traumatic deaths of the sharp and blunt force varieties. In sharp force trauma, homicides and suicides are the most common cause of death from stabbing or cutting inflictions.[2] On the other hand, blunt force trauma involves direct and indirect stress propagations that are typical of car accidents or beatings.[3] Recently, research was completed to show that patterns are exhibited in the hair post- trauma; however, this study was unable to define specific characteristics and was unable to link statistics to this pattern-based analysis.[4]

During this research, trauma recreation was completed for both sharp and blunt force trauma with the use of varying weapons on hair in attempt to define traits that are unique to each type of trauma. Statistics can then be applied to provide measures of consistency and repeatability of this experiment. Each hair was photographed and evaluated under crossed polars before and after recreation with a photomicrograph. One strand of hair was then taped onto a wooden circular post meant to mimic a human skull. [5] Cuts were inflicted for sharp force with the following weapons: a box cutter, glass, serrated and non-serrated knives, scissors, and flathead and Phillips-head screwdrivers. Blows were administered for blunt force trauma with a metal baseball bat, a crowbar, a hammer, a hollow copper pipe, a metal pipe, jagged and smooth rocks, and a piece of wood. Based on the makeup of these weapons, it was expected that sharp force implements would leave behind a smooth straight pattern, while blunt force weapons would exhibit a jagged pattern due to a more forceful blow needed to break the hair. This was completed for the three common race categories of hair studied in forensic science (Caucasoid, Mongoloid, and Negroid) to evaluate the similarities and differences in the trauma patterns both across and within races.

Based on the 90 hairs evaluated to date, a majority of sharp force cuts exhibited smooth characteristics with the exception of the serrated knife, which appeared as jagged due to the uneven cutting edge of the knife. The blunt force weapons exhibited much more variability, with most being smooth or a combination of smooth and jagged. The screwdrivers displayed the most combination of traits, which is not surprising as they have both a sharp edge for cutting and a blunt force component due to compression. Additionally, damage was only evident away from the break in blunt force trauma, which provides an important method to differentiate between the two traumas. Further, when damage was present, color changes under the polarized light microscope were sparse. Comparison across trials of the same race showed much more consistency within the same weapon for Negroid and Mongoloid compared to Caucasoid.

Future research will attempt to study the influences hair dyes, shampoos, and age may have on breakage characteristics. Furthermore, hair samples will be acquired from actual traumatic fatalities to compare to the above recreations, then blind samples will be given to volunteers to determine if they can differentiate sharp and blunt force trauma via the established pattern types.

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Hair, Trauma, Pattern Analysis

H136 Violence Against Vulnerable Persons: The Death of a Transgendered Individual in Mississippi

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After attending this presentation, attendees will understand the variation observed in homicide cases as it pertains to violent acts committed against vulnerable individuals (e.g., transgendered) in the state of Mississippi.

This presentation will impact the forensic science community by discussing a case of homicide in which the violence was perpetrated by a 20-year-old male against a transgendered individual.

In 2016, the body of a 28-year-old pre-operative male-to-female transgendered individual was found dead in a hotel room located on the Mississippi Gulf Coast. It was learned that the decedent had traveled to the area with friends and planned to attend a sporting event.

External examination observation revealed female undergarments and accoutrements and the presence of male genitalia; the testicles were descended into the scrotum and appeared atrophic. Upon postmortem examination, multiple sharp force injuries were identified. Approximately 190 individual stab and incised wounds were noted in the head, face, neck, axillary region, torso, chest and shoulders, back, and an extremity. Injury of the left lung, the right external jugular vein, the right subclavian vein, the larynx, and the left axillary vein were identified. There was also multifocal subarachnoid hemorrhage. Abrasions were noted on the face, neck, and chest. Defense-type injuries were present on the left hand and left upper extremity.

The skull was retained and fixed in formalin for subsequent anthropological analysis.

Images released from the decedent's hotel surveillance cameras led to the arrest of a suspect who was charged with capital murder and robbery. It was established that the decedent had prior contact with the killer before her death and had arranged to meet with him instead of attending the rodeo event with her friends. The suspect, stationed at a local military base, eventually entered into a plea agreement with prosecutors after investigators learned he had borrowed a knife from someone on base the day of the murder. He claimed that he and the victim had been chatting online for several months, but decided to meet when she and her friends traveled to Biloxi, MS, for the rodeo. The suspect said that immediately upon returning to the victim's hotel room, they engaged in anal sex. When he subsequently learned of the victim's transgender status, the suspect said he "lost it" and that he did not remember much after that.

After capital murder charges were reduced, the former Navy seaman pleaded guilty to second-degree murder and robbery. He is currently serving a total sentence of 48 years in prison.

Transgender, Homicide, Sharp Force Injury

H137 Establishing Organ Weight Norms for Caucasian and Minority Populations Using Autopsy Data From Two Institutions and the Evaluation of Autopsy Reports Using a Novel Free-Text Analysis Tool

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The goal of this presentation is to review organ weight data from adult autopsies performed across multiple demographics at two different institutions. A reference range of the expected weight for each organ examined, taking into account Body Mass Index (BMI), age, race, and sex will be presented. In addition, this presentation will illustrate the utility of macros to automate the review of autopsy reports for natural disease diagnoses.

This presentation will impact the forensic science community by providing a reference range of normal expected organ weights for unique demographic populations. This presentation will also underline the utility of developing custom programs to analyze reports.

Methods: Demographic and organ weight data was collected from 13,283 autopsies performed between 2013 and 2016 in the state of New Mexico, Office of the Medical Investigator (OMI) and from 2011 to 2016 from Birmingham, AL, Jefferson County Coroner/Medical Examiner Office, University of Alabama at Birmingham (UAB). Organs examined included brain, heart, lungs, kidneys, spleen, and liver. To evaluate adult organ weight norms, cases were excluded if they fell into any of the following categories: age less than 18 years, natural, pending or undetermined manner of death, documented postmortem changes, or greater than 48 hours between death and autopsy. In total, 2,552 cases were included in this study. Specific organs were excluded if there was evidence of trauma or natural disease that could influence organ weight (i.e., myocyte hypertrophy and heart weight). Lung weights from overdose cases were removed because of the frequency of pulmonary edema. Additionally, to better accomplish the task of removing natural disease, 400 OMI autopsy reports from 2013 were reviewed manually to flag diagnoses that would impact organ weight. After a list of diagnoses was established, a script (macro) in Visual Basic for Applications (VBA) was developed in Excel® to evaluate the same group of autopsies for natural disease. This macro was evaluated for accuracy against the manually flagged cases. Once the list of cases with normal organs (no disease, no trauma) was identified, Statistical Analysis System (SAS) was used to perform statistical analyses on the demographics and organ weights, with p-values of 0.05 or less considered statistically significant.

Results: Preliminary data indicate that OMI and UAB serve different populations and that all races, except Asian/Pacific Islander, were represented: OMI had 44.4% White non-Hispanic, 38.5% White Hispanic, 14.6% American Indian, and 2% African American, while UAB had 58.7% White non-Hispanic, 39.3% African American, and 2% White Hispanic. The OMI decedents were older than those at UAB ($p<0.0001$) and were significantly more obese ($p<0.0001$). Manner of death was statistically significant with a higher percentage of homicides at UAB (24.1%) than OMI (11.6%). BMI significantly affected organ weights, especially the liver and heart, which tended to be markedly heavier. Male organ weights were significantly heavier than females, regardless of race. African Americans had the largest mean heart weight (364 grams) while White non-Hispanics had the heaviest livers (1,709 grams).

The VBA script evaluated OMI autopsy reports and was in strong agreement (94.9% across flagged organs) with the method of manually flagging cases for natural disease. A sampling of 20 UAB autopsy reports correlated similarly. Kidney conditions were missed most often (92% agreement). Missed diagnoses were typically the result of a missing phrase in the program's vocabulary describing a diagnosis; for example, fatty liver was initially missed since the program scanned for steatosis. The script also struggled with misspelled words in reports. Of note, manually flagging cases missed several diagnoses (21 on heart alone) that the macro identified.

Conclusion: This study is the first to analyze how healthy organ weights are affected by multiple demographics, including race, BMI, age, and sex. Organ weights were most significantly increased with higher BMI and male sex. Additionally, this study developed a macro that was effective at flagging natural disease within organs. With this information, equations can be developed to predict normal weights on the majority of organs within these demographics and can establish average weights on a plethora of natural disease flagged by the macro. Additionally, there are histologic correlations for each organ; for example, correlations between histologic evidence of hypertrophic myocytes and interstitial fibrosis can be made to heart weight. Finally, future studies are planned to incorporate this organ weight data with Computed Tomography (CT) imaging data.

Organ Weight, Reference Calculator, BMI

H138 The Thanatotranscriptome: An Assessment of Messenger RNA (mRNA) Abundances in Cadaver Prostate Tissues

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After attending this presentation, attendees understand how to use the thanatotranscriptome of prostate tissues to gain further insight into apoptotic pathways after death. Precisely, attendees will understand that RNA is stable in cadavers' prostate tissues and is sufficient for profiling gene expression for up to 60 hours.

This presentation will impact the forensic science community by providing information on transcriptional mRNA abundance profiles of postmortem prostate tissues in which there is a paucity of previous studies.

Molecular autopsy has the potential to provide valuable information to establish the cause and manner of death in medicolegal investigations. Gene expression studies are well-established components of antemortem research with broad applications ranging from elucidating basic mechanisms responsible for physiological processes to ascertaining therapeutic targets in pathophysiological conditions; however, gene expression studies and their applications in the medicolegal field are still in their infancy. The thanatotranscriptome, or "transcriptome of death," involves the study of mRNA transcripts occurring in human tissues after death (*thanatos*, Greek for death). The identification of transcript abundances in human body tissues through mRNA-based profiling is potentially useful for forensic investigations.

It was hypothesized that there are detectable and significant disparities in transcript abundances in cadaver tissues from homicide and suicide victims compared to individuals who succumbed to natural causes. The intent of this study was to determine molecular markers (i.e., mRNAs) that provide accurate information regarding the cause and/or manner of death. This study investigated genetic studies involving organ- and manner-of-death-specific profiling of apoptosis gene expression panels through the application of Polymerase Chain Reaction (PCR). This procedure was performed using postmortem prostate tissues from actual criminal cases from the University of Pavia, Italy. Complementary DNA (cDNA) was synthesized and RNA concentrations were measured. The goal of this study was to provide detailed insight into expression of 84 key genes involved in complex apoptotic pathways from prostate tissues.

The results demonstrate that at 38hrs Postmortem Interval (PMI), most of the genes related to induction and positive regulation of apoptosis, Caspases, are upregulated more at 60hrs PMI. This finding suggests that apoptosis is upregulated compared to the control group. Several antiapoptotic genes, such as BCL2 and BCL2 A genes, are more expressed in 38hrs, but at 60hrs, they are drastically reduced in expression and, in some cases, to the insignificant fold-change level. These outcomes propose that initially, in the control group, cells fight with their antiapoptotic machinery; however, later, proapoptotic machinery takes over.

In conclusion, this study demonstrates that RNA molecules are stable in postmortem prostate samples, which makes RNA a sufficient molecule for gene expression studies. This study design validates a technique that will meet the demand for rapid and reproducible thanatotranscriptomic methods. These novel techniques will correlate apoptotic gene expression patterns as possible biomarkers compared to classic methods by expanding the capacity of molecular autopsy techniques.

Thanatotranscriptome, Prostate, RNA

I1 Elder Abuse: Perception and Knowledge of the Phenomenon by Healthcare Workers From Two Italian Hospitals

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After attending this presentation, attendees will better understand the importance for forensic and healthcare practitioners to recognize early signs of physical, psychological, and emotional abuse on elderly people.

This presentation will impact the forensic science community by demonstrating that the absence of early recognition of elder abuse can be a major disadvantage for the community.

Background: With a rapidly aging population in countries worldwide, the number of elderly adults vulnerable to abuse, neglect, and exploitation is expected to grow. Hospital personnel usually play a crucial role in identifying mistreatment and making appropriate referrals as they are usually the first people with a medical background to see these victims. Physicians and nurses are also mandatory reporters of elder abuse in accordance with state requirements.

Goals: To establish a level of awareness and perception of elder abuse by healthcare workers, to understand if these workers are able to recognize and report elder abuse properly, and to identify the physical elements of abuse and neglect.

Methods: The research tool was a survey of 35 questions. Selected questions were chosen from previously validated questionnaires used in other similar published survey studies. Participants were represented by physicians, nurses, and care assistants working in the internal medicine and geriatric services of two different University Hospitals, representative of the Italian public health system in Southern Italy (Cardarelli Hospital of the University of Molise and Policlinico of the University of Bari "Aldo Moro").

Results: The results included 98 of 142 administered questionnaires (69.0% response rate). All data were further analyzed, taking into account age, gender, work experience, and qualifications. For the majority of all personnel, neglect represents a type of abuse; however, approximately 40% of the physicians and 37% of the nurses considered the concept of abuse to be false. The surveyed population was aware that many seniors are victims of abuse and that elder abuse is a form of violation of personal rights, but 46.94% were unsure about the existence of standard procedures for reporting abuse and/or treatment, suggesting low attention paid to the problem and little information provided by institutions on abuse reporting procedures. Regarding whether participants suspected abuse, most nurses (45.7%) and care assistants (68.8%) declared they had never had suspicions of abuse, while 48.7% of physicians stated suspecting abuse on one to three occasions in their lifetime. Only 23.9% of the nurses, 22.4% of the physicians, and 18.8% of the care assistants stated they had witnessed abuse between one and three times in their lives. Surprisingly, in both suspected or witnessed cases, the health care personnel did not take any action and did not report the abuse to public authorities or to adult protective service agencies.

Conclusions: The results of the present survey on health care professionals demonstrate that there is still a strong need for education and specific training programs on elder abuse.

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Caregivers, Elder, Abuse

I2 Financial-Psychological Crime: The Madoff Case

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After attending this presentation, attendees will better understand the link between financial fraud and psychopathy.

This presentation will impact the forensic science community by exploring the psychological and forensic profiles of victims and perpetrators, with the goal of demonstrating how some individual attributes and characteristics, such as a respectable front, charisma, or strategic presentation of a self-image, are positively correlated with the fraudster profile.

In 1920, Charles Ponzi was the first to conduct a large-scale tax fraud scheme. The basis of a Ponzi scheme relies on individuals entrusting a portion of their capital to a subject mediating between the individual and the market, in a process known as financial mediation. The trigger of the scheme is the faith the client has in the intermediary and his superior knowledge in this field. In the fraud mechanism, the perpetrator needs to convince future victims to allow him/her to handle their savings in order to make investments that do not actually exist, promising relatively large returns in a limited period of time. The client/victim, who first obtains financial gains that satisfy his/her expectations, continues to invest and convinces other people to do the same.

At the end of 2008, a famous New York broker and ex-president of the National Association of Securities Dealers Automated Quotation (NASDAQ) named Bernard L. Madoff was accused of fraud and was arrested. Madoff succeeded in perpetrating the fraud thanks to his ability to handle first impressions. In other words, he possessed the specific communication trait that can manage the fundamental human tendency to imagine, plan, and regulate social behavior in such a way as to leave a strong impression on others, convincing them of the truth of the self-image presented. In psychological terms, what convinces people to invest in such plans is the fraudster's ability to exploit people's weak points, such as greed, trust, and fear. According to Schiller's theory of the positive feedback investment cycle of large-scale bubbles, when many people believe they will receive good profits, they communicate their belief to other potential investors, making it seem like a mistake to miss out on the investment.[1] This triggers phenomena such as familial bias and imitation: the stories and perceptions of the profit to be gained from a given investment make it appear to be affected by a minimum or negligible risk. As persuasion tools, these are even more effective than the fraudster's own persuasive strategies. In fact, according to Greenspan, the success of the Ponzi scheme was attributable to the human tendency to model actions on those of others, especially when dealing with matters that the client knows little about.[2]

Typical traits of the victims of these schemes are a limited knowledge of financial matters, little available money, older age, or a low level of education (excluding those affected by mental disease).[3] Characteristics of the "fraudster" are being male, between the ages of 35 and 65, an advanced education, "a respectable front" (strictly connected to the self-image presentation strategy), charisma, and a marked ability to handle social relationships.[4] One of the most devastating consequences of such schemes is the fraud trauma syndrome that engenders emotions such as rage, pain, anxiety and fear, mistrust of the future, isolation and devastation, as well as symptoms such as insomnia, panic, anxiety attacks, or depression.[5] The line between fraud trauma syndrome and post-traumatic stress disorder is becoming less and less clearcut.[6] The sense of betrayal of the trust placed in the fraudster provokes a feeling of abuse and violence in the victim. Many victims report that they have even considered committing suicide, or have suffered a worsening of preexisting medical conditions. Similar to victims of rape, victims of fraud tend not to denounce the crime they have suffered due to feelings of guilt induced by the social and legal systems.

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Criminalistic, Financial Fraud, Persuasive Strategies

I3 Sudden Death of a Child: What Could Have Happened in a Family With a Different Socioeconomic Status?

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After attending this presentation, attendees will better understand the importance of the diagnosis of malnutrition and failure to thrive in a child death investigation.

This presentation will impact the forensic science community by providing elements to diagnose the exact cause of death in a child neglect or abuse case as well as the legal outcomes which may result.

Fatal child starvation is uncommon in Italy, and, according to the National Statistical Data, malnutrition is related to being overweight and morbid obesity.

Child abuse and maltreatment are defined as a series of deliberate actions and/or omissions that are conducted by adults (parents, relatives, caretakers), or other children or adolescents, that result in physical or emotional damages or the imminent risk of serious damage or death. Maltreatment/abuse can be expressed as: a failure to provide age-appropriate care, spousal abuse in the child's presence, psychological maltreatment, physical maltreatment, and sexual abuse. Classifying various forms of child maltreatment is useful for exemplifying goals, but children are usually victims of different types of maltreatments simultaneously. The neglect of a child without appropriate care is an ongoing pattern of inadequate care by parents and caregivers. It includes child neglect, lack of care, inappropriate care at the time, and excessive care.

Child neglect is defined as a type of maltreatment related to the failure of parents to provide for the child, which could cause serious damage. The signs of child neglect are a sense of abandonment, refusal, failure to thrive, or other forms of abuse (which may be life threatening). Neglect has received less attention than physical and sexual abuse, probably because it is difficult to identify and often includes other forms of maltreatment.

This case study describes a 2-year-old girl who was found dead on the sofa by her mother's partner. The child was known to social services for suspected abuse after being admitted to the hospital for a fracture of an upper limb and bruises, which the mother reported as an accidental fall. Furthermore, the child was suffering from psychomotor retardation associated with serious self-inflicted injuries with bites, hitting her head against the wall, and pulling out her own hair. Consequently, she was transferred to neuropsychiatry, but the drug therapy (periciazine) administrated by her mother was ineffective. A week before her death, the child was admitted to a pediatric hospital with severe anemia, failure to thrive below the third percentile of growth, and electrolyte imbalance; however, the mother decided to take the child back home, against the advice of doctors.

The case had multiple risk factors leading to neglect: the mother's young age, low socioeconomic status and education, dysfunctional family characteristics (child's parents were half-siblings, adopted by two different families), the presence of an adult unrelated to the child (mother's new partner), and parental stress.

The case study included the scene investigation, autopsy, toxicology, and police investigation. The external examination was remarkable for multiple limb bruises, bitemarks on her hands and upper limbs, and scratches on the face. The autopsy was negative. There was no chest or head trauma, only the results of the known upper limb fracture. The gross examination of the heart revealed left ventricular hypertrophy and histology exhibited the usual finding of restrictive cardiomyopathy, such as interstitial fibrosis. The toxicological test was positive for a therapeutic range of periciazine.

Finally, the cause of death established was cardiac arrest due to restrictive cardiomyopathy, which is known for having a poor prognosis in children. This case met legal challenges due to disagreements regarding the cause of the child's failure to thrive. In other words, the legal question was whether malnutrition was due to neglect or to cardiomyopathy. In the end, although neglect was not the cause of death, it certainly was related to it. In any case, studies reveal there is poor knowledge of child neglect among medical resources, which leads to a higher rate of sickness and death among these children. This case report illustrates that even in highly suggestive cases of abuse or neglect, it is necessary to refer to the report of the child's death investigation and pay attention to risk factors in the family context; beginning from the crime scene investigation and medical records to the autopsy and histological findings.

Establishing the cause and the manner of death may lead to different legal outcomes in cases that clearly involve child abuse or child neglect.

Child Neglect, Cardiomyopathy, Maltreatment

I4 Self-Cutting and Suicide Risk Among Adolescents: The Case of the "Blue-Whales"

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After attending this presentation, attendees will understand the importance of training healthcare staff to promptly recognize suicidal risk behaviors among adolescents.

This presentation will impact the forensic science community by providing information regarding the media phenomenon known as the "Blue-Whale-Challenge," which includes self-cutting; this carries a high potential risk of suicide.

The Blue-Whale-Challenge is a dangerous internet phenomenon that consists of a series of duties, imposed by an administrator to players who must complete a list of actions involving self-mutilation. The game lasts 50 days, with players usually completing one duty per day, and ends with the suicide of the player.[1] The term "Blue-Whale" results from the similarity of suicidal behavior among beached whales. The phenomenon began in 2013, in Russia, on the "VKontakte" social network, with the first case of suicide in 2015. The inventor was a psychology student, expelled from his university. The student said that his purpose was to clean society of people of no value.[2] The phenomenon spread among teenagers in Russia, then in other areas, due to the media resonance caused by television broadcasts and newspaper articles that featured information regarding the game. After a wave of panic caused by an article concerning the many suicides related to the Blue-Whale phenomenon in Russia, the inventor was arrested in 2016.

Around the world, the phenomenon accounts for many cases, some of which have often turned out to be hoaxes or emulators. In Italy, the first news about the Blue-Whale appeared in June 2016 in a national newspaper, but only in May 2017 did a well-known television program deal with the subject, using reports that did not quote official sources. From that moment on, the police began receiving calls and newspapers published alarming news concerning Blue-Whale incidents, which were often quickly denied. Between May and June 2017, five suspected cases of Blue-Whale have been managed by the officers of the unit. The officers were dedicated to the evaluation of suspected abused children ("Bambi") of the "Ospedale Infantile Regina Margherita" (Turin).

The data collected during the multidisciplinary evaluation of these cases was reported: all patients were female; one case involved a 17-year-old girl, while the other four cases involved 14-year-old girls; all families experienced critical economic, social, or psychological issues; in two cases, the girls' parents were separated; in one case the father had been sent away from home as he beat his wife and daughter; and the last girl described her father as extremely aggressive and oppressive. During the psychological evaluation, all girls recounted difficulties in integration at school and anxiety. One girl confessed to having purposely taken an excessive dose of alprazolam. In three cases, the medical examiners identified scars related to previous self-cutting. In one case, this activity was recent and the lesions on the forearm were thought to resemble a whale. There was no evidence to sustain an involvement in the Blue-Whale-Challenge or the influences of an administrator in any of these cases, but rather emulative behavior caused by psychological issues. Only one of these girls was hospitalized, while psychological help programs were prescribed for the other girls.

Self-harm is the strongest predictor of suicide among young people. Between 40% and 80% of suicide victims had self-harmed in the past.[3,4] In particular, longitudinal data indicated that self-cutting is a significant risk factor for complete suicide in children and adolescents.[5] Young people who self-harm report that it is difficult to talk about their suicidal feelings and they do not really feel "listened to" when they do.[6] Indeed, clinical staff often has a negative attitude toward self-harm and may not possess the ability to deal with it effectively. In this series of cases, there was a strong demand for attention, illustrated through self-cutting and simulation of participation in a life-threatening game. In accordance with the literature, all of these young people experienced negative life events and had difficulties in relationships with families and friends.[7] Given these premises, it is crucial that frontline medical staff receive training about self-harm and suicidal behaviors to reduce suicide rates. It has been demonstrated that even short-term training can significantly improve staff attitudes.[8] This presentation provides attendees with a better knowledge of behavioral and psychological factors that highly increase the risk of suicide among adolescents.

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I5 Acute Stress Disorder (ASD) Symptomatology and Crime in a Nationally Representative Sample of Youth

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After attending this presentation, attendees will appreciate the correlation between ASD symptomatology and criminal behavior in youth and will use this knowledge to implement early interventions for traumatized youth.

This presentation will impact the forensic science community by presenting data that will encourage clinicians and policy-makers to pay close attention to symptoms of ASD in youth, as early intervention may help prevent the development of Post-Traumatic Stress Disorder (PTSD) and potentially may deter youth from committing crimes.

No previous studies have examined the relationship between ASD and criminal behavior among youth. This study looked at data from the National Comorbidity Survey-Adolescent Supplement (NCS-A). Participants in this survey, which took place between February of 2001 and January of 2003, consisted of 10,148 youths between the ages of 13 and 18 years. Interviews were conducted using computer-assisted personal interviews, computer-assisted telephone interviews, and telephone interviews.[1] This study hypothesized that analysis of a nationally representative sample of youths would reveal a relationship between symptoms of ASD and criminal behavior. Since the NCS-A was conducted prior to the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*), symptoms of PTSD from the *DSM-IV* were mapped to *DSM-5* criteria for ASD. Due to changes in the *DSM-5* criteria for ASD, the youths in this study were described as having "ASD symptomatology." Data were analyzed using the Statistical Package for the Social Sciences (SPSS) to estimate prevalence rates of ASD symptomatology and examine the relationship between ASD and criminal involvement.

Youths with ASD symptomology were significantly more likely to report arrest-related crimes than youths without any lifetime diagnosis. Youths with ASD symptomology had 25.4 greater odds of a report arrest for a violent crime when compared to youths who did not meet criteria for any lifetime diagnosis. Additionally, youths with ASD symptomology had 8.3 greater odds to have reported arrest for property crimes and 17.9 greater odds to have reported arrest for "other" crimes compared to youths without any lifetime diagnosis. Youths with ASD symptomology had 11.6 times greater odds of reporting, but not being arrested for, a property crime, 24.2 times greater odds for a violent crime, and 10.9 greater odds for any other crime when compared to youths who did not meet the diagnostic criteria for any *DSM-IV* lifetime diagnosis.

The data presented here provide a more accurate picture of the relationship between ASD and related crime, allowing for prevention and intervention strategies to be potentially developed. Focusing on at-risk youths to provide support and education is crucial. Due to the inevitable nature of certain trauma, intervention strategies must be catered to those youths who are experiencing ASD symptoms. Without addressing youths who are experiencing symptoms of ASD, their conditions may progressively worsen into more severe trauma-related disorders.[2,3] The quality of life for these untreated youths decreases severely and serves as an increased risk factor for criminal involvement, suicide, and other comorbid psychiatric disorders.

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Acute Stress Disorder, Crime, Adolescents

I6 Female-Perpetrated Sexual Abuse on Children: A Five-Year Long Italian Experience

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After attending this presentation, attendees will better understand the gender-specific features of child sexual abuse perpetrated by women compared with male-perpetrated abuse.

This presentation will impact the forensic science community by providing tools to recognize a form of sexual abuse often difficult to identify because of its intrinsic characteristics.

In the literature, little attention has been paid to females who sexually abuse as most of the studies focused on male perpetrators.[1] The phenomenon has typically been reported as relatively rare with a male-to-female sexual offenders ratio equal to 20:1. Available data indicate that women constitute approximately 5% of all sexual offenders.[2]

Despite this, female-perpetrated abuse is responsible for a non-negligible number of victims and offenders who need clinical attention. In addition, the phenomenon is certainly underestimated because it is difficult to diagnose females for several reasons: sexual abuse is often perpetrated by women who care for the child during routine daily activities, such as bathing or dressing, so sexual offending is often hidden behind the woman's caretaking behavior.[3] Moreover, in most cases, a strong affective bond between the victim and the offender is created, so that the child does not reveal the abuse. The emerging cases that are reported for investigation appear to be the "tip of the iceberg" with respect to cases that are not reported. Finally, there are usually no clinically detectable lesions because sexual abuse is typically not conducted via violent acts.

In order to contribute to the knowledge of this topic, this study reports the data collected from January 2012 to June 2017 in the multidisciplinary unit called "Bambi," dedicated to the evaluation of suspected abused children, of the "Ospedale Infantile Regina Margherita" (Turin). Among 474 cases of suspected child sexual abuse, in nine cases (1.9%), the potential perpetrator was a woman: one child was male and the others were female. The children were between 3 and 9 years of age (four 4-year-olds, two 5-year-olds, two 9-year-olds, and one 3-year-old). In five cases, the parents of the children were divorced. The perpetrators in two cases were the mothers of the children, in four cases the paternal grandmother, in two cases a female neighbor, and in one case the babysitter. In only one case was the sexual abuse conducted in association with other perpetrators. One of the perpetrators already had a diagnosis of psychiatric disorders (borderline disorder and depression). In all cases, the perpetrators committed the abuse by touching and licking the ano-genital area, and in four cases, by also penetrating the vagina or anus. In one case, the culprit forced the child to watch porn videos. All children, except one, spontaneously described to the medical staff members the abusive actions perpetrated by the offender. More detailed information on the clinical and judicial path of each case will be provided during this presentation.

Although it is a common belief that if a female is involved in a sexual abuse, she must have been forcefully coerced by a male partner, the reported cases, in accordance with previous studies, suggest this is often not true.[4,5]

Considering the unusualness of the phenomenon, although many treatment requirements appear to be similar to those of male sexual offenders, it is crucial that the gender-specific features of the phenomenon be taken into account, rather than attempting to fit female sexual offenders' treatments to existing male models.[1]

This presentation provides attendees with additional knowledge regarding female-perpetrated sexual abuse on children in order to understand gender-specific criminal offending patterns and provide an important tool in the development of prevention and rehabilitative strategies.

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Child Abuse, Female Perpetrators, Sexual Abuse

I7 Sexual Offending and IQ: What Is the Relationship?

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The goals of this presentation are to: (1) inform those involved in assessments of persons who have sexually offended of considerations of intellectual functioning as it relates to etiology and, in turn, risk of reoffending; and, (2) provide treatment providers with data on the potential impact of intellectual functioning for treatment planning use.

This presentation will impact the forensic science community by improving the ability of evaluators of sexual offending behavior to determine etiology. This presentation will also impact attendees by helping risk and treatment providers to develop treatment plans with likelihoods of successful outcomes.

The current proposed presentation will focus on the range of intelligence estimates in a sample of adult men convicted of sexual offenses. Specifically, this presentation will include the data of approximately 1,040 individuals who were serving time for a sexual offense conviction and being assessed for potential civil commitment under the South Carolina Sexually Violent Predator (SVP) Act. A previous meta-analysis of 25,146 adult male sex offenders reported that sexual offenders obtain lower Intelligence Quotient (IQ) scores than non-sexual offenders, but that this difference is likely accounted for by the large proportion of individuals with sex offenses against children in those studies.[1] Additionally, a relationship between IQ score and the presence of pedophilic disorder was noted.[1] Based on previous research, it was hypothesized that as the offenders' intellectual functioning (i.e., IQ score) decreased, so did the age of the victims. [2,3] This study hypothesized that offenders with below-average IQ would have younger (i.e., at least pre-adolescent age range) victims than those with average or above-average IQ scores. It was also hypothesized that as IQ score decreased, the report of childhood sexual abuse victimization of the offender would increase. This study hypothesized that the offenders with above-average IQs would be more likely to have no other criminal history beyond the sexual offense(s). Finally, it was predicted that the presence of a pedophilic disorder would be associated with lower IQ scores than of those offenders not meeting diagnostic criteria for pedophilic disorder. Preliminary analyses indicate that IQ scores in this sample ranged from 43 to 164, with a mean IQ score of 91.88 (*Standard Deviation (SD)*=16.00). Approximately 35% of the sample (*n*=364) had been diagnosed with at least one mental illness, to include paraphilic disorders (*n*=25). The sample had an average of 2.17 (*SD*=2.57) victims and the number of victims ranged in age from 1 to 45 years. This presentation will also address the challenges of providing effective sex offender treatment to offenders with intellectual impairments.

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Sexual Offenders, Intellectual Functioning, Sexually Violent Predators

I8 Do Sex Offenders Secretly Reoffend During Treatment?

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The goals of this presentation are to provide insight on: (1) the likelihood of unreported sexual reoffenses occurring during treatment; (2) which sexual reoffenses are more likely to occur; (3) the likelihood of sex offenders disclosing their reoffenses to health care professionals; and, (4) the period sex offenders are more likely to reoffend.

This presentation will impact the forensic science community by debunking misconceptions of persons who have committed sexual offenses. Attendees will become more aware of the types of reoffenses that are most likely to be secretly committed by sex offenders. Persons undergoing treatment for problematic sexual behaviors will be given hope that the treatment outcome is usually positive.

It was hypothesized that if reoffenses were reported, hands-off reoffenses were more likely to occur than hands-on (contact) reoffenses. The other hypothesis was that the majority of relapses and/or reoffenses would have occurred between six months and one year of treatment, if any were reported.

The methodology of this study is unique in the sense that it is virtually untraceable and provides complete anonymity and confidentiality to its participants. An anonymous poll booth was set up in a room where group therapy normally takes place and was located away from cameras and possible prying eyes. Participants were instructed to complete the survey during the break or discreetly during group therapy. They were to go behind the cardboard trifold and had the option of wearing cotton gloves in case of concerns regarding tracing their responses through fingerprint analyses. Participants were also instructed to use the felt-tipped pen provided so all answer sheets would look identically filled and their responses would be untraceable through any form of handwriting analyses. After completing the survey, they were to drop their responses through the slit of a sealed cardboard box. Before starting the survey, a deck of cards was passed out to all participants and they were to choose one card from the deck. The research assistant had a second deck of cards, which was identical to the first deck given to participants. The second deck was shuffled in front of the participants and a card was randomly selected from the deck. The group was told that the participant with the matching card was told not to reveal himself/herself and was also told to complete the survey with false responses and claim that he/she had reoffended. This would provide assurance to participants that if they had disclosed reoffenses, it would be virtually impossible to distinguish their disclosure from the assigned liar's disclosure. Participants then completed the survey discreetly during group or during the break.

Results of the study revealed that the majority of sex offenders did not reoffend while in treatment. When reoffenses did occur and were not reported, the majority of those reoffenses were breaches of conditions. The least likely re-offense to occur, even in secret, were contact offenses that involved sexual touching of a child and/or sexual touching of an adult. Re-offenses were also most likely to occur between the first six months and one year of treatment. Those who had secretly reoffended were also unlikely to disclose their relapses to their doctors.

Given that most reoffenses reported were non-sexual breaches, professionals in charge of supervising and/or re-integrating offenders back into the community can improve on preventing breaches. Since pornography-related offenses were the second-most likely to occur, prevention methods may need to be installed to reduce accessibility of illegal pornography. Furthermore, these results inform professionals that treatment can prevent reoffenses, but when reoffenses occur, it is unlikely their patients will disclose those incidents. It also cautions professionals to be vigilant of potential reoffenses during the first six months to one year of treatment. Future research should investigate factors that increase the likelihood of disclosure to health professionals or disclosure of a pending relapse.

Sex Offender, Paraphilia, Sexual Reoffenses

I9 The Unfolding Development of Forensic Behavioral Science

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After attending this presentation, attendees will apply a deeper understanding of the underpinnings of their fields to their work and, as a result, will enjoy more satisfying conversations with colleagues of other disciplines.

This presentation will impact the forensic science community by increasing appreciation for gathering and applying sound information — both new and old.

The founding of the American Academy of Forensic Sciences (AAFS) marked the commitment of serious professionals in each of the forensic sciences to contribute to the unending progress in the quality of their work. Their intention applied to both the reliability and validity of the methods and procedures to use in each field. In doing so, our founders expected to promote justice and thereby serve not only parties in litigation, but all humanity as well.

Regarding the behavioral forensic sciences, it is useful to understand their history as developing over a four-stage course, beginning with a time of genuine, although poorly acknowledged, ignorance. In time, it became difficult to demonstrate that behavioral experts' opinions offered anything better than random chance; the equivalent of a coin toss.

Experiencing the painful impact of even moderately good scientific methodology on their theory-based testimony sufficed for most experts to usher in the second developmental stage. By roughly the early 1970s, working groups from several universities began the publication of statistical scales, based on subjects' behavioral histories, medical and social histories, and varied additional data yielding estimates of probabilities of future behaviors.

Meanwhile, the age of scans, the third developmental stage, was getting underway. In the United Kingdom during the mid-1970s, engineers working for Electronic Music Incorporated (EMI) developed crude planar images of living human subjects' brains. To do this, they took advantage of technical refinements in the generation and detection of X-rays. Soon enough, these "EMI scans" became valued for their clinical utility. In the forensic arena, their use has demonstrated both the value and the danger of the saying that a picture is worth a thousand words. Having a basic understanding of the workings of the more recent scanning technologies, especially Magnetic Resonance Imaging (MRI), only strengthens this recognition.

The final phase, for the present at least, is that of genetics. Here, the behavioral forensic specialist needs to comprehend at least the elementary jargon of the DNA expert and related forensic specialists. Any efforts to do so are soon handsomely repaid. As progress in this fourth stage continues, we may look forward to important conversations among forensic experts as they recognize the many diverse likenesses in their professional DNA.

Development, Interdisciplinary, Progress

I10 Application of the Equivocal Death Psychological Autopsy for Investigation: A Case Study

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After attending this presentation, attendees will better understand the role of the Equivocal death Psychological Autopsy (EPA) for investigation.

This presentation will impact the forensic science community by discussing the specific technique of EPA as applied during the investigation process in cases registered as a suicide and reinvestigated from the point of view of homicide.

Forensic psychological evaluation has contributed significantly as an aid to investigation in many sensitive cases. There are various subfields of forensic psychology and one such technique is that of the psychological autopsy. A psychological autopsy is an extremely important tool to ascertain the mental status of any individual before his or her death; it throws light on various facts that may have been missed during the investigation. An EPA is useful in aiding the investigation process, especially in controversial deaths. The technique of EPA is relatively new in Italy. This particular technique was applied during an investigation for a referred case in which there was a controversy in terms of the investigating agency and the family in deciding between suicide and homicide.

Introduction: Forensic psychology deals with the application of principles of human behavior and cognition to the legal, civil, and criminal delivery system. It is also the scientific discipline dealing with the understanding of factors that culminate in the expression of violent and legally unacceptable behavior; this brings the perpetrator of the actions under the focus of law and the need for specialized rehabilitation. A forensic psychologist tries to understand the causes of criminal behavior and tries to establish a link between the crime, crime location, the victim, and the offender; however, forensic psychologists also try to work in areas related to victimology or the victim's psychology. Forensic psychologists also attempt to understand why a particular victim was chosen, as this aspect throws light on the offenders modus operandi. Similarly, forensic psychologists also perform psychological autopsies in equivocal deaths. Equivocal death analysis is by far the most demanding work. An equivocal death analyst requires extensive information about the victim and circumstances surrounding his or her demise before rendering a knowledgeable opinion of the victim's personality and behavior. The goal of equivocal death analysis is not to prove the manner of death, but to arrive at an informed opinion as to whether a homicide, a suicide, or an accident most likely occurred. Psychological autopsy is a retrospective psycho-social examination of a descendent to the time of his or her death. It is an extension of victimology that reconstructs the deceased's psychological state before his or her death. This presentation attempts to portray the importance of the EPA technique in an equivocal death case of a young man whose death was initially registered as a suicide and was reinvestigated as a homicide due to various controversial aspects in the overall case.

Case Report: This case has been tested through the method of psychological autopsy. The case involves a 48-year-old male who was found dead (by hanging) by his best friend. The case was initially declared to be a suicide; however, his family insisted that it was a homicide and not a suicide. The case was thus referred for an EPA to better understand the entire case and to check for investigative leads. The EPA was conducted using a detailed study of his personal diary, his postmortem report, and the court petition filed by the family. Information gathered from interviews with key informants by means of direct interviews and past photographs were also utilized as sources of information for this report. The psychological autopsy method entailed reconstructing the biography of the deceased through psychological information gathered from personal documents; police, medical, coroner records, and first-person accounts, either through depositions or interviews with family, friends, coworkers, school associates, and physicians.

Results: The results revealed that, with deep psychological investigations, the possibility of homicide cannot be ruled out completely.

Conclusions: Psychological autopsy is an important and valuable tool for aiding an investigation. This presentation provides detailed information regarding an individual's death using various sources and reveals new points that could have been missed during the initial investigation process. This technique is an investigative approach that provides direction in equivocal deaths and attempts to bring justice to the victim.

Equivocal Death, Psychological Autopsy, Forensic Psychology

I11 A Review of More Than 20 Parricides and Crime Scene Behaviors: Does It Differentiate Mental Illness, Psychopathy, or Abuse as the Reason for Killing Parents?

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The goal of this presentation is to further attendees' understanding of parricide and crime scene analysis to better understand the motive of the crime.

Parricide, while a rare event, has devastating impact on families and others. The crime scene could show details as to the motive of the crime. This presentation will impact the forensic science community by demonstrating how, with further analysis of the crime scene, the motivation could be better extrapolated to improve treatment of cases with mental illness or abuse as compared to psychopathic intentions.

Children killing their parents is a rare event that falls into three areas or profiles: (1) children that have mental illness; (2) children that are abused; and, (3) children that are psychopaths. Parricides, while rare, are one of the most sensational crimes that attract the attention of the media, clinicians, and researchers worldwide. Much research has been conducted on the topic of parricide despite only being approximately 1.5% to 2.4% of all homicides yearly. Often research focuses on the offender and the victim's characteristics to be able to evaluate the crime and motive; however, there is very limited research into the crime scene behaviors. When looking at more than 20 cases of parricide and reviewing the crime scene information from case information in addition to researching data from public records, data has shown that one-third of the children who kill their parents are abused, one-third are mentally ill, and one-third are psychopathic killers. When looking at crime scenes, there is information that can be helpful to both police and mental health providers about the motive of the crime.

This study, after observing the patterns of the crime scene, anticipates demonstrating that children who are abused or mentally ill are more likely to cover the body or even stay in the home with their deceased parents' bodies. Psychopathic offenders will demonstrate less care at the crime scene and not have the empathy to cover or disguise the bodies due to their lack of connection to the parents. Most research has focused on the demographics, motives, social, legal and psychological factors, such as mental health and abuse history. Minimal research of crime scene behaviors in parricide cases have been explored. Crime scene profiles or analyses have been examined when investigating other cases, but not specifically for parricides. When the research has been examined at parricide cases, the focus has been on age and weapons were used, not the basic crime scene information. Often, the basic crime scene details are dismissed, but they can be an invaluable tool for investigators and police in helping to understand the offender of violent crimes. By scrutinizing data from crime reports, publications, and cases, the data on the crime scene can be used to better evaluate the connection of the crime scene data to the reason for the parricide. This concept has not been examined in parricide cases, which could help better understand the events and circumstances that lead an individual to taking their parents' lives.

To examine the components of crime scene behaviors and their implications within parricide, data from adolescent parricides will be presented. This study will present preliminary data concerning parricide offenders and crime scene behaviors, such as the condition of the body when found (covered, face covered, moved, or hidden); the type of attack (blitz, surprise, conned/deceived); the presence of defensive wounds; signs of overkill; and the weapon used.

Parricide, Psychopathy, Crime Scene

I12 Narcissism and Violence: Criminological Understanding in a Homicide Case of Complete Decapitation

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After attending this presentation, attendees will be able to recognize some of the characteristics of the relationship between narcissism and violence as well as the role of self-conscious emotions, including problems of self-esteem, in the etiology of a homicide case involving complete decapitation.

This presentation will impact the forensic science community by demonstrating: (1) how narcissistic behaviors have an important influence on forensic psychiatric evaluation results; and, (2) the need for reliable and more careful attention to both the subject's history as well as investigation and evaluation results.

According to the literature, narcissists tend to become violent when they are confronted with a threat to the self. Nevertheless, the role that provocation plays regarding the personality of the aggressor has not been studied very much from a clinical criminological perspective. In order to better assess the importance of understanding the findings of forensic psychiatric evaluations in such circumstances, this study provides a case of intra-familial homicide in which a nephew shot his uncle with a firearm due to an apparent territorial dispute, after which he completely decapitated the victim and disposed of the head.[1]

The information acquired during psychiatric investigation revealed a man who was deeply troubled by family conflicts that began in childhood; his relationship with his parents was characterized by deep ambivalence, as well as a lack of stable and well-defined object relations. These elements conditioned the development of thought processes and behavior, which are characterized by intense feelings of inadequacy toward one's fellow man. The offender coped with these emotions by avoiding social relationships and having the tendency to be highly controlling of the world around him. The subject also exhibited a deeply fragile identity that was brought about by the ambiguity he experienced during his childhood. It was for this reason that he felt empty and "out of place" and sought out idealized figures who would provide him with an apparent sense of stability that would allow him to construct an acceptable ego. Among these figures, one uncle stood out who was a role model for the subject from when he was very young. Later, the uncle became very strict in addition to being an obstacle to the offender's ability to develop a healthy self-esteem. This uncle also threatened to expropriate a piece of land that was allegedly owned by the nephew.

This homicide presents an important paradox: the gap between the obvious horror and high level of destructiveness of the act committed and the apparent banality of the motives and the reason he killed in such a manner. In fact, expert testimony has demonstrated that violent acts directed toward the uncle were neither premeditated nor the result of psychotic elements. On the criminological level, understanding the motive can be found in both the combination of the killer's personality (depression and narcissism) and the triggers for his violent behavior (the grudge he held against the victim, who was guilty of closing the door on their relationship). This was a source of great humiliation and embarrassment for the perpetrator as he believed this abandonment to be both unjust and unfounded. The ensuing suffering he endured triggered a desire for revenge related to the offenses he endured (the uncle's unacceptable behavior) and for the latest narcissistic wound that was inflicted. In other words, the key to reading the crime lies in comprehending how the perpetrator's personality not only developed but was also grafted onto the victim's.[2,3]

This homicide has its origins in the distorted relationship between the two subjects, one of whom was convinced to have incurred irreparable damage. The other is merely the projection of the perpetrator's true enemy, who, in reality, does not exist and is imaginary in nature. In cases such as this one, unlike in serial murders, there is a scapegoat onto which the accumulated anger and aggression may be directed. The victim becomes the unsuspecting symbolic intermediary and symbolic message of the murderer.

One wonders about the nature of the relationship between the perpetrator and the victim. What could have motivated such violence in which the nephew (the offender) first shoots a pistol at his uncle and subsequently decapitates him using a machete? Following psychiatric evaluation, the perpetrator was found to have a narcissistic personality.

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Narcissism, Violence, Decapitation

I13 Holy Crime: Sexual Abuse of Minors by Priests

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After attending this presentation, attendees will better understand the psychopathic mechanisms underlying a phenomenon that spread in some realms of the Catholic church, namely the sexual abuse of minors.

This presentation will impact the forensic science community by serving as an example on which to base a scientific explanation of the phenomenon not only in terms of the victims, but also of the offenders.

The John Jay Report of 2004 revealed that in most cases of sexual abuse of minors that came to light concerning the Catholic church in the United States between 1950 and 2002, the episodes involved puberal, preadolescent, and adolescent minors, and so should really be referred to as ephebophilia (offence against minors aged 10 to 17 years). In total, 4,329 priests (accounting for 4.3% of parish priests and 2.5% of priests belonging to religious communities) were accused, on plausible evidence, of the sexual abuse of minors.[1]

Sexual abuse by priests seems to be a reactive behavior likely generated by insufficient emotional, affective, sexual, and relational maturity; in other words, a compensation mechanism that attempts to fill a void of affection, erotism, and sexuality. The abuse is seen and rationalized as similar to masturbation or pornography so that it does not tarnish the public function of a church minister. The choice of a minor is also due to the fact that satisfaction is thus sought outside the commitment of a relationship.

The John Jay College of Criminal Justice reported (referring to 2004) that 64% of the accused priests had abused males only, 22.4% females only, and 3.6% had abused both sexes. This has very often led to attributing the plague of sexual abuse to an increase in the number of homosexual priests.[1]

In fact, it is sexual immaturity, due to entering a seminary at an early age, and the lack of any sexual education, together with a strong vulnerability to narcissism (in relational terms), that leads the subject to turn his attentions to young people of both sexes. The youths are perceived as psychosexual peers. The sexual choice (mostly male) largely reflects opportunity rather than sexual leanings. It is easier to approach boys and there can be no fear of unwanted pregnancy, as is risked in relations with puberal or postpuberal girls. Finally, some priests conceive of celibacy as the abstention from sexual relations with women. Therefore, they convince themselves that sexual relations with boys do not contravene their vow of celibacy. The boy is seen simply as a means for obtaining pleasure in all safety.

Those most likely to suffer abuse by ministers, the victims, are generally young people with some social or physical lack, who are therefore vulnerable. At first, they feel "special" because they have been chosen by the charismatic figure of the priest, but later they develop conflicting, confused feelings during the episodes of abuse, and, finally, they feel betrayed and alienated from the church.

Sexual abuse by a priest is a sexual and relational betrayal perpetrated by a Father Confessor of the community — a man the child has learned to trust more than any other since birth. Therefore, it is a psychological shock for the victim, provoked by a violent overstimulation and personal betrayal that triggers the activation of various defense mechanisms. As a result of the sexual abuse, the child loses faith in the world as a relatively safe, foreseeable place, is unable to build a positive, confident self-image, and can no longer trust in relationships with others.[2]

In order to contain the phenomenon, it is not sufficient for physicians and psychologists to study only the victims; they need to analyze the perpetrators as well and conduct a scientific study of their traits, personalities, and internal operative models. This is not easy, because the institutions and public opinion are ill-equipped to deal with crimes that arouse such strong, aggressive reactions. The expert approaching the study of such phenomena could run into difficulty when faced with feelings and behaviors that test his/her empathic powers and so run the risk of collusion. The adoption of a scientific approach in the attempt to help the perpetrator, too, may interrupt the cycle of violence and thus offer real help for victims, past, present, and future.[3]

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Children, Sexual Crimes, Priests

I14 Are There Similarities Between Forensic Technician and Sworn Peace Officer Stress?

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After attending this presentation, attendees will better understand the physical, psycho-social stress and traumatic experiences endured by forensic technicians and sworn peace officers. Moreover, this survey examined the levels of perceived job-related stress and mental health concerns in forensic technicians and sworn peace officers, both on duty and off duty.

This presentation will impact the forensic science community by promoting stress and mental health awareness for forensic technicians who investigate crime scenes and encouraging research into this relatively unexplored area.

The results of occupational stress, especially in forensic technicians, has not yet been fully appreciated by law enforcement agencies or human resources departments involved in recruiting and training these professionals. Understanding the lasting impact of occupational stress on forensic technicians will enable law enforcement agencies and their families to proactively train, recognize, and help forensic technicians cope with many job-related stressors.

This study hypothesized that sworn peace officers and forensic technicians would report similar levels of overall job stress while on duty and that both occupations would report significant physical and psychological impacts as a result of their crime scene-related occupations. Forensic technicians provide field services at every possible type of crime scene with varying levels of direction and support from sworn peace officers. The primary field services assigned to forensic technicians includes identifying, documenting, collecting, preserving, and conducting preliminary analysis of physical evidence in relation to criminal investigations. They are constantly exposed to the stressful world of violent crime. As a result, forensic technicians can also experience physical and psychological stress, violence, and security vulnerability similar to other law enforcement first responders; however, unlike the latter, there is a lack of research regarding the impact of the stress and danger experienced by forensic technicians on their mental and physical health.

This has now been addressed in this study through the development and administration of an anonymous survey that gathered scaled, yes/no, and fill-in-the-blank type choices to 25 basic questions regarding perceived stress, physical danger on and off duty, physical and psychological life impact of job-related duties, preferred stress management networks, and coping strategies. Participants included forensic technicians and sworn peace officers employed at California law enforcement agencies with one or more years of experience processing major crime scenes.

Forensic technicians (N=37) and sworn peace officers (N=36) submitted qualifying surveys. The results indicated that perceived on-duty stress was significantly higher ($P \leq 0.05$) for the forensic technicians (M=3.50, S*tandard Deviation (SD)*=.99) compared to the sworn peace officers (M=2.99, SD=.97) based on the scaled (1-5) responses. The off-duty stress rating was higher for forensic technicians than sworn peace officers, but was not statistically significant. It was noted that of the 54 pre-selected stressors common to law enforcement, forensic technicians and sworn peace officers shared 10 of the top 20 ranked stressors. Two-thirds of all stressors were ranked statistically similar between the two occupations. Forensic technicians reported negative job-related impact responses in 14 out of 17 physical and psychological impact categories, whereas sworn peace officers reported negative job-related impacts in only 8 of the categories. Furthermore, forensic technicians and sworn peace officers exhibited statistically similar career-related impacts in 10 of the 17 physical and psychological categories. Perceived danger was experienced less frequently by forensic technicians compared to sworn peace officers, but was not statistically different. Last, both forensic technicians and sworn peace officers reported using friends and family for stress management more frequently, followed by peer support, and ,last, mental health resources. When compared to sworn peace officers, forensic technicians reported lower availability, awareness, and utilization of agency mental health support services, something this study suggests needs to be addressed.

The survey responses from this study suggested a similarity of work-related stressors that are shared by both forensic technicians and sworn peace officers; however, it appears that the perceived effects of stress for forensic technicians exceeded that of sworn peace officers. These findings indicate that the initial hypothesis, in part, can be rejected.

Forensics, Crime Scene Investigator, Stress

I15 Variation in Genes Affecting Dopamine Turnover, Oxytocin, and Serotonin in Inmate and Student Populations

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After attending this presentation, attendees will gain knowledge concerning the relationship between Single Nucleotide Polymorphisms (SNPs) associated with genes of oxytocin, serotonin, and dopamine as well as specific behavioral traits. Furthermore, attendees will learn about these genetic differences in an inmate population compared to a student control population.

This presentation will impact the forensic science community by demonstrating the genetic influence on aggressive and antisocial behavior. These behaviors have become a major problem as the United States currently has the highest incarceration rate in the world. Moreover, antisocial and aggressive behavior are two of the leading causes of mental health referrals. The strong heritability and environmental issues surrounding criminal activity indicates that a genetic underlying can help explain at least some features related to these behaviors.

Behavior is a complex process influenced by both genetics and the environment. Some neurotransmitters have been associated with social behavioral traits, including: Oxytocin (OXT), Serotonin (5-HT), and Dopamine (DA). Certain genes (such as genes of receptors, transporters, and enzymes involved in metabolic pathways of these neurotransmitters) are associated with these neurotransmitters. These genes contain polymorphic sites that can be studied to relate or link them to certain behavioral traits. SNPs are single-base variations found at a specific location on the genome and are considered to be the most abundant type of polymorphism in humans. While some associations between SNPs and behavior have been made, this study analyzes multiple SNPs within the three most common ethnic groups in the United States (Caucasian, Hispanic, and African American) in both inmate and student populations.

This study analyzed a total of 17 SNPs: 12 SNPs associated with DA turnover (rs2283739, rs1799836, rs3788862, rs909525, rs979605, rs740603, rs737865, rs739388, rs1611115, rs165599, rs4680, and rs129882), two SNPs associated with the OXT gene (rs877172 and rs4813625), two SNPs related to the serotonin receptor (5HTR2A) (rs6314, and rs6311), and one SNP located within the serotonin transporter gene (5-HTT) (rs25531) using Single-Base Extension (SBE). A student sample set (N=200) and inmate sample set (N=100) were genotyped, and individuals participated in a survey designed to assess 31 behavioral traits.

Significant associations were found within the control population for two SNPs associated with OXT and 5-HT: rs6314 and antisocial behavior in Hispanics (p=0.008), and rs877172 and antisocial behavior in Caucasians (p=0.001). Furthermore, statistically significant differences in haplotype frequencies were observed in inmate vs control populations in SNPs associated with dopamine turnover (monoamine oxidase; MAOA). These results indicate that these SNPs play an important role in social behavior, including antisocial behavior.

The results of this study provide some evidence that OXT, 5-HT, and enzymes related to DA turnover can influence behavior. It was found that SNPs associated with these neurotransmitters influence antisocial behavior. These behavioral SNPs may be used in early prevention or treatment of psychiatric disorders, which have a large impact the medical field and criminal justice system. Furthermore, understanding the influence of OXT, 5-HT, and DA on behavior may help explain the etiology of aggressive and antisocial behavior.

Single Nucleotide Polymorphism, Oxytocin, Dopamine

I16 Phenotypic Characteristicts in Different Groups of Psychopathic Individuals

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After attending this presentation, attendees will be able to recognize phenotypic characteristics in different groups of psychopathic individuals.

This presentation will impact the forensic science community by helping identify any phenotypic gender-specific factors related to psychopathy.

Psychopathy in a personality disorder entailing traits and behaviors that have a negative impact on individuals and society. The diagnostic criteria for psychopathy has a long history in psychiatry and overlaps with criteria of other personality disorders, especially antisocial personality disorder and narcissistic personality disorder. These include egocentricity, superficial charm, a grandiose sense of self-worth, the need for stimulation, pathological lying, a manipulative approach to relationships, a lack of remorse and guilt, callousness, a lack of empathy, impulsivity, irresponsibility, and an inclination toward criminal behaviors. The confusion in the use of the term "psychopathic" could be explained by different phenotypic characteristics in different groups of psychopathic individuals and by gender differences.

The Psychopathy Checklist-Revised (PCL-R) is one of the most commonly used measures of psychopathy.[1] The total score of PCL-R is composed by interpersonal and affective (factor 1) scores and lifestyle scores (factor 2). The current study investigated discrepancies in scoring of PCL-R between a male psychiatric forensic sample, a female psychiatric forensic sample, a female prisoner sample, and female prisoners who had been convicted for Mafia-related crimes. Prior research on psychopathy has primarily focused on the problem in men. Only a few studies have examined whether psychopathy even exists in women, and, if so, how the disorder manifests itself within them. Research on differences between the sexes has suggested that psychopathy is less frequent in women than in men; however, it is debated whether the observed differences in the occurrence of male and female psychopaths reflect actual physical differences in the frequency of psychopathy, or whether those differences reflect factors related to aspects of the diagnostic tools and the terminology used, which surface when these criteria for evaluating psychopathy are applied to women. This study has shown how the psychopathic individuals in the different female samples demonstrated similar phenotypic characteristics. On the contrary, differences between the sample of women and that of men were observed: female sufferers more often seem to demonstrate emotional instability, verbal violence, manipulation of social networks, and, to a lesser degree than male psychopaths, criminal behavior and instrumental violence.

The importance of a correct diagnosis of "psychopathy" relates to its potential usefulness with regard to issues such as the choice of treatment strategies, treatment evaluation, risk assessment, and the prediction of future violence. If one assumes that the same research results achieved in studying men are automatically transferable to women, one does risk misjudgments of enormous consequence. For instance, within the field of forensic psychiatry, the diagnosis of psychopathy is often used to justify the length of prison terms. In some countries, indefinite prison terms can be given to criminals with this diagnosis. The diagnosis of psychopathy may also be used to justify patients' exclusion from treatment programs as well as other punitive measures.

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Psychopathy, Gender-Specific Factors, Phenotypic Characteristics

I17 The Psychopathic Semantics of Serial Killer Theodore Robert Bundy

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The primary objective of this study is to perform an exploratory analysis using quantitative linguistic analysis software, Linguistic Inquiry and Word Count (LIWC), to study how one prototypical psychopathic serial killer used language within various contexts over time.

This presentation will impact the forensic science community by providing exposure to a novel and relatively affordable way of gleaning insights into psychopathology by looking at individual differences in language use.

Theodore Robert Bundy (1946-1989), or "Ted," was the quintessential psychopathic serial killer, prototypical of how the clinical construct was extensively detailed *qualitatively* by psychiatrist Dr. Hervey Cleckley[1] and later further refined and *quantified* by psychologist Dr. Robert Hare.[1-3]

Psychopathy is a clinical construct loosely characterized by a cluster of severe affective, interpersonal, and behavioral components that include, but are not necessarily limited to, traits such as: superficial charm, egocentrism, glibness, conning/deceptiveness, pathological lying, poor behavioral controls, callous lack of empathy, and/or lack of remorse. Although related, psychopathy is often incorrectly equated with the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*) diagnosis of Antisocial Personality Disorder (ASPD), a relatively heterogeneous categorical construct.

One of the many characteristics that distinguish psychopaths from non-psychopaths is the way they employ language in unusual ways.[4-7] Their words are sometimes perceived as hollow shells devoid of affective content, a phenomenon that Cleckley referred to as "semantic aphasia."[1] Language is the most common and reliable manner to convey our thoughts and emotions so that others can understand us.[8] Our words reflect who we are and how we relate to others. Researchers have long studied the semantics of subjects with various psychopathologies to glean more insights into them and their behaviors; however, such endeavors often proved tedious, time consuming, expensive, and often had problematic interrater reliability.[9] With the advent of more affordable and powerful computers, we can now empirically and reliably accomplish in seconds or minutes what may have previously taken months or years. One tool that circumvents the aforementioned issues is the LIWC software.[9]

LIWC (pronounced "Luke") is a transparent text analysis program developed by psychologist Dr. James Pennebaker and his students at the University of Texas at Austin.[9] It counts words and places them in psychologically meaningful categories.[10,11] These empirical dimensions have been extensively studied, well validated, and applied to detect meaning in a wide variety of circumstances, such as predicting mild cognitive impairment, detecting deception in written and spoken language, and understanding individual differences between attempters and completers of suicide.[12-16] To date, relatively few studies have used LIWC to understand psychopathy, and, as of this writing, no published research has used it to study serial killers.

The primary objective of this study is to perform an exploratory analysis using LIWC to study how one prototypical psychopathic serial killer (Bundy) used language within various contexts over time. The dataset includes transcripts of police interrogations, personal correspondence, courtroom testimony, and interviews with the press collected by this study from books, video footage, and original documents from state archives in both Florida and Washington. The goal of this presentation is to introduce attendees to the powerful and affordable potentials of using LIWC in forensic investigations by illustrating how it can reliably relate language patterns to psychopathology.

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14. Ali, Mohammed and Timothy Levine. 2008. The Language of Truthful and Deceptive Denials and Confessions. *Communication Reports.* 21 (September 2014): 82-91.

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Ted Bundy, Psychopathy, LIWC

I18 The Psychopathological Profile of the Female Serial Killer: From Homicide to Cannibalism — The True Story of the "Soapmaker of Correggio"

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After attending this presentation, attendees will better understand the role of the psychopathological profile in female serial killers and, in particular, the role that gratification plays in inducing the serial killer to commit murder.

This presentation will impact the forensic science community by highlighting that, for the female serial killer, a psychopathic personality and child sexual abuse are common features.

In most cases reported in the literature, serial killers are men. This study sought to analyze cases of female serial killers. A literature review was performed using the Pubmed NCBI search engine with "female serial killer" as the key word. There are few cases in the literature concerning female serial killers. This work seeks to tell the story of a female Italian serial killer.

An investigation of the mental processes with the analysis of her memoir, *Leonarda Cianciulli, An embittered soul's confessions,* attributed to this serial killer, was performed and the data compared. This study reports the case Leonarda Cianciulli. She was born in 1892, in Mantella, Italy. Her childhood was difficult: "I was a weak child … my parents treated me like a weight … I attempted to hang myself twice …." She had 17 pregnancies, but only four sons survived. These children became an obsession with her.

Cianciulli attended three friends, who were single, middle-aged women. The first victim was Faustina Setti. Leonarda told her she had found a husband for her. Since Faustina was semi-literate, Leonarda volunteered to help her write a letter. The same day, she killed her friend with an ax, then dissected the corpse. She wrote, "I threw the pieces into a pot … I made a lot of pastries … also Giuseppe (her son) and I ate them." The second victim was Francesca Soavi, who wanted to find an occupation. Cianciulli claimed to have found her a job and convinced her friend to write to her acquaintances regarding her departure. Leonarda rushed the woman with an ax and killed her. The third victim was Virginia Cacioppo, an opera singer who lived in poverty. Leonarda claimed to have found her a job. About her, Cianciulli said, "She ended up in the pot, like the other two ... after a long time on the boil, I was able to make some most acceptable creamy soap. I gave bars to neighbors and acquaintances."

After numerous investigations (victims' blood and dentures were found in her home), it was considered certain that the woman had committed the crimes. She was sentenced to 30 years in prison and 3 years in a criminal hospital. The woman, in the Aversa mental hospital, said, "I killed … only for mother's love." Cianciulli died of cerebral apoplexy in prison.

From a psychiatric point of view in serial killing cases, the murders are premeditated, based on obsessions that become an irresistible impulse to kill. The necessary conditions for homicide are the fantasy, the symbolism, the ritualism, and the compulsion. The killer takes with him/her something belonging to the victim. In fact, Cianciulli confessed that she killed the women, destroyed the bodies by boiling them, made soaps, and kept the blood to mix with milk and chocolate to make cookies for her sons, believing this would save them from a mysterious death. Leonarda identified herself with the goddess Thetis, who wanted to give her sons immortality. The victims always have features in common. The perpetrator has a criminal record and prefers victims who are geographically close. According to scientific studies, serial killers are victims of childhood traumas. In these murderers, psychopathological personality disorders, a desire for control, delusions of omnipotence, and sexual motives are common denominators.

This historic case is unique for manners; the sexual motive is absent, but it clearly represents a profile of the female serial killer. The feelings of parental abandonment and the sons' deaths caused frustrations and a desire to control of events. The choice of weapons, such as hatchets or axes, was related to her constitution of being a small woman who needed harmful weapons. The dissection of the bodies was performed for two reasons: to dissolve the traces and to retain fetishes as rituals.

Forensic Science, Female Serial Killer, Homicide

I19 Empathy for the Psychopathic Patient

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The goal of this presentation is to provide a brief review of the literature on this subject, along with the means to cross barriers of negative countertransference when navigating similar cases.

This presentation will impact the forensic science community by demonstrating how positive countertransference has been directly correlated to improved patient outcomes, particularly in psychiatry, as the therapeutic alliance remains a central focus in treatment.

Literature on this topic is extensive; however, literature on positive countertransference for criminals and psychopaths is scarce. Developing positive countertransference remains challenging for some clinicians when faced with particularly difficult patients. When clinicians are noted to struggle in their supervision to develop positive relationships with patients, supervisors recommend empathizing with such patients as a way of developing positive feelings toward them; however, certain patients, particularly psychopaths, challenge a clinician's ethics and fundamental beliefs. Hence, developing rapport with such patients remains difficult for many clinicians. Lastly, it is important to differentiate between what is positive countertransference induced by the patient's attempt to manipulate, appeal, and please the provider versus identification of true and genuine empathy on the provider's part.

The phenomenon of countertransference was first defined publicly by Sigmund Freud in 1910 as being the result of a patient's influence on the psychiatrist's unconscious feelings. The concept of countertransference was originally considered a barrier in psychotherapy, whether positive or negative. Freud originally thought it should be identified and rooted out; however, it is now considered very important in the therapy process. Otto Kernberg provided psychiatry with a totalistic way of looking at countertransference. When juxtaposed with Freud's theory, it encompasses the complete emotional reaction of the therapist toward his patient. Heinrich Racker linked countertransference with empathy. This study would like to implement Racker's model of countertransference for the purpose of this presentation.

Most of the available literature on this topic suggests that empathy for psychopathic patients among psychiatrists is quite rare and only occurs in the context of voyeuristic curiosity or envy of the criminal's ability to cross social and moral barriers. In this presentation, it is argued that genuine empathy for such patients is possible. Here is described the experience of two residents, both pursuing careers in forensic psychiatry, who provided care for such a patient with concordant rapport. This particular patient had a violent background with severe psychopathic traits; however, both providers were capable of appreciating the patient's pathology, and identifying his feelings of loss, isolation, illness, and decay. This presentation will also provide a brief review of the literature on this subject, along with the means to cross barriers of negative countertransference when navigating similar cases.

Psychopathy, Countertransference, Empathy

I20 How Just Is Manifest Injustice (MI)?: Evaluating the Use of Manifest Justice in the Washington State Juvenile Rehabilitation Administration

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After attending this presentation, attendees will be able to: (1) appreciate the juvenile justice system's mission to provide rehabilitation and treatment for youths, as well the existence of policies that support this mission (i.e., MI); (2) recognize the disproportionate minority contact and racial inequality in youth sentencing that exists within the juvenile justice system; (3) understand the circumstances that are contributing to the sentencing disparity identified with the use of MI and the importance of adequate community services for these youths; and, (4) inspire efforts to engage in advocacy for youths and families engaged in the juvenile justice system.

This presentation will impact the forensic science community by illuminating ongoing sentencing disparities within the juvenile justice system. The hypotheses generated to explain the findings of this study point to critical system-wide injustices that need to be addressed and better understood. Further research is needed to ensure laws are being used justly across all ethnic and minority groups.

Objectives: In the Washington (WA) State Juvenile Code, a provision called Manifest Injustice (MI) allows judges to sentence youths outside of the standard sentencing range guidelines. Racial inequality in juvenile justice sentencing is well established.[1] This investigation evaluates how MI is used across racial groups among WA youths involved in the Juvenile Rehabilitation Administration (JRA). It was hypothesized that MI would be used more frequently to decrease sentences of Caucasian youths and to increase sentences of minority youths.

Methods: De-identified and aggregated administrative data of the JRA residential population (n=436) was obtained for youths in custody on 1/11/16. The goal was to compare rates of JRA involvement and MI between racial minorities and Caucasian youths. Rate ratios were used to compare the proportions of WA state and JRA-involved minority youths who received MI Down or MI Up/In with Caucasian youths.

Results: African American (AA) youths were more than seven times as likely to be involved in the WA Juvenile Justice System than Caucasian youths (RR=7.85, p=<0.0001), while Mixed youths were three times more likely (RR=3.17, p=<0.0001), and Hispanic youths were 40% more likely (RR=1.40, p=0.0131). Although results did not meet statistical significance, there was a trend toward AA and Mixed youths having MI used to decrease their sentence less than Caucasian youths. AA youths were about half as likely to have MI used to increase or intensify their sentence compared to Caucasian youths (RR=0.49, p=.002), whereas Mixed youths were 42% less likely (RR=0.58, p=.04).

Conclusions: Finding that Caucasian youths were more likely than AA and Mixed youths to have their sentences increased or intensified was contrary to what was hypothesized. More AA youths reside in the urban and more liberal parts of the state where judges may be more progressive and less likely to use MI to intensify sentences. More diversion programs are available in the urban areas of the state, some target minority youths, and more AA youths are transferred to adult court; all of these actions reduce the likelihood of minority youths receiving sentence intensification. Judges in the rural areas of the state, which are Whiter and have fewer treatment resources, may be using MI to send youths into facilities to access treatment. It is imperative that community behavioral health services are available so youths and families can be justly served.

Reference(s):

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Juvenile Justice, Mental Health, Disparities

I21 Potential Effects of Legalized Recreational Cannabis on Youth

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After attending this presentation, attendees will appreciate the growing national trend toward legalization of recreational cannabis and understand the potential effects that acceptance of cannabis use and the growing cannabis industry may have on the vulnerable population of children and adolescents.

This presentation will impact the forensic science community by increasing awareness of the specific vulnerabilities of children and youths to cannabis products that are transforming due to a changing legal stance toward recreational use. Clinicians will be more aware of changes in Tetrahydrocannabinol (THC) concentration, means of consumption, and packaging that potentially have an impact of pediatric use of cannabis. Clinicians may be better able to counsel their patients on marijuana use and preventing inadvertent consumption of cannabis products by children.

Currently, recreational cannabis for adults is legal in eight states and the District of Columbia, and the list is growing.[1] This presentation explores current trends in legalization of recreational cannabis, the growing cannabis industry, and changes in THC potency and means of consuming that have resulted from this legal shift; however, a recent report by Han et al. examining data from the National Survey on Drug Use and Health from 2002-2014 found that youth cannabis use has been declining since 2011.[2,3] This data is in contradiction to what might be expected with increasing acceptance of cannabis use.

As more states begin to legalize recreational marijuana, it remains to be seen if this downward trend in youth cannabis use will continue. This presentation will examine how some of the marketing efforts of the cannabis industry for new forms of cannabis, such as edibles, are being packaged to appear nearly indistinguishable from candy products and may target children or be mistakenly consumed by children. The current trend of legalizing recreational marijuana use may have an adverse effect on the vulnerable youth population. The potential effects of the higher visibility of cannabis products, as well as higher potency products, is also explored in the context of research that suggests that younger populations may be more vulnerable to cognitive impairment and more susceptible to developing psychosis with cannabis use.[4]

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4. Volkow N.D., Baler R.D., Compton W.M., Weiss S.R.B. Adverse health effects of marijuana use. *The New England Journal of Medicine.* 370 (2014): 2219-2227.

Cannabis, Legalization, Adolescents

I22 Females Who Sexually Offend: Characteristics and Behaviors

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The goal of this presentation is to add to the empirical literature on sexual offending by females, given the paucity of such resources available to those involved in assessment, treatment, and prevention.

This presentation will impact the forensic science community by increasing empirically based understanding of females who sexually offend, which, in turn, will improve clinicians' abilities to effectively evaluate clients for diagnosis, treatment, and risk reduction.

Female sex offenders are an understudied population and are often regarded as being rare; however, as a recent meta-analysis reported, sex offenses committed by women are more prevalent than it previously appeared.[1] Specifically, Cortoni and colleagues reported that although only approximately 2% of sex offenses reported to law enforcement are perpetrated by women, victimization surveys report a much higher (approximately 12%) instance of sexual offending by women.[1]

This presentation will include data on 13 women who were convicted of sexual offenses and were assessed for civil commitment under the South Carolina Sexually Violent Predator (SVP) Act. The mean age was 32.85 (*Standard Deviation (SD)*=6.50, range 23-44) at time of assessment. The sample had one to two victims (*M*=1.23, *SD*=0.44) each and 30.8% had female-only victims, 61.5% had only male victims, and 7.7% had both female and male victims. The victims' ages ranged from 3 to 17, with the mean age of the offenders' youngest victim being 12.54 (*SD*=4.41). The convictions included Criminal Sexual Conduct with a Minor, Lewd Act on a Child, Criminal Solicitation of a Minor, Promoting Minor Prostitution, and Sexual Exploitation of a Minor. Two of the women had prior convictions for sexual offenses and four had prior convictions for felony offenses. Nine (69.2%) had victims who were unrelated, three (23.1%) had related victims, and one (7.7%) had both related and unrelated victims. None of the women had been diagnosed with a paraphilic disorder. Nearly 70% (*n*=9) had been diagnosed with a mental illness to include schizophrenia, bipolar disorder, anxiety disorder, substance use disorders, post-traumatic stress disorder, and an unspecified depressive disorder. More than 75% (*n*=10) reported experiencing childhood sexual abuse. Only one of the women was referred for further review for potential civil commitment under the SVP Act. A comparison with a matched set of male sex offenders will be included, noting similarities and differences, with discussion of likely etiologies of each.

Practical application of findings for diagnostic and risk assessments and treatment planning is presented. Next steps in developing this area of limited evidence base are outlined for consideration.

Reference(s):

1. Cortoni F., Babchishin K.M., and Rat C. (2017). The proportion of sexual offenders who are female is higher than thought. *Criminal Justice and Behavior. 44*(2), 145-162.

Female Sexual Offenders, Sexually Violent Predators, Women

I23 High-Functioning Autism and Violence Risk

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After attending this presentation, attendees will better appreciate how Autism Spectrum Disorder (ASD) may be an underrecognized condition requiring different approaches to the assessment and mitigation of violence risk. Attendees will also gain awareness of special considerations in the assessment and treatment of potentially violent patients with high-functioning autism.

This presentation will impact the forensic science community by helping clinicians who routinely assess patients for violence learn to incorporate assessments for autism spectrum disorder (i.e., Autism Spectrum Quotient). Clinicians will learn to utilize recommended strategies for mitigating risk specific to high-functioning autistic individuals.

This presentation describes a case of a man admitted to an acute inpatient psychiatry ward with vague symptoms of anxiety and difficulty controlling his anger, and indirectly alluding that he was a threat to harm himself or others. Prior to his admission, he had his first visit with his outpatient psychiatrist, where he reported that he was receiving "ideas" to harm himself and others. The outpatient psychiatrist diagnosed him with likely autism spectrum disorder and recommended he be hospitalized for further assessment. On admission to the inpatient ward, the patient was fixated on themes of government conspiracies and rights to bear arms, making it difficult to obtain a reliable history. He also reported trouble connecting with others, and on further evaluation, reported that he had been diagnosed as being on the autism spectrum as a child. The patient completed the Autism Spectrum Quotient, and scored in the low range of the autism spectrum (formerly Asperger's Disorder). He was treated with a low dose of risperidone (titrated to 1mg twice daily), which resulted in improved mood and less irritability. He no longer reported thoughts or messages to harm himself or others at the time of discharge, and a course of psychotherapy aimed at improving social skills was recommended.

The relationship between high-functioning autism and violence is poorly understood. Media representation of recent mass killers, such as perpetrators of the shootings in Sandy Hook Elementary School, Newtown, CT, in 2012 and Santa Barbara, CA, in 2015, would seem to suggest a link between violence and individuals who appear to be on the autism spectrum. Research to date has not supported this link. Daniel Im reviewed the literature to explore ASD and violence from 1943 to 2014 and found no conclusive evidence of a connection.[1] In this review, he did identify some risk factors specific to individuals with ASD that may increase violence risk among these individuals, such as problems with emotional regulation and deficits social-cognitive functioning. This presentation proposes that early identification of ASD may assist in preventing violence by allowing the identification and treatment of these unique risk factors, which otherwise may be missed if the diagnosis is not considered. Assessment and treatment strategies for patients with possible ASD who are at a risk for violence will be discussed.

Reference(s):

1. Im D.S. Template to perpetrate: An update on violence in autism spectrum disorder. *Harvard Review of Psychiatry.* 23 (2016), 14-35.

Autism Spectrum Disorder, Violence, Risk Assessment

I24 Factitious Disorder Imposed on Another: A Life-Threatening Italian Case

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After attending this presentation, attendees will better understand Factitious Disorder Imposed on Another (FDIA), also termed Munchausen Syndrome by Proxy in some jurisdictions, which should be one of the possible differential diagnoses of recurring health issues in children.

This presentation will impact the forensic science community by emphasizing the importance of a multidisciplinary evaluation of suspected somatoform disorders perpetrated by caregivers.

FDIA continues to mystify health care professionals, law enforcement officials, and the judicial system.[1] Even though the first cases were described in 1977, it remains puzzling as to why a parent would want to induce fictitious symptoms and illnesses onto a child.[2] Many professionals do not consider FDIA as a diagnosis because the parent, usually the mother, is extraordinarily able to convince them that she is a "good" mother and wants the best for her child.[3]

This study reports a severe case of FDIA managed in the multidisciplinary unit dedicated to the evaluation of suspected abused children ("Bambi") of the "Ospedale Infantile Regina Margherita" in Turin, Italy. After an 11-year-old boy had several prior hospitalizations in multiple other facilities over a period of eight years, he was hospitalized in the Endocrinology Department by his mother. The child was treated for various problems, including Hirsprung Disease and diabetes mellitus type 1 with life-threatening ketoacidosis. He was also subjected to invasive procedures (cystoscopy with bladder neck incision, rectal biopsy, intestinal resection with coloanal anastomosis, and botulin toxin injections). In the consultation processes with the "Bambi" team, the mother and the boy were interviewed separately. The child described himself drawing "Pinocchio" (a notorious character known for being a liar). In interactions with the mother, he always appeared dominant, blackmailing, and manipulative. The mother displayed a facility with medical language, even though she was not a health professional, and strongly identified with the role of therapist. She also displayed tentative and inconsistent parental behaviors and inefficient coping skills. The mother conveyed that she was the only caregiver and refused support from her partner, who had left her and their children a few months earlier. The woman had always cared for sick relatives and showed pride for this role. Her whole life had been focused on the illnesses of others, and she displayed considerable expertise in working with the social workers to obtain economic and other welfare benefits. A discrepancy between the severity of the reported facts and the woman's emotional state was evident. A careful global assessment of clinical and family history found the heterogeneity of the child's symptoms and their escalation over time. There was a lack of correlation between the symptoms' progressions and therapies. There was always a temporal correlation between the stressful life events of the mother (death of her mother, abandonment by her husband) and the subsequent clinical deterioration of the child. Furthermore, when the woman was engaged in taking care of other relatives, the clinical condition of her son improved and his hospitalizations decreased.

After a comparison between the "Bambi" unit personnel (pediatrician, medical examiner, psychologist, and trained nurse) and physicians of the Endocrinology Department, the situation was referred to the judicial authority, formulating the hypothesis of FDIA. Thanks to a brief period of intense observation, it was documented that a ketoacidosis crisis had been induced by incongruous insulin delivery by the mother. The subsequent psychiatric evaluation confirmed the mother's diagnosis. The overall assessment of the case by a multidisciplinary team was fundamental to formulating the proper diagnosis of the mother's psychological pathology. For many years, the child had been a victim of this particularly subdued form of maltreatment and, upon several occasions, had been subjected to unnecessary life-threatening interventions. In fact, the child's false symptoms (in particular, the ketoacidosis crises) were an expression of the mother's need to maintain the only function she had in her life: to be a caregiver. The diagnosis was further complicated by the fact that the child was actually suffering from various diseases, which were overtreated by the mother.

This presentation provides attendees with a better knowledge of FDIA. This diagnosis should always be kept in mind when health care professionals evaluate cases of chronic pediatric diseases in the presence of an inappropriate correlation between symptoms and therapeutic efforts.[4]

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Child Abuse, Differential Diagnosis, Munchausen Syndrome By Proxy

I25 Clinical Psychiatry and Neuropsychiatry in the Forensic Context

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The goals of this presentation are to: (1) educate mental health professionals in three important medicolegal areas; (2) provide greater understanding of Tardive Dyskinesia (TD) and of Traumatic Brain Injury (TBI); and, (3) demonstrate methods to practice better psychiatry and more properly evaluate patients clinically and forensically. This presentation will provide a greater understanding of clinical psychiatry and neuropsychiatry in the forensic context and consists of lectures on the forensic aspects of TD, TBI, and proper evaluations.

This presentation is applicable to all psychiatrists and mental health professionals. This presentation will impact the forensic science community by raising awareness of the need for careful clinical care, prophylaxis, and proper evaluations and will highlight principles generalized to other areas of civil and criminal forensic practice.

Psychiatric evaluations include appropriate examination, ongoing assessments, diagnosis, management, prognosis, attributions of proximate causality, and damages. Prior medical histories are critical. Forensic data has an even higher standard emphasizing causality attributions and prognoses.

Two key common illustrative medical conditions are: (1) Tardive Dyskinesia (TD) — a complex neuroleptic-prescription-induced, sometimes irreversible, movement disorder. Here, physicians commonly err in management, and the pharmaceutical industry may not properly put "warnings" on drug labels; and, (2) traumatic brain injuries — these could be repeated and catastrophic. Proper acute and chronic care is complex and sometimes additive to previous events.

TD is possibly the most well-known but often missed drug-induced neuropsychiatric forensic condition. TD is sometimes incurable and induced by long-term prescription neuroleptic treatment (antipsychotic medications as well as gastro-intestinal medications, such as metoclopramide). Civil litigation against prescribers (physicians, particularly psychiatrists) and the pharmaceutical industry is a common, major consideration. Several steps for ensuring ongoing proper clinical evaluation and management are often neglected, including early detection, appropriate follow-up, including testing, outside specialized expert referral, differential diagnosis, and recognition of patients at risk. Evaluations include an effective specific TD scale (Neppe's STRAW scale) with other formal examinations (AIMS, Simpson-Angus and possibly SCT Hans). Videotaping monitors progress. Management requires prophylaxis, early recognition, and ongoing interventions. It has been found that off-label, high-dose buspirone treatment (1989-2017 experience) is extraordinarily successful, efficacious, cost-effective, and safe. This study regards it as far preferable to expensive, tetrabenazine derivatives (e.g., valbenazine), with theoretically significant side-effects and ostensibly incomplete control long-term.

Traumatic brain injury (TBI) is very common with variable symptomatology: (1) non-recognition of the blow, but still having subtle changes; (2) concussion is common with several presentations, but sometimes incorrectly labeled; (3) unrecognized, seldom diagnosed yet treatable focal cerebral abnormalities; this includes particularly temporal or frontal lobes dysfunctions and uncommonly, subtle changes; (4) ranges through to prolonged deep coma, where acute lengthy hospitalization and rehabilitation is specialized. Management clearly varies acutely compared with the subacute and chronic residual phases; (5) recently, Chronic Traumatic Encephalopathy (CTE) with repetitive TBI has become increasingly recognized in contact sports and has major potential medicolegal implications; and (6) subtle differences must be recognized. To facilitate, the presentation has classified head injury forensically and clinically.

Some management nuggets include: (1) missing the subtle focal injuries can be disastrous in clinical and forensic consequences, and yet can be commonly helped with appropriate, but often unprescribed, medications (including anticonvulsants and azapirones); (2) cognitive rehabilitation (previously expensive and lengthy) has largely been replaced by appropriate computer programs facilitating easier, often effective, management and rehabilitation (important medicolegally); (3) certain less well-known tests, such as the Inventory of Neppe of Symptoms of Epilepsy and the Temporal Lobe (INSET) and Soft Organic Brain Inventory of Neppe (SOBIN) are very important, structured ways of monitoring symptoms clinically and in follow-up; (4) costly, sometimes beneficial, specialized evaluations (e.g., home ambulatory electroencephalography, head Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) with contrast, **Positron Emission Tomography** (PET) scanning, Single-Photon Emission Computed Tomography (SPECT) scan, and polysomnography amplify diagnoses. Civil litigation may involve contradictory clinician and forensic roles.

Testing in Clinical and Forensic Psychiatry and Neuropsychiatry: Every clinical patient *could* be a potential forensic case, because a major basis of litigation is substandard care. Applying standardized techniques while recognizing individual differences facilitates the appropriate clinical and forensic evaluations. Treatments are sometimes necessarily off-label while repeatedly assessing progress and ongoing management revisions. Clinical and forensic evaluations must include appropriate diagnostic, symptom, and risk assessments. Particularly, medicolegally appropriate data correlations, all pertinent records (medical and psychosocial), and outsider validations by family members, friends, and sometimes law-givers are important. Standardized neuropsychological testing has significant strengths by applying comparative norms. Accounting for baseline data — education, background, and previous test exposures — is essential. Yet, unrecognized testing weaknesses can imply over-inclusiveness and ignore significant individual differences. The clinical neuropsychiatric evaluation with repetitive individualized longitudinal monitoring is essential. Repeated follow-ups, including reviewing critical neglected areas (e.g., subtle malingering, motivation and fatigue), are ideal. These evaluations also require individual clinical tailoring of broad structured psychiatric and neurological questionnaires. The Pacific Neuropsychiatric Institute (PNI) has developed and meaningfully applied many such medicolegal neuropsychiatric screens. These include the detailed "Diagnostic-Screen questionnaires" ("DS-10"), the INSET and SOBIN, plus the BROCAS Screening Cerebral Assessment of Neppe (SCAN) cognitive examination.

Tardive Dyskinesia, Traumatic Brain Injury, Evaluations

I26 Contribution of the Psychiatrist in the Evaluation of Fitness for Detention While in Custody in France

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After attending this presentation, attendees will better understand the evaluation of fitness for custodial detention in France, which allows, in some cases, a direct intervention at the police station by a forensic psychiatrist.

This presentation will impact the forensic science community by illustrating the in-custody management of mental health issues of a police station in France.

Each year in France, several hundred thousand individuals who are suspected of having committed a criminal offence are arrested and retained in custody in a police station. As soon as the measure begins, the individual is allowed several defense rights, including the right to see a defense attorney and the right to have access to medical care at any moment while in the custody. A medical examination can also be solicited by the police officer or by a member of the individual's family. The physician is always appointed by the police officer.

These examinations are performed either by general practitioners or by emergency room doctors, but, in 2011, French forensic medical units were proposed to have a supplementary budget to perform these examinations in the geographic area closest to them. As a result, physicians with forensic training are performing these evaluations in some geographic areas. This report presents the organization of an institute that is located in an area of 600,000 inhabitants. Approximately 1,500 medical evaluations of persons in custody are performed each year. As French medicolegal physicians may have different professional courses, a psychiatrist is working full-time at the unit and is frequently solicited in this context.

In general, medical examinations are requested in five circumstances: (1) when the arrestee declares he/she is suffering from a particular disease and requires a specific treatment that needs to be continued during police custody; (2) when recent traumatic lesions need to be treated and recorded; (3) when the arrestee complains of acute symptoms that appeared during police custody; (4) when a mental disorder could lead to an involuntary hospitalization requested by a state representative — this usually concerns individuals whose behavior has disturbed public order; and, (5) when it is imposed by law — for minors, for instance. It should be noted that the individual may still refuse the examination.

Intervention of a psychiatrist to evaluate the fitness for detention is of particular interest in two situations, not exclusive from one another: (1) when the individual is suffering from alcohol and/or substance use; and, (2) when the individual needs a mental health assessment. In the first situation, the question of delivering a psychotropic medication may arise, either because the individual requests a substitutive treatment to avoid withdrawal symptoms or because the individual presents symptoms of anxiety or suicidal risk. In the second situation, the psychiatric interview allows determination as to whether or not there is a mental issue that could lead to the deliverance of a specific treatment or an observation in a psychiatric ward.

This report suggests that direct evaluation by a psychiatrist in the police station allows better collaboration with the police officers to perform surveillance and administer medication to these individuals. The psychiatric interview also provides a filter before a more complete evaluation in the emergency room (if this is necessary).

Forensic Science, Police Custody, Psychiatric Evaluation

I27 Impartiality and Forensic Psychiatry: How Forensic Psychiatry Specialists Consider the Concept of Impartiality

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The goal of this presentation is to determine knowledge and representation regarding impartiality and which factors could change this.

This presentation will impact the forensic science community by increasing knowledge of the factors that influence impartiality.

Introduction: When it comes to justice, an expert psychiatrist has a different role than a therapist. In the first case, the psychiatrist works only when asked by authorities to testify. Impartiality is a priority for the expert psychiatrist.[1] This survey was initiated to better understand how forensic psychiatry specialists consider the concept of impartiality and also asks which factors can influence impartiality.

Material and Methods: An online survey was created that included a clinical case, some general issues about impartiality, and questions on personal and professional specifications relating to the persons who answered. This survey was sent to psychiatrist members of the World Psychiatrist Association of the European Psychiatric Association and of the American Association of Forensic Psychiatry.

Results: One hundred thirty-one psychiatrists from 18 countries (66% from the United States) participated in this survey with a rate of 94.5% answers (103-131 answers by question). The sample consisted of 74% men and 94.5% of graduate psychiatrists. The average age was 53.4 years and the average forensic psychiatry experience was 18.3 years. More than 80% of the sample had an additional forensic psychiatry certification. In the clinical case, personal convictions were identified as a factor that called into question their impartiality in 80% of the cases. Impartiality pertains primarily to ethics and legal topics, according to the survey. The most important factors that influenced personal impartiality were: (1) being the treating psychiatrist of the assessed person in the past (97%); (2) personal past experiences (92%); and, (3) personal convictions (90%). The most frequent factors that strengthened personal impartiality were: (1) forensic psychiatric training (94%); and, (2) professional past experiences (77%). The most important factors that question legal impartiality were: (1) already being the treating psychiatrist of the assessed person in the past (93%); (2) personal convictions (78%); and, (3) already having performed a psychiatric assessment on the person (70%). Comparisons reveal very few differences between answers provided by men and women, the doctor's age had an influence on the factors questioning legal impartiality, and there were very few differences between the experienced psychiatrists and those who did not have a lot of experience. It was noted that the choice of factors that strengthen and call impartiality into question were influenced by the psychiatrists' work countries. Finally, the survey compared the different types of forensic psychiatric training. Results reveal that psychiatrists trained in forensic psychiatry within a general psychiatry program feel that personal convictions and being the treating psychiatrist for the assessed person in the past strengthen impartiality. Psychiatrists with additional training in forensic psychiatry consider impartiality more as an ethical notion and that additional training in forensic psychiatry strengthens impartiality. Psychiatrists who were supervised during their assessment consider that the fact that they have already completed a psychiatric assessment affects impartiality — that this could also question it *and* that professional past experience calls impartiality into question.

Conclusion: The psychiatrists sampled have a good knowledge of impartiality. The principal factors they identified as being able to affect, strengthen, and call into question are the same factors mentioned in scientific articles and international recommendations.[2] The high participation rate of American psychiatrists could be explained by a large diversity of organizations and the recognition of forensic psychiatry in European countries. As expected, having additional training in forensic psychiatry did not change the perception of impartiality. Moreover, years of experience in forensic psychiatry had a small influence on the answers, whereas psychiatrists older than 65 years of age were most likely to identify some factors that call impartiality into question.

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Impartiality, Forensic Psychiatry, Psychiatric Assessment

I28 Racial Trauma: Its Mental Health Manifestations in Racial Minorities Involved in the Legal System and Incorporating Findings in Forensic Psychiatric Assessments

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After attending this presentation, attendees will be able to: (1) understand the concept of "racial trauma"; (2) realize how it could apply specifically to racial minorities involved in the legal system; (3) better understand its psychiatric manifestations; and, (4) value its importance in the forensic psychiatric assessment.

This presentation will impact the forensic science community by exposing how racial harassment and discrimination, in its numerous forms, against minorities in American society could lead to emotional and psychiatric manifestations due to traumatic stress. Awareness of this topic will enhance the efficacy of the forensic psychiatrist's evaluation and treatment plan. In some cases, it may also strengthen the therapeutic alliance between the treating psychiatrist and the client.

The information presented is obtained from previously conducted studies and literature that examined the link between cultural competency regarding racial discrimination and the criminal justice system. Additional sources include current events reported by the media that relate to perceived racial injustice. The fact that many members of racial minorities believe there is racial injustice makes it imperative that forensic psychiatric evaluators address and include these issues in their examinations.

Researchers have noted that, in recent decades, one area of inquiry that needs increased attention is racial discrimination, specifically as perceived by non-dominant group members (African, Latino, Asian, and Native Americans). Carter and Forsyth define racial discrimination as a "form of avoidant racism, reflected in behaviors, thoughts, and policies that have the effect of maintaining distance or limiting contact between dominant and non-dominant racial group members" and racial harassment as a "form of racism that involves feelings, thoughts, and actions intended to communicate a target's subordinate status due to membership in a non-dominant racial group."[1] Multiple studies have found that "between 40%-98% of racial minority participants reported that they had experienced racial discrimination."[1]

Currently, there are limited resources to assist psychiatrists in appropriately assessing racial trauma. Psychological reactions to racial discrimination often do not fit the specific criteria in the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-V*) for post-traumatic stress disorder; however, researchers have documented statistically significant relationships between "perceived experiences with racism" and mental disorders, such as adjustment, stress reaction, mood, and anxiety.[1]

According to the United States Census Bureau, the 2010 population of Whites (72.4%) far outnumber Black (12.6%) and Latino (16.3%) populations. Yet, in the same year, the national prison population consisted of 38% Black and 22% Latino men and women.[2] Race has been used as a political and media tool and, consequently, has engendered fear within the dominant group toward the minority group and vice versa. This has been a contributing factor to the minority groups' overall distrust of the legal system. The following are some examples: the murder of unarmed Black males by law enforcement in which the perception in the Black community is that justice hasn't been served, the outcry for the construction of a "wall" between the United States and Mexico with mass deportation of illegal immigrants, or the imposition of a Muslim ban on entry to the United States from Islam-dominant countries. These events have led to severe emotional and psychiatric stress (racial trauma) in these target communities that should be identified.

This presentation seeks to: further analyze racial trauma; explore how these reactions to perceived racial injustice could be expressed emotionally, psychologically, cognitively, or behaviorally; and provide a guideline in performing a thorough forensic psychiatric evaluation. In addition, consideration of perceived racial trauma will ultimately lead to enhancing the therapeutic alliance between client and psychiatrist.

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Racial Trauma, Guideline, Forensic Evaluation

I29 Mental Wellness and Suicide Prevention Programming Among United States Police Agencies

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After attending this presentation, attendees will better understand the availability and use of police officer wellness promotion and suicide prevention programs implemented nationally as well as the perceptions of program effectiveness expressed by employing departments. Attendees will be able to identify and describe various difficulties encountered with, and the need for research within, the area of police mental wellness and suicide prevention.

This presentation will impact the forensic science community by discussing perceptions of effectiveness in mental wellness and suicide prevention programming utilized by police departments.

Officer suicide is a major issue affecting police: the 2015 President's Task Force on 21st Century Policing reported on the necessity of research in this area, stating officers were more than twice as likely to die from suicide as from homicide.[1] It has been recommended that training and programming should educate officers on emotional strength, and that the provision of relevant resources are essential to reducing officer suicide.[2] A dearth of research exists in the area of police mental wellness and suicide prevention, especially regarding wellness programs utilized by police departments. By better understanding the state of police officer wellness promotion, it may be possible to determine the most effective programming for officer wellness and suicide prevention. To date, there has not been a comprehensive list of available programs or an examination of their effectiveness.[3] This study is important to law enforcement as mental health issues may negatively impact cognitive abilities, job performance, and the likelihood of post-traumatic stress disorder and suicidal ideation.[1,4]

This presentation will examine data collected from a national sample of city police departments and sheriff's offices describing any mental wellness and/or suicide prevention programs implemented. Using the most recent Census of State and Local Law Enforcement Agencies, police departments and sheriff's offices with more than five full-time, sworn officers were compiled.[5] These departments were then stratified into three groups based on the number of full-time, sworn officers: 5-20 (small), 21-100 (medium), and 101+ (large). The ten largest departments, and those recognized for wellness programming by the Destination Zero Program of the National Law Enforcement Officers Memorial Fund, were targeted for sampling.[6] Other departments to be sampled were chosen randomly from within these strata. The phone interview, adapted from Kuhns, Maguire, and Leach, asked questions about available programming and perceptions of utilization and effectiveness of those programs.[3] Most respondents have been ranking officers with some mental health training or civilian psychologists/counselors employed by the agency. Data collection ended in December 2017. Thirty departments from each stratum (90 total) were targeted, and to date, 13 departments have been interviewed (response rate of 25.5%; 13 respondents out of 51 contacted).

Several logistical issues have arisen, most notably, difficulty in making contact with departments. The telephone-based recruitment procedure was chosen to avoid the non-response bias inherent in email or mail-based procedures.[7] Nevertheless, difficulties in making contact with the appropriate potential respondents within these departments have occurred (i.e., some departments were unfamiliar with who should be contacted for the request at hand, resulting in the need to contact multiple people from within one agency). Some agencies that declined to participate noted policy against research participation and/or not enough time or officers to respond to the interview; others did not provide a reason for the refusal or never responded to repeated requests for participation. Most respondents willing to participate expressed pride in their department's programming and had dedicated some effort to mental health issues in police work.

Preliminary data indicated peer support groups are the most common programs used; 61.5% of responding departments use them. These programs were considered to be the most highly effective of utilized wellness or suicide prevention programming as well. Some respondents considered peer support programs to be ineffective, citing stigma in asking for help, and thus the programs are being underutilized. Other employed programs identified by departments included employee assistance programs, resiliency training, wellness campaigns/symposia, and use of critical incident response teams. Most agencies recommended peer support programming of all programming utilized. Future directions for research, including further discussion of the methodological and practical implications for generating empirical knowledge, will be addressed.

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I30 Determining Implicit and Explicit Attitudes of Hiring Non-Violent Ex-Offenders

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After attending this presentation, attendees will better understand the everyday practices of hiring non-violent ex-offenders and the impact of employment opportunities available to those who have been convicted of a non-violent crime as compared to non-offenders.

This presentation will impact the forensic science community by explaining reductions in recidivism rates among non-violent ex-offenders (e.g., harsh penalties for drug charges). Offenders who are incarcerated for drug charges are in need of substance abuse treatments instead of imprisonment, while those found not guilty by reason of insanity need treatment for psychological disorders within a secure environment. Both populations have commonalities such as treatment, reintegration back into their community, and productiveness within their society. An integral part of success is gaining employment. The purpose of this study is to examine the implicit and explicit attitudes individuals have toward non-violent ex-offenders and the effect attitudes may have on the hiring process. Implicit and explicit attitudes would have the same impact on hiring decisions and could also aid in understanding discrimination in the hiring process.

In 2015, the Bureau of Justice Statistics stated there were approximately 6,741,400 individuals (approximately 2.7%) under some form of correctional supervision (e.g., parole, probation). In 2011, nearly 660,000 people were released from prison. In mid-October 2015, the Federal Bureau of Prisons released another 6,000 offenders to help with the dilemma of population overcrowding and reduced the time served for non-violent offenders with harsh sentencing for some drug-related offenses.[1] The drop in numbers has been attributed to lower incarceration rates (down 2.3%), as well as a community supervision decrease (down 1.3%).[2] Another reason for the decrease in prison populations was overcrowding in prisons. Attitudes that people have toward ex-offenders have a significant impact on their lives, including social inclusion and employment. Attitudes can be explicit (consciously aware) or implicit (unaware), and each type of attitude may or may not affect behavior in the same way.[3,4,5] Wilson, Lindsey, and Schooler demonstrated that explicit and implicit attitudes can have different outcomes on behavior.[6] Explicit attitudes have been shown to have a greater influence on well-thought-out decisions. Conversely, implicit attitudes are more difficult to control and monitor; thus, these affect decisions when a person does not know what the response should be. Former President Barack Obama supported a law reform for hiring practices involving ex-offenders. The "Ban the Box Campaign" would ban the criminal information questions from federal employment in the *initial* hiring stage.[7] There are 25 states, (including Oklahoma), and more than 150 counties and cities across the United States that have adopted the "Ban the Box" from the initial job application, which delays background checks until a later stage in the hiring process. The Fair Chance Act protects ex-offenders' rights to have their employment history based on experience rather than viewed as an ex-offender on the first page of an application. The act provides ex-offenders the same chance as everyone else who is applying for the same position.[8]

In conclusion, this study could educate employers on the significance of implicit attitudes and their impact. Many people are not aware of how influential these attitudes can be. If they are aware of the impact, they may think more conscientiously about employment decisions. Hopefully, this study will also influence interviewees to request a reason for their employment denial; which is usually offered in the application paperwork.

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Attitudes, Offenders, Employment

I31 A Complex Case of Psychosis and Factitious Disorder

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The goals of this presentation are to highlight the diagnostic challenges of recognizing factitious disorder and to separate this diagnosis from malingering and psychosis in a clinical setting.

This presentation will impact the forensic science community by improving the ability of clinicians to understand and recognize the diagnostic challenges in separating factitious disorder from malingering and psychosis. This presentation will also improve the ability of clinicians to manage this disorder.

Factitious disorder is a *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-V*) -classified condition characterized by an individual's intentional deception of medical professionals and others to present themselves or another as ill, injured, or otherwise impaired, motivated by a desire to assume the "sick role."[1] Factitious disorder can pose as a diagnostic challenge, as the patient will often go to great lengths to convince others they are ill, whether by feigning symptoms or by self-inflicting illness or injury, often leading to unnecessary and, in some cases, costly and invasive tests and procedures.[2,3] After medical illnesses have been ruled out, factitious disorder may also be confused with other psychiatric disorders, including somatic symptom disorder and conversion disorder (both of which can be distinguished from factitious disorder by the absence of intentional falsification of symptoms) or malingering (characterized by patients feigning symptoms for secondary gain).[1]

Due to the fact that factitious disorder remains so diagnostically elusive, the exact prevalence is unknown. Studies have suggested that between 0.3% and 1% of patients admitted to general medicine services, who also have had consults placed to psychiatry, may actually have had diagnoses of fictitious disorder.[3-5] The majority of patients with factitious disorder present feigning medical conditions, with endocrinologic, dermatologic, and cardiac complaints being the most prevalent.[6] Among patients on psychiatric units, studies have estimated between 0.5% and 8% may have diagnoses of factious disorder with a primary psychological complaint.[7,8] This occurs most commonly with co-morbid diagnoses of substance use disorder, depression, and cluster B personality traits.[6,9-11] There have also been past reports describing factitious disorder patients presenting with primary symptoms of bereavement and post-traumatic stress disorder.[12-15] Factitious disorder patients presenting with primary symptoms of psychosis are less common, though there have been sporadically documented case series and reports.[16,17] This study presents a diagnostically challenging case of a patient with an unusual presentation of factious disease, with primary presenting symptoms in psychotic and somatic spheres.

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Mental Health, Psychosomatics, Malingering

I32 Criminological Analysis of Human Smuggling and Migrant Trafficking Into Italy

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After attending this presentation, attendees will understand the identification and criminal characteristics of human traffickers, as well as the organizational structure of trafficking operations into Europe.

This presentation will impact the forensic science community by elucidating the findings of a recent study investigating migrant smuggling and human trafficking operations into Europe in an effort to propose empirically based identifiers to assist naval intervention. Furthermore, this presentation will define the role of the forensic and mental health advisor in operations designed to thwart these crimes.

With increasing frequency, migrants have been entering Europe. The use of human traffickers is a common method used by migrants to achieve these goals, despite being subjected to dehumanizing treatment and perilous journeys. Previous studies regarding human trafficking have primarily highlighted that the smuggling of migrants is operationalized within a stable, hierarchical, and transnational structure, using non-banking, intermediary, financial circuits called Hawala as payment. These organized crime efforts have been difficult to study because of variable investigative methods. In addition, traffickers make efforts to avoid detection, and migrants, typically instructed to avoid identification photographs, are difficult to identify.

The goal of this research study, conducted on behalf of the European Union Naval Force Mediterranean/Operation Sophia (EU NAVFOR MED), was to identify criminological aspects of migrant smuggling and the characteristics of traffickers in order to aid in halting human trafficking in Italy.

This study examined a large number of relevant court cases and reviewed national and international literature on the topic with the goal of identifying potential criminal traffickers, from a criminological perspective, during the first moments of migrant survivor rescue. This study found that traffickers are generally male, between 25 and 35 years of age, speak several languages, have good persuasive communication skills, live in shelters, have legal residency paperwork, and are sometimes married.

This study also proposes a scientific investigative protocol, based on holistic and systemic methods, to better characterize and study the *modus operandi* and style of the perpetrators of these crimes. Strategic investigation may be conducted on two levels, direct and indirect, while migrants are detained and recovering from rescue.

A direct investigative approach involves the use of digital audio and video recordings of survivor behaviors; these materials are often requested by the judicial authorities. Alternatively, an indirect investigative approach focuses on interviews of survivors, specifically women with minor children, who generally have fewer ties to the traffickers. This method would be managed through the use of behaviorally trained forensic consultants.

This study proposes that forensic consultants, used to evaluate these survivors, employ a questionnaire that evaluates the communication methods used by traffickers. The Self-Administered Interviews (SAI) questionnaire was found to be most efficient for this task. In addition, a direct interview to reconstruct the context and the actions of traffickers would be helpful using a Cognitive Interviewing (CI) technique. This technique provides validation for the concerns of migrant survivors and provides investigators with a wealth of information on human traffickers.

The use of these investigative methods in a systematic manner will aid in accumulating relevant data regarding this phenomenon.

Criminological Analysis, Human Smuggling, Smuggling

I33 Live Streaming Suicide and Murder on Facebook®: Can Someone Be Held Liable?

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After attending this presentation, attendees will better understand the unique legal considerations related to the live streaming of suicide or murder on the social media site, Facebook®. Attendees will also become familiar with freedom of speech on social media platforms and the associated liability.

This presentation will impact the forensic science community by: (1) presenting a review of the current laws regarding the liability of live streaming suicide and murder; and, (2) discussing the possibility of a duty by the social media site and viewers to protect others and prevent harmful acts.

Facebook® Live is a service that allows Facebook® users to create and broadcast real-time videos to their followers. It became publicly available in January 2016. Since then, a string of suicides and some murders have been broadcast over Facebook® to a wide audience. These incidents raise questions regarding legal liability related to freedom of speech, trauma inflicted on viewers, and obligations to protect and prevent persons from engaging in harmful behavior.

Does Facebook® have a legal responsibility to censor disturbing content, such as live broadcasts of suicide or murder? The first amendment gives Facebook® and its users the right to freedom of speech; however, there are instances when freedom of speech does not protect all content. For example, obscene content (material that offends the sexual morality of its viewers) is federally banned from the internet. Although suicide and murder do not fall under this category, is there another category that would ban such disturbing content?

If such videos are not monitored and removed from viewing on Facebook®, can Facebook® observers of these acts of suicide and murder sue the media site for trauma inflicted while watching these videos? There have been cases in which relatives present at the time of death successfully sued for emotional distress after witnessing their family member being killed; however, in these lawsuits, the negligent infliction of emotional distress required that the plaintiff was physically near the scene of the incident. Negligence has been hard to prove and often unsuccessful in cases viewing death from a distance as well as on televised media.

After many suicides were live streamed, Facebook® launched tools for viewers to report suicides. Given this ability, would the viewer have a legal obligation to report suicidal behavior? The law traditionally does not impose a general duty on the public to prevent another person from taking his/her own life; however, the case of a "special relationship" (e.g., mental health professional-patient relationship) can give rise to a responsibility where none would otherwise exist. For example, if a psychiatrist were to see a patient live streaming suicidal behavior, then the psychiatrist potentially would have a duty to protect the patient. If it is a murder that is being live streamed, certain states require any person who reasonably believes that he/she has observed the commission of a murder to notify a Peace Officer.

Facebook® currently has teams designing artificial intelligence algorithms for identifying users who may be at risk for suicide before it becomes too late. If this algorithm could match or even surpass a physician's diagnostic abilities, what duties would Facebook® owe to the individuals its algorithm identifies or overlooks? Could Facebook® be found negligent if it does not do enough to prevent suicide?

This presentation will review the literature on live streaming suicide and murder with respect to key legal considerations that pertain to liability for viewers and media sites. In addition, possible recommendations toward reducing the incidents of harmful behavior that is live streamed will be provided.

Live Streaming, Death, Liability

I34 Do Evidence Submission Forms Expose Latent Print Analysts to Task-Irrelevant Information?

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The goals of this presentation are to educate attendees regarding the nature and quantity of potentially biasing, task-irrelevant information routinely requested by crime laboratories before latent print analyses are conducted and to provide insight into appropriate countermeasures.

This presentation will impact the forensic science community by providing results of a study examining the type and relevance of information requested by evidence submission forms in laboratories across America. Further, this presentation will apply the recent literature demonstrating contextual effects in forensic sciences to the evidence submission process and offer recommendations for countermeasures.

In 2009, the National Academy of Sciences (NAS) released their congressionally mandated Report, *Strengthening Forensic Science in the United States: A Path Forward.*[1] Detailing a variety of problems in the wide-scale practice of forensic science, this influential Report prompted media attention and widespread calls for reform. The concern that forensic science findings may be influenced by *contextual effects* (i.e., extraneous data and pressures that are unnecessary and potentially biasing to scientific analysis of fingerprints, firearms, DNA, and other evidence) was a primary identified problem. For example, forensic scientists who perform circumscribed procedures such as analyzing latent fingerprints may receive superfluous information regarding the criminal suspect or crime scene details; such contextual information is unnecessary to the task of comparing fingerprints and has the potential to bias the examiner toward a particular finding.

Concerns regarding contextual effects are clearly consistent with a rich body of research in cognitive and social psychology.[2] Moreover, several seminal studies specifically addressing contextual effects among forensic science procedures recently raised concerns throughout the forensic science community.[3-5] Although limited, this growing body of research has substantial implications for policy and justice — many advocates have already urged substantial reforms.[6]

One of the primary recommendations offered in the NAS Report was to identify sources of bias and develop appropriate "countermeasures."[1] This current study sought to clarify the nature and quantity of potentially task-irrelevant information that is routinely requested before latent print analyses are conducted in forensic laboratories. Moreover, this study seeks to identify explicit requests for potentially biasing information in order to provide insight into appropriate "countermeasures."

In this study, 183 crime laboratories accredited by the American Society of Crime Laboratory Directors – Laboratory Accreditation Board (ASCLD-LAB) for the analysis of latent prints were first identified. An additional 24 laboratories accredited by ANSI-ASQ National Accreditation Board (ANAB) (a unified list of accredited laboratories was not available at the time of data collection) were identified in addition to three other laboratories that were either unaccredited or had recently stopped conducting latent print analyses. This study only identified laboratories accredited in latent print analysis for simplicity, clarity, and due to recent research specifically demonstrating significant contextual effects in latent print analyses.[3,7] Each laboratory was asked to provide a blank evidence submission form used in latent print analysis requests. Two weeks after the initial request, a reminder email request was sent to all laboratories that did not respond. All remaining laboratories are now being contacted by telephone to personally request blank evidence submission forms.

To date, 76 laboratories responded to this study's request and the provided submission forms represent at least 105 laboratories across America. The submission forms from 68 laboratories were sufficient to be fully coded. These forms represent at least 97 laboratories in 39 states. Descriptive analyses focus on information requested by submission forms regarding the offense, suspect, victim, and other seemingly task-irrelevant and potentially biasing subjects. For example, approximately 96% of all forms request information regarding the type of offense, whereas only 20% specifically request a police or incident report be provided. Approximately half of all forms request information regarding the suspect's race and criminal history. Moreover, while some task-irrelevant prompts appear to have practical purposes (e.g., approximately half of the forms request information regarding the location of the offense and most request suspect's name), others have no overt practical purpose (e.g., victim sex and race). Moreover, approximately 18% of forms request seemingly task-irrelevant information that appears likely to bias latent print analysts (e.g., "Is suspect serious violent felon?"; "Please indicate if item was the probable-cause evidence in your case"). This presentation will provide much more descriptive information regarding the type of task-irrelevant information being requested and exposed to latent print analysts.

In conclusion, this presentation will discuss the potential influence of task-irrelevant information currently being requested before latent print analyses are performed. This presentation will also relate current findings to extant literature demonstrating contextual effects in forensic sciences and latent print analysis specifically.[3,5,7] Finally, there will be discussion regarding methods of achieving the correct balance of information necessary for latent print analysis (e.g., case management, linear sequential unmasking, Laboratory Information Management Systems (LIMS)) to prevent subconscious bias.

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Contextual Effects, Bias, Latent Print Analysis

I35 Jury Instructions on Insanity Acquittal Disposition

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The goals of this presentation are to: (1) provide a summary of federal and state laws on jury instructions on insanity acquittal disposition; (2) review the primary reasons for and against jury instructions on what happens to a person acquitted by reason of insanity; and, (3) review the empirical evidence on juror knowledge concerning insanity acquittal disposition.

This presentation will impact the forensic science community by reviewing the arguments for and against jury instructions on insanity acquittal disposition and will provide attendees with a review of the empirical research on juror knowledge concerning the consequences of a successful insanity defense.

Jurors generally know that persons found guilty of a crime are punished and persons found not guilty are set free. Although jurors may not be aware of sentencing guidelines applied to a particular defendant, they generally have a basic understanding regarding the range of criminal punishments afforded to persons found guilty of a crime and understand that most convicted defendants serve time in jail or prison.

Perhaps less obvious to jurors is what happens to defendants who are found Not Guilty by Reason of Insanity (NGRI). Because of uncertainty regarding NGRI disposition, some jurors may believe that criminally insane defendants are set free upon an NGRI verdict or may have unreasonable expectations about the duration of confinement.

An important topic concerning the insanity defense is what jurors should be told about the disposition of a defendant acquitted NGRI. In the federal system, jurors are not told about the consequences of an insanity verdict under *Shannon v. United States.*[1] State courts are divided on the issue.

This presentation will review the current status of jury instructions — in the federal system and among the states — on the consequences of an NGRI verdict. The most recent legal cases on this topic will be discussed. The role of the jury will also be reviewed. Historically, juries have decided on guilt without knowledge of the consequences to the defendant.

In this presentation, principle arguments for and against a jury instruction on NGRI disposition will be reviewed. Some courts address this issue by emphasizing the differences between the role of the jury and that of the judge — that judges are responsible for applying the law and imposing sentences, not the jury.[2] Other state courts have held, at least as a matter of policy, that a jury instruction on NGRI disposition is necessary to prevent juror confusion and misguided verdicts. In states that have both NGRI and Guilty but Mentally Ill verdicts, instruction(s) about the verdicts and disposition outcomes are likely to be particularly important to reduce juror confusion.

Of particular interest, this presentation will provide a review of the empirical evidence on juror knowledge regarding insanity acquittal disposition. Although the studies are limited in number and scope, they provide relevant information concerning juror knowledge and attitudes about the insanity defense. The weight of the studies confirms juror misunderstanding about a defendant's disposition upon an insanity acquittal. In one study, a juror commented that he voted for a guilty verdict (in contrast to NGRI) because he "did not want a mad dog released."[3] This comment exemplifies the reason to give an instruction on the consequences to the defendant following acquittal by reason of insanity.

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Insanity, Jury Instruction, Diisposition

I36 Compassionate Care for the Criminal Courts

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The goal of this presentation is to educate defense attorneys, district attorneys, judges, and mental health professionals who work with the criminal court system on the benefits of compassionate care and enhancing empathy in a system that is traditionally adversarial.

This presentation will impact the forensic science community by integrating concepts in contemplative science with issues in forensic science.

People who are involved with the criminal justice system are under an extreme amount of stress. Defendants are facing possible lengthy incarcerations, defense attorneys are often overworked and underpaid, and judges have an overwhelming number of cases on their docket. Prosecutors are concerned with protecting the community and are not usually as interested in issues pertaining to a suffering individual who has committed a crime. When a defendant is mentally ill and needs treatment to be able to become competent to stand trial, this can delay cases for months and sometimes years. Legal professionals who are not trained in mental illness often have a difficult time understanding how mental illness can affect criminal responsibility and could interfere with someone's ability to understand their charges and assist in their own defense.

Mental health professionals and officers of the court often speak different languages. Issues pertaining to the law are focused on responsibility and blame. Psychiatrists and other clinicians are more concerned with diagnosis and cure and how to help someone out of their suffering.

Concepts from Compassionate Care in contemplative science may be helpful in laying common groundwork between mental health professionals and legal professionals that could benefit their own wellbeing as well as their understanding of mentally ill criminal defendants.

There are numerous studies on the psychological and neurological health benefits of meditation, yoga and other relaxation techniques that could help educate professionals in the criminal justice system on how to take care of their emotional health in a manner that results in more energy and understanding for another's suffering. Teaching officers of the court to take time to engage in self-care could help them develop the capacity to better understand and appreciate people who are mentally ill and suffering. Meditating and slowing down at times during the day could also be helpful.

Numerous case examples illustrate the need for better understanding of the difficulties mentally ill people face when they are in the criminal justice system. Enhanced empathy does not change the adversarial nature of the court system, but could provide each side with more information in order to better understand each position and the individual's unique set of circumstances. As a result, they work together to help the defendant/client receive appropriate treatment/rehabilitation. Ultimately, this allows the criminal court system to function in a fairer and more efficient manner.

Contemplative, Criminal, Court

I37 The Patient Can Leave: Involuntary Hospitalization of Non-Psychiatric Patients Who Lack the Capacity to Refuse Medical Treatment

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After attending this presentation, attendees will have gained knowledge of California's current laws regarding involuntary hospitalization of medically ill patients who do not meet criteria for an involuntary psychiatric hold.

This presentation will impact the forensic science community by illuminating the quandary the medical professional may be in when a patient who lacks capacity to refuse treatment chooses to leave the hospital. As most of these cases involve individuals with cognitive impairment, this will become an even greater issue as the proportion of the aged population increases.

Generally, there is no legal mechanism to hospitalize medically ill patients against their will unless they are psychiatrically ill, have a legal conservatorship, or have certain contagious diseases. Possible solutions to this dilemma will be discussed.

The need for involuntary hospitalization occasionally arises for medically hospitalized patients. Most of these cases pertain to patients with an acute mental illness. In California, if a person is mentally ill and a danger to self and/or others, or gravely disabled (unable to provide food, clothing, or shelter), the patient can be involuntarily hospitalized for psychiatric treatment for a 72-hour period. California law allows for additional periods of involuntary hospitalization of psychiatric patients, if indicated.

However, at times there are medically ill patients who do not have a psychiatric illness but may require involuntary hospitalization for medical treatment purposes. An example may be a patient with delirium who lacks the capacity to refuse treatment and wants to leave the hospital. When an individual requires inpatient medical treatment, but lacks the capacity to refuse the treatment, a petition can be filed under California Probate Law. If the court finds that the patient lacks capacity, the court can authorize treatment and appoint an individual to make health care decisions on behalf of the patient for that specific medical problem.

It is important to recognize that when this health care decision-making is granted to another, the law does not explicitly grant that individual the authority to involuntarily hospitalize the patient against their will. Consequently, the patient could choose to leave prior to administration of the treatment. Given this situation, staff members at some hospitals are instructed to refrain from physically restraining these patients if they try to leave the hospital. The hospital is in a difficult situation. If they prevent the patient from leaving, they are doing so illegally. If they allow the patient to leave, they are possibly exposing the patient to a serious threat to their physical health.

The situation mentioned above presents medical professionals with a significant dilemma. Changes to hospital policy or legislation could provide much needed direction.

This presentation will provide a general overview of California statutes related to involuntary psychiatric hospitalization, guardianships, conservatorships, and other protective proceedings. A case example will be used to illustrate the application of these laws and current hospital policies.

Involuntary Hospitalization, Capacity, Leaving

I38 Capacity to Consent to Psychiatric Treatment in Ontario, Canada: Perspectives From a Forensic Psychiatry Program

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After attending this presentation, attendees will: (1) understand the legal concepts of the capacity to consent to treatment in Ontario, Canada; (2) understand the challenges that clinicians have when someone is refusing psychiatric treatment (including risk of violence); and, (3) be able to discuss ethical aspects of forcing someone to receive psychiatric treatment.

This presentation will impact the forensic science community by increasing awareness of a different legal framework and what the impact is on both the patients and the community.

Introduction: Pharmacological treatment can be one of the major components of the mental stability of individuals suffering from a psychotic disorder or a bipolar disorder; however, as per the nature of their psychotic or mood symptoms, these patients may lack insight into their condition and refuse to take psychiatric medication. In that situation, these persons may remain acutely psychotic or manic for months and even years if no action is taken to force medication. Most of the legislation authorize but limit the use of forced treatment. The most common method in place is when a Substitute Decision Maker (SDM) is named; in that case, the SDM will make the decision on behalf of the patient as he has the capacity to understand the nature of the mental disorder and the consequences of taking or not taking psychiatric medication. Also, many legislations allow the patient to appeal the decision of the physician who found his/her patient incapable of consenting to treatment.

In Canada, each province and territory has developed its own legal framework for consenting to treatment. In Ontario, the legal process is deemed under the Consent and Capacity Act. It appears that the Ontario process has the particularity of not allowing any treatment until the appeal process is extinguished, either because of the patient not having appealed the decision within the allowed time or because no further appeal is possible (the last decision from the Supreme Court of Canada). Some legal procedures may be quick (a few weeks) and some may be extremely long (one or more years), during which time the patient remains acutely unwell. The individuals in favor of such a procedure often argue that each patient should be allowed a freedom of choice.

Methods: In the St. Joseph's Healthcare Forensic Psychiatry Program, patients who have gone the route of appealing the decision of capacity to consent to treatment were identified. Several factors were reviewed, such as how long the process took place, how far the patient appealed the decision, and the change of their mental status before and after receiving treatment (including their risk factor, their aggressive incident, and their access to privileges)

Results: Ten patients were identified who appealed their treatment capacity. Only three of them went to the Supreme Court of Canada. The longest appeal was 1.5 years. All had a significant decrease of their aggressive incidents after receiving treatment and were allowed to use off-unit, unaccompanied privileges shortly thereafter.

Discussion: The data related to the significant behavioral improvement after receiving treatment was expected; however, obtaining these data may help provide some guidance to enhancing a different legal process and help these patients recover more quickly. It also raises ethical questions and legal issues, notably when these patients are part of the forensic system and their main psychiatric disorder is associated with an offense. All of these points will be detailed in the presentation.

Capacity to Consent, Psychiatric Treatment, Forensic Psychiatry

J1 An Examination of Highly Deceptive Counterfeit Currency

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After attending this presentation, attendees new to the field will have gained a useful learning experience regarding security features present in Pakistani currency notes, while practicing questioned document examiners will have a better understanding of those security features that require special attention when dealing with the detection of high-technology counterfeit currency.

This presentation will impact the forensic science community by illustrating various security features that have been emulated to a high degree of success by counterfeiters and those qualities that could not be replicated.

Counterfeiting of currency has been occurring for ages, and there has been a continuing race between certifiers and counterfeiters for all that time. The state always attempts to introduce security features cannot be forged in their currency notes, but the problem of counterfeits has persisted. Counterfeiters attempt to duplicate the overt features and appearance of genuine currency notes and include at least some of the required security features in their counterfeit products. The original security features that require uncommon high-technology facilities cannot be duplicated in the same way as they appear in genuine notes; however, the introduction of sophisticated color printers, color photocopiers, and scanners in the early 1990s brought a dramatic shift in counterfeiting technology. This new technology has made it possible for counterfeiters to produce imitation currency of a higher quality. A number of counterfeit detection methods are being used by crime laboratories and other law enforcement agencies, but some are time consuming, some are imprecise, and some are destructive. In this presentation, a non-destructive method of counterfeit Pakistani currency note detection that does not involve specialized equipment, except for the readily available video spectral comparator and stereomicroscope, will be demonstrated.

A case study involving the examination of Rupees 5000 and Rupees 1000 Pakistani currency notes for their genuineness was performed in a non-destructive manner by using a Video Spectral Comparator (VSC6000) and a stereomicroscope. Both reference and questioned currency notes were examined for all available security features, including Ultraviolet (UV) -luminescent features, intaglio printing, watermarks, denomination printed with see-through register, and the flag printed with Optically Variable Ink (OVI). This comparative study revealed that the questioned currency notes included security fibers, security thread, see-through register, watermarks, UV-luminescent printing, and the OVI-printed flag appeared similar to those in genuine currency notes, but the quality of the watermark, UV-luminescent printing, and positioning of the see-through register was found to be different. A detailed examination of the inks used to produce micro-printed features revealed that a four-color printing process had been used instead of monochromatic printing inks and the rainbow printing process. Keeping in view all previous observations from counterfeit currency notes submitted at different time intervals for examination, it can be concluded that micro-printing features and rainbow printing are the most difficult to be introduced into counterfeit currency notes, so these features must be observed carefully by the examiners.

Counterfeit Currency, Questioned Documents, Security Features

J2 Questioned Document Examination of Two-Source Traced Forgery in Signatures: A Case Study

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After attending this presentation, attendees will have learned how to examine two-source tracing in signatures. Traced forgery is performed using tracing paper, carbon paper, transmitted light, indented tracing, or a scanned image, but two-source signature tracing in questioned documents has great importance in solving questioned documents signature cases.

This presentation will impact the forensic science community by introducing a unique case study in which two-source signature tracing in questioned documents was analyzed with the comparison of known routine signatures of the deceased.

Pakistan has been affected by the worst form of terrorism and militancy. The scourge of terrorism has taken its toll on every aspect of life. One of the better ways to help overcome these difficulties is through education, and, in particular, high-level research and development in the field of forensic sciences. Signatures have great importance today, as every person signs his or her name or mark once, twice, or numerous times a day. Signature forgery can occur in many ways, including freehand simulation, tracing, and image transfer. Alternatively, a signature may be written with some disguise with a view to disavowing it at a later time.

Due to the regular use of signatures, the problem often arises of determining whether these signatures are genuine, disguised, or traced. Signature forgery means changing any signature with the intent to deceive, which could be accomplished in a number of ways. One of the methods most often used is discussed in this case study, that of two-source traced forgery, which means reproducing an exact copy of the genuine signature by using two sources of an original signature. In this type of forgery, most signatures that are traced have two parts. One part of the signature is traced from a document with an original signature and the second part is traced from another document with a second original signature to reveal the natural variation in signatures. Traced forgery is performed by using tracing paper, carbon paper, transmitted light, indented tracing, or scanned images. The forensic questioned document examiner examines every detail of the traced signature and concludes whether or not the questioned signature exhibits the characteristics and mathematical measurements with respect to the specimen and admitted routine signatures. In this presentation, a case will be discussed in which questioned signatures were examined and conclusions provided when the questioned author was deceased; the forensic document examiner relied only on admitted routine signatures. The comparison/examination of questioned signatures was performed, followed by the comparison/examination of the admitted routine signatures in order to locate the origin of the questioned signatures. The result in this particular case revealed that the disputed signature exactly superimposed with a slight adjustment with the admitted routine signature.

Signatures, Tracing, Forgery

J3 The Significance of Electrostatic Detection Apparatus (ESDA) in the Determination of Tampering

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After attending this presentation, attendees will better understand the importance of ESDA in determining alleged tampering in cases in which the conventional use of a video spectral comparator does not produce promising results sufficient to prove alleged tampering.

This presentation will impact the forensic science community by demonstrating the use of ESDA as a significant tool in the determination of tampering in cases in which no significant visually apparent signs of tampering were present.

Background: One of the most demanding fields of forensic document examination is proving alleged tampering in a disputed document using a variety of techniques. When examining a piece of writing for alleged tampering, one of the items the examiner looks for is any difference in the writing instrument/ink used. This will consequently result in one of two findings: the writing instrument(s) used in the area of the questioned writing is similar/same, or more than one (different) writing instrument has been used. In the latter scenario, the interpretation is usually easier, whereas in the former, there may be no tampering or tampering using the same or similar writing instrument. The examination may become complicated when the suspected tampered area of writing involves writing instruments having ink composition that is similar enough to evade differentiation by commonly used non-destructive methods. Such disputed documents prove to be more demanding for the document examiner, and the use of alternate techniques accompanied by problem-solving approaches becomes essential.

Method: This presentation discusses a case that involves successful detection of tampering (alteration) in a disputed notebook. The case presented a common problem involving the use of the same or very similar inks/writing instruments for tampering that the conventional use of a Video Spectral Comparator (VSC6000) was unable to prove tampering in the disputed notebook. Based on preliminary optical examination of the disputed entry, the examiner was sure that "something" was suspicious about the questioned entry in the disputed notebook and continued to investigate. The questioned entry was also examined from the rear using a VSC6000 to discover any information, but resulted in failure. At last, the examiner processed the page underneath the disputed page of the notebook by using ESDA^2. Use of ESDA^2 resulted not only in a number of findings sufficient to prove tampering in the questioned notebook but also provided probative evidence to the investigation.

Conclusion: Use of ESDA^2 seems to be an ideal technique in cases in which no significant visually apparent signs of tampering are present. Tampering (alteration) done with the same and/or a similar type of ink/writing instrument but lacking normal and natural writing flow, consistent writing pressure, and involving suspicious pen lifts may be successfully revealed by using ESDA^2.

Tampering, ESDA, VSC

J4 Rubber (Hand) Stamps: A Long-Term Study Into Stamp Wear and Damage, Stamp Impression Variation, and Incompatible Inks

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After attending this presentation, attendees will be familiar with: (1) examples of stamping impression variation in addition to the progressive wear/damage to stamps over a long period of time; and, (2) the effect that an incompatible ink has on polymer stamping materials.

This presentation will impact the forensic science community by providing information concerning: (1) the preliminary results of long-term experiments regarding progressive wear/damage to rubber (hand) stamps; (2) the variation in stamp impressions; and, (3) the effects of incompatible inks on polymer stamping materials.

The topic of stamp wear/damage, stamp impression variation, and incompatible ink effects is described in different examination standards and some publications; however, there is less published on the documented progression of wear/damage to stamps and the degree to which variation in stamp impressions may occur from different stamping actions.[1-4] Similarly, there is little published on the progressive damage to stamp dies over a period of time when an incompatible ink has been used.

Part 1 of this study involves getting stamp impressions from different-sized stamps and from different individuals. Three different sizes and types of stamp were used: a large-area rubber stamp (38mm by 72mm), a self-inking round rubber stamp (diameter 24mm), and a mass-produced, factory-made stamp with a combination of date and selectable word (55mm by 5mm). Each of these stamps used a separate inking pad.

To obtain impressions with variation from the stamping process, a variety of volunteers were sought. The volunteers were attendees at two forensic science meetings: the Australasian Society of Forensic Document Examiners Inc. (ASFDE Inc.) Meeting held in Melbourne, Australia, during May 2017, and the International Association of Forensic Sciences (IAFS) Meeting in Toronto, Canada, during August 2017. Impressions were made by attendees of the meetings during different times onto pre-numbered and configured sheets designed for each stamp type. Variation was present due to the differences in the biomechanics of stamping from participants and from the number of impressions taken in a session. For example, in some stamping sessions, participants made many impressions in a short period of time; this is in contrast to when a single impression was made.

The collection of additional stamp impressions is ongoing, with an opportunity to participate at this meeting by making impressions. Only the number of impressions made on a given day will be recorded, with no information about anyone making impressions retained.

Part 2 of this study is an experiment to determine the effect of an incompatible ink on polymer stamping materials over a long period of time. Incompatible inks are those with chemical formulations that may degrade stamping materials for which they were not designed. This experiment involves different stamps that are not part of the wear/damage and variation study. One stamp and one unmounted die for both polymer and rubber-stamping materials were made using the same graphics. Impressions were made on a regular basis using a fast-drying (alcohol-based) ink. Alcohol-based inks are not designed to be used with polymer stamps but can be used with rubber-based stamps. An equal number of impressions were produced over the period of approximately one year with the polymer and rubber stamps; ink was applied to the unmounted dies, but no impressions were taken.

The presentation on Part 1 of the study will focus on the variation in impressions with secondary discussion on the observed wear/damage. For the Part 2 experiment, the degree of degradation for the polymer stamp compared to the rubber stamp will be presented and discussed.

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2. Maureen A. Casey. The individuality of rubber stamps. *Forensic Science International*. 12 (1978): 137-144, doi:10.1016/03790738 (78)90022-1.
3. A. Herkt. Rubber Stamps, Manufacture and Identification. *Journal of the Forensic Science Society*. 25 (1985): 23-38, doi:10.1016/ S0015-7368(85)72359-6.
4. *SWGDOC Standard for Examination of Rubber Stamp Impressions.* Scientific Working Group for Forensic Document Examination, accessed 8 July 2017, http://swgdoc.org/images/documents/standards/SWGDOC%20Standard%20for%20Examination%20of%20Rubber%20Stamp%20Impressions.pdf.

Rubber (Hand) Stamps, Document Examination, Variation

J5 An Analysis of Indented Writing Impressions in Questioned Documents Using Flatbed Scanners in Pakistan

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After attending this presentation, attendees will better understand how to decipher indented writing on different types of paper without using the Electrostatic Detection Apparatus (ESDA). Indented writing on documents has great importance in solving questioned documents cases. Therefore, in this research lecture, different techniques will be discussed relating to how results can be obtained without using an ESDA.

This presentation will impact the forensic science community by demonstrating a non-destructive process that leaves no marks on the document and has significant results.

Pakistan has been affected by the worst form of terrorism and militancy. The scourge of terrorism has taken its toll on every aspect of life. One of the better ways to help to overcome these difficulties is through education, and, in particular, high-level research and development in the field of forensic sciences. Indented writing may have significant value in resolving questioned document issues. As the term implies, indented writings are non-visible indentations applied to a sheet of paper positioned below the page actually written upon. While a specialized laboratory instrument can be used by document examiners to recover indented writing, the indentations may be made visible by using side light and flatbed scanners with the use of software. This is a non-destructive process in which the questioned documents do not have any type of change or effect. In this study, different types of techniques were used on different weights of paper.

Forensic document examination is the application of forensic science in analyzing questioned documents. Indented writing impressions can be seen by using an angled, oblique light with high intensity, or more accurately, by using the ESDA; however, because of the limited resources in Pakistan, the accessibility of costly equipment such as the ESDA in forensic labs in all provinces of Pakistan is limited. This suggests that an instrument with equal performance and lower price should be a substitute. In this study, various types of papers found at crime scenes that had indented impressions were analyzed using the ESDA and commercial flatbed scanners with image enhancement software (Adobe® Photoshop® CS-8). This study was divided into three experiments: (1) dissimilarity of interleaving paper; (2) paper quality test; and, (3) variation of writing pressure. The results of this study reveal that flatbed scanners can be used as an alternative instrument for recovery and revealing of indented writing impressions.

Questioned Documents, Indented Writing, Adobe® Photoshop® (CS-8)

J6 The State of the Art in Computational Forensic Document Examination

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The goal of this presentation is to provide attendees with a better understanding of the document research work being conducted that may not initially have a forensic intent but may ultimately be applicable to further development of the profession.

This presentation will impact the forensic science community by alerting practitioners to tools and techniques they would not normally find within the forensic community.

Forensic Document Examination (FDE) is a broad discipline that, while necessarily being firmly tied to traditional technologies, is greatly affected by modern advancements in instrumentation and analytical techniques. FDEs are quite familiar with databases for examined subject matter, such as inks and typewriters, and digital tools, such as laser microscopes, spreadsheets, and advanced image enhancement; however, there is another parallel world of work conducted on documents in academia that FDEs should find beneficial.

The Pattern Recognition (PR) research community has been working on applying PR techniques to document and handwriting problems as they have been presented in the world of archiving and commerce. While these subjects tend to focus on digitizing historical tomes or modern business documents, a subset of PR researchers have worked on automating handwriting authorship determinations, the same analyses that FDEs claim as expertise.

The International Association for Pattern Recognition (IAPR) has a number of Technical Committees (TC); number 11 (TC11 – Reading Systems) concerns the analysis and recognition of document-based information, namely image processing, Optical Character Recognition (OCR), and handwriting recognition. TC11 publishes the *International Journal on Document Analysis and Recognition*. There are two recurring conferences and one workshop which are held biannually and in alternate years.

The International Conference on Document Analysis and Recognition (ICDAR) is held on odd calendar years and is geared primarily, but not exclusively, toward non-handwriting document issues. On even years, the International Conference on Frontiers in Handwriting Recognition (ICFHR) is held, as is the main TC11 workshop, Document Analysis Systems (DAS).

Smaller workshops held in conjunction with these conferences have included Historical Image Processing (HIP), the International Workshop on Camera-Based Document Analysis and Recognition (CBDAR), the International Workshop on Multilingual OCR (MOCR), and the International Workshop on Robust Reading (IWRR). New for 2017 are the Historical Book Analysis (HBA) workshop and the First International Workshop on Computational Document Forensics (IWCDF). Additionally, the Automated Forensic Handwriting Analysis (AFHA) group was well-attended by FDEs when it was co-located with the meeting of the American Society of Questioned Document Examiners (ASQDE) in 2014.

These gatherings typically make use of competitions whereby interested parties will acquire a common dataset of known ground truth and apply their algorithms against the problem at hand. Winners are announced after the submissions have been evaluated for efficiency and accuracy.

In addition, the forecast of the paperless office has largely failed to materialize and the amount of hardcopy documents used in society remains large, while we have simultaneously seen a swelling of digital document quantities. Outside of PR, the task of organizing and navigating such cross-media collections is a point of important research as institutions see the reduction of librarians as a cost-cutting measure, while the work is downloaded to the subject matter expert. Researchers are at work integrating large collections and speeding the re-finding of hardcopy documents through searching a digital collection. Moreover, as cases grow in size and complexity, such interfaces could aid the FDE in an examination capacity so that comparing handwriting and other features across thousands of documents becomes a more attainable feat.

These products and techniques may prove useful to FDEs in future casework situations or in the organization of vast reference materials and databases. A review of the state-of-the-art in computational document forensics is presented, in addition to a recap of known technologies, highlighting capabilities and limitations. The potential uses of such advanced computational tools will be discussed.

Documents, Computation, Pattern Recognition

J7 Deployment of the Counterfeit Detection Training Electronic Learning Course

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After attending this presentation, attendees will be aware of the deployment strategy for the Document Security Features electronic learning course and how government agencies may request access.

This presentation will impact the forensic science community by providing a resource for comprehensive, broad, and scalable training on security feature technologies used in passports, identity cards, birth records, and other security documents worldwide.

In the United States Department of State, the Bureau of Consular Affairs and the Bureau of Diplomatic Security have developed a broad strategy for deployment of fraud document detection training to a geographically diverse mix of consular and law enforcement staff that must assess a broad range of national identity and travel documents. To address this need, the Bureaus jointly authored and deployed the e-learning course Document Security Features to audiences throughout the Department of State in 2017.

Document Security Features is composed of a series of electronic learning modules that cover a variety of anti-counterfeiting and anti-alteration technologies used in documents, including substrate features used in paper and plastics, printed security features, specialty inks, optically variable devices, and features based on lens technologies. Users are trained to recognize and authenticate these technologies independently of document type or the issuing source agency. Because this broad scope familiarizes users with discrete technologies rather than their specific deployments in individual national documents, users can rapidly assess and self-train on the security features in an unfamiliar document even where no additional background information can be obtained.

It is in the interest of both Bureaus to encourage better understanding of document security technologies within other government agencies at the United States federal, state, and local levels, and also among our partners in the international community. One of the key advantages of e-learning is that once the training is developed, delivery can be scaled in ways that are simply impossible for classroom-based training, because no instructors, classrooms or scheduling logistics are required. Accordingly, once the internal Department of State deployment was completed, the Bureaus commenced work on a second version of Document Security Features that could be offered to external audiences.

The external version of Document Security Features includes information on all of the same topics as the internal version, though some content has been adjusted for non-Department of State audiences. During this presentation, the course content, teaching format, and exercises will be demonstrated using the externally approved version of Document Security Features. For government agencies interested in using Document Security Features to train their own staff, procedures for requesting a copy and completing the required Memorandum of Understanding with the Department of State will be discussed.

Counterfeit, Training, E-Learning

J8 Security Document Artwork That Resists Reverse Engineering

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After attending this presentation, attendees will understand how design strategies for security document artwork can play a key role in the prevention of artwork reverse engineering by sophisticated traditional counterfeiters.

This presentation will impact the forensic science community by demonstrating novel strategies for the design of artwork in security documents and how forensic examination of document artwork could be impacted in the future if there is some adoption of this novel design paradigm.

Counterfeiting can be accomplished via one of two basic workflows: digital and traditional. The easier scan-and-print workflow of digital counterfeiting has made counterfeiting easier and more accessible to criminals with limited graphic art skills because it does not require counterfeiters to redraw document artwork, manufacture printing plates, or operate a printing press; however, digital counterfeiting processes are only capable of simulating document artwork using inkjet or toner devices. This places limitations on the quality of the counterfeits that can be produced by digital counterfeiting because the printing process characteristics of the letterpress, intaglio, and lithographic printing processes used to produce genuine security documents cannot be fully replicated with computer printers, office copiers, or multifunction devices.

In contrast, traditional counterfeiters follow a more sophisticated and technically demanding workflow that has the potential to produce counterfeits of higher quality. Traditional counterfeiters try to mimic not just the basic appearance of the document but also the combination of printing processes used in its manufacturing. Before any redrawing or replication of document artwork can proceed, traditional counterfeiters must reverse engineer the artwork to determine how many different printing plates were used, the specific artwork featured on each plate, and other production parameters. Once the counterfeiter understands how a genuine document was printed, it becomes possible to replicate the document artwork, even if executing the replication is made more difficult because of printed security features such as line art, spot colors, microprinting, guilloche patterns, split fountain printing, latent images, transparent register, and similar features.

The security artwork strategies described above all increase the difficulty of either generating the artwork or physically printing the artwork on press. Importantly, not one of these strategies is deliberately targeted at the prepress process of separating the individual printing plate images, which means that a number of theoretical strategies that do target this particular process remain largely unexplored in the contemporary practice of security document design. If a counterfeiter is unable to determine the number of printing plates used or the specific artwork present on each plate, then there is no pathway toward accurate replication of the individual plate artwork. At best, the counterfeiter abandons the attack or, at worst, must compromise the quality of the counterfeit. The question is how to use design to force traditional counterfeiters to face these complications.

To understand how a genuine document was printed, traditional counterfeiters rely on a series of four visual cues that are present in the artwork of most historical and contemporary security documents. These cues include the printing of different colors of ink from each separate plate, the use of continuous line patterns that can be traced, delivery of complete artwork visual elements from a single printing plate, and the use of different artwork styles on different printing plates. Therefore, purposefully designing artwork that does not contain these four cues can produce security designs that can be originated and printed by genuine document issuers, but which are very challenging to reverse engineer if starting only from the printed hardcopy document, as a counterfeiter must. Such designs are essentially the opposite of the four cues described above; they contain the same color of ink on each printing plate, continuous line patterns are eliminated or minimized, most artwork elements require multiple plate images to produce, and the same basic artwork style is used across all printing plates.

This presentation will describe these design techniques, provide examples of security artwork concepts that comply with these principles, and explain ramifications for forensic examination of security document artwork.

Counterfeit, Artwork, Design

J9 Distinguishing Characteristics of Robotic Writing

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After attending this presentation, attendees will be familiar with the characteristics observed in machine-simulated writing.

This presentation will impact the forensic science community by alerting the forensic document examiner of the advances of robotic writing technology and the features that distinguish robotic writing from genuine handwriting.

Robotic handwriting has been long in the making, dating back to the 18th century. Since the 1940s when the Robot Pen became commercially available, autopen devices with advanced features have been used to reproduce an exact copy of a person's signature. In the field of forensic document examination, the term "autopen" has become a standard for all signature-duplication machines. These autopen devices are used to replicate signatures of government officials and also major corporate companies, so these influential leaders and business people can apply their time elsewhere without removing the touch of personalized correspondence and the authenticity of official documents.

Today, in an era of rapid proliferation of digital devices, handwriting has become an unconventional, vintage skill. But, with the advent of new technology, it is possible to mimic an individual's handwriting to generate customizable written documents with the use of digitization, mechanics, and software programming. Programming and mechanics work together to encompass pen positions, speed, and letter form, then integrate that into writing onto paper via a robot. The robot can simulate pen movements, including pen lifts and touches, by operating along three linear axes that move simultaneously. To an untrained eye, the product of this machine is a document that can be mistaken for a genuine, handwritten correspondence by the individual whose handwriting is being simulated.

To reveal the distinguishing characteristics of such technical reproductions of one's handwriting, a study was conducted. Robotic writing samples of six individuals were compared to known, genuine writing samples of the same individuals, including directed and collected samples. Distinct features observed in the robotic samples included even pen-pressure, variable sequence of up strokes and down strokes, and the superimposition of letter forms. Examination, comparison, and evaluation of these features in both the questioned and known samples revealed substantial and significant dissimilarities and resulted in an opinion of non-genuineness.

Technology can certainly assist humans, but it cannot entirely replace humans in the area of writing. Handwriting is an acquired, perceptual motor skill requiring the melding of the mind and body. A person's handwriting is made up of a complexity of habitual patterns and can be identified based on the presence of individualizing features. Careful examination of the structural features as well as dynamic features of a particular writing can aid an examiner in recognizing when a robot is being used. This presentation will confirm the inconsistencies between the human hand and the robotic arm.

Reference(s):

1. McCarthy F., Winchester J. The Autopen. *Journal of Forensic Sciences*. 1973, 18(4), 441-447.
2. *History of Computers, Computing and Internet History*. Accessed July 31, 2017, http://history-computer.com/Dreamers/Knauss.html.
3. *Send Handwritten Notes*. Accessed July 31, 2017, https://bond.co/.

Questioned Documents, Robotic Writing, Autopen

J10 An Analysis of Forensic Document Examiner (FDE) Aptitude in Determining and Comparing Velocity Rates of Handwritten Strokes

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After attending this presentation, attendees will better understand the aptitude of FDEs in comparing and determining the velocity of handwritten strokes. This presentation will provide training information for journeyman and trainee FDEs.

This presentation will impact the forensic science community by providing empirical data on the aptitude of FDEs in discriminating velocity of handwritten pair samples. This discrimination is one part of the examination process and can be vital when determining genuineness in handwriting and signatures. The results will provide insights into the ability of FDEs in this area of examination, and the study will be an impetus for future research.

Proper evaluation of the speed of a written stroke is an important parameter for FDEs in determining whether or not signatures or handwriting are genuine. FDEs make this evaluation based on line gradation, variations in pen pressure, and initial and terminal strokes. FDEs have been shown to make reliable evaluations of line speed from early generational photocopied samples and from online samples. This study was conducted by extracting 60 original handwritten letters ("l") from a large database of 16-bit digitized samples and presenting them to 50 forensic document examiners in the form of sample pairs. Each pair included samples of the letter "l" selected to range in known stroke velocity from 10mm/s to 150mm/s. The average velocity of a handwritten stroke is 100mm/s. Pairwise comparisons of letters executed at various speeds were then presented to the FDEs who were asked to provide an opinion as to which of the two samples of the pair was faster. The opinions were a forced call and no inconclusive opinions were allowed. FDE judgments were evaluated to determine the reliability of the FDEs in the task of comparing line velocity using blinded repeat pairs.

The FDEs evaluated the line velocity using online samples. Once these examiner classifications were performed and results received, they were evaluated against the known velocity differences between the sample pairs. That point along the continuum of velocity difference scores where 95% or more of the FDEs accurately selected the faster sample served to operationalize the Just Noticeable Difference (JND). Further analyses were performed to examine effects of years of experience on the magnitude of the JND.

This study processed FDE judgments for accuracy and their ability to identify the JND in perceived stroke velocity based on ground truth of known velocities. This study has implications for validating claims by FDEs about reliably estimating handwriting speed. Identifying the perceptual JND for stroke velocity is relevant to questions of whether experienced FDEs can distinguish important features in handwriting that fall within or outside the writer's natural variation.

This study provides information on the ability of FDEs to determine the velocity of handwritten strokes and their ability to differentiate strokes based on velocity ratings from digitized samples. It should also provide training samples for FDE journeyman examiners and trainees and an impetus for other studies to expand on this one.

Forensic Document Examination, Velocity, Kinematics

J11 The Influence of Terminal Illness and Prescription Medications on Patient Signatures

Jan Seaman Kelly, BA, 9360 W Flamingo Road, #110-400, Las Vegas, NV 89147*

After attending this presentation, attendees will have a better understanding of the influences that opiate pain killers, benzodiazepines, and illnesses may have on a patient's handwriting or signatures.

This presentation will impact the forensic science community by educating attendees that opiate and benzodiazepine drugs act on the brain and nerves, producing a calming effect in relieving pain or anxiety that may result in the patient's signature containing characteristics of distortion. In cases involving such a deviation, attendees will understand the importance of obtaining contemporaneous signatures and a list of medications the patient (writer) was taking.

Every forensic document examiner is aware of the complexities within an individual's handwriting. All physical repetitive actions, including handwriting and the execution of signatures, are achieved through the Central Nervous System (CNS) using the neuromuscular system. Eighty percent of the cerebral cortex is used to produce a handwritten text.[1] Naturally written handwriting and signatures contain a combination of characteristics that can individualize the writing to a specific writer; however, the central nervous system is susceptible to prescription medications designed to relieve pain, anxiety, depression, etc. The patient's writing or signatures may be distorted when under the influence of prescription medications that act on the CNS. Obtaining information regarding the health of the writer and his or her prescribed medications taken during the timeframe of the production of the signature can assist the forensic document examiner in understanding the physiological process involved in the production of distorted writings or signatures.[2]

A 69-year-old male patient was diagnosed with terminal small-cell carcinoma of the lungs on May 3, 1996, and was advised he had four to six months left to live. The patient was asked if he would provide at least one signature each day during his illness as this would assist forensic document examiners in understanding the influence of illness and medications reflected in his known signatures. The patient agreed and the following information was to be recorded: date and time of each signature; the date, time, and name of medication; and the patent's comments regarding pain or anxiety.

Sixty-five signatures were recorded, with the last one written two weeks prior to the patient's death on June 22, 1996. The patient's pain was managed with a variety of opiate pain killers. Benzodiazepines were prescribed to relieve the patient's anxiety and depression. A comparison of the timeline of pain, anxiety, and prescription medications to the timeline of the signatures will provide forensic document examiners with the opportunity to gain a better understanding of the influence these factors may have on the writings and signatures executed by terminally ill patients.

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2. Michael Caligiuri and Linton Mohammed. *The Neuroscience of Handwriting Applications for Forensic Document Examination*. (Florida; CRC Press Taylor & Francis Group ,2012), 168.

Prescription Medications, Central Nervous System, Signatures

J12 Measuring the Frequency Occurrence of Handwritten Numerals

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The goal of this presentation is to provide attendees with objective pre-established frequency of occurrence proportions that provide the soundest foundation for authorship determination and probability estimations.

Pre-established frequency of occurrence proportions is generally acknowledged to be the most objective form of rarity determination available to the forensic document examiner. This presentation will impact the forensic science community by addressing the issue of handwritten numerals and is designed to serve as a sister study to "Measuring the Frequency Occurrence of Handwriting and Handprinting Characteristics" published in January 2017 in the *Journal of Forensic Sciences*.[1]

This presentation describes the results of a statistical research study. The motivation was to strengthen the statistical basis for overall handwriting comparisons in conjunction with the previous paper on which this study expands.[1] Multiple studies and recommendations for purposes of strengthening forensic science have highlighted the need for more comprehensive foundational studies.[2-4] In response to these and other requests, the objectives of this study were to develop statistically valid frequency occurrence proportions for handwritten numeral specimens from sampling representative of the United States adult population for the purposes of providing forensic document examiners with additional objective, statistical information for reliability and measurement validity and to provide courts with additional foundational and supporting data.

This study produced an initial set of 32 handwritten numeral features that were subsequently reduced to 25 characteristics (78%) that passed an Attribute Agreement Analysis (AAA) and were utilized in this study. By passing an AAA, these characteristics are shown to be unambiguously identified by forensic document examiners. Handwriting specimens from 1,197 participants were collected based on the parameters set forth by Johnson et al.[1] Meeting the prescribed population representation through paring led to the selection of 849 numeral specimen forms that closely approximate the demographic proportions represented in the United States. The analysis of these specimens yielded 25 specific frequency occurrence proportions. This study relied on the same protocols applied by Johnson.[1]

There are several studies relative to numeral characteristics.[5-7] Ahola delved into frequency of occurrence in Canada but used a modest, non-stratified sampling number and concentrated on styles of numerals as opposed to specific design structures referenced herein.[5]

Forensic document examination, along with its sister disciplines within the forensic sciences, is undergoing exponential changes in the basic fiber of its foundation. One such change is the recognition that objective, pre-determined frequency of occurrence proportions is the most objective form of weighing the significance of characteristics noted in an examination. In recognition of this, much time has been spent in establishing estimates and this work must continue.

The product of this study is data. Forensic document examination must now collectively decide the best ways in which to utilize this data. Johnson provided several suggestions and ideas, but ultimately it will require a discipline-wide consensus as to the best way to move forward. Frequency occurrence issues are now appearing in reports and in court testimony, so the need for some levels of standardization is becoming increasingly important.

This study is the second in what is anticipated to be a series of studies. The purpose of this study is to fill a gap from the original study in which none of the numeral characteristics passed the AAA. It is anticipated that the next study will further fill gaps from the first two studies. Subsequent to that, it is imperative that these studies be maintained and expanded. By the collection of additional specimens and the identification of additional characteristics that pass AAA, forensic document examination will have an ever-increasingly valuable tool for use in casework and in presenting to courts the foundational research that provides the basis from which we operate.

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Frequency Occurrencce, Statistics, Numerals

J13 Initials: Value for Identification

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After attending this presentation, attendees can expect to better understand the identification value of initials in 21st-century handwriting.

This presentation will impact the forensic science community by increasing appreciation for the individuality and limitations of the examination of initials representing abbreviated signatures that forensic document can expect to encounter in their casework today.

A paucity of research on 21st-century initials along with a case involving disputed initials on a loan application motivated this study. Computer keyboarding, diminished teaching of cursive writing to school children, and more use of hand printing in modern writing may have an impact of the use of initials in everyday business documents. This study was conducted to determine the individuality of handwritten initials and their similarities or differences when compared to the capital letters representing the first letter of each name in a signature. Initials are condensed signatures. Forensic document examiners use the same methodology in examining initials as they do for signatures and extended hand printing or cursive writing. Appreciation for the individuality of initials and their consistency or departure from the first letter of each name in a signature will help forensic document examiners correctly identify or eliminate writers of initials in disputed documents.

Forms using letter-sized paper were created to collect four initial samples and two signature samples from each writer in 2017. Sections requesting age, gender, and country of origin of the writer were also included. The goal was to collect samples from at least 200 writers. Evaluation of the collected forms revealed the individuality of many of the basic letter forms of initials in cursive and hand-printed styles. Individuality was also revealed in the punctuation, connectedness, spacing, and size of initials that forensic document examiners can expect to see in cases. Sets of initials composed of two to three letters were compared to the signatures by the same person to discover instances of consistency and differences between the two in basic letter construction, spacing, size, slant, and punctuation.

This study sought to provide an evaluation of modern initials, their general format, and individuality. It also sought to illustrate that initials can conform closely to the capital letters of a signature, but also may be constructed quite differently. This study revealed that the number of letters in a group of initials and their complexity will determine their value for identification. Forensic document examiners must also consider that since letters used to write initials do not always conform to the capital letters of each initial of a signature, known initials must be collected for comparison purposes in casework involving questioned initials.

Initials, Handwriting, Identification

J14 Competency for Chinese Handwriting Examination

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The goals of this presentation are to: (1) introduce the basic principles and methods for the examination of Chinese handwriting to the international forensic community; (2) introduce activities organized to assist the in sharing of the latest developments; and, (3) evaluate the competency of the practitioners.

This presentation will impact the forensic science community by allowing overseas document examiners to better understand the principles and methodology in Chinese handwriting examination and how to demonstrate their competency in this area.

Chinese characters were invented approximately 4,000 years ago, and, unlike English words that are composed of linearly arranged discrete units (alphabet letters and characters), all Chinese characters are written within a framework of an imaginary box with component radicals arranged in a two-dimensional array. Do these disparities in structure and formation between Chinese and Latin-script writing give rise to significant differences in the methods of examination between the two types of handwriting?

Chinese handwriting and signatures are used by more than 1.3 billion people all over the world; document examiners will have the chance to encounter cases involving the examination of Chinese handwriting and signatures. Extensive studies on the examination of English handwriting and signatures can be found in the literature, whereas publications on the identification of Chinese handwriting are relatively limited. Would it be possible for a document examiner who is unfamiliar with Chinese characters to apply the principles of identification and elimination of authorship described in the various literature sources for the examination of English handwriting to the examination of Chinese handwriting?

Hong Kong is an international city where both English and Chinese writing are used in commercial and legal documents. Document examiners of the Government Laboratory, Hong Kong Special Administration Region (HKSAR) have ample opportunity to examine both Chinese and English handwriting and are provided with the opportunity to compare the methodology used in mainland China and overseas. A series of research projects on the examination of Chinese handwriting has been conducted since the late 1980s. In this presentation, the basic principles and methods for the examination of Chinese handwriting will be introduced to the international forensic community.

This presentation will include a review of Chinese handwriting examination that includes: (1) a brief introduction to the history and development of Chinese characters; (2) methods of Chinese handwriting examination as compared to the examination of English handwriting; (3) the significance of various writing characteristics in Chinese handwriting useful for the identification and differentiation of authorship; (4) methods of disguise in Chinese handwriting; and, (5) a study into the individuality of Chinese handwriting.

Numerous international reviews on forensic science stressed the importance of competency and expertise of the forensic practitioners. How can document examiners demonstrate their competency for conducting Chinese handwriting examination? Over the decades, various activities have been organized with a view to providing an analytical forum for participants in the field of document examination to evaluate the competency of participants and share the latest development for seeking further development. Activities such as Chinese handwriting workshops and proficiency testing programs will also be described in this presentation.

Competency, Chinese Handwriting, Proficiency Testing Programs

J15 Research on the Mechanism of Paper Burning by Thermal-Gravimetry and the Handling Methods for Charred Documents

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After attending this presentation, attendees will understand the mechanism of paper burning, which can be summarized by four stages. Additionally, a new handling method with softening and flattening solutions will be introduced.

This presentation will impact the forensic science community by increasing knowledge regarding charred document examination, especially on the use of suitable handling methods based on the stage of the charred paper.

In questioned document examination, a charred document is a piece of burned paper or its fragments containing a message. The examination of such a document and deciphering the writing thereon are important for gaining information, usually demanding a careful application of certain scientific methods and techniques because of its unstable property. To address this issue, the mechanism of paper burning needs to be studied in order to discover the essential and key factors. Here, Thermal-Gravimetry (TG) was applied to investigate five types of paper with their TG and Derivative Thermo-Gravimetric (DTG) curve observed at different atmospheric conditions. The results demonstrated that the shape of curves, albeit similar, varied with the physical and chemical composition of paper. In the burning process, dehydration and de-polymerization are the two main pathways for cellulose — the major ingredient of paper. The heating rate indicated little influence on the curves while the type of atmosphere was strongly influential. The reason is due to the lack of tar oxidation when nitrogen is used as the atmospheric environment. At a moderate temperature, de-polymerization prevails and the tar can be observed. With increasing temperatures, the tar and cellulose are further decomposed, leading to products of a high boiling-point. According to the results, the charred document can be classified as one of dehydrated, tarred, charred, and ashed: (1) Dehydrated — below 100°C, paper is slightly yellow. In this stage, water absorbed in cellulose is gradually lost, and the basic physical property stays intact; (2) Tarred — from 150°C to 250°C, paper color changes from yellow to brown, the edges become curly, and the dimension diminishes. In this stage, although cellulose is not completely decomposed, tar is obtained through de-polymerization; (3) Charred — from 250°C to 350°C, paper changes from dark brown to black and becomes much curlier. In this stage, residual tar continues to oxidize, thus solid char can be observed; and, (4) Ashed — above 350°C, paper color turns from gray-white to ash. In this stage, char continues to oxidize, leaving the high boiling-point products, mainly the filler materials (mostly Calcium Carbonate ($CaCO_3$)).

Except for the ashed document, the other three can be handled and deciphered to collect information. A new softening solution (glycerinum-water) and flattening solution (polyvinylpyrrolidone-ethanol) with a new handling method can be used for subsequent examinations. The results of this study may provide a fundamental method for examining and deciphering charred documents.

Charred Document, Thermal-Gravimetry, Handling Method

J16 The Evaluation of Method Parameters Affecting Magnetic Flux Measurement of Toners as a Screening Tool for Casework Application

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After attending this presentation, attendees will better understand the potential use of a quantitative magnetic flux measuring device to differentiate between black and white toner-printed documents from different sources. Attendees will gain an understanding of the magnetic characteristics exhibited by toner-printed documents and learn how to employ magnetic flux measurement techniques during comparative examinations between questioned and reference printed texts.

This presentation will impact the forensic science community by providing a method for quickly screening black and white toner-printed documents, reducing the need for more time-consuming or destructive methodology.

This study was created to address questions raised by previous research into magnetic properties of toner. Specifically, this research addresses questions related to the optimization of instrumental parameters to maximize repeatability of results and ensure that variability due to the user may be minimized.

Toward this goal, a trial was conducted on seven randomly selected samples from incoming mail collected between April 2007 and January 2016 at the University of Lausanne, Switzerland. A series of three trials were conducted across the span of one week, and a total of 63 magnetic flux measurements were collected from each of three different areas of text per sample. The text areas were selected to include one sample that completely filled the sensor area, one that filled approximately half of the sensor area, and an intermediate sample with an area between the two extremes. The area of each measurement was determined individually using image processing software, and the units were normalized to nWb flux/mm^2 of toner to facilitate comparison.

The primary objective was to determine if the placement of the text within the sensor field affected the precision of measurements. The hypothesis for the experiment based on instrument manuals was that if the text occupied the periphery of the sensor area, there would be distortions in the measurements and the results would become increasingly inaccurate and repeatability would decrease. This is because the sensor is unable to completely image the vector for the magnetic induction of the pixels at the periphery, which leads to inaccurate interpretations of the numerical value for the flux field for those peripheral pixels. The secondary objective was to optimize the protocol for the area determinations. It was determined in a previous study that the mean gray value of the pixels selected appeared to have an impact on reproducibility of measurements. If the standard deviation of the mean gray value was greater than 1 for a sample set, the variance of the results for that sample set appeared to increase. The hypothesis for this linkage was tested by performing the area determinations for the same sample set of interest until the variance for the nWb flux/mm^2 was observed to stabilize at its lowest possible value.

The results of this study indicate that repeatability of magnetic flux measurements of toners can be improved by selecting text areas that do not intersect with the peripheral edges of the sensor and by maintaining a mean gray value standard deviation of .25 or less within the same sample set. This finding is useful to aid in optimizing a method that can be used in a forensic laboratory setting, which will allow for quick screening of toner-printed documents without the need for further testing, which may be destructive.

Questioned Documents, Toner, Magnetic Flux

J17 The Application of t-Stochastic Node Embedding and Random Forest Statistical Methods to Classify Raman Spectra of Inkjet Printer Inks for Purposes of Identification and Production of Investigative Leads

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After attending this presentation, attendees will gain knowledge regarding the application of a relatively novel statistical approach to Raman patterns gathered *in situ* from micrometric colored spots of inkjet-printed documents from different sources.

This presentation will impact the forensic science community, with an emphasis toward questioned document examiners, by evaluating the utilization of a statistical approach to extract information from Raman patterns for investigative purposes. Although the main goal of this research is the improvement of the current examination of inkjet-printed counterfeit banknotes, the proposed approach is definitely relevant to areas of forensic science where spectroscopic data are used.

The printing process by means of inkjet technology involves the production of a constellation of ink spots of micrometric size. Raman spectroscopy proved to be a suitable method for rapidly obtaining a chemical signature *in situ* from the three main colored components (cyan, magenta, and yellow) of inkjet printer inks. The present step of this study seeks to evaluate whether the statistical methods of t-Stochastic Node Embedding (t-SNE) and random forest are suitable for classification to produce investigative leads in cases in which a suspected printer needs to be developed based on its detected Raman profiles.

One hundred fifty Raman spectra were captured from the cyan, magenta, and yellow spots of ten inkjet printer ink samples provided by the Treasury Obligations Section of the United States Secret Service, using a Near-Infrared (NIR) laser wavelength at 785nm. Given that inkjet ink samples generate Raman spectra that often can be differentiated on the basis of the presence of minor peaks only, a sensible classifier is then required for conducting spectral comparisons.

t-SNE is a dimension reduction technique that is primarily used for visualization of high-dimensional data. This technique seeks to preserve low-dimensional (potentially non-linear) groupings that may exist in high-dimensional data. Linear methods such as Principal Component Analysis (PCA) are unable to preserve these non-linear relationships. The method proceeds by converting Euclidean distances to conditional probabilities that represent similarities. These probabilities are computed in the original dimension and in the proposed low-dimensional representation. The optimal lower dimensional representation is the one that minimizes a well-known information theoretic criterion known as the Kullback-Leibler divergence. Van der Maaten and Hinton proposed a variation to t-SNE in which the Gaussian distribution used to compute the probabilities with a Student t-distribution is replaced and the joint probability distribution of pairs of points (rather than the conditional probabilities of one point given another) is used.[1] Both of these proposals lead to improved performance. The method of random forest is an ensemble approach to classification in which a group of low-dimension classifiers can be combined to provide a strong classifier (or learner). A random forest takes random subsets of the available variables to produce a tree, then aggregates (usually by averaging) the classifications over the trees to produce a classification.

The t-SNE visualization and PCA biplots of the Raman data reveal that in general, measurements from the same source (ink cartridge) cluster together. These methods also reveal observations that are behaving poorly. It is interesting to contrast the plots for all dye colors using t-SNE and PCA methods. As one might expect, the PCA plots emphasize the gross differences between the dye colors, whereas the t-SNE plots emphasize the clusters of samples within colors. Inspection of PCA biplots (using pairs of the first three principal components) reveal strong local structure aligned with the source of the measurement; that is, measurements from the same source appear to be close together in Euclidean space. This is an indication that classification techniques should be able to make a reasonable classification; in nearly each case, there are five measurements on each of ten sources. A 60:40 split for training and testing of a random forest model was used. The overall classification rate varied between 75% and 95%, providing at least initial evidence that this technique is a promising classifier for the samples of this study.

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1. Van der Maaten L.J.P, Hinton G.E. Visualizing High-Dimensional Data Using t-SNE. *Journal of Machine Learning Research*. 2008; 9: 2579-2605.

Inkjet, Raman, Statistics

J18 Inks Examination Using a Combination of Video Spectral Comparator (VSC) Spectra and Color Deconvolution

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The goal of this presentation is to conduct ink examinations using a combination of VSC spectra and color deconvolution.

This presentation will impact the forensic science community by providing Forensic Document Examiners (FDEs) a quantifiable means to examine inks and to better discriminate different inks.

Ink examination is usually performed either by: (1) using non-destructive methods such as visual and microscope examination and VSC examination; (2) destructive methods such as Thin-Layer Chromatography (TLC), Raman spectroscopy; or, (3) a combination of both non-destructive and destructive methods. If possible, FDEs would prefer non-destructive over destructive methods in their examinations. Often, the infrared reflectance and luminescence examinations using VSC are sufficient to differentiate the inks; however, issues arise when the difference observed in VSC results is subtle and requires examiners to interpret and determine if these subtle differences (e.g., a small difference in intensity of luminescence of the inks) are sufficient to determine if the inks are different or insufficient not to exclude them. It is this type of interpretation that differs from examiner to examiner that, in the end, could result in different conclusions between examiners. Yet, without using destructive methods such as TLC or Raman spectroscopy, which could provide a more conclusive finding, it is difficult for examiners to make any effective conclusion solely based on observations of when the inks exhibit subtle differences in luminescence at the various VSC settings.

With the goal of resolving the ambiguity that may arise due to the subtle differences observed for inks, a combination of two non-destructive methods of VSC spectra and color deconvolution is proposed. These two methods utilize different working principles and, hence, are complementary to each other in the examination of inks.

VSC spectra: The inks are subjected to various settings of the VSC under both the reflectance and luminescence modes and the results are then tabulated in the format of spectra. The spectra provide examiners a more objective interpretation of the results based on the profiles of the spectra and do not require examiners to agree or disagree if the extent of luminescence is sufficient or not to determine if they are different.

Color deconvolution: The inks are scanned, then processed using the image processing tool, which helps to separate the color of the different inks. The image processing tool utilizes mathematical algorithms that can define each image pixel as a vector of red, green, and blue components, then uses these vectors to help make subtle color differences obvious.

By using the combination of the spectra obtained by VSC and color deconvolution, examiners would have a more quantifiable means in the interpretation of results.

Inks Examination, VSC, Color Deconvolution

J19 The Evolution of Documents and Their Security Submitted for Examination Using a Video Spectral Comparator (VSC®80)

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This goal of this presentation is to discuss how the type of documents encountered by questioned document examiners has changed over the last few decades. The increasing pace of developments in security features and the corresponding advances in Video Spectral Comparators (VSCs) for their examination will be examined, as will how these changes affect document examiners today and how they may be affected in the future.

This presentation will impact the forensic science community by alerting attendees to the evolution of documents and to the progress in the development of VSC instrumentation for the examination of falsified and/or altered documents.

After attending this presentation, attendees will understand how a new technology that allows Video Spectral Analysis (VSA), especially using the VSC®80, can enhance and facilitate the investigation and examination of altered security documents, such as identity documents, banknotes, travel documents, and security features, as well as the more routine questioned document problems submitted for forensic document examination to the laboratory, such as those involving alterations due to erasures and additions made to questioned documents.

Personal computers, such as desktops, laptops, and notebooks, have become a part of the VSC systems — initially, this was primarily a method to save images or connect to a printer, but increasingly by taking over control of the instrument by automating tasks, recording settings, monitoring lamps, running self-diagnostic tests, etc. With many instrument capabilities now controlled by software, they can constantly evolve. It is therefore more important than ever for forensic document examiners to keep up to date with technology and its capabilities.

At the same time, digital camera technology has progressed rapidly, as have the associated hardware- and software-driven capabilities, enabling the achievement of higher resolutions, faster operation speeds, and greater image quality. Document security features and devices have followed a similar path. As some types of optically variable features such as holograms lost their effectiveness against ease of compromise, security printers introduced new technologies, such as anti-Stokes inks, micro-taggants, and others. Documents have become more sophisticated. To appreciate this, one has only to compare the latest driver licenses with those issued ten years ago.

Threats have become more sophisticated also — counterfeit driver's licenses, easily available online, are able to match most security features. VSCs closely follow these trends. They are supplied to a great variety of end users, such as Departments of Motor Vehicles, banks, lotteries, universities, insurance companies, and security printers, as well as to the more traditional forensic document examiners. This presentation will conclude with the revealing of a new VSC and its capabilities for the examination of security features in documents — the VSC®80.

Documents, Security, Video Spectral Comparator

J20 A Physical-Chemical Study of Crossed Line Intersection

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The objectives of this presentation are to answer the many questions regarding whether or not: (1) non-visible ink migration can be used for ink dating; (2) fading of luminescence can be used to determine the production time gap (Dt) between two intersecting lines; and, (3) there is the possibility of identifying luminescent compounds by utilizing various chemical methods.

This presentation will impact the forensic science community by providing a proposed methodology that will enable forensic experts and investigators around the world to conduct examinations at globally accepted standards.

The INTERPOL Counterfeit Currency and Security Documents Branch (CCSD) is responsible for establishing programs that provide forensic support, operational assistance, and technical databases to assist the 190 member countries of INTERPOL regarding counterfeit currency, security documents, and addressing border security issues by improving the integrity of travel and security documents.

The CCSD frequently encourages the incorporation of science into its police work and recognizes the importance of science in the development of technology and investigations. The Physical-Chemical Study of Crossed Line Intersection project began in 2010 at the initiative of the CCSD and in partnership with the International Academy for Handwriting and Documents (AIEED). This project provides a proposed methodology that will enable forensic experts and investigators worldwide to conduct examinations at globally accepted standards. Gaining a better understanding of the sequencing of line-crossings will help forensic document examiners to identify falsified documents, which will assist criminal investigations and combat and prevent future crimes.

For this project, inks were chosen in which migration of ink components has already been observed as well as such inks with unknown behavior. Non-intersecting lines can also be considered for migration studies, if they are positioned close to each other and if luminescence appears in this area. In some cases, invisible migration can be measured/observed outside of intersections.

Shared with participants at the regular INTERPOL meetings on Physical-Chemical Study of Crossed Line Intersection, the "proof of concept," which was established following a year-long forensic analysis of the protocol conducted by laboratories in 13 countries, is to now be tested in the field with the goal of training forensic document examiners to assist in fraud or forgery investigations. This project will foster the development of new research in this domain and expertise to facilitate knowledge-sharing between international laboratories.

This study has been presented to nearly 120 forensic document examiners from 54 INTERPOL member countries who have been regularly attending working group meetings at the INTERPOL Secretariat General.

Migration, Luminescence, Ink Dating

K1 Drivers Under the Influence of Alcohol and Drugs: An Eight-Year Retrospective Analysis in a Southern Italian Region

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After attending this presentation, attendees will better understand the Italian trend of alcohol and drug use among motor vehicle drivers involved in Road Traffic Crashes (RTC).

This presentation will impact the forensic science community by providing alcohol and drug results of biological samples collected from 1,797 drivers at the request of police from 2009 to 2016 that were processed at a major forensic toxicological laboratory in southern Italy.

Operating a motor vehicle while Driving Under the Influence Of Alcohol (DUIA) or Drugs (DUID) is considered a crime worldwide because of the risk to traffic safety. Based on the recent report by the National Institute for Statistics (ISTAT) in Italy, there were 173,892 traffic accidents resulting in personal injury in 2015. From 2013 to 2015, an average of four to six deaths and 20 injured drivers per 100,000 people were recorded by ISTAT in the Campania region, the third most-populous region in Italy. This region has a population of 5,869,965 people, with 4,434,136 inhabitants living in the Naples metropolitan area alone, the second most-populated metropolitan area in Italy, after Milan.

A recent Italian Road Traffic Law (IRTL) (L. 41/2016) just updated the crimes related to DUIA and DUID with the penal sanctions having been generally increased. If a driver causes the death of one person and injury to another, he can be punished with 18 years in prison and at least 5 years disqualification from driving. In the Campania region, the Forensic Toxicology Unit (FTU) of the University "Luigi Vanvitelli" of Campania represents the "reference laboratory" of the entire region, performing all of the confirmation toxicological analyses for medicolegal purposes. The toxicology lab is accredited to perform the analytical work on postmortem samples as well as on hospitalized drivers injured because of RTC. According to the sampling protocol established by the current IRTL, when drivers are injured in an RTC, a medical evaluation must be performed first. Immunochemical screening tests on biological samples must follow in order to find evidence of alcohol/drug effects on the driver's performance. Only positive blood and urine samples collected from injured drivers are forwarded to the FTU for confirmation of the toxicological analyses.

To assess the trends in the use of alcohol and drugs among motor vehicle drivers, a retrospective analysis was performed based on drivers involved in RTC and admitted to 16 Emergency Departments (ED) located in the different provinces of the Campania region from 2009 to 2016. An additional goal of the study was to collect data useful to the improvement of toxicological analytical work and preventive policies with regional relevance. Confirmation tests of positive toxicological screening analyses were performed on biological samples (blood/urine) collected from 1,797 hospitalized drivers. The analyses were performed on a total of 780 blood samples: 609 cases were referred for suspected DUIA and 171 cases for suspected DUIA and DUID; 1,017 urine samples were also collected from DUID cases when the blood test was denied by drivers. All blood and urine samples were collected at admission to the ED within two hours of the accident. Blood Alcohol Concentration (BAC) on whole blood was analyzed by Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID). Qualitative and quantitative analyses for drugs were accomplished by Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

Results: BAC greater than 0.5g/L (the legal limit in Italy) was observed in 91.5% of drivers suspected for DUIA and in 93% of drivers suspected for DUID. In particular, BAC >1.5g/L were found in 308 suspected cases of DUIA out of 609 (50.5%) and in 66 suspected cases of DUID out of 171 (38.6%). RCT occurred mostly in drivers with BAC >1.5g/L, while in cases of DUID, BACs between 0.5g/L and 1.5g/L were most common. Toxicological analyses for drugs in blood were negative in 51 drivers out of 171 DUIA and DUID cases total (29.8%). Cocaine and Δ^9-Tetrahydrocannabinol (Δ^9THC) were the drugs most commonly associated with alcohol, followed by poly-drug abuse, a combination of different drugs among which, again, cocaine and THC were the most represented, followed by methadone and Benzodiazepine (BDZ). Among positive urine analyses, 11-nor-9-Carboxy-Δ^9-Tetrahydrocannnabinol (THCCOOH) was the most frequently identified compound, alone or in association with other drugs, followed by poly-drug>cocaine>BDZ>opiates. It is worth mentioning that negative confirmation tests were obtained in 14.5% of the drivers previously recognized as positive in screening analyses. Therefore, an improvement in the protocols currently applied to DUIA and DUID assessment is needed, and confirmation tests on the blood should be considered mandatory in demonstrating a violation of the Road Traffic Act.

Driving Under the Influence, Blood Alcohol Concentration, Road Traffic Crashes

K2 Surface-Enhanced Raman Spectroscopy (SERS) -Based Screening Test for Synthetic Cannabinoids in Oral Fluid

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After attending this presentation, attendees will better understand SERS and how it can be used to retrieve structural information from small molecules at very low concentrations. Attendees will also understand how this approach can be applied to the detection of xenobiotics in oral fluid.

This presentation will impact the forensic science community by demonstrating how SERS is a fast, selective, and sensitive approach for synthetic cannabinoids screening, as an alternative to immunoassays.

Synthetic cannabinoids are New Psychoactive Substances (NPS) that represent a worldwide issue due to unknown toxicological effects and widespread use among the young population. Moreover, the rapidity by which these compounds are modified and introduced into the illegal market makes it difficult to detect them using standard screening methods, such as immunoassays. Indeed, cross-reactivity between different species and false negative responses severely limit this approach.

SERS has been shown by this study to have great potential to solve these problems by providing a sensitive and selective screening approach that can provide fingerprint signals from xenobiotics at toxicological concentrations. This was achieved on benzodiazepines, both as standard solutions and in spiked urine matrices, and more recently, on standard solutions of synthetic cannabinoids. The latter included JWH-018, JWH-030, JWH-073, JWH-081, JWH-122, JWH-175, AM-2201, MAM-2201, with typical Limits of Detection (LODs) ranging from 20ng/mL for JWH-018 to 140ng/mL for JWH-081 and AM-2201. By translating this strategy to biological matrix analysis, the forensic and emergency medical field would benefit from a sensitive and selective alternative to current immunoassays. Because the procedure provides a spectral fingerprint, SERS can be seen as a complementary tool to other structure elucidation techniques, such as mass spectrometry.

SERS is a surface spectroscopy that amplifies Raman scattering by several orders of magnitude via the addition of metallic nanoparticles capable of producing Localized Surface Plasmon Resonance (LSPR). In this method, citrate-reduced gold nanospheres were prepared as LSPR-bearing substrates and later aggregated through the addition of $MgCl_2$. The aggregation process red-shifts the frequency of the LSPR and produces strong electromagnetic fields where the particles interact. The result is a rapid method for detection with exceptional sensitivity.

This presentation will focus on the development of an optimal extraction technique to detect synthetic cannabinoids in oral fluids. Fortified oral fluid samples were pretreated via centrifugation in the presence of methanol, which yielded protein sedimentation. Throughout the course of this work, thiocyanate anions were found to be a critical interfering species, as they strongly interact with the colloidal gold. Therefore, a variety of different desalting procedures were examined prior to SERS analysis, including ion exchange and solid phase extraction. The SERS signal was also increased through various wash steps conducted on the gold colloid, prior to its use as an enhancing substrate. This reduced residual citrate molecules carried over from the synthetic process, leaving the nanoparticle's surface more available for analyte adsorption.

The optimized methodology was developed using JWH-018 as a model target drug, then extended to other naphthoylindole synthetic cannabinoids. Detection was achieved using a portable Raman spectrometer operating at 785nm. This new procedure has great potential in forensic analysis, both as a more specific and flexible replacement for immunoassay and as an orthogonal method for analysis that is compatible with downstream mass spectral detection.

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SERS, Synthetic Cannabinoids, Oral Fluid

K3 Will the Real "Molly" Please Stand Up? N-Ethyl Pentylone-Related Deaths in Alabama

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The goals of this presentation are to: (1) review n-ethyl pentylone-proposed pharmacology; (2) describe the n-ethyl pentylone case facts; and, (3) recognize the increasing prevalence of n-ethyl pentylone and the potential trend of combining it with cocaine.

This presentation will impact the forensic science community by increasing knowledge and awareness of n-ethyl pentylone through communication of its increasing prevalence as observed in the Birmingham, AL, area and highlighting the potential new drug trend of combining n-ethyl pentylone and cocaine.

Hypothesis: N-ethyl pentylone, a Novel Psychoactive Substance (NPS), has been observed in high concentrations and mixed with cocaine, which may represent a new drug combination (i.e., speedball (cocaine and heroin), twisters (crack and methamphetamine), el diablo (cocaine, heroin, and marijuana)).

Statement of Content/Methods: Three death cases were investigated by the Jefferson County Coroner and Medical Examiner's Office (JCCMEO) between February and May of 2017; each involving n-ethyl pentylone. N-ethyl pentylone (i.e., bk-EDBP, ephylone, Mercedes, Mitsubishi, Lacoste) is classified as a psychostimulant related to cathinone. Stimulants modulate neurotransmitters (i.e.; serotonin, dopamine, and norepinephrine) through increased release or reuptake inhibition. Being structurally related to cathinone, n-ethyl pentylone is proposed to modulate multiple neurotransmitters with overdoses resulting from serotonin syndrome. Effects related to n-ethyl pentylone use range from euphoria, increased alertness, and talkativeness to agitation, tachycardia, hyperthermia, rhabdomyolysis, hypoglycemia, renal failure, and cardiac arrest. Users describe routes of administration as insufflation, intravenous, and oral with the onset of effects occurring within 30 minutes and lasting three to five hours. Due to its current legal status, n-ethyl pentylone can be purchased via the internet in either powder or pill form and has been observed as a component of Neuregulin 1 (NRG-1) and as "Molly."

The first n-ethyl pentylone case encountered by the JCCMEO was a 34-year-old White male who had been observed using "Molly." According to the witness, the decedent became erratic and paranoid. The decedent was later located underneath a car in an auto garage with the scene in disarray -— overturned items, including chairs and stepladders, and papers scattered around the room. The second occurrence was a 34-year-old Black male. This decedent was at a party when he entered into cardiac arrest and was transported to the emergency room. The decedent transpired a few hours later. The third n-ethyl pentylone case was a 25-year-old Black male found lying in the street suffering multiple gunshot wounds. According to neighbors, the decedent was in a feud with the suspect. In all three cases, postmortem specimens (blood, urine, vitreous, bile, liver, and brain) were collected and submitted to the toxicology laboratory. Analyses performed included: volatiles testing, immunoassay screening, and Gas Chromatography/Mass Spectrometry (GC/MS) confirmation/quantification.

Summary of Results: Postmortem toxicology results for the first n-ethyl pentylone encounter were trace levels (<0.01mg/L) of cocaine and cocaethylene and n-ethyl pentylone at 0.953mg/L. Cause Of Death (COD) and Manner Of Death (MOD) were reported as "Acute n-Ethyl Pentylone Toxicity" and "Accident," respectively. The toxicology results for the second occurrence were cocaine at 0.033mg/L, fentanyl at 0.003mg/L, n-ethyl pentylone at 0.121mg/L, methamphetamine at 0.938mg/L, and amphetamine at 0.086mg/L. COD and MOD for case 2 were "Multiple Drug Toxicity from Methamphetamine, Cocaine, Fentanyl, and n-Ethyl Pentylone" and "Accident," respectively. The third case was the result of multiple gunshot wounds (COD) and determined to be a homicide (MOD) but resulted in toxicological findings, including trace levels of hydrocodone, alprazolam at 0.030ng/mL, and n-ethyl pentylone of 0.045mg/L.

Conclusion: Presented here are the first three occurrences of deaths related to n-ethyl pentylone by the JCCMEO. With these deaths occurring in such a short time period, it is shown that the prevalence of n-ethyl pentylone is increasing in the Birmingham area. Furthermore, a potentially deadly combination of n-ethyl pentylone and cocaine has been observed in two of the cases, with both psychological (paranoid and agitated) and physiological (cardiac arrest) effects witnessed. In short, n-ethyl pentylone is a dangerous NPS that should be included in toxicological analyses.

N-Ethyl Pentylone, Novel Psychoactive Substances, Molly

K4 Carfentanil-Induced Fatalities: A Case Series

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The goal of this presentation is to investigate several fatalities associated with carfentanil and to provide a template for medical examiner offices to monitor "designer" opioid deaths through standardized toxicological screening.

This presentation will impact the forensic science community by providing novel carfentanil toxicity levels, considering that the human biological effects of the illicit opioid are unknown.[1]

Carfentanil is a μ-opioid receptor agonist that induces respiratory compromise and central nervous system depression. Its potency is 10,000 times greater than morphine.[2] Used as an elephant tranquilizer, the schedule II drug has concealed itself within the street drug market in North America. Fatalities continue to climb, considering there has been only one published instance of human exposure to an illicitly manufactured version of carfentanil with a successful medical outcome.[3]

This case series will investigate 17 fatal intoxications involving carfentanil, considering the postmortem findings, autopsy results, and toxicological screenings.

A retrospective review of 2,807 deaths was conducted through the Oakland County Medical Examiner's Office database to investigate all potential deaths of carfentanil use. Every public carfentanil-related death in Oakland County, MI, over a six-month period is included in this study. Postmortem specimens for toxicology measurement were extracted from the matrix source of the femoral artery, except for instances of heart blood when no femoral blood was present. Each decedent received a volatile screen through a fentanyl enzyme-linked immune absorbance assay test kit. With a positive confirmation of a fentanyl metabolite, an expanded postmortem, forensic, quantitative blood test was ordered through the National Medical Services Laboratories (NMS Labs). Carfentanil spectra was matched through a spectral library and measured through Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). A standardized case review form was used to characterize each carfentanil-positive death. All available medical and legal history was reviewed to interpret the death.

Sixty-one percent of the decedents were below the age of 35 years. Other than two females, every decedent was male. The cause of death deemed by the medical examiner was drug abuse (n=0), drug intoxication (n=6), and drug overdose (n=1). The manner of death was undeterminable in 16 of the cases, with one exception of suicide by overdose. Only five of the decedents had pre-existing medical conditions that could be speculated as contributory to cause of death. The rest of the decedents were otherwise healthy individuals with no anatomical evidence of trauma or pre-existing disease. Eight of the decedents underwent endotracheal intubation and six were administered naloxone. Five cases were found with a syringe in hand or within arm's reach. Of 11 chronic substance users, seven either actively abused heroin or used within the past six months per family members and friends. Four presented with biventricular hypertrophy and three possessed left ventricle myocardium hypertrophy. The mean combined lung weight was 1,536 grams. Every case possessed moderate to severe pulmonary edema, with remarkable pulmonary congestion. As reported by NMS Labs, every case presented with a unique panel of drugs with a mean carfentanil concentration of 0.384ng/mL. It was previously suggested that 20 micrograms of carfentanil could induce death, although the concentrations reported in the bloodstream at time of death were as low as 10ng/mL.[4] Five cases were found to have 6-monoacetylmorphine, with a mean concertation of 17.66ng/mL. U-4700 was found in conjunction with carfentanil in three instances.

There has been only one other study which provided results from a comprehensive and sensitive screening method, through Ultra High-Performance Liquid Chromatography (UHPLC) -Ion Trap-MSn, to identify carfentanil.[5] That study only provided detection results, without quantification values. The methods presented in the current study could be employed as a template for other medical examiner offices, as LC/MS/MS can reduce toxicological discrepancies between cases and detect low concentrations.

Hopefully, the cases presented in this study will provide a foundation for further studies examining carfentanil's toxicological characteristics. Future studies are warranted to investigate the epidemiological trends of this perilous opioid.

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Carfentanil, Postmortem Concentration, Overdoses

K5 An Assessment of the Incorporation of Amphetamine and Diazepam Into Human Head Hair for the Preparation of Hair Reference Material

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After attending this presentation, attendees will better understand the process of preparing Hair Reference Material (HRM) with incorporated xenobiotic substances.

This presentation will impact the forensic science community by contributing to a body of research targeted at better understanding the interactions between drugs of abuse with different physiochemical properties and hair that would be considered during routine analysis in forensic laboratories.

HRM is essential for the development and validation of methodologies used in forensic hair analysis. At present, HRM containing selected xenobiotic substances is only available on a limited basis. In addition, most xenobiotic substances relevant to forensic casework are not available as incorporated drug standards in human head hair. Additionally, little is known about the incorporation process that occurs when human head hair is soaked in a xenobiotic-containing buffer solution. This body of work demonstrates the intentional incorporation of Amphetamine (AMP) and Diazepam (DZP) into human head hair for the development of "in-house" HRM by exploring the relationship between time and the concentrations of drug detected in the incorporation buffer solution, daily hair extracts, and solutions used to wash daily hair aliquots. The hypothesis was that, over time, the concentration of xenobiotic detected will decrease in the buffer solution, increase in the hair extracts, and remain relatively constant in the wash solutions.

Purchased human head hair was soaked in 1X Phosphate-Buffered Saline (PBS) spiked with either AMP or DZP at 800pg/mL for five days (120h). Each day, aliquots of hair and spiked PBS solution were taken to monitor incorporation. The hair aliquots were washed using both organic (2-propanol) and aqueous (1X PBS) solvents, then pulverized using a Retsch® MM200 ball mill. Subsequently, the pulverized hair was incubated in a mixture of organic and aqueous solvents (methanol:cetonitrile: 2mM ammonium formate in water; 1:1:2) for 18h to extract incorporated drug from the matrix. After incubation, the samples were centrifuged to separate the hair particulates from the solvent mixture containing recovered drug. The resulting solution was subjected to online Solid-Phase Extraction (SPE) cleanup and Liquid Chromatography/Triple Quadrupole/Tandem Mass Spectrometry (LC/QqQ-MS/MS) analysis. Mass Spectrometry (MS) analysis was performed on the spiked PBS solutions, extracted samples, and wash solutions. A 1µL injection of each sample was introduced to an Agilent® 1290 Infinity Flexible Cube to perform online SPE. A reversed-phase LC column (Agilent® ZORBAX® Rapid Resolution High-Definition Eclipse Plus C18, 2.1 X 50mm, 1.8µm) was used as the analytical column on an Agilent® 1290 Infinity® HPLC system. A gradient elution was used over 8min using 5mM ammonium formate in water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B). Analysis was performed with positive mode Electrospray Ionization (ESI) on an Agilent® 6460 QqQ-MS instrument.

Quantitative results demonstrated the successful incorporation of AMP and DZP into blank human head hair. Contrary to the hypothesis, the amount of drug extracted from the hair aliquots was not significantly different between the 24h and 120h time points for either drug. Final incorporated drug levels were approximately five-fold higher for AMP than DZP. The organic wash did not remove a significant quantity of AMP, but did remove DZP from the surface of the hair. The three aqueous washes each removed AMP from the hair surface, with decreasing concentration in each wash. In contrast, the organic wash and the first of the aqueous washes removed DZP, while subsequent aqueous washes did not remove any additional drug. These results suggest that the maximum transfer of drug from the incorporation buffer into hair occurs within the first 24h of incubation for both drugs. In addition, AMP may be more prone to contaminating the surface of the hair or it may be more loosely bound to the hair matrix than DZP. The differences between incorporation, decontamination, and extraction of these two drugs may be attributed to differences in their physiochemical properties.

Hair Analysis, Hair Reference Material, Incorporation of Xenobiotics

K6 A Mass Spectrometric Approach to the Analysis of Covalent Modifications of Blood Proteins by Drugs of Abuse

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After attending this presentation, attendees will better understand *in vitro* formation of covalent protein modifications formed by reactive drug metabolites, as well as the Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analytical approach required for detection of these adducts.

This presentation will impact the forensic science community by demonstrating that covalent protein adducts formed *in vitro* provide the necessary framework for an *in vivo* detection method under development for the retrospective detection of drugs of abuse in human blood.

Hemoglobin (Hb) and serum albumin (SA), two prevalent proteins in human blood,- contain unbound cysteine thiol moieties, creating a nucleophilic site with the potential for covalent modification by reactive chemical species. These covalent modifications, called "adducts," are stable entities that accumulate during acute and chronic exposure and remain covalently bound for the life-span of the protein. Despite their current use as exposure markers for a variety of compounds, the use of adducts in assessing exposure to drugs of abuse has not yet been explored. The goal of this work is to examine the *in vitro* adduct-forming capability of selected drugs of abuse with Hb and SA to provide additional proof of principle for the development of a real-world detection and monitoring analysis method. Use of protein adducts as biomarkers of drug exposure will allow for an increased window of detection, from several days to several months, as compared to current blood analysis methods. The drugs examined in this study cover a wide range of abused drugs, including cocaine, methamphetamine, and Δ^9-THC, and have all been shown in previous work in the laboratory to form adducts with glutathione and/or other thiol-containing peptides.

For this research, a new assay procedure was created to facilitate recovery of modified protein by combining published methods with existing methodology used in the lab. The new assay utilized a dialysis membrane to maintain separation of proteins of interest from the microsomal components, while allowing for small molecules (i.e., stable and reactive metabolites) to pass through, resulting in a decrease in the number of steps required to extract the modified protein of interest. For the metabolism/adduction assay, each drug was added to a plastic microfuge tube with residual solvent removed via vacuum centrifuge. Human liver microsomes were added to the tube and combined with Nicotinamide Adenine Dinucleotide Phosphate (NADPH) in the presence of a regeneration system containing glucose-6-phosphate and glucose-6 phosphate dehydrogenase, in sodium phosphate buffer (pH 7.4). The protein of interest was then added; the tube was incubated at 37°C for 18h, then centrifuged. An aliquot of supernatant was removed and added to a clean LC/MS vial for analysis. Instrumental analysis of modified protein was performed using positive Electrospray Ionization (ESI) on an Agilent® 1290 Infinity® Ultra High-Performance Liquid Chromatography (UHPLC) coupled to an Agilent® 6530 MS and chromatographic separation utilized an Agilent® ZORBAX® Rapid Resolution HD Eclipse® Plus C8 column. Data were collected using full MS scan mode, to allow for necessary analysis of all protein components. The mobile phases used were as follows: (1) water with 0.1% trifluoroacetic acid; and, (2) 95% acetonitrile, 4.9% water, 0.1% trifluoroacetic acid. The total run time was 16 minutes with a 2-minute post-run for column re-equilibration. Initial analysis of MS data obtained was performed using Agilent's® MassHunter™ Qualitative Analysis software, followed by MassHunter™ BioConfirm software for protein deconvolution and proteomic analysis of adducts formed.

The use of protein adducts for retrospective drug detection represents a novel and useful advance in drug testing and analysis. The characterization of covalent adducts formed *in vitro* shown in this research provides the necessary framework for future examination of a more complete set of abused drugs. The confirmation and subsequent analysis of these covalent protein adducts reinforces the need for the development of a real-world applicable method to screen for drug exposure utilizing a longer window of detection than is currently available for most drugs and matrices.

Protein Adducts, Drugs of Abuse, LC/MS

K7 Hydrogen Sulfide (H_2S) Poisoning in the Workplace: Toxicological Investigations in a Fatal and Non-Fatal Accident

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After attending this presentation, attendees will understand that in cases of occupational asphyxiation, it is important to both conduct a complete postmortem toxicological analysis and to perform accurate scene investigations supported by environmental-toxicological monitoring.

This presentation will impact the forensic science community by highlighting the usefulness of the measurement of thiosulfate that represents an important step in a toxicological investigation of H_2S poisoning because it can supply information regarding the time of death and toxicokinetics.

H_2S is a toxic gas involved in deaths in the workplace. Few cases have been reported in the literature and the accident occurs predominantly in the sour gas industry and in other industrial settings.

The reported case regards fatal and non-fatal intoxications due to H_2S asphyxiation involving six seamen who were working on a ferryboat.

Accident scene reconstruction revealed that three workers had to remove the waste fluid from a bilge of the ferryboat. The first victim unscrewed bolts of the bilge manhole and in a few minutes fell unconscious. Two other seamen who saw what happened asked for help and approached the subject, but they too fell unconscious. Three other workers arrived to move the three unconscious seamen and only one had a protective mask. The first victim died in the workplace, the second one died in the ambulance, while the third one died in the emergency room. Of the remaining three workers, one had a severe pulmonary edema that required intensive treatment, the second had pulmonary injuries also involving the heart, and the third, who wore the mask, had lung injuries.

Toxicological environmental analyses were performed two hours after the accident on air samples from the bilge, the area in front of the bilge manhole, and on the waste fluid collected from the bottom of the bilge. The analyses revealed high levels of H_2S in all specimens.

Autopsies were performed after 48 hours and the findings were the same for all the victims, showing edema of the lungs and multi-organ congestion.

Toxicological analyses were performed both on venous blood taken from the living subjects on arrival at the emergency room and on femoral blood and urine taken during autopsy to evaluate the presence of volatile hydrocarbons, carbon monoxide, hydrogen cyanide, and H_2S. Results from the surviving workers were negative and, in particular, H_2S concentrations were below the quantification limit, being already biotransformed and eliminated in the urine, either in the form of unmodified sulfide or as thiosulfate. In the deceased workers, the toxicological investigation was positive for high levels of H_2S; thus, thiosulfate research was performed to distinguish between the H_2S concentrations in blood secondary to lethal poisoning and those produced by a putrefactive phenomena. Thiosulfate evaluation revealed significant levels in femoral blood (45.7µg/ml) and even more in the urine (510.1µg/ml) belonging to the first decedent. Lower levels were observed in samples from the second decedent (blood: 35.5µg/ml; urine: 10.4µg/ml). Even lower levels were determined in the blood of the third decedent (21.5µg/ml) and no measurable concentrations were in the urine.

These findings demonstrated that the seamen died from asphyxia due to H_2S poisoning. Moreover, the toxicological results allowed for the evaluation of several different survival times. In fact, the first seamen survived for a longer time because metabolism of much of the inhaled sulfides had occurred and a high amount of metabolite (thiosulfate) had eliminated in the urine. The second worker survived briefly and was able to metabolize a smaller amount of sulfides and to eliminate a small amount of thiosulfate in the urine. Finally, the third survived for a very short time because the urine thiosulfate was negative.

This presentation also highlights the importance of safety devices used as well as compliance to the relative Italian legislation.

Hydrogen Sulfide, Toxicological Analysis, Occupational Accident

K8 The Development of a Rapid Multi-Target Screening Method for Emergency Toxicology by Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will understand a rapid simultaneous screening method using GC/MS and LC/MS/MS for the determination of multiple toxicants in urine samples collected from intoxicated patients in emergency rooms.

This presentation will impact the forensic science community by informing attendees of the rapid method for simultaneous screening of multiple toxicants by GC/MS and LC/MS/MS that were established to determine targeted and unknown toxicants in urine.

From February 2015 to March 2017, 265 urine samples were collected from the Chungnam University Hospital emergency room. Urine samples were cleaned by using Waters® Ostro™ (pass-through type) and examined by GC/MS and LC/MS/MS. After analysis by GC/MS, the library search for unknowns was conducted by in-house mass spectral databases with the Automated Mass spectral Deconvolution and Identification System (AMDIS). In addition, Chemstation® software was mobilized to identify toxicants. For LC/MS/MS analysis, the 3200 QTRAP® LC/MS/MS and Cliquid® software was used for a simultaneous multi-targeted screening.

A rapid multi-target screening method by GC/MS and L/-MS/MS was developed to determine toxic substances in urine. By using Ostro™ extraction and an in-house database, it was possible to screen urines for toxic substances within three hours. With this method, 265 urine samples were examined and it was noted that zolpidem, acetaminophen, and citalopram were detected in 49, 29, and 16 cases, respectively, and those were the most frequently encountered drugs in emergency room patients. The targeted and unknown toxicants were well searched by in-house and commercial mass spectral databases in all specimens studied. AMDIS & Chemstation® software were used for GC/MS analysis and Cliquid® 2.0 software was used for LC/MS/MS analysis.

The rapid multi-target screening methods by GC/MS and LC/MS/MS developed in this study proved to be applicable to the actual hospital poisoning samples. This method can be efficiently used to detect toxic substances within three hours in emergency cases.

Multiple Toxicants, Targeted and Unknown Toxicants, Emergency Toxicology

K9 A Concentration of Biomarkers in Vitreous Humor for the Estimation of Postmortem Interval (PMI) in South Korea

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After attending this presentation, attendees will understand which biochemical markers in vitreous humor can be measured by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) for the PMI and the correlation between the levels of biochemical markers and PMI in South Korea.

This presentation will impact the forensic science community by examining the correlation between the concentrations of Hypoxanthine (Hx), potassium, and time after death. This will be useful information for the estimation of PMI.

To choose the possible biomarkers for PMI, concentrations of Hx and lactic acid in vitreous humor were measured by LC/MS/MS. Hx is the terminal stage of purine catabolism in man and is known to be highly correlated to PMI. Also, in mammalian cells, lactate is formed from pyruvate in carbohydrate metabolism under anaerobic conditions. Lactic acid is chiral, consisting of two optical isomers. Because L-lactic acid is known to be correlated with PMI, it was targeted in this study.

A vitreous humor was collected from a cadaver with a known time of death between 18 hours and 103 hours at the National Forensic Service (NFS) in Korea. Twenty-one samples were comprised of 16 males and 5 females with an age range of 27-84 years. Vitreous humors were extracted by a solid-phase extraction with Oasis® MAX cartridges. Agilent® 1260 infinity HPLC system and Sciex® 3200 QTRAP® MS were used for the quantification of Hx, uric acid, and lactic acid in vitreous humor. Chromatographic separation was performed by using 0.1% formic acid in water and methanol as the mobile phase. The Multiple Reaction Monitoring (MRM) of ion transitions monitored was m/z 137.0>110.0, 119.0 for Hx and 5-(p-methylphenyl)-5-phenylhidantoin 267.2>163.3 as the Internal Standard (IS). Lactic acid was separated into L-lactic acid and D-lactic acid by derivatization, and (+)-O,O`-diacetyl-L-tartaric anhydride (≥97%) (DATAN) were used as derivatization reagents. The MRM of ion transitions monitored was m/z 308.0>89.0 for L-, D-lactic acid and L-lactate-3,3,3,-d3 308.1>92.1 as IS for lactic acid.

Analysis of 21 vitreous humor samples revealed that the concentration of Hx ranged from 327μM to 1,780μM and well correlated with the PMI. The Hx concentrations increased gradually until 96 hours, indicating the concentration of Hx and time after death are well matched. The differences in HX concentration between gender and age were not noted. The concentration of potassium was also well related with PMI, while creatinine, Blood Urea Nitrogen (BUN), sodium (Na), and chlorine (Cl) were not correlated with PMI. Lactic acid was separated into L-lactic acid and D-lactic acid through its derivatives.

The correlation between the concentrations of Hx, potassium, and time after death will be very useful information for the estimation of PMI.

Postmortem Interval, Hypoxanthine, Potassium

K10 Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Extraction of Novel Psychoactive Substances (NPS) From Biological Matrices

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The goal of this presentation is to describe the findings of using QuEChERS as a quicker and less expensive alternative for the extraction of NPS from biological matrices. This presentation will cover a broad range of drug classes and the trends observed. An optimized extraction method that encompasses as many NPS as possible will also be presented.

This presentation will impact the forensic science community by presenting an alternative to currently used extraction techniques for NPS that includes added benefits, such as decreased costs and time spent on sample preparation.

This presentation is intended to demonstrate the potential of QuEChERS ("catchers") as an extraction technique for various NPS in biological fluids (urine and whole blood). Although extraction techniques for common drugs of abuse are well studied, developing extraction methods specifically targeting NPS is needed due to the high prevalence of NPS in forensic casework. A validated screening/confirmatory triggered Multiple Reaction Monitoring (tMRM) method for 826 NPS by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) recently developed in the lab is being used to analyze all extracts for this study. The method allows for the screening of a wide variety of NPS drug classes and metabolites, with a focus on synthetic stimulants and cannabinoids due to their current importance in forensic toxicology casework.

Most biological fluids require an extraction step before analysis to avoid unwanted matrix effects and to protect instrumentation. Some commonly used extraction/purification techniques are Solid Phase Extraction (SPE), Liquid-Liquid Extraction (LLE), and dilute/crash and shoot. These techniques can be expensive, time consuming, and may not eliminate all matrix effects. Extraction methods ideally should be inexpensive and relatively fast to allow for high throughput in forensic toxicology labs.

QuEChERS has the potential to be a desirable alternative to these techniques because it can decrease overall costs, matrix effects, and time. QuEChERS was originally developed to simplify and speed up sample prep to extract pesticides from fruits and vegetables. QuEChERS uses a dispersive SPE (d-SPE) technique that increases the sample's contact with the sorbents. This increased contact allows for a more effective extraction than classic SPE. It is specifically designed for highly aqueous matrices and, therefore, can be a very useful technique for forensic toxicology work. While QuEChERS has been previously used as a technique for extracting common drugs of abuse from urine and blood, to date it has not been applied to extraction of NPS on this scale.

In this study, a modified Bond Elute kit was utilized. Blank human urine spiked with 29 different NPS at three different concentrations (5ng/mL, 20ng/mL, and 80ng/mL) was used for this work. The mixture included NPS from different drug classes, including synthetic cannabinoids, synthetic cathinones, tryptamines, and phenethylamines. The range of concentrations was selected to test the utility of the technique for authentic samples, which may contain very low concentrations of these compounds. The extraction process consisted of two major steps: a drying step with salts, followed by d-SPE. First, 3mL of the spiked urine was combined with 3mL acetonitrile, followed by the addition of salts (magnesium sulfate and sodium acetate) to dry the sample, which was then centrifuged at 4,400rpm for 5min. Then, 1mL of the acetonitrile layer was used for d-SPE. The acetonitrile was added to a centrifuge tube containing primary secondary amines, magnesium sulfate, and CI8 and centrifuged at 4,400rpm for 5min. A 100µL aliquot of the resulting supernatant was diluted with 100µL of internal standard mix and 300µL of High-Performance Liquid Chromatography (HPLC) water for analysis by LC/QqQ/MS. The total time for extraction was 15min, which is an improvement over most other extraction methods. An Agilent® 1290 Infinity® HPLC system and Agilent® 6460 QqQ/MS with Jet Stream Technology ESI was used with an Agilent® ZORBAX® Rapid Resolution HD Eclipse® Plus C18 column for LC/MS/MS using the tMRM method.

Results demonstrate that QuEChERS is a promising technique for the extraction of tryptamines, phenethylamines, and synthetic cannabinoids, all of which exhibited recoveries >85% at all three concentration levels. This work is being continued with whole blood and additional NPS to further assess the technique's potential. QuEChERS is capable of extracting NPS from multiple drug classes in a single urine specimen and represents an appealing alternative to other extraction methods due to fewer transfer steps, quick extraction time, and decreased cost.

QuEChERS, Novel Psychoactive Substances, LC/MS

K11 A Validated Method for the Quantitative Determination of Zolpidem, Zopiclone, and Zaleplon (ZZZ Drugs) in Blood, Stomach Contents, and Liver by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will better understand a validated method for the quantitation of ZZZ drugs in blood, stomach contents, and liver by basic Liquid-Liquid Extraction (LLE) and LC/MS/MS.

This presentation will impact the forensic science community by describing a method validation to rapidly and simultaneously confirm all three ZZZ drugs with matching deuterated internal standards (zolpidem-D6, zopiclone-D4, and zaleplon-D4).

Zolpidem, zopiclone, and zaleplon are sedative hypnotics.[1] Due to their rapid onset of action and short half-lives, ZZZ drugs have become the standard alternative to short-acting benzodiazepines for the treatment of onset and maintenance forms of insomnia.[2] ZZZ drugs are GABA agonists and their poly-use with benzodiazepines, ethanol, or other Central Nervous System (CNS) depressants can increase impairment and lead to toxicity or death.[3] If taken in compliance, the drugs should exist at or below therapeutic concentrations with little to no residual effects upon waking. ZZZ drugs are commonly detected both independently and in conjunction with benzodiazepines and ethanol in Driving Under the Influence of Drugs (DUID) and postmortem cases.[4] Current methods for the detection of ZZZ drugs in blood are generally performed with Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) either in tandem with benzodiazepines or are not quantitative for all three analytes.[1,3,5] The goal of this work was to validate a method for the quantification of zolpidem, zopiclone, and zaleplon by LC/MS/MS.

Both acidic and basic LLE methods were investigated during method development. The basic extraction yielded higher area counts, more uniform peak symmetry, and easier isolation of the organic layer. The basic extraction method utilized saturated sodium borate (pH 12), ethyl acetate, and matching deuterated internal standards. An Agilent® 1290 Infinity® II Stack and 6460 Triple quadrupole/Mass Spectrometry (QqQ/MS) system was employed in positive electrospray ionization mode with Multiple Reaction Monitoring (MRM) transitions selected by the Agilent® Optimizer program. Separation was achieved on an Agilent® InfinityLab Poroshell 120 EC-C18 column (3.0mm x 100mm, 2.7µm) with a 0.6mL/min flow rate of 0.1% formic acid in H_2O (A) and 0.1% formic acid in CH_3CN (B). The gradient was initialized at 20% B for 0.8min, increased to 45% B over 0.8min, and 95% B over 1min. An isocratic hold was placed at 95% B for 1.5min, followed by a decrease to 20% B over 0.3min, for a total run time of 4.7min. Method validation was conducted according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines.

Seven non-zero calibrators were used to establish a 10ng/mL-1,000ng/mL working range with a 1/x weighting factor. Standard residual plots indicated that zopiclone was best fit with a linear model while zolpidem and zaleplon required a quadratic model. Studies assessing the limit of detection are currently underway and the Limit Of Quantitation (LOQ) was set at the lowest non-zero calibrator (10ng/mL) as ZZZ drugs tend to be found, in blood, at concentrations exceeding the lowest calibrator.[2,6] Carryover following the 1,000ng/mL calibrator was determined to be 0.35%, 0.23%, and 1.28% of the LOQ for zolpidem, zopiclone, and zaleplon, respectively. Three concentration levels (25ng/mL, 400ng/mL, 750 ng/mL) in triplicate were used to determine the bias and precision. Bias and precision were calculated at ≤15% for all three analytes, concentrations, and matrices, with the exception of zopiclone in stomach contents at 17.3% CV. Ion suppression was assessed by a post-extraction spike of mobile phase and negative blood, stomach content, liver, and urine matrices at low and high Quality Controls (QCs). Although ion suppression was present at values exceeding SWGTOX guidelines, it had no impact on accuracy or precision of quantitation. No significant interferences were present in mobile phase samples spiked with common drugs of interest (fentanyl, opiates, cocaine, diphenhydramine, trazodone, buspirone, PCP, 3-MeO-PCP, dextromethorphan, ketamine, duloxetine, venlafaxine, tramadol/ nortramadol, amitriptyline/ nortriptyline, clozapine, doxepin/nordoxepin, fluoxetine/norfluoxetine, olanzapine, quetiapine/norquetiapine). The selected MRM transitions were not triggered by endogenous compounds in negative human blood, stomach contents, liver, or urine matrices. Bias and precision of dilution integrity fell ≤20% and was monitored by preparing a 1,500ng/mL ZZZ calibrator and diluting in triplicate (x25, x10, x4) in negative blood. The preceding validation method meets the requirements of SWGTOX guidelines.

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K12 Performing Retrograde Extrapolation of Blood Alcohol in Driving Under the Influence (DUI) Trials

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After attending this presentation, attendees will understand the need to consider relevant factors before performing retrograde extrapolation in DUI trials.

This presentation will impact the forensic science community by increasing awareness that retrograde extrapolation must be performed with caution to ensure that a reliable calculation is obtained. Data from a Las Vegas Metropolitan Police Department (LVMPD) drinking study will be used to illustrate this point.

In the Illinois case of *People v. Floyd*, the defendant was convicted of aggravated DUI and resisting arrest.[1] The defendant's DUI conviction was later reversed and remanded for a new trial by the appellate court. The decision to reverse the conviction was due to an assumption made by the State's expert witness while performing retrograde extrapolation that the defendant was in the post-absorptive phase without considering all relevant factors. Although the ruling recognizes that it does not "[create] a blueprint or a bright-line rule for the admissibility of retrograde extrapolation evidence," it emphasizes the fact that retrograde extrapolation must be carefully conducted.[1]

To demonstrate this point, a drinking study involving 12 subjects (6 males and 6 females), between the ages of 23-35 years, was conducted. Nine of 12 subjects consumed food within 2.5 hours of the start of drinking. Two subjects consumed food more than four hours prior to the start of drinking, and the time of the last meal was not provided for one subject. Hard liquor was consumed over 1.5-3 hours. Three blood draws were taken from each subject approximately 1, 2, and 3 hours following the end of drinking.

Each blood sample was collected in a 10mL gray-stoppered glass blood tube and was stored at 2ºC-8ºC from the time of collection to the time of analysis. The blood samples were analyzed using a dual column headspace gas chromatograph with two flame ionization detectors. The method was validated following the Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices.

A plot of Blood Alcohol Concentration (BAC) as a function of time was generated for each subject, and the elimination rate was determined through linear regression analysis. Of the 12 subjects, 2 appeared not to be in the post-absorptive phase at the time of the first blood draw. Elimination rates for these 2 subjects were determined through linear regression analysis using only second and third blood draw data. Mean elimination rates of 0.020g/100mL/h (range: 0.015g/100mL/h-0.024g/100mL/h) and 0.018g/100mL/h (range: 0.007g/100mL/h-0.027g/100mL/h) were obtained for male and female subjects, respectively.

For retrograde extrapolation, the elimination rate and rate range considered were 0.015g/100mL/h and 0.010g/100mL/h-0.035g/100mL/h.[2] Assuming that each subject was in the post-absorptive phase at the time of the first blood draw, extrapolating to that time using the second blood draw data overestimated the BAC of one subject. This was the case whether the 0.015g/100mL/h or 0.010g/100mL/h-0.035g/100mL/h elimination rate/rate range was used. Furthermore, retrograde extrapolation to the time of the first blood draw based on the third blood draw data, using the 0.015g/100mL/h elimination rate, marginally overestimated BACs of 2 subjects; however, similar retrograde extrapolation using the 0.010g/100mL/h-0.035g/100mL/h elimination rate range did not overestimate any BAC.

Based on the LVMPD drinking study data and the *People v. Floyd* appellate ruling, it may not always be appropriate to assume that a subject is in the post-absorptive phase at the time of the incident. The BAC may be overestimated if relevant factors, such as the drinking scenario, food consumption, and circumstances surrounding the incident, are not considered. Retrograde extrapolation is an effective tool in determining the BAC at an earlier time when it is carefully conducted.

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Forensic Toxicology, Retrograde Extrapolation, DUI

K13 The Identification of Five Kratom Alkaloids Using High Resolution Mass Spectrometry

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After attending this presentation, attendees will be able to identify and separate kratom alkaloids using Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). The influence of mobile phase additives and adduct formation will be discussed and common fragmentation pathways of corynanthe-type alkaloids will be explored.

This presentation will impact the forensic science community by highlighting the importance of mobile phase selection, optimization of ionization conditions, and structural identification of fragment ions using high-resolution MS.

Mitragynine (MG) (9-methoxycorynantheidine, kratom) and 7-hydroxymitragynine (MG-OH) are naturally occurring corynanthe-type indole alkaloids present in the leaves of *Mitragyna speciosa*. This flowering plant of the *Rubiaceae* genus contains more than 20 alkaloids, of which mitragynine is the principal pharmacologically active component, with 7-hydroxymitragynine being a minor psychoactive constituent. Mitragynine and 7-hydroxymitragynine are μ-opioid agonists. Kratom also contains two diastereoisomers of mitragynine (speciociliatine and speciogynine) and paynantheine. Although these three compounds are not known to be psychoactive, their presence in biological specimens may indicate kratom use. Although not yet federally regulated, kratom's dual stimulant and opiate-like effects are somewhat unique, making it an ideal candidate for misuse among recreational drug users.

Separation and identification of MG, MG-OH, Speciociliatine (SC), Speciogynine (SG), and Paynantheine (PY) in biological samples presents a significant analytical challenge. LC/qTOF/MS is a high-resolution MS technique that offers high sensitivity and significant benefits in terms of mass accuracy and structural identification. Mobile phase composition and optimization of the ionization conditions is essential in order to achieve high sensitivity and adequate chromatographic separation. Tandem Mass Spectrometry (MS/MS) spectra can provide valuable structural information. Characterization of fragmentation pathways and identification of ions is important for new assay development.

During the development of an analytical method for MG, MG-OH, PY, SC, and SG in urine, a total of three mobile phase additives were evaluated in deionized water/acetonitrile: 0.1% formic acid; 10mM ammonium formate, and 5mM ammonium acetate. Chromatographic resolution, ionization efficiency, and the formation of adducts were investigated. Fragmentation pathways for MG, MG-OH, PY, SC, and SG were elucidated. MS/MS spectra were used to identify fragments and make mass assignments. Ultimately, this process plays an important role in the selection of highly specific precursor ion transitions. A total of three transitions were selected for each of the compounds.

The most abundant product ions for all compounds were associated with C-ring cleavage and the loss of the substituted piperidine (D-ring) between C2 and C5. The abundance and specificity ultimately led to this being selected for quantitation purposes for MG (399→174), MG-OH (415→190), SC (399→174), SG (399→174), and PY (397→174). Variations of C-ring cleavage predominated for all other major product ions, as well as formation of intact substituted piperidine ions.

Chromatographic separation and mass spectral acquisition are particularly important analytical variables due to the potentially large number of structurally similar alkaloids and diastereoisomers found in *M. speciosa*. LC/qTOF/MS and other high-resolution MS techniques are particularly useful for complex analytes such as these.

Kratom, Fragmentation, LC/qTOF/MS

K14 Conformational Considerations of Ethylenediamine Opioids AH-7921 and U-47700

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After attending this presentation, attendees will be able to recognize potentially confounding spectra attributable to conformational preference or slow conformation interchange of the amide bond in the AH- and U-series opioids.

This presentation will impact the forensic science community by informing attendees regarding spectroscopic issues that arise from conformational aspects seen in the related AH-7921 and U-7700 series opioids.

The published Structure Activity Relationships (SAR) for the AH-7921 series opioids demonstrate a preference for N-monosubstituted benzamides with hydrogen attached to the amide nitrogen. These types of analogs should favor a *trans* amide bond preference.[1] Conversely, U-47700 analog (SAR) indicates a preference for a methyl group on the corresponding amide nitrogen, which allows for *cis* and *trans* amide conformations.[2] The hypothesis is that this preference may be due to each series having the opposite amide bond configuration when bound to the mu-opioid receptor. Preliminary molecular modeling studies that explore this hypothesis by looking at preferred conformations for examples for each of the series and the potential for overlap of key functional groups between the AH-series and the U-series compounds will be presented.

The studies presented will highlight issues that an analyst may encounter that may cause confusion due to data that could be misinterpreted as a mixture. For example, peak doubling in the Nuclear Magnetic Resonance (NMR) due to slow interchange between *cis* and *trans* amide conformations may mislead one into thinking the sample is impure. It has long been documented in the literature that unsymmetrically N,N-disubstituted alkyl amides can exhibit both *cis* and *trans* amide bond conformations due to the lower barrier for rotation and diminished steric preference compared to N-monosubstituted alkyl amides.[3]

Analogs within the AH-7921 and U-47700 series will be analyzed by a variety of methods, including NMR, Gas Chromatography/Mass Spectrometry (GC/MS), Infrared (IR), Raman, etc., particularly regarding indications of conformational preference and potential for rotational interchange. It is well known that simple N-monosubstituted amides that are not conformationally restrained prefer the *trans* amide configuration. The AH-7921 analogs fall into this category. The U-47700 analogs are N,N-disubstituted alkyl amides and demonstrate both *cis* and *trans* configurations as evidenced in their NMR spectra, since the rotational interchange of the amide bond is slower than the NMR time scale. The conditions and the degree to which this phenomenon is observed will be described. The binding of the respective *cis* vs *trans* isomers to the mu-opioid receptor is not known, but it is likely one of the isomers would be preferred by the receptor over the other just as any flexible drug molecule binds to its receptor with a preferred conformation. Also, it is known from structure activity relationships for the U-series analogs that small changes in structure can lead to significant changes in receptor selectivity (e.g., phenylacetamides as kappa-receptor agonists vs. benzamides as mu-receptor agonists).[2]

In conclusion, occasionally even simple compounds can present the analyst with potentially confusing data, so awareness of not only stability issues (e.g., UR-144 degradation in the GC), but also conformational influences on chromatography and spectroscopy is important.

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Synthetic Opioids, AH-7921, U-47700

K15 The Transformation of Drug/Metabolite Ratios: An Objective Assessment of Toxicity

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After attending this presentation, attendees will be aware of the use of a simple mathematical transformation of urine test results to afford an objective assessment of toxicity through the ratio of drug to metabolite (e.g., fentanyl to norfentanyl). Comparison of postmortem drug(s) to metabolite ratios from urine to a large database of such ratios from urine drug testing can help provide this objective assessment of toxicity.

This presentation will impact the forensic science community by demonstrating that the mathematical transformation described in this report can provide a tool to help with the assessment of toxicity in postmortem examinations. These data are necessarily from urine in which "normal" population data exists from pain medication testing. This will require a testing paradigm shift in postmortem samples in which often only the parent drug is tested in various bodily fluids.

Forensic assessment of toxicity is often subjective. For example, it is expected intuitively that fentanyl concentrations from postmortem samples are higher than corresponding therapeutic levels, whether in blood or urine. In fact, many forensic laboratories only test for fentanyl and not the metabolite, norfentanyl. Ruan et al. suggested that the absolute value of the ratio of norfentanyl to fentanyl could be related to the probability of "acute fentanyl toxicity."[1] This work reports that a simple logarithmic transformation of the ratio of parent drug concentrations to metabolite concentrations from a large body of therapeutic test results in urine can afford a stable database for comparison with forensic (postmortem) sample results treated the same way. The stable database for comparison is readily available from urine data obtained during pain medication monitoring testing.[2] Similar databases from blood testing are not available. While this approach can work for any number of potentially toxic drugs, the transformed ratios of fentanyl to norfentanyl in urine from several postmortem cases were compared with the "normal" therapeutic distribution of such transformed ratios to confirm this model/hypothesis. Urine fentanyl/norfentanyl data from overdose cases reported by Coopman et al., Peer et al., and Poklis et al. were similarly transformed to compare with the existing pain monitoring population (Figure 1).[3-5] There is some apparent overlap between the populations, but the overdose population is higher than the "normal" therapeutic results as predicted by Cummings et al.[6] Additional fentanyl/norfentanyl urine data from postmortem samples is expected to more clearly delineate that population of results in contrast to the therapeutic distribution. Thus, this simple transformation of the test results can provide an indicator of toxicity. Of course, the total setting of the death must be taken into consideration, but this approach could serve as an additional tool for forensic scientists in their investigations.

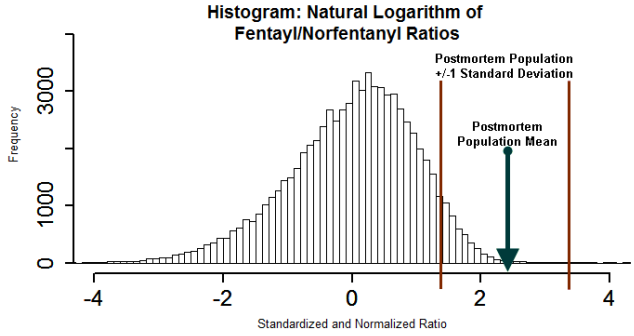

Figure 1.

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Fentanyl, Transformation, Postmortem

K16 The Electroanalytical Identification of 25I-NBOH and 2C-I Via Differential Pulse Voltammetry: A Rapid and Sensitive Screening Method to Avoid Misidentification

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The goal of this presentation is to present a new electrochemical method to identify 25I-NBOH, a new, potent serotonin 5-HT2A receptor agonist usually identified in blotter paper.

This presentation will impact the forensic science community by introducing a new, selective, and sensitive method for the identification of 25I-NBOH, a compound that is usually misidentified by routine Gas Chromatography/Mass Spectrometry (GC/MS) methods.

Recently, a new potent serotonin 5-HT2A receptor agonist was identified in blotter paper seizures in Brazil.[1] This compound, named 25I-NBOH, is a label molecule that undergoes degradation when examined under routine GC/MS conditions, leading to misidentification as it degrades into 2C-I, an amphetamine-type stimulant.[2] The prevalence of this substance on the Novel Psychoactive Substances (NPS) market can be underestimated under GC/MS conditions, the most widely and routinely utilized analytical technique for drug sample analyses, as it can misidentify 25I-NBOH because of its degradation into 2C-I (and corresponding 2C for the other members of the series).[2] Despite many attempts in adjusting GC/MS conditions and even changing the extraction solvent, Coelho Neto et al. stated that degradation could not be avoided.[2] The degradation takes place inside the GC/MS injector and appears to be caused by the high temperature inside the injector with the degradation products reacting with the alcohol used in the extraction procedure.[2] Another recent study described the analytical determination of phenethylamines derivatives; compounds of the NBOMe group via cyclic and differential pulse voltammetry.[3] Noting that 25I-NBOH has only a single modification regarding 25I-NBOMe, a substitution of a metoxy group for a hydroxy group in the position 3 of the secondary aromatic ring, a very sensitive and specific method to identify 25X-NBOH avoiding misidentification as 2C-X of this class of compounds was developed.

The voltammetric behavior of 25I-NBOH and 2C-I were investigated and their electroanalytical characteristics determined. The investigation of the electrochemical behavior by Cyclic Voltammetry (CV) using a carbon Screen-Printed Electrode (SCPE) showed two oxidative waves observed at +0.74 V and +1.09 V for 25I-NBOH and one single oxidative wave at +1.20 V for 2C-I. The first oxidative peak is a result of the electrochemical oxidation of the secondary amine present in the NBOH compound and the second oxidative wave is due to the halogen oxidation to a hydroxyl group and subsequent oxidation to a ketone (quinone/catechol equilibrium). The effect of scan rate (v) on the peak current (ip) and the peak potential (Ep) upon the electrochemical oxidation of both drugs were also examined. The slope values observed were close enough to the theoretically expected value of 0.5 for a purely diffusion-controlled current. The pH analyses revealed a linear dependence in the order of magnitude to that expected for a monoelectronic/monoprotonic reaction. To achieve unmistakable identification, differential pulse voltammetry was also used. The method uses the electrochemical oxidation of these molecules to produce an analytical signal that can be related to each compound concentration with an average lower limit of quantitation of 0.01mg/mL. The analytical identification for 25I-NBOH, 25I-NBOMe, and 2C-I was performed using the second oxidation wave, although the first oxidation wave was used in the quantification analysis.

A novel, fast, and sensitive electrochemical method for detection of 25I-NBOH using SCPE was achieved and all method characteristics demonstrated the method to be analytically valuable. The method is selective enough to identify the three compounds individually, even given the great similarity in their structure. The method is selective and achieved full differentiation between 25I-NBOH, 2C-I, and 25I-NBOMe.

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25I-NBOH, Electrochemical Identification, NPS

K17 Death From Poppy Tea Consumption

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After attending this presentation, attendees will understand the toxicological implications of home brewing poppy beverages and the biological concentrations associated with these types of deaths.

This presentation will impact the forensic science community by increasing awareness of the toxicity of this natural high by educating forensic investigators, pathologists, and postmortem toxicologists.

The historical practice of brewing poppy tea for its opioid-like effects is making a comeback with modern-day substance abusers. Whether it is brewed as an attempt to get high, as a source of natural pain relief, or to alleviate opioid withdrawal symptoms, the desired effects among users include euphoria, sedation, or the lessening of any negative symptoms of withdrawal, such as anxiety, nausea, and sweating.

This study presents three postmortem cases with opiate toxicology results that can serve as excellent case studies for debate on the hazards of poppy-drink ingestion. Enough attention has not been given to the dangers of this practice due to the variability of the morphine content of the opium exuded from the plant. While internet tea recipes offer guidance, differences in poppy seed/pod cultivation, washing, and infusing time are some of the reasons why beverages may contain different alkaloid concentrations from brew to brew. Variability in individual opioid tolerance in addition to other drugs also taken will impact the degree of toxicity of the opiates in the tea.

Free opiates (morphine, 6-acetylmorphine, codeine, hydrocodone, oxycodone, hydromorphone, and oxymorphone) in blood and urine are analyzed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) following Solid Phase Extraction (SPE) using a method validated against the Scientific Working Group for Forensic Toxicology (SWGTOX) validation standard. The blood concentrations of free morphine and codeine were 0.94mg/L and 0.11mg/L in case one, 0.62mg/L and 0.034mg/L in case 2, and 0.16mg/L and 0.010mg/L in case 3, respectively. Urine was submitted to the laboratory for two of the three cases. The urine concentration of morphine and codeine in case 1 were 10mg/L and 0.98mg/L and in case 2 were 13mg/L and 1.7mg/L, respectively. None of these cases were positive for 6-acetylmorphine. The minor opium alkaloids thebaine and laudanosine were identified by routine basic drug extraction in the urine of cases 1 and 2.

A review of the scene evidence from all three cases will help practitioners understand the investigatory clues leading to probable poppy drink consumption.

Poppy Tea, Poppy Seed, Forensic Toxicology

K18 The Impact of Storage Temperature, Glucose, and Microorganisms on Blood Alcohol Concentration in Non-Decomposed Whole Blood

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After attending this presentation, attendees will have a better understanding of how storage temperature, time, and the presence of excess glucose, bacteria, fungi, and yeast have an impact on Blood Alcohol Content (BAC) in non-decomposed whole blood.

This presentation will impact the forensic science community by illustrating the effects of glucose and microorganism contamination on BAC over a six-month storage period.

The presence of microorganisms has been studied in biological matrices, particularly in urine, but to date there are no comprehensive, longitudinal studies that demonstrate the impact of microorganisms and excess glucose in whole blood. Current research has revealed that some bacteria and yeasts, particularly *Candida albicans*, can produce ethanol in blood samples through a fermentation pathway. Additionally, storage of samples in warmer temperatures can increase the bacteria's ethanol production.[1] Conversely, degradation of ethanol in blood alcohol samples can be caused by storage temperature, time of storage, and sample volume.[2] Currently, an international standard for the collection, handling, storage, and testing of blood alcohol samples has not been established. If samples need to be re-analyzed or significant time elapses between evidence receipt and analysis, the samples must be stored correctly to ensure accurate results.[3] This study illustrates the effects of sample volume, storage temperature, and presence or absence of excess glucose and microorganisms on BAC over a six-month period.

Two sets of stock solutions of seven different ethanol concentrations were prepared in defibrinated sheep's blood: 0g/dL, 0.05g/dL, 0.08g/dL, 0.10g/dL, 0.15g/dL, 0.20g/Dl, and 0.30g/dL. D-glucose was added to one set of stock solutions in sufficient quantity to result in a blood glucose measurement of at least 240mg/dL. The appropriate blood was then added to 10mL gray-stoppered BD Vacutainer™ blood collection tubes in varying amounts (2.5mL, 5mL, 7.5mL, and 10mL) by removing the stopper and adding the blood via a syringe. For each BAC, four groups of eight samples were prepared. Group 1 included tubes of each volume as described, with and without excess glucose stored at room temperature (25°C). Group 2 was stored refrigerated at 4°C. Groups 3 and 4 were inoculated with a mixture of *Saccharomyces cerevisiae, Candida albicans, Acinetobacter johnsonii, Fusarium oxysporum,* and *Staphylococcus aureus* to simulate microbe contamination by improper collection (these strains were chosen as some of the most abundant on skin and/or having been indicated as common sources of contamination).[4] All four sets were made at each BAC for monthly analysis (months 0-6), for a total of 1,568 tubes. Each month, samples were analyzed in duplicate with an internal standard of 0.005% 2-butanone in water by an Agilent® 7820AGC and 5977EMS with headspace after instrument calibration, with 0.10g/dL standards run every 24 vials. Additionally, samples from each BAC level were streaked on blood agar plates and incubated to determine viability of the bacteria, yeasts, and fungus. If excess glucose was present, tubes inoculated with the microorganism mixture produced ethanol. Refrigerated samples experienced less degradation than samples stored at room temperature. Sample volume affected the rate of decomposition; smaller sample volumes experienced greater amounts of sample degradation. At BAC greater than 0.20g/dL, the microorganism survival rate was lower.

The results of this study indicate that the presence of microorganisms, particularly in the presence of excess glucose, can negatively affect the accuracy of ethanol analysis and that storage conditions and sample collection conditions, if known, should be considered when analyzing BAC data obtained from casework.

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BAC, Contamination, Longitudinal

K19 An Analysis of Drugs and Their Metabolites in Saliva and Urine Using Various Swabs in Conjunction With Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

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After attending this presentation, attendees will be able to: (1) understand the application of DART®-MS to the analysis of illicit drugs and their metabolites on various swabs used in routine drug testing applications; (2) understand the pros and cons of toxicology analysis of buccal swabs and urinary swabs by DART®-MS; and, (3) understand the effects of swab composition, drug type, drug polarity, and collection time on the sensitivity of drug detection in biological samples.

This presentation will impact the forensic science community by improving examiner knowledge of the variables involved in the analysis of oral fluid and urine for the detection of drugs of abuse using DART®-MS as a detection technique for swab analysis.

Hypothesis: Application of DART®-MS is gaining momentum in the forensic sciences due to its fast analysis time and minimal sample preparation. In toxicology casework, various swabs may be used for oral fluid collection and analysis. Though not popular, urine may also be sampled by a swabbing technique after being donated by the testee to concentrate any drugs present in the urine onto the swab tip.[1-3] These swabs may vary in their performance based on the method used for identification of drugs on these swabs, the composition of the swabs (hydrophobicity/hydrophilicity of the fibers), the physical properties (solubility, acidity/basicity, etc.) of the drug, and other factors.

Methods: Positive ion mass spectra were acquired using a DART® ion source interfaced to an AccuTOF™ mass spectrometer. To test the utility of swab sampling techniques for the analysis of drugs by DART®-MS, four different types of swabs were used: CVS™ brand cotton swabs, 155C rayon swabs, and two polypropylene applicators, 4508C FLOQSwabs™ and 4504C FLOQSwabs™, with different tip shapes. Solutions of lidocaine, procaine HCl, diphenhydramine HCl, and quinine monohydrochloride dihydrate were prepared in concentrations ranging from 1mg/ml to 1ng/ml. Specificity, Limit of Detection (LOD), and Linear Dynamic Range (LDR) were established for each swab variant. Next, the method was applied to multiple illicit drugs and metabolites, including cocaine, Δ^9-tetrahydrocannabinol, benzoylecgonine, and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol. These drugs were used to determine the correlation between: (2) swab fiber polarity; (2) drug polarity; and, (3) sensitivity. These drugs were spiked into synthetic urine or synthetic oral fluid in concentrations ranging from 100ug/ml to 1ng/ml. Each swab was submerged in the solution for 3s and analyzed via DART®-MS.

Results: There was a positive correlation between swab fiber polarity, drug polarity, and sensitivity of drug analysis. When urine or oral fluid solutions of polar drugs were sampled with polar rayon or cotton swabs, sensitivity was an average of ~10X-20X compared to polypropylene applicators. When urine or oral fluid solutions of non-polar drugs were sampled with the non-polar polypropylene applicators, sensitivity was ~10X-20X lower. When polar drugs in urine or oral fluid were sampled by the swabs, peak intensities were greatest for non-polar swabs, followed by more polar swabs. Non-polar drugs gave the highest peak intensity when cotton swabs were used. The two polypropylene applicators are composed of the same fibers, but have different tip shapes; 4508C is slightly more rounded than 4504C, and 4508C always had a higher peak intensity versus 4504C, showing the impact that swab tip shape has on sensitivity. The application of these techniques to the analysis of various synthetic drugs will be presented.

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DART®-MS, Swabs, Forensic Toxicology

K20 The Concentration and Distribution of Methamphetamine (MA) and Amphetamine (AM) in MA-Related Postmortems

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The goal of this presentation is to inform attendees that concentrations of MA and AM in the blood could be estimated with the postmortem concentrations in Bile Juice (BJ), when Peripheral Blood (PB) or Cardiac Blood (CB) cannot be taken.

This presentation will impact the forensic science community by presenting a study that is expected to play a major role in estimating the postmortem concentration of MA and AM in MA-related death cases.

MA is a frequently abused drug in southern parts of South Korea due to the nature of the port area. Also, MA-related deaths often occur in this area more than in other areas of South Korea. This study compared the concentrations and distributions of MA and AM in MA-Related Postmortems (MRPs). Gastric content (GS), CB, PB, BJ, and urine were analyzed in 20 autopsy cases of MRPs.

A Forensic Toxicant Screening Test (FTST) for medicine, pesticides, and cyanide in GS, CB, and urine was conducted by Gas Chromatograph/Mass Spectrometry (GC/MS) or liquid chromatograph/tandem mass spectrometry.

A Forensic Drug Screening Test (FDST) for MA, delta-9-carboxytetrahydrocannabinol, cocaine, and benzodiazepine in urine was conducted by immunoassay, and ethyl alcohol screening in PB by GC. MA and AM, if detected by the FDST, were subsequently confirmed and quantified by re-extraction and re-analysis in CB, PB, and BJ by GC/MS.

The postmortem MA and AM concentrations (mg/L) ranged from 0.42-204.10 (average 18.46) and 0.001-8.70 (average 0.68) in CB (n=15), 0.11-194.40 (average 13.29) and 0.001-7.30 (average 0.54) in PB (n=15), and 0.09-149.40 (average 27.46) and 0.001-4.20 (average 0.83) in BJ (n=9). The ratios of CB to PB (n=12) for MA and AM were 0.79-6.59 (average 1.92) and 0.21-6.67 (average 1.87), BJ to PB (n=8) were 0.77-10.50 (average 4.62) and 0.58-12.67 (average 5.36).

These data suggest that the postmortem MA and AM concentrations in CB are approximately two times (average 1.92 and 1.87) higher than those in PB, and four to five times (average 4.62 and 5.36) higher in BJ. The CB and BJ to PB ratios less than five are consistent with little to no propensity for postmortem redistributions; these data demonstrate that MA and AM are unlikely to exhibit significant redistributions.

According to these detection ratios, the concentrations of MA and AM in the blood could be estimated with those in BJ of the postmortem, which cannot take PB or CB.

Therefore, this study expects to play a major role in estimating the postmortem concentration of MA and AM in MA-related death cases. Additionally, it could assist in or predict the interpretation of MA intoxications and redistributions for the MRPs.

Methamphetamine, Postmortem, Concentration

K21 The Effects of Kambo: The First Case of Sudden Death in Forensic Literature

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After attending this presentation, attendees will be able to describe the effects of Kambo as a concurrent cause of death in users with heart disease.

This presentation will impact the forensic science community by explaining Kambo's biological effects and the need to control its sale.

Kambo is a substance obtained from the skin secretions of a frog, *Phyllomedusa bicolor*. The secretions are used during a purification ritual common in some regions of South America. After the skin is burnt, the secretions are applied, causing a type of poisoning in a process not yet fully understood. No fatalities associated have yet been reported. In the case reported, the biological effects of Kambo with the potential to cause death were analyzed.

A 42-year-old man was found dead in his house. The external examination showed recent burns on the left arm. A wooden stick, approximately 10cm long and burnt on one end, was found near the body. A plastic box labeled "*Kambo Sticks*" was also found. From his medical history, it emerged that the man was a chronic consumer of this substance. There was no history of drug use nor a family history of sudden premature death or ischemic heart disease.

A toxicology analysis was performed on biological fluids for alcohol, prescription medication, and illicit drugs. A toxicological study was also conducted on the Kambo sticks and on biological fluids (blood and vitreous humor). The analysis consisted of reverse phase High-Performance Liquid Chromatography (HPLC) and mass spectrometry with TripleTOF® 5600+ System. Internal analytical standards were used for the research of dermorphin, deltorphin A, phyllocaerulein, phyllokinin, sauvagine.

The heart exhibited left ventricular concentric hypertrophy. There were also apparent petechiae on the subpleural area and a subconjunctival hemorrhage. Histological examination revealed moderate coronary artery disease with a reduction of approximately 65% of the left coronary branch by the atheroma. The brain displayed intraparenchymal microhemorrhages. The lungs had subpleural petechiae and bullous emphysema, with fibrotic thickening of the septal interstitium and sporadic micro-granulomas. The myocardium exhibited fragmented myofibrils and areas with marked inter-myofibrillar connective tissue.

The toxicological screening was negative for cannabinoids, opiates, cocaine metabolites, benzodiazepines, and ethanol. The investigations performed on the sticks found in the house demonstrated the presence of deltorphin A, phyllocaerulein, and phyllokinin; however, regarding biological fluids, deltorphin A was isolated exclusively in blood.

Kambo is comprised of a peptide mix. The peptides contain opioids including dermorphin and deltorphins, vasoactive molecules including phyllocaerulein, phyllomedusin, phyllokinin, sauvagine, and antimicrobials including dermaseptins. The peptides affect the body both at a central and peripheral level. The effects of each peptide are not yet fully understood. In the case reported, the man died suddenly after the substance was applied. In this case, as noted by the clinical data reported by the family doctor, the young man didn't appear to have any diseases or symptoms, and didn't take any medicine for treatment or other drugs intravenously. From the testimony of his mother, death happened approximately 30 minutes after the application of the drug. The autopsy revealed a left ventricular hypertrophy. By analyzing the action of these peptides on the body, it is possible to assume that the chronic consumption of some of the cardioactive peptides could lead to the development of left ventricular hypertrophy. Coronary artery disease was also found, most likely associated with the man's lifestyle (smoker and overweight). Certainly, the more likely effect would be hypotension. Death might therefore result from hypoperfusion of the heart which, in this case, could well be exacerbated by the increased left ventricular mass and moderate coronary artery disease. In a heart exhibiting moderate coronary artery disease and left ventricular hypertrophy, this generated an acute cardiac ischemia and consequent ventricular fibrillation. The following pressure peak generated was demonstrated by diffuse cerebral micro-hemorrhages, subpleural petechiae, and subconjunctival hemorrhage. The positive results of the toxicological investigation made it possible to state that the substance could be one of the concurrent causes of death. It is also important to consider that this substance can easily be ordered and obtained from many websites without any controls and/or prescriptions.

Forensic Science, Kambo, Sudden Death

K22 An Evaluation of Alcohol Concentrations in Samples Referred to the Forensic Laboratory in Baghdad

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After attending this presentation, attendees will be aware of the minor impact of the problem of alcohol drinking in causing and contributing to the cause of death in all autopsy cases in Baghdad. The goal of this paper is to detect and measure the concentration of alcohol, tackle this issue, and reveal its scope.

This presentation will impact the forensic science community by revealing the size of the problem of alcohol intake in contributing to or causing death.

Alcohol is one of the world's leading risk factors for morbidity, mortality, and disability. In 2012, 5.9% of all global deaths were attributed to alcohol and 5.1% of all global diseases and injuries were attributed to its use as well. Its effect was more pronounced from neuropsychiatric disorders.[1] Annually, 88,000 people die from alcohol-related causes in the United States and it is considered the fourth-leading preventable cause of death. It is blamed for 31% of all driving fatalities.[2]

Alcohol is also related to many crimes. In 2013-2014, 53% of violent crimes were committed under the effect of alcohol, including assaults, wounds, sexual offenses, homicides, criminal damages, theft, and robbery.[3]

This study was a prospective study within the first six-month period of 2015 on postmortem blood samples referred to the main forensic toxicology laboratory in the medicolegal directorate in Baghdad for the detection and measurement of alcohol; 5ml to 10ml of blood was withdrawn for each sample and a Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID) from Agilent 7890A was used.[4]

A fifty-milligram percentage of alcohol was considered the cut-off point and every result above 50% was considered to be a positive sample. In general, traumatic death was predominant among all victims.[5] From the total 1,275 samples, only 112 (8.8%) were positive, with males more than five times more frequent than females. There was a significant relation to alcohol intake with traumatic causes of death, yet in only 13 victims were the concentrations fatal.

This study also revealed that traumatic causes of death decreased significantly with advancing age. Only 12 positive samples were attributed to natural causes of death. In those victims, alcohol probably factored with their diseases in precipitating death.

Alcohol drinking is a minor problem, as it was detected in a small group of all cases, yet its association with traumatic death was significantly higher than natural death and its consumption was more than five times higher in males. Only in a limited number of cases was the concentration fatal.

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Alcohol, Ethanol, Postmortem

K23 The Evaluation of Direct and Indirect Biomarkers of Ethanol Consumption: A Likelihood Ratio (LR) Approach to Identify Chronic Alcohol Misusers for Forensic Purposes

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After attending this presentation, attendees will understand how to interpret and evaluate the values of direct biomarkers of ethanol consumption to be detected in hair and keratin matrix for forensic purposes.

This presentation will impact the forensic science community by informing attendees that the LR models proved to be capable of significantly discriminating non-chronic from chronic alcohol consumers.

The determination of direct ethanol metabolites, such as Ethyl Glucuronide (EtG) and Fatty Acid Ethyl Esters (FAEEs), to be quantified in keratin matrix samples is indicated as the gold-standard approach to efficiently identify chronic alcohol drinkers.[1,2] Even if cut-off values have been established by the Society of Hair Testing (SoHT) to interpret EtG and FAEEs results, it has been documented that several confounding factors may alter the correlation between alcohol consumption and biomarkers' concentration in hair (e.g., cosmetic treatments).[3] As a consequence, the adoption of the traditional univariate interpretative approaches seems not to be the best option, as it may lead forensic experts and physicians to infer misleading conclusions, thus triggering the possibility of employing alternative multivariate data interpretation approaches.

Due to the fact that the LR models overcome the drawbacks of the traditional univariate approaches, as no cut-off values are involved during the process of evidence evaluation, several LR models have been developed and tested.[4] In the practice, LR values reveal the support to be delivered to the evaluated propositions and LR results can be expressed by means of verbal scales. An LR approach evaluates the Evidence (E) in case of two different, and mutually exclusive, hypotheses by examining the collected data. In the present case, the first hypothesis (H1) is that the individual under examination is a non-chronic alcohol consumer. Otherwise, the second hypothesis (H2) states that the examined subject is a chronic alcohol misuser. At the current stage, the collected data consists of direct (FAEEs, EtG) biomarkers of alcohol consumption from more than 150 scalp hair samples of different individuals, representing both chronic and non-chronic alcohol drinkers target categories. Different multivariate LR models have been evaluated and their ability to discriminate chronic alcohol misusers from non-chronic alcohol consumers were examined. The performance of each model was evaluated in terms of rates of correct classification (%) and empirical cross-entropy parameters. Since satisfactory reduction of information loss have been observed, together with correct classification rates close to 100%, LR validated models proved to be capable of discriminating non-chronic from chronic alcohol consumers. Similar results have been observed when employing further multivariate data analysis strategies such as Partial Least Squares Discriminant Analysis (PLSDA).[5]

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Chronic Alcohol Drinkers, Hair Samples, Likelihood Ratio

K24 2017 Novel Illicit Opioids: Trends and Toxicological Insights

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After attending this presentation, attendees will be able to describe the changes in novel illicit opioid positivity in forensic casework over a 6- to 12-month time period in 2017.

This presentation will impact the forensic science community by detailing trends in the use of novel illicit opioids in 2017, including change in positivity over time, user demographics, and quantitative data from casework, all of which underscore the need for comprehensive toxicological testing with a dynamic scope for novel opioids and vigilance by investigators, forensic scientists, and legislators.

Novel illicit opioids are a major component of the opioid epidemic that has become a major public health crisis. Increased misuse and diversion of pharmaceutical fentanyl in the early 2000s has given way to exponential growth since 2013 with the appearance of illicitly synthesized fentanyl and the introduction of additional novel illicit opioids, such as U-47700, furanyl fentanyl, and carfentanil, which have subsequently been identified in toxicological casework. Toxicological identification involved either Liquid Chromatography/Time Of Flight (LC/TOF) or fentanyl-based immunoassay screening and targeted confirmations using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) or a Gas Chromatography/Mass Spectrometry (GC/MS) database match.

Positivity data was obtained from toxicology casework (death investigation, impaired driving, and hospital admissions) in which the commonly encountered novel opioids furanyl fentanyl, U-47700, carfentanil, butyryl fentanyl, para-Fluoroisobutyryl Fentanyl (FIBF), and acryl fentanyl were detected in blood samples (Table 1). Percent positivity indicates the proportion, relative to the totals for these drugs encountered by month.

	January		**February**		**March**		**April**		**May**		**June**		**TOTAL**
	N	%Pos	N	%Pos	N	%Pos	N	%Pos	N	%Pos	N	%Pos	
Furanyl Fentanyl	122	41.4	178	49.3	164	39.0	114	39.6	120	33.7	90	28.1	**788**
U-47700	34	11.5	31	8.6	53	12.6	47	16.3	68	19.1	45	14.1	**278**
Carfentanil	79	26.8	51	14.1	31	7.4	46	16.0	51	14.3	73	22.8	**331**
Butyryl Fentanyl*	6	2.0	17	4.7	45	10.7	18	6.3	23	6.5	7	2.2	**116**
FIBF*	34	11.5	28	7.8	55	13.1	29	10.1	63	17.7	80	25.0	**289**
Acryl Fentanyl	20	6.8	56	15.5	72	17.1	34	11.8	31	8.7	25	7.8	**238**
TOTAL	**295**		**361**		**420**		**288**		**356**		**320**		**2040**

Table 1: *Change in positivity from January 2017 to June 2017.*

**%Pos = %Positivity for month specified. *Butyryl Fentanyl and FIBF are not differentiated from their isomers isobutyryl fentanyl and para-fluorobutyryl fentanyl, respectively.*

During the same time period, a total of 5,589 fentanyl cases were encountered, in addition to 2,458 cases containing the heroin metabolite 6-monoactylmorphine. Carfentanil, furanyl fentanyl, and U-47700 became popular in 2016 and maintained popularity in 2017. Positivity of furanyl fentanyl has been declining as positivity for acryl fentanyl and FIBF have been increasing from 6.8% to 7.8%, and 11.5% to 25%, respectively, in casework between January and June. Several other fentanyl variants are also being detected in toxicology casework to a lesser extent. 3-methyl fentanyl has continued to be seen (70+ detections total) in 2017, after re-appearing in 2016 following a roughly 30-year hiatus. There have also been sporadic detections of valeryl fentanyl (*n*=1), 4-methoxybutyryl fentanyl (*n*=1), and fluoro fentanyl analogs (*n*=20). An additional "U" series compound, U-49900, has been confirmed in two postmortem cases and both cases were also positive for Tetrahydrofuran Fentanyl (THF-F), a new fentanyl derivative first seen in 2017. Additionally, methoxyacetyl fentanyl (*n*=7) and cyclopropyl fentanyl (*n*=1) are being detected with increasing frequency as of July 2017.

These new substances challenge the forensic toxicology and chemistry communities because they pose all the same risks of routine opioids while going undetected. The data also illustrates the short cycle time of many of these drugs as they can come and go before a toxicology laboratory can develop and validate a method for their detection. In addition, the potency of these derivatives and correspondingly low concentrations in biological fluids challenge the capabilities of routine analytical methodology, forcing laboratories to seek new technologies to keep abreast of the trends.

Novel illicit opioids have been confirmed by the reporting laboratory in 42 states and Canada. Although certain states, especially the Northeast United States, have greater prevalence for certain compounds, they are not isolated to one part of the country. The forensic science community in general needs to be aware of the impact of novel illicit opioids.

Opioids, Novel Psychoactive Substances, Methoxyacetyl Fentanyl

K25 The Morphine in Your Pantry — Understanding the Overdose Risk of Home-Brewed Poppy Seed Tea

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After attending this presentation, attendees will understand the risks involved with consuming home-brewed poppy seed tea, including death. Attendees will learn that regardless of extraction conditions, it is possible to rinse opium alkaloids from poppy seed coats by home-brewing methods. In particular, attendees will learn that it is possible to rinse lethal amounts of morphine from unprocessed poppy seeds at home.

This presentation will impact the forensic science community by demonstrating the need for further consideration regarding the legality of purchasing unprocessed poppy seeds in bulk.

Morphine, codeine, and thebaine are naturally occurring opiates found in the latex of the opium poppy (*Papaver somniferum*). This latex is harvested worldwide for both licit and illicit opioid production. Poppy seeds are commonly harvested for baking, and opium alkaloids are transferred onto poppy seed coats during the harvesting process. Bulk, unprocessed poppy seeds can be purchased online with no current legal repercussions.

Recently, a medical examiner reported the case of a 24-year-old male who died of morphine intoxication with aspiration pneumonitis; however, neither licit nor illicit sources of morphine were found at the scene. It was suspected that the decedent ingested a lethal amount of morphine from home-brewed poppy seed tea. A 5-pound bag of poppy seeds and a 33-fluid-ounce bottle filled with seeds and water were found at the scene.

Due to the recent increase in poppy seed tea-related deaths, this research proposed that lethal amounts of morphine could be extracted from poppy seeds by home-brewing methods. Codeine concentrations were investigated because codeine is another opium alkaloid with analgesic properties, and codeine and morphine can have compounding effects when both drugs are taken simultaneously. Thebaine concentrations were investigated because, although it has no analgesic properties, its presence in biological specimens is indicative of poppy seed consumption.

No studies to date investigated opium alkaloid content that can be extracted from poppy seeds by home-brewing methods. For this reason, 22 samples of poppy seed products were purchased from online sources and extracted with four home-brewing methods representative of recipes found on drug user forums. Poppy teas were produced in room temperature and heated water, with and without lemon juice (acid) modifier over 10min with gentle agitation.

Extracts were analyzed in triplicate, and morphine, codeine, and thebaine were quantified in these extracts by liquid chromatography-tandem mass spectrometry using a validated analytical method. Precision and accuracy were 4.2%–7.7% and 92.3%–103.4%, respectively, with minimal matrix effects (95.1%–103.7%). Morphine, codeine, and thebaine concentrations were <1mg/kg–2,788mg/kg, <1mg/kg–247.6mg/kg, and <1mg/kg–124mg/kg, respectively. Many samples yielded morphine levels that would correlate to lethal concentrations if moderate volumes of tea were consumed. Yield varied between all extractions for all analytes in all samples.

Forensic Science, Forensic Toxicology, Poppy Seed Tea

K26 Carfentanil-Related Deaths in Wayne County, Michigan: Epidemiology and Toxicology

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After attending this presentation, attendees will be able to describe the toxicological and epidemiological aspects of a series of 129 carfentanil-related fatalities in Wayne County, which includes the city of Detroit, MI.

This presentation will impact the forensic science community by providing attendees with the carfentanil concentrations observed in medicolegal death cases related to the drug in addition to the demographic information associated with the decedents, which will assist with the interpretation of drug concentrations in future carfentanil deaths.

Introduction: Carfentanil, a synthetic opioid with an analgesic potency estimated to be 10,000 times that of morphine and that is approved for veterinary use in the sedation of large animals, was analytically confirmed in blood samples from 129 death investigation cases investigated by the Wayne County Medical Examiner's Office between August 2016 and June 2017. Details of the findings in these cases are presented and discussed.

Methods: All cases presented were submitted to NMS Labs in Willow Grove, PA, for comprehensive toxicological analysis by a Liquid Chromatography/Time Of Flight/Mass Spectrometry (LC/TOF/MS) screen for approximately 250 drugs and their metabolites, which includes carfentanil and several other emerging novel opioids in an additional secondary targeted accurate mass database. Confirmatory testing for carfentanil was performed by quantitative Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) with a quantitative reporting limit of 0.10ng/mL and limit of detection of 0.03ng/mL. Carfentanil-positive cases were reviewed for other drug findings, as well as demographic case data.

Results: Carfentanil concentrations ranged from 0.1ng/mL to 14ng/mL (*N*=127) with two cases <0.1ng/mL. The mean and median concentrations were 0.75ng/mL and 0.44ng/mL, respectively. It was found that 87% of cases had a carfentanil concentration less than or equal to 1.0ng/mL. There were only three cases in which carfentanil was the only drug detected. In 87.6% of cases, another opioid was detected, including morphine (57%), fentanyl (43%), 6-acetyl morphine (38%), furanyl fentanyl (28%), and U-47700 (12%). Other common findings were ethanol (50%), cocaine and/or benzoylecgonine (45%), cannabinoids (31%), and alprazolam (21%). Death locations were at home (37%), at a scene (32%), or in a hospital (31%). Naloxone was detected in 78% of hospital deaths compared to 17% at home and 7.3% at a scene. Decedent demographics were mostly male (73%) and White (63.5%). Black or African American (32.5%) and other (4%) were slightly underrepresented compared to overall Wayne County demographics (52% White, 31% Black or African American, 7% Other). Total cases peaked between November 2016 and February 2017, after which there was a sharp decline in deaths related to carfentanil. In one case, an antemortem whole blood specimen was collected at the hospital approximately 14 hours prior to death. The antemortem blood and postmortem femoral blood concentration was 0.93ng/mL and 0.34ng/mL, respectively. The half-life of carfentanil in female elands was reported to average 7.7 hours.[1] This single human case appeared to have a half-life of 8.1 hours. While there are certainly caveats to this extrapolation of kinetic data, it does suggest that a one-time dose of naloxone (half-life 64 +/- 12min) would be insufficient as an antagonist. To various degrees, all of the reported cases involved police, first responders, investigators, morgue assistants, and pathologists. There was not a single incident of any adverse effects to any individual in any case involving incidental exposure to carfentanil.

Conclusions: Carfentanil was detected as an opioid of abuse in 129 cases Wayne County, MI, between August 2016 and June 2017. Although it was detected with other opioids in more than 80% of cases, it contributed to the cause of death in all cases that were considered drug-related fatalities. The decrease in the incidence of carfentanil toward the end of the reporting period suggests the possibility of new novel opioids that may be contributing to drug-related deaths.

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Carfentanil, Opioids, Fatalities

K27 A Field Performance of the DrugTest 5000® and DDS®2 Onsite Oral Fluid (OF) Devices by Oregon and Vermont Drug Recognition Experts (DREs)

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After attending this presentation, attendees will be able to describe the field performance of the Draeger DrugTest 5000® (DT5000®) and Alere™ DDS®2 Onsite OF devices compared to Mass Spectrometry (MS) OF confirmatory tests and understand the usefulness of drug tests in Driving Under the Influence of Drugs (DUID) cases.

This presentation will impact the forensic science community by informing attendees regarding the use of onsite OF tests in identifying drug use.

Background: OF is easily collected and tested at the roadside to rapidly identify recent drug intake. Collection is non-invasive and gender neutral, with results available in minutes rather than hours for urine or invasive blood collection. During this delay, blood drug concentrations may decrease greatly, especially Δ9-Tetrahydrocannabinol (THC), hampering identification of recent drug consumption.

Methods: OF was collected with the DT5000® in Oregon ((OR), N=57) and Vermont ((VT), N=35), and the DDS®2 (N=23) in VT. Only one device was utilized per individual. Cutoff concentrations and performance for the combined OR and VT DT5000® data and the VT DDS®2 data are in the tables below. NMS Labs performed confirmation testing on OF collected with Immunalysis™ Quantisal® devices. All OR samples were collected in DUID cases, while 49 VT cases were from a court-ordered rapid intervention program and 9 from DUID cases.

Results:

DT5000 (OR & VT)

Drug, cutoff ng/mL	TP	FN	FP	TN	Sensitivity %	Specificity%	Efficiency %	PPV %	NPV %
THC, 5	47	1	0	44	97.9	100	98.9	100	97.8
Cocaine, 20	5	0	1	85	100	98.8	98.9	83.3	100
Amphetamine, 50	23	7	2	60	76.7	96.8	90.2	92	89.6
Methamphetamine, 35	34	0	2	56	100	96.6	97.8	94.4	100
Benzodiazepines, 15	2	0	0	90	100	100	100	100	100
Opiates, 20	31	1	3	56	96.9	94.9	95.6	91.2	98.2
Methadone, 20	3	0	0	89	100	100	100	100	100
Overall	145	9	7	480	94.2	98.6	96.9	95.4	98.2

DDS®2 (VT)

Drug, cutoff ng/mL	TP	FN	FP	TN	Sensitivity %	Specificity %	Efficiency %	PPV %	NPV %
THC, 25	3	2	0	15	60	100	90	100	88.2
Cocaine, 30	2	0	0	21	100	100	100	100	100
Amphetamine, 50	3	0	3	17	100	85	87	50	100
Methamphetamine, 50	0	0	0	23	n/a	100	100	n/a	100
Benzodiazepines, 20	0	0	0	23	n/a	100	100	n/a	100
Opiates, 40	3	1	1	18	75	94.7	91.3	75	94.7
Overall	11	3	4	117	78.6	96.7	94.8	73.3	97.5

Discussion: For the DT5000® device, sensitivity, specificity, and efficiency exceeded 94.9%, except for the amphetamine assay, which had 7 FN tests. This could have been due to the much lower 10ng/mL OF amphetamine confirmation test, and the 2 FP tests could have been due to cross-reactivity of the DT5000® antibodies with other sympathomimetic amines. For 641 OF samples and seven drug classes, the DT5000® had sensitivity, specificity, and efficiency of more than 94.2%, with high Positive Predictive Values (PPV) and Negative Predictive Values (NPV) of ≥95.4%. The DDS®2 device had 78.6% sensitivity, 96.7% specificity, and 94.8% efficiency, with a high NPV of 97.5% and a lower PPV of 73.3%. The poorer THC DT5000® results may be the result of a higher THC cutoff or that there were only five confirmed positive THC samples or that different individuals were tested. The amphetamine assay specificity was problematic with a PPV of only 50%. Although there were many cocaine tests for the DT5000® evaluation, there were too few positive cocaine, benzodiazepines, and methadone cases to draw conclusions about sensitivity, and for the DDS®2, there were only 11 positive cases in the entire set. For DUID cases, PPV is important because of the consequences on the driver from an FP test. There were only 1.1% FP tests for the DT5000® and 3.0% FP for the DDS®2. In drivers with negative field tests or when results are inconsistent with observed intoxication, supplemental tests should be ordered because the onsite OF devices test for a limited number of drug classes.

Conclusion: The devices achieved good specificity, with better sensitivity for the DT5000® as compared to the DDS®2 device. In addition, savings on cost of transport time, officer time, phlebotomist costs, and a reduction in the number of witnesses required for testimony may be substantial.

Oral Fluid, Onsite, DUID

K28 An Analysis of Ethanol in Blood and Oral Fluid Samples From Dosed Individuals by Headspace Gas Chromatography

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After attending this presentation, attendees will better understand the application of headspace gas chromatography to the determination of ethanol in blood and oral fluid. Attendees will also understand the relationship between ethanol concentrations in blood, oral fluid, and breath samples during a human dosing study.

This presentation will impact the forensic science community by providing evidence of a validated method for the analysis of both blood and oral fluid. The validated method was utilized in the analysis of samples from individuals dosed with ethanol in order to assess the viability of oral fluid as a matrix in Driving Under the Influence (DUI) applications. The use of headspace gas chromatography for this toxicological analysis allows for minimal sample preparation and little devaluation of the column with repeated usage.

Oral fluid has become a matrix of interest for forensic toxicological analysis, mainly for the qualitative and quantitative analysis of various drugs of abuse. Oral fluid is an ideal matrix for forensic analysis due to its lack of invasive collection procedure and ease of collection and monitoring, making the sample difficult to adulterate.[1-3] The findings of this study have the potential to impact current policy and forensic sample collection in suspected DUI cases. The use of oral fluid as a forensic specimen that is collected carside after probable cause of driving while impaired has been determined to have the potential to improve the quality and accuracy of the forensic toxicological analysis. The ability to collect oral fluid at the scene can potentially reduce the lag time between the traffic stop and obtaining the toxicological sample.

A method for the analysis of ethanol in blood and oral fluid was developed. In this study, a Perkin Elmer® HS-Clarus® 580 headspace gas chromatograph with two flame ionization detectors and a TurboMatrix™ 40 autosampler was utilized. A single headspace injection was split between two columns, Elite-BAC1 (30m x 0.32mm x 1.8µm) and Elite-BAC2 columns (30m x 0.32mm x 1.2µm). Helium carrier gas at a flow rate of 12.30mL/min was utilized, and the column temperature was set to 70°C. The method was validated using aqueous solutions, bovine blood, human blood, and human oral fluid with Lower Limit of Detection (LLOD) and Lower Limit of Quantification (LLOQ) values of 0.01% for ethanol. Calibration curves demonstrated good linearity for the BAC1 and BAC2 column where the r^2 values exceeded 0.999.

A controlled dosing study was performed utilizing subjects who consumed a pre-determined amount of wine (11.5%) to reach a target blood alcohol concentration of 0.05g/dL. Blood, breath, and oral fluid samples were collected from subjects prior to the consumption alcohol. Blood and breath samples were collected every 15 minutes over 3 hours; oral fluid samples were collected every 5 minutes for the first 30 minutes post-consumption and every 15 minutes following for 3 hours. Blood and oral fluid samples were prepared using 3mL of internal standard (0.016% *n*-propanol), 300µL of sample, and ¼ teaspoon of NaF/NaCl salt mix. Breath samples were measured with a portable breath-testing device. Results revealed the ethanol concentration profiles correlated well between blood and oral fluid. The Pearson correlation values between samples of oral fluid and blood were 0.92–0.97.

In conclusion, the validated method for the analysis of ethanol in blood and oral fluid samples illustrates the utility of oral fluid samples as a matrix in DUI investigations. The ease of collection of oral fluid and the fast and simple sample preparation for analysis makes this method viable for implementation in a forensic toxicology laboratory for analysis of DUI samples.

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Forensic Toxicology, Blood Alcohol, Oral Fluid

K29 Alcohol Extrapolations: Scientific, Legal, and Ethical Considerations

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After attending this presentation, attendees will better understand the various methods for performing alcohol extrapolation, issues with using only the Widmark formula, and the scientific and legal expectations when performing alcohol extrapolations.

This presentation will impact the forensic science community by discussing a more scientifically robust approach for performing alcohol extrapolations by accounting for individual differences in volume distribution and elimination rates, thus representing Blood Alcohol Concentration (BAC) extrapolations as a range of values rather than a single point estimate.

Forensic science laboratories are often faced with Driving Under the Influence of Drugs (DUID) and Drug-Facilitated Sexual Assault (DFSA) cases involving alcohol. Forensic toxicologists and the justice system are frequently faced with questions pertaining to the BAC, or level of intoxication, experienced by an individual at a particular time in which an incident occurred. Direct measurements of the blood are typically taken at a time after the incident occurred and forensic toxicologists are frequently tasked with the determination (estimation) of the actual BAC at the time the incident occurred using a combination of chemical analyses of the blood or breath and any of the various empirically derived extrapolation methods. Many experts attest to BAC extrapolations using the original Widmark factor for estimating volume distribution without consideration for successive improvements to the formulas by Watson, Forrest, Ulrich et al., and Seidl et al., as well as assumptions that all of the alcohol had been absorbed at the time of the incident and the individual exhibits an average rate of elimination.[1-5] These simplistic assumptions and use of a single coefficient by Widmark are likely due to perceptions of the complexity to employ the more complicated algorithms, which account for gender, age, weight, height, water content, and Body Mass Index (BMI) as well as typical variations in absorption and elimination rates.

While simplistic, the single-point BAC result derived by limiting the calculation to a single method does not reflect the entire range of possible values at the time of the incident. Limiting the calculation to an average of the physiological ranges without consideration of a bounded interval of possible BAC values does not address individual differences and, therefore, could present incomplete and potentially misleading information to a fact-finder when evaluating whether a specific individual's BAC was greater than a statutory level at a particular time prior to the direct measurements. A more scientifically robust approach to alcohol extrapolations by expressing the full range of possible BAC values not only does provide a more thorough representation of the BAC, it provides a standardized framework for evaluating results across different laboratories, and cases in which assumptions and input parameters may otherwise vary. In *State v. Read*, the courts ruled that evidence is inadmissible if unfairly prejudicial — if it has the capacity to skew the truth or prejudice the truth finding process itself.[6] In "The Admissibility of Novel Scientific Evidence: *Frye v. United States*, a Half Century Later," Giannelli states that "the major danger of scientific evidence is its potential to mislead a jury; an aura of scientific infallibility may shroud the evidence and thus mislead the jury to accept it without critical scrutiny."[7] In *State v. Fausto*, the court asserted that, "When a witness is sworn in, he or she most often swears to 'tell the truth, the whole truth, and nothing but the truth.' In other words, a witness may make a statement that is true, as far as it goes. Yet there is often more information known to the witness, which if provided, would tend to change the impact of the information already provided."[8]

A review of court rulings clearly demonstrates the expectation, albeit the requirement, for clear expert testimony that in no way misleads a jury. The ANSI-ASQ (American National Standards Institute-American Society of Quality) National Accreditation Board's (ANAB's) document, *The Guiding Principles of Professional Responsibility for Forensic Service Providers and Forensic Personnel,* also speaks to the use of clear communications and the presentation of expert testimony that is not misleading to the judge or jury.[9] This presentation will discuss the methods for performing alcohol extrapolation, issues with using only the Widmark formula, and scientific and legal expectations when performing alcohol extrapolations.

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Alcohol Extrapolations, Legal, Scientific Formula

K30 Quantification of Minor Blood Cannabinoids and Their Utility as Recent Cannabis Use Markers in Driving Under the Influence of Drugs (DUID) Investigation Cases

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After attending this presentation, attendees will be able to adopt and validate a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for quantitation of minor cannabinoids, including Cannabidiol (CBD), Cannabigerol (CBG), and Cannabinol (CBN). As CBG and CBN were reported as recent cannabis use markers, attendees will be able to apply the findings to improve the interpretation of recent cannabis use.

This presentation will impact the forensic science community by providing information on a quantitative method for minor cannabinoids as recent cannabis-use markers and their utility to aid in the interpretation of blood cannabinoid results in DUID cases.

It has been demonstrated that cannabinoid blood concentrations following smoking are not well correlated with effects because their concentrations are a function of various factors (dose, potency, route of administration, users' experience level, frequency of drug use, etc.), making interpretation of results challenging. Determination of the presence of minor cannabinoids in blood can add value to the interpretation by providing a time frame on last cannabis use.

This study describes a sensitive LC/MS/MS method for quantification of minor cannabinoids (CBD, CBG, and CBN). Cannabinoids were extracted from 100µL of whole blood using liquid-liquid extraction, separated in a two-dimensional LC system with an Agilent® Poroshell 120 PFP (4.6mm x 5mm; 2.7µm) as a guard column and an Agilent® Poroshell 120 Bonus RP (2.1mm x 50mm; 2.7µm) as an analytical column, in a 5min run time and detected by an AB SCIEX™ 6500 system with turbo ion spray operating in positive ion mode with scheduled mass spectrometric Multiple Reaction Monitoring (MRM). The method validation protocol was based on the Scientific Working Group for Forensic Toxicology (SWGTOX) guideline to include linearity, Limit Of Detection (LOD), Lower Limit Of Quantitation (LLOQ), precision and accuracy, interfering substances, extraction efficiency, matrix effect, stability, dilution of samples, matrix matching, and carryover.

The calibration for CBD, CBG, and CBN was linear from 0.1ng/mL to 50ng/mL. Minimum extraction efficiency and the maximum observed matrix effect were 97.4% and 2.8% suppression, respectively. The method also met validation criteria for precision and accuracy at the LLOQ, low and high controls, dilution of samples, matrix matching, interference, and carryover.

Between January and March 2017, NMS Labs received 2,787 cases for a basic DUID panel consisting of TEN common drugs of abuse. Cannabinoids were presumptively positive by Enzyme-Linked Immuno-Sorbent Assay (ELISA) in 52% (*n*=1,450) of cases and the presence of Δ^9-tetrahydrocannabinol (THC) was further confirmed in 1,202 cases (83%) by LC/MS/MS with a Reporting Limit (RL) 0.50ng/mL. Of those cases, 98 samples with positive THC at various concentrations were additionally tested for CBG, CBN and CBD using the method described. Table 1 summarizes these findings.

	CBG (ng/mL)	CBN (ng/mL)	CBD (ng/mL)
Mean (±SD) (ng/mL)	0.44 (±0.51)	0.28 (±0.15)	0.25 (±0.33)
Median (ng/mL)	0.26	0.23	0.12
Range (ng/mL)	0.10 – 3.3	0.10 – 0.74	0.10 – 1.1
% positive	74 (*n*=72)	67 (*n*=66)	10 (*n*=10)

Table 1. *Blood concentrations and positivity rates for CBG, CBN, and CBD*

To assess the correlation between concentrations of THC and three minor cannabinoids, the correlation coefficient (*r*) values were calculated and analyzed. The analysis demonstrated that both CBG and CBN have *r* values greater than Critical Values when *p*=0.05, suggesting a statistically significant positive correlation between THC and CBG as well as THC and CBN. The results from the same analysis between THC and CBD and CBG and CBN showed no correlation.

CBD had a significantly lower positivity rate compared to CBG and CBN. This, partly because of its short detection window and the various concentrations depending on the growth environment and strains, excluded CBD as a reliable recent cannabis use marker.

Using the incident and blood collection times provided in 42 cases with positive CBG and/or CBN at a RL of 0.1ng/mL, the calculated time difference (Δt) ranged from 0.033 to 5.4 hours. Of those cases, 11-hydroxy-THC (11-OH THC) was positive above RL of 1.0ng/mL in 39 cases (93%) with the mean and median concentrations of 5.1ng/mL and 3.9ng/mL, respectively (range: 1.4-22).

The detection windows were also evaluated for CBG and CBN at the previously studied RLs. Of 72 CBG-positive cases, a majority (>90%, *n*=65) were in between 0.1ng/mL and 1.0ng/mL; CBG was above 1.0ng/mL in seven cases with an average Δt of 1.1 hours (*n*=3; time information provided). Smoking higher doses of cannabis may explain the longer detection window for CBG in this study compared to the previously reported 0.5hr. Of 66 CBN-positive cases, six cases had CBN above 0.5ng/mL (RL 0.1ng/mL) with an average Δt of 0.78 hours (range; 0.68–1.13 hours, *n*=5; time information provided). This was consistent with the previous finding.

Recent Cannabis Use Markers, CBG, CBN

K31 A Study of an Active-State CB1 Receptor Model and JWH Synthetic Cannabinoids

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After attending this presentation, attendees will better understand the interactions between synthetic cannabinoids from the JWH family with an active-state CB1 receptor model.

This presentation will impact the forensic science community by contributing to the understanding of essential interactions between specific substituents of JWH synthetic cannabinoids with specific CB1 receptor residues through molecular modeling. The knowledge of these key interactions can help forensic chemists predict the structure of new and undiscovered families of synthetic cannabinoids.

Synthetic cannabinoids have emerged onto the drug scene as an alternative to illegal marijuana.[1] Like delta-9-tetrahydrocannabinol (THC), the main psychoactive ingredient in marijuana, synthetic cannabinoids interact with G-coupled protein receptors found in the brain, immune system, and peripheral organs.[2] There have been two cannabinoid receptors identified: CB1 and CB2. The binding of THC and synthetic cannabinoids to the CB1 receptors that are prevalent in the brain, activating the receptors, is believed to be the cause of the drugs' psychoactive effects. In the 1990s, John W. Huffman et al. developed a large series of synthetic cannabinoids. (These compounds were all given the name JWH-XXX, after Huffman.) Many JWH compounds have been found to have similar effects as THC, functioning as CB1 receptor agonists.[3] These JWH compounds are seen in many synthetic cannabinoid or "Spice" drugs and have become an important area of research in the forensic science community.

In this study, an active-state CB1 receptor model, prepared by the Doerksen lab, was used to compare the ligand-receptor interactions between the CB1 receptor, the JWH synthetic cannabinoid family, and the THC compound. This study was conducted using Schrödinger's Maestro molecular modeling software. Synthetic cannabinoids from the JWH family were selected based on their affinity to bind to the CB1 receptor. The docking of the ligands to the receptor took place after both the synthetic cannabinoid ligands and CB1 receptor model were prepared for docking and a grid of the active site was generated. In order to increase understanding of the interactions between cannabinoids and the CB1 receptor, parameters can be set to provide the five best possible poses, or positions, for the ligands. Once the ligands were docked to the CB1 receptor model, the interactions were thoroughly analyzed. The information collected from this study includes: (1) the amino acid residue interactions with the ligands and the bond distances of these interactions; (2) the docking score of each ligand and each pose; and, (3) estimated binding affinities. This study revealed: the specifics of the interactions, such as the presence of π-π stacking; which interacting residues are hydrophobic, charged, or polar; and whether solvent exposure was important for parts of the molecules.

Results from this study reveal which residue interactions with the CB1 receptor are important for the JWH compounds and how these interactions vary between compounds within this family. Identifying the key interactions between the synthetic cannabinoids and the CB1 receptor is a step toward a better understanding of the effects of these drugs, including toxicity and potential for abuse. The long-term goal is to develop a database and computer program to help predict new structures and different classes of synthetic cannabinoids that have not previously been identified. Future research will include studying all classes of synthetic cannabinoids and other synthetic drugs in addition to the metabolites of these substances.

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Synthetic Cannabinoid, CB1 Receptor, Molecular Modeling

K32 The Evaluation and Preservation of Urine Specimens in Forensic Toxicology

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After attending this presentation, attendees will better understand how the pH of urine changes over time, the effect temperature has on pH change, the need for a preservative in urine samples, and the recommended minimum concentration of preservative required to stabilize urine pH. This research will directly benefit stability studies conducted on urine samples, as well as benefiting forensic and clinical laboratories analyzing for illicit substances in urine.

This presentation will impact the forensic science community by providing urine pH stability for 200 days as well as urine pH stability at room temperature from a multitude of participants to illustrate how variable the matrix is. Attendees will also understand the effects of added buffers, as well as preservatives, in varying concentrations in order to maintain the pH of a urine sample. The results of this study will allow toxicologists to better understand why some of their extractions have failed and also allows urine samples to be stored for longer periods of time to decrease the number of samples canceled for matrix stability problems. These results were evaluated for clinical and postmortem situations so a standardized method could be applied to both fields.

Urine is a commonly encountered matrix when screening for illicit substances in Driving Under the Influence of Drugs (DUID) cases within forensic laboratories and for clinical testing within hospital laboratories. Once a sample is received, after a period of storage, it generally undergoes an extraction procedure to remove any interferences from the matrix prior to instrumental analysis. Solid phase extraction techniques require pretreatment of samples to achieve an appropriate pH so the analyte of interest is in the appropriate form. This is generally achieved by use of a buffer. If the sample is not in the proper form, poor or no recovery may be a result.

During method development for a range of cathinones, it was determined that the pH of urine was changing over time and affecting these processes. It was determined that as the time a sample remained at room temperature increased, so did the urinary pH. This was hypothesized to be due to the breakdown of urea and creatinine into ammoniated compounds. This hypothesis was tested using Nessler's reagent and the Jaffee test. Nessler's reagent was used to measure the amount of urea present in a sample by scanning all wavelengths and reporting the absorbance at a specific wavelength. The Jaffe test was conducted in the same manner, but this test measured the amount of creatinine present in a sample. Appropriate calibration curves were made and urine samples were monitored over time to determine how the levels of these two compounds changed.

The addition of an appropriate preservative or buffer that can be added to urine to stabilize the pH of the matrix was investigated to help decrease the number of failed extractions and increase the stability of drugs present in urine over time. Sodium fluoride at 0.2% and 2.5% weight by volume (w/v) were added to urine samples and the pH was monitored to see if this was appropriate. Buffers of varying molarity were also evaluated. Samples were monitored in duplicate when stored in both the refrigerator and at room temperature for a period of 200 days. It was found that sodium fluoride at 0.2% w/v helped to maintain the pH with the necessary pH range required for successful extraction for a period of 90 days. Urine samples containing sodium fluoride at 2.5% w/v were found to be significantly less stable than that of the 0.2% w/v, but more stable than that of unpreserved urine. Without the addition of the preservative, urine pH is only stable for approximately two weeks. With the implementation of this research into case work, laboratories have the potential to extend the viability of the matrix and decrease the number of specimens canceled due to matrix instability. This research also provides the potential for detecting illicit substances for longer periods of time because pH is not adding to the degradation.

Urine, pH, Preservative

K33 Novel Stimulants N-Ethyl Pentylone and Dibutylone: Case Reports, Quantitative Confirmation, and Metabolic Profile Determination

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After attending this presentation, attendees will be able to evaluate N-ethyl pentylone and dibutylone concentrations in postmortem and Driving Under the Influence of Drugs (DUID) cases. In addition, attendees will be able to describe the metabolic biotransformation of N-ethyl pentylone and dibutylone and identify the metabolites in toxicological casework.

This presentation will impact the forensic science community by characterizing biomarkers of two emerging stimulants and providing analytical data for use in qualitative and quantitative interpretation.

Novel stimulants, like other Novel Psychoactive Substances (NPS), have been subject to various chemical modifications resulting in the appearance of a rapid succession of novel substances. Information related to the metabolism of these substances is often limited due to the lack of *in vitro* and/or *in vivo* studies, or unreported identification in authentic human specimens. In addition, uncharacterized chromatographic retention times, lack of identified target ions, and undetermined recreational or toxic concentration ranges create challenges for analytical detection and toxicological interpretation.

N-ethyl pentylone and dibutylone have been identified as emerging stimulants in impaired driving and death investigation casework, as well as in recreational drug users. The metabolic pathways of neither have been previously characterized.

Separate *in vitro* incubations of N-ethyl pentylone and dibutylone were performed with pooled human liver microsomes in duplicate over three days. Biotransformations identified for N-ethyl pentylone included demethylenation, ketone reduction, and hydroxylation. Biotransformations identified for dibutylone included demethylenation, ketone reduction, hydroxylation, and N-demethylation, forming butylone. After characterization of *in vitro* metabolic pathways, *in vivo* verification of these metabolites was accomplished using authentic specimens from toxicological casework or drug user studies.

Blood specimens (*n*=20) were quantitatively analyzed for N-ethyl pentylone by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) from postmortem cases (*n*=12), DUID cases (*n*=5), and cases with unspecified history (*n*=3). The mean (± Standard Deviation (SD)), median, and range for N-ethyl pentylone concentrations are shown in Table 1. One postmortem blood specimen was positive for pentylone (200ng/mL), a species not identified as a metabolite during microsomal incubations. In total, five metabolites of N-ethyl pentylone were confirmed in these specimens.

	Postmortem Cases (*n*=12)	**DUID Cases (*n*=5)**	**Unspecified Cases (*n*=3)**	**Overall (*n*=20)**
Mean	247 (±259)	41 (±26)	813 (±618)	280 (±374)
Median	155	34	1,140	95
Range	12-833	21-87	100-1,200	12-1,200

Table 1: *N-ethyl pentylone concentrations (ng/mL).*

Blood (*n*=4), urine (*n*=3), and vitreous (*n*=1) specimens were quantitatively analyzed for dibutylone and butylone by LC/MS/MS. All specimens were analyzed from postmortem cases (*n*=4), with overlap in specimens collected from the same individual. Specific case concentrations are shown in Table 2. In total, five metabolites of dibutylone were confirmed in these specimens.

	Blood (*n*=4)		**Urine (*n*=3)**		**Vitreous (*n*=1)**	
	Dibutylone	**Butylone**	**Dibutylone**	**Butylone**	**Dibutylone**	Butylone
Case 1	383	130	3100	69	250	108
Case 2	<10	385	16500	3060	-	-
Case 3	61	<10	2140	149	-	-
Case 4	1400	600	-	-	-	-

Table 2*: Dibutylone and butylone concentrations (ng/mL).*

N-ethyl pentylone and dibutylone were detected in combination in blood specimens (*n*=5) from death investigation cases. In four cases, mean (±SD), median, and range for N-ethyl pentylone concentrations were 479 (±316), 545, and 38-790ng/mL, respectively, and for dibutylone were 18 (±14), 12, and 10-40ng/mL, respectively. One additional blood specimen was positive for N-ethyl pentylone at 50,000ng/mL and dibutylone at 14ng/mL. Butylone was quantitatively confirmed in only two cases, above the analytical threshold.

Additional NPS identified in these cases included methylone, dimethylone, ethylone, 4-fluoroamphetamine, 4-chloro-alpha-PVP, acryl fentanyl, tetrahydrofuranyl fentanyl, carfentanil, para-fluoroisobutyryl fentanyl, U-47700, and U-49900. Causes of death included drug overdose, homicide, suicide, and vehicular crash. Reports of suspected "Molly" and "bath salt" use were noted in two cases. Specimens originated from Pennsylvania, New Jersey, New York, Florida, Texas, Utah, Vermont, Illinois, Missouri, and the District of Columbia. The majority of individuals were male (86%).

A comprehensive analytical approach is necessary to confirm novel stimulants and NPS in biological specimens, as drugs are often found in combination. Specimen concentrations for novel stimulants can vary, as high as µg/mL; therefore, appropriate dynamic range, detection limits, and dilution capabilities should be assessed. Rapid identification of biomarkers can be useful in the determination of unique and/or common metabolites between related substances, possibly providing additional information about ingestion and prolonging detection windows.

N-Ethyl Pentylone, Dibutylone, Postmortem

K34 Pharmacokinetic and Pharmacodynamic Differences Between Paramethoxymethamphetamine (PMMA), Paramethoxyamphetamine (PMA), 3,4-Methylenedioxymethamphetamine (MDMA), and Amphetamine in a Mouse Model

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After attending this presentation, attendees will be able to describe the pharmacokinetics of PMMA and related drugs. Attendees will also recognize the differences in behavior the drugs induce in a mouse model.

This presentation will impact the forensic science community by adding pharmacokinetic and pharmacodynamics data for PMMA and related drugs in a mouse model.

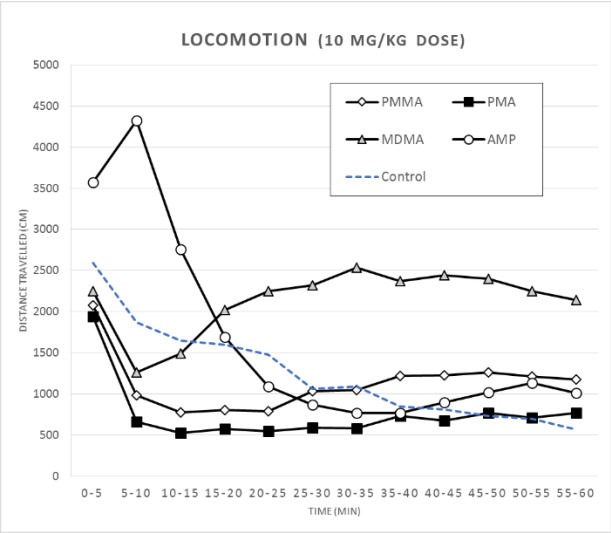
Background: Controlled study data regarding the pharmacology of PMMA and PMA in humans are lacking, but some data is available for the rat, pointing toward MDMA-like effects. Also, studies have suggested there is a delay in brain uptake that may trigger the user to take another dose because of absence of effect.

Goals: The goals of this study were to investigate the pharmacokinetics of PMMA and PMA in a mouse model and to compare their pharmacodynamics with MDMA and amphetamine.

Methods: The experiments were approved by the Animal Ethics Committee in Linköping, Sweden. Male C57/BL6 mice, 8-12 weeks old, weighing 25±1g were used for the experiments. The behavioral experiments were performed in an open field model. A video camera recorded the movements of the mouse during 60min and the movements were analyzed using EthoVision XT 9. Total distance travelled and time spent in the central zone were measured. In the behavior experiments, the animals (*N*=10) were dosed via intraperitoneal injection (i.p.) with either 0, 1, 5, or 10mg/kg of each substance immediately prior to the open field session.

Two pharmacokinetic experiments were conducted. First, dose concentration relationships were investigated using the same doses as in the behavior experiments with animals (*N*=5) sacrificed at 60 minutes. In addition, blood and brain kinetics were investigated for PMA and PMMA at 5mg/kg and 10mg/kg, respectively. The higher PMMA dose was chosen to increase the possibility of also measuring PMA formed from PMMA. Samples were obtained at 5, 10, 20, 40, 60, 80, and 120 minutes after injection.

Blood and brain concentrations of the substances were determined by Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) using an AB Sciex™ 4500 coupled to a Shimadzu® LC-30AD liquid chromatograph. The column used was an Acquity® UPLC® BEH Phenyl (2.1mm x 50mm, 1.7µm). In brief, 100µL whole blood was fortified with internal standard, precipitated, then further diluted 10 times before analysis. The whole brain was weighed and homogenized in 0,075% HFo in acetonitril/ethanol (90:10), an aliquot fortified with internal standard and then diluted 20 times.



Results: There was a good positive correlation between dose and both blood and brain concentrations for all four substances with Pearson's *r* between 0.90 and 0.99.

The kinetics of PMA and PMMA were slightly different. PMMA distributed equally fast to blood and brain whereas PMA demonstrated a delay in maximum brain concentrations. Also, the disappearance of PMA from the brain was slower than for PMMA. The brain concentrations correlated well with the effects from the behavior experiments, with a longer duration of locomotor suppression for PMA. As can be seen in the figure, both PMA and PMMA resembled MDMA in their temporal pattern but with less pronounced effect, whereas amphetamine exhibited quite the opposite. The time spent in the center zone is a measure of anxiety. The only significant result was PMA at the 10mg/kg dose, which acted anxiolytic with more time spent in the center zone.

Conclusion: The findings suggest that the behavior effects are correlated to brain concentrations of PMMA and PMA and that the effects resembled those of MDMA, rather than amphetamine.

PMMA, Open Field, Pharmacology

K35 The Development of a High-Resolution Mass Spectrometry (HRMS) Library and Method Validation for Screening and Confirmation of 800+ Novel Psychoactive Substances (NPS) by Liquid Chromatography/Quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS)

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After attending this presentation, attendees will better understand the development of a compound database and spectral library containing more than 800 NPS, metabolites, and related compounds from a wide variety of drug classes with a particular focus on synthetic cannabinoids and stimulants. In addition, this presentation will demonstrate the use of standard mixtures rather than individual compounds to validate a comprehensive screening/confirmation method for the detection and identification of these NPS in biological fluids using LC/qTOF/MS.

This presentation will impact the forensic science community by presenting a developed HRMS library that can be used to help identify NPS.

NPS are of great interest to forensic toxicology labs due to their potentially high potency and ability to evade detection by many current screening methods. These compounds can also be rapidly developed to avoid current scheduling laws, causing a need to develop comprehensive detection methods covering a wide variety of drug classes.

An Agilent® 1290 Infinity® HPLC system and Agilent® 6530 QTOF-MS with Jet Stream technology Electrospray Ionization (ESI) source was used for this research. A total of 826 compounds to be included in the final method were analyzed using Flow Injection Analysis (FIA) to create an HRMS library with spectral data collected at three collision energies (10eV, 20eV, and 40eV) for each compound. Once the spectral data were collected, all compounds were run through an Agilent® ZORBAX® Rapid Resolution HD Eclipse® Plus C18 column (3.0mm x 100mm; 1.8µm particle size) to obtain retention times. LC was performed with a gradient of 95% A (5mM ammonium formate in HPLC water with 0.1% formic acid) and 5% B (methanol with 0.1% formic acid) from 0min–1min, increasing to 90% B over 1min–9.5min, then held at 90% B for the remainder of the 20min run. All retention times were used to create the final method for validation. The collected HRMS data and retention times were curated into a database/library using the MassHunter™ Personal Computer Database Library (PCDL) Manager software. Each compound entry also contained the following information: compound name, chemical formula, monoisotopic mass, chemical structure, and International Union of Pure and Applied Chemistry (IUPAC) name. Chemspider and Chemical Abstracts Service (CAS) numbers were also included, when available. The developed HRMS library is used to help identify NPS in real-time analysis, as well as to retrospectively search previously collected data.

In order to fully validate the method, calibration curves were created for each drug standard. Completing individual calibration curves for each of the 826 NPS included would be extremely time consuming and inefficient; therefore, an approach using a series of standard calibration mixes was investigated. Validation of the proposed method for 826 compounds involved the creation of 25 mixes containing between 29–37 different compounds each. The compounds selected for each mix were selected so that no compounds had the same retention time and had a minimum of 0.2min between compound peaks in the mix. Seven different calibration levels were chosen for method validation: 1, 2, 5, 10, 20, 50, and 100ng/mL. All calibrators also included an internal standard "supermix" made of 22 deuterated standards representing multiple NPS drug classes. Calibrations were performed with both methanol-based and spiked matrix (urine) mixtures for method optimization. For calibrations completed in urine, a simple "dilute and shoot" approach was used in which a 1:5 dilution was directly injected into the instrument.

To date, individual calibration curves have been created for nine different NPS mixtures representing nearly 300 of the 826 proposed NPS for the final validated method. LC chromatograms were analyzed using MassHunter™ QTOF Quantitation software. This approach was capable of identifying all compounds in each mixture. The results of these experiments clearly demonstrate the value of using standard mixes for method validation in comprehensive toxicological analysis in conjunction with an HRMS library. Work is continuing to create calibration curves for the remaining compounds using mixtures containing a maximum number of compounds to limit the number of mixtures needed for full validation.

LC/qTOF/MS, Novel Psychoactive Substances, Method Validation

K36 6-Monoacetylmorphine (6-MAM) Positivity: A Comparison of Two Methods

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After attending this presentation, attendees will better understand the impact of methodology and reporting limits on the ability to confirm heroin use through the detection of 6-MAM in forensic cases.

This presentation will impact the forensic science community by comparing the ability of two different methods to confirm heroin use through the evaluation of 6-MAM positivity.

With opiate use and abuse increasing, laboratories are under increased pressure to distinguish licit opiate use from illicit use; however, confirming the presence of heroin (6-diacetylmorphine) can be challenging due to the pharmacokinetics of the drug. Heroin is rapidly metabolized to 6-MAM and eventually to morphine. Confirming 6-MAM is essential to determining the presence of heroin in body fluids. Laboratory methodology and reporting limits can vary greatly in their ability to confirm and quantitate 6-MAM. This work compares the ability of a Gas Chromatography/Mass Spectrometry (GC/MS) method with a reporting limit of 10ng/mL and a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method with a reporting limit of 1ng/mL to confirm and quantitate 6-MAM.

Method: Antemortem Driving Under the Influence of Drugs (DUID) and postmortem blood specimens submitted for opiate confirmation by GC/MS from April 2013 through April 2014 were reviewed. These results were compared to blood specimens submitted for opiate confirmation by LC/MS/MS from April 2014 through April 2015. Submissions in which 6-MAM confirmation was not performed for various reasons and cases that were none-detected for morphine were excluded. Percentages were rounded to the nearest whole number using conventional rounding rules. Statistical analysis was performed using student *t*-tests assuming unequal variance and that the populations were independent.

Results: A total of 14,932 cases that were submitted for opiate confirmation between April 2013 and April 2015 were included in this review. The overall 6-MAM positivity based on method increased from 15% by GC/MS to 32% by LC/MS/MS, an increase of 18% ($p < 0.05$). Submissions were also evaluated based on submission type: DUID (n=3,072) or death investigation (n=11,859). 6-MAM values ranged from 10ng/mL to 160ng/mL (mean 31ng/mL) by GC/MS confirmation and from 1ng/mL to 6,000ng/mL (mean 27ng/mL) by LC/MS/MS confirmation in DUID cases. 6-MAM positivity increased from 4% by GC/MS to 16% by LC/MS/MS, an increase of 12% ($p < 0.05$). Further investigation revealed that 87% of the DUID 6-MAM confirmations on LC/MS/MS were below 10ng/mL during this time period. Death investigation 6-MAM values ranged from 10ng/mL to 26,000ng/mL (mean 65ng/mL) on GC/MS and from 1ng/mL to 830ng/mL (mean 17ng/mL) on LC/MS/MS. 6-MAM positivity increased from 16% by GC/MS to 38% by LC/MS/MS, an increase of 22% ($p < 0.05$). In addition, 64% of the 6-MAM confirmations on the LC/MS/MS were below 10ng/mL in death investigations.

Conclusion: A comparison of these two methods demonstrated that a GC/MS method with a reporting limit of 10ng/mL could fail to confirm a large majority of heroin use in DUID and death investigation cases. In this particular review, a 6-MAM positivity rate increase of 18% was observed between the two methods.

Heroin, LC/MS/MS, GC/MS

K37 The Development and Validation of a Method for the Analysis of Novel Emerging Opioids

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After attending this presentation, attendees will be able to describe a Scientific Working Group for Forensic Toxicology (SWGTOX) - compliant approach to method validation for the analysis of designer opioid compounds in a variety of forensic samples using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) technology.

This presentation will impact the forensic science community by demonstrating the capability of an analytical method that can be used to simultaneously analyze 17 novel emerging opioid compounds, many of which have only recently appeared on the market.

In recent years, an increasing number of novel opioids have appeared on the illicit drug market and have been linked to the growing opioid crisis in the United States. In 2015, butyryl fentanyl was ranked in 12th place of the most frequently identified narcotic analgesics category in the National Forensic Laboratory Information System (NFLIS). By mid-year 2016, butyryl fentanyl was replaced by furanyl fentanyl, U-47700, and 3-methylfentanyl, which were ranked 12th, 13th , and 14th, respectively.

A method is described for the analysis of 19 of the most current novel opioid drugs in whole blood and serum, and 17 analytes in urine using LC/MS/MS. Blood and serum were analyzed quantitatively for butyryl fentanyl/isobutyryl fentanyl, MT-45, AH-7921, furanyl fentanyl, para-fluorofentanyl, ortho-fluorofentanyl, para-fluorobutyryl fentanyl/FIBF, 4-methoxybutyryl fentanyl, 4-ANPP, alpha-methyl fentanyl, 4-methylphenethyl acetyl fentanyl, U-47700, U-50488, acryl fentanyl, valeryl fentanyl, carfentanil, and beta-hydroxythiofentanyl. Urine samples were analyzed qualitatively for butyryl fentanyl/isobutyryl fentanyl, MT-45, AH-7921, furanyl fentanyl, para-fluorofentanyl, ortho-fluorofentanyl, para-fluorobutyryl fentanyl/FIBF, 4-methoxybutyryl fentanyl, 4-ANPP, alpha-methyl fentanyl, 4-methylphenethyl acetyl fentanyl, acryl fentanyl, valeryl fentanyl, carfentanil, and beta-hydroxythiofentanyl. The isomer pairs butyryl fentanyl/isobutyryl fentanyl and para-fluorobutyryl fentanyl/FIBF are not chromatographically separated in this method and are reported as a pair.

The method was validated according to a SWGTOX-compliant procedure, which for the quantitative portion evaluated precision and accuracy, limit of detection, lower limit of quantitation, linearity, stability in matrix and on-instrument, robustness, an evaluation of interfering compounds, matrix matching, dilution integrity, carry-over, matrix effect, and extraction efficiency. The validation for the qualitative portion of the method evaluated precision around the decision concentration (cut-off) stability in matrix and on-instrument, sensitivity and specificity, robustness, evaluation of interfering compounds, matrix effect, and extraction efficiency.

Sample preparation consisted of protein precipitation followed by solid phase extraction using Agilent® Plexa™ PCX 3mL/60mg extraction columns. The analytical method consisted of separation using a ZORBAX® RX-SIL (3mm x 100mm, 1.8 micron) column coupled with an Optimize EXP filter (0.2 micron) and a gradient elution utilizing ammonium formate, pH 4.0 (Mobile Phase A), acetonitrile (CH3CN), LC/MS grade (Mobile Phase B and weak wash), and formic acid in deionized water, 0.1% (strong wash). The analysis was performed on a Waters® ACQUITY® TQD MS/MS with a Waters® ACQUITY® Ultra Performance LC system.

This method produced data that met the acceptance criteria established for the validation. The quantitative portion of the analysis produced controls within 25% of target value, while the qualitative portion produced 94.1% sensitivity and 100% specificity during the validation. During the validation, it was determined that all analytes were stable in blood at room temperature for at least two weeks, and at refrigerated and frozen conditions for 30 days, with the exception of acryl fentanyl, which was only stable for one day at room temperature and one week refrigerated. In serum, it was determined that all analytes were stable at room temperature for at least two weeks, and 30 days refrigerated and frozen, except acryl fentanyl, which was stable for two days at room temperature, and MT-45, which was stable for one week at room temperature. In urine, it was determined that all analytes were stable for a minimum of one week at room temperature, and 30 days refrigerated and frozen.

Designer Opioids, Forensic Toxicology, LC/MS

K38 A Semi-Quantitative Retrospective Method Validation for Three Synthetic Cannabinoids With Analytical Confirmation in Toxicology Casework

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After attending this presentation, attendees will be able to discuss the importance of implementing analytical testing methods that allow for quick updates for continuously evolving synthetic cannabinoids and will be able to understand the benefits of performing a retrospective method validation to determine the concentrations of these analytes in biological fluids.

This presentation will impact the forensic science community by describing a retrospective method validation protocol used to determine semi-quantitative results of the synthetic cannabinoids 5F-AMB, 5F-ADB, and FUB-AMB, the most commonly seen analytes in toxicology casework from May 2016 through July 2017.

Since 2009, synthetic cannabinoids have presented a challenge to toxicology laboratories. As new compounds become available within the recreational drug market, labs are required to update their analytical methods to stay relevant. The development and validation of quantitative methods can be a long and demanding process, especially when there is a lack of deuterated internal standard for every analyte in the panel. Development of a qualitative confirmation method allows for faster incorporation of new compounds. Since these newer drugs are not part of many laboratories' routine testing procedures, there is limited information available on their expected levels in casework, making interpretation difficult; however, observed concentrations in biological fluids can help provide insight into the toxicity of these compounds. Therefore, a retrospective method validation of a qualitative method was performed for three compounds with a high positivity rate to acquire semi-quantitative data.

The qualitative assay was developed to detect 5F-AMB, 5F-ADB, and FUB-AMB, in addition to 24 related synthetic cannabinoids. During method development, it was noted that running a calibration curve improved the precision around the cut-off concentration; however, the quantitative controls for several analytes were not meeting the stringent requirements required by quantitative validations. Therefore, the method was validated qualitatively according to laboratory Standard Operating Procedure (SOP), including the evaluation of the cut-off concentration, sensitivity/specificity, carryover, matrix effect, interfering substances, and stability. Whole-blood samples were extracted using a liquid-liquid extraction, and analytes were detected using positive mode Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Separation was achieved on a BEH C18, 2.1mm x 100mm column with mobile phases consisting of water containing 0.1% formic acid and an acetonitrile and methanol mixture (80:20).

A retrospective method validation was performed based on laboratory SOP and Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. The Limits Of Quantitation (LOQ) were 25pg/mL for 5F-AMB and FUB-AMB and 50pg/mL for 5F-ADB. Linearity was established across two ranges (25-200pg/mL, and 50-400pg/mL) using five calibration points (n=5) with correlation coefficients ≥0.990 for all analytes and all back-calculated calibrators within 13% of target. The three synthetic cannabinoids were measured at three different concentrations for 15 separate days to provide acceptable between-run precision (13.5% Coefficient of Variation (CV)) and accuracy (±10.6%).

Based on the results of the retrospective method validation, all data that was still available on the laboratory instruments was reprocessed to determine the concentrations of 5F-AMB, 5F-ADB, and FUB-AMB. Data was included for all runs that met the following criteria: calibration curve correlation coefficients >0.990; back-calculated calibrators within ±20% of target and controls within ±20% of target. In this data set, there were 15, 97, and 112 cases above the limit of quantification for 5F-AMB, 5F-ADB, and FUB-AMB, respectively. Of these, 47% 5F-AMB, 24% of 5F-ADB, and 38% of FUB-AMB were below the reporting limit of the qualitative assay and thus had been reported "None Detected." The concentrations of all cases that fell within the calibration range are provided in the table below.

Analyte	Concentration (pg/mL)	# Cases >ULOQ
5F-AMB	68 ± 54 (n=10)	5
5F-ADB	200 ± 90 (n=52)	45
FUB-AMB	100 ± 60 (n=74)	38

The development of a qualitative method to detect synthetic cannabinoids allows for ease of updating the scope to remain relevant within the designer drug market. The ability to obtain semi-quantitative data through a retrospective method validation offers the forensic toxicology community information concerning the toxicity and expected concentrations of 5F-AMB, 5F-ADB, and FUB-AMB in whole blood samples.

Synthetic Cannabinoids, Retrospective Validation, Semi-Quantitation

K39 Fully Automated Detection and Quantification of Insulin Analogs by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) in Postmortem Vitreous Humor

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After attending this presentation, attendees will be informed about the current state of insulin analysis and the challenges surrounding its detection by LC/MS/MS in forensic samples. In addition, attendees will be able to implement a forensically validated LC/MS/MS method.

This presentation will impact the forensic science community by providing a novel approach for the simultaneous detection and quantification of human insulin and five pharmaceutical analogs and by describing its application in a series of forensic death investigations.

The analysis of biological specimens for the presence of exogenous insulin is of special interest in select postmortem investigations. Like other drugs and chemical agents, insulin may be implicated or suspected in the cause of a death; however, toxicological analysis is challenging due to complexities associated with immunoassay screening (cross-reactivity between endogenous and pharmaceutical analogs), challenges regarding multistage sample preparation (protein precipitation coupled to solid phase extraction or antibody immunopurification), as well as difficulties with mass analysis (poor fragmentation, low specificity transitions, analog/isotope coelution, and a reliance on low-flow microbore or nanobore chromatography). As a consequence, the determination of insulin in postmortem cases is not routinely performed. The work described here enables unambiguous differentiation of human insulin as well as five pharmaceutical analogs, including insulin glargine, glulisine, lispro, aspart, human, and detemir, through the use of robotic immuno-microchromatography coupled with insulin β-chain detection by LC/MS/MS.

Insulin extraction was performed on the Agilent® AssayMAP Bravo robotic platform using protein-G cartridges. Before extraction, 150µL of human vitreous humor is diluted 1:1 with Phosphate Buffer Saline (PBS) and fortified with porcine insulin as an internal standard. Cartridges are primed and conditioned with PBS prior to loading two mouse anti-insulin monoclonal antibodies (Santa Cruz SC-377071 and BioRad 5329-3806) to generate anti-insulin immunoaffinity microchromatography cartridges. Diluted vitreous humor is then loaded onto the immunoaffinity cartridges, washed sequentially with 4xPBS, 1xPBS, and 20% acetonitrile in 50mM ammonium bicarbonate, and eluted with 2% acetic acid into an existing volume of 40mM Tris(2-Carboxyethyl)Phosphine Hydrochloride (TCEP-HCL) in 30% acetonitrile. Following a brief incubation, insulin beta chains are analyzed in positive Multiple Reaction Monitoring (MRM) mode on an Agilent® 6495 triple quadrupole mass spectrometer coupled with a 1290 series Ultra High-Performance Liquid Chromatography (UHPLC). Chromatographic separation is performed using an Agilent® RRHD 300Å SB-C18 1.8µm, 2.1mm x 50mm analytical column with a stepwise gradient at 0.4mL/min over nine minutes.

Method validation was performed in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for Standard Practices for Method Validation in Forensic Toxicology. All analogs performed within criteria for acceptable performance. Parameters evaluated included linear range (500pg/mL–25,000pg/mL), limit of quantitation (500pg/mL), limit of detection (500pg/mL for insulin detemir and 125pg/mL for all other analytes), accuracy and precision (within and between run Coefficient of Variation (CV) <20%), interference, carryover, and stability (4°C and -20°C up to 30 days). In addition to the validation results, samples from five cases involving a suspected death by insulin have been analyzed. Of these, one case was positive for insulin aspart (743pg/mL) and one for insulin lispro (2,003pg/mL). A summary of the case history as well as an interpretation of findings will be discussed.

Insulin, LC/MS/MS, Overdose

K40 The Effect of Sample Preparation Techniques on Matrix Effects and Absolute Recovery of Opiates in Liver Tissue Using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS): Part 1

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After attending this presentation, attendees will better understand the effectiveness of the various sample preparation techniques for the extraction of opiates from liver tissue in order to determine which method may be suitable for their own implementation.

This presentation will impact the forensic science community by increasing knowledge regarding sample preparation techniques for the forensic pathology and postmortem toxicology communities. Many sample preparation techniques are primarily designed for the extraction of drugs from blood or urine, and the adoption of these techniques for difficult matrices, such as liver tissue, has occurred without complete understanding of the effects of the matrix on the analysis of the analyte(s) of interest.

In this presentation, an evaluation of the effect of sample preparation techniques on matrix effects and absolute recovery of opiates in liver tissue will be presented. In postmortem toxicology, the concentration of drugs in the blood is often used to assist in the determination of the cause and the manner of death; however, central cavity drug blood concentrations can be unreliable because of the phenomenon of postmortem redistribution. This can be combated by evaluating liver concentrations, as drug concentration in liver is fairly stable after death. While liver is a valuable tissue for postmortem toxicology, the protein, fat, and phospholipid components of the matrix can interfere with analysis, and thus the drug must be isolated from the matrix prior to analysis. If proper and effective sample preparation and clean-up are not performed, matrix effects, such as ion enhancement or suppression, can hinder analysis and affect recovery of the drug. To limit matrix effects, it is necessary that the preparation technique used has the ability to extract the analyte as completely as possible while limiting the extraction of any interfering compounds.

There are many different approaches to sample preparation for drug extraction. The three traditional types of techniques are Solid-Phase Extraction (SPE), Liquid-Liquid Extraction (LLE), and filtration. A growing number of simple and rapid sample preparation techniques have become commercially available in recent years. These new techniques are commonly based on the traditional techniques but have added features to improve the extraction process. While these newer techniques have the ability to make sample preparation both easier and faster, there are still limitations. A majority of the user guides and technical notes for these new products focus on either blood or urine sample matrices. There is limited published data regarding tissue matrices, such as liver. For these techniques to be effectively used for liver samples, the matrix effects, absolute recovery, and process efficiency for extractions from liver must be evaluated.

These sample preparation techniques were evaluated for matrix effects and recovery by extracting opiates from homogenized liver tissue. Liver tissue was homogenized in saline at a ratio of 1:4. Homogenates were fortified with six opiates, at two concentrations (n=6), and their respective isotopic derivatives. The opiates analyzed were codeine, hydrocodone, hydromorphone, morphine, oxycodone, and oxymorphone. Three sets of samples were analyzed: neat, fortified before, and fortified after. Sample preparation was performed following manufacturer's guidelines (Waters® Oasis™ PriME HLB cartridge, Biotage® ISOLUTE® SLE+, and Biotage® ISOLUTE® PLD+) and using a laboratory validated LLE technique. Samples were analyzed using a previously validated UPLC-MS/MS method.

Results varied greatly between the methods evaluated. For Waters® Oasis™ PriME HLB, the observed matrix effects were between -35% and +5%, and recoveries were between 100% and 122%. For Biotage® ISOLUTE® SLE+, the observed matrix effects were between -18% and 0%, and recoveries were between 86% and 109%, with the exception of morphine, which had recoveries between 30% and 32%. For Biotage® ISOLUTE® PLD+, the observed matrix effects were between -16% and +50%, and recoveries were between 55% and 94%. For LLE, the observed matrix effects were between -59% to -37%, and recoveries were between 39% and 82%.

Liver is a difficult matrix to analyze. Sample preparation is not as simple as it is for blood or urine. It was observed that not all sample preparation techniques are effective or reliable for the extraction of opiates from liver tissue. Of the techniques evaluated, the Biotage® ISOLUTE® SLE+ was more effective at removing matrix effects and improved recovery.

Opiates, Liver, Sample Preparation

K41 The Identification of Adulterants in Preliminary Drug Analysis

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After attending this presentation, attendees will understand the implications of drug adulteration for urinalysis and how commercially available and common household adulterants can affect results in preliminary drug screening techniques, such as Enzyme-Linked Immunosorbent Assay (ELISA).

This presentation will impact the forensic science community by demonstrating how these immunoassay-based screening techniques are prone to producing false positive and negative results in the presence of adulterants. Adulteration of urine samples can circumvent current preliminary screening protocols (i.e., ELISA) and even common adulterant test strips. These results may affect criminal proceedings that are reliant on drug tests to determine convictions, compliance in probation, and court-based treatments.[1]

Despite drug abuse being one of the major issues that has plagued society for centuries, the technology to detect drugs and their metabolites in bodily fluids has only been accessible for less than 50 years.[2] Drug testing always begins with a screening technique in the form of immunoassays, such as ELISA, and adulterant testing strips may also be utilized to ensure the sample has not been manipulated.[3] Although response accuracy of immunoassays have increased drastically over the years, they only remain accurate approximately 95% of the time for the detection of drugs of abuse and their corresponding metabolites in urine samples.[4] This value decreases when samples have been adulterated.[2]

Approximately 30 urine samples were collected from anonymous volunteers. Each participant was required to complete surveys detailing the frequency of their drug use in the week prior to providing a sample. Based on this information, samples that may contain significant concentrations of common drugs of abuse and their metabolites were identified (i.e., THC, cocaine, amphetamine, and benzodiazepines). Aliquots of these urine samples were adulterated at different levels (i.e., 5, 10, 25, and 50% volume/volume (v/v)) with common and commercially available adulterants, including bleach, vinegar, eye drops, Drano®, nitrite, table salt, hydrogen peroxide, and hand sanitizer. Preliminary research using ELISA revealed that some adulterants (e.g., bleach, eye drops, Drano®) drastically reduce the concentrations of detectable drugs/metabolites in comparison to the unadulterated urine samples.

Adulterant test strips were also utilized to determine if, and at what level, they were able to detect when a urine sample had been tainted. Preliminary data revealed that most of the adulterants were not able to be detected at concentrations less than or equal to 10% v/v. Eye drops, specifically those that contain benzalkonium chloride, were not detected in the adulterated urine samples, even at high levels. This is of great concern, considering that eye drops drastically reduced the detection of THC, cocaine, and amphetamine by ELISA. These results suggest that new pre-screening techniques may need to be identified to combat and detect the presence of adulterants in urinalysis.

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ELISA, Adulterant Test Strips, Adulterants

K42 Using Medical Examiner Case Narratives to Improve Opioid Overdose Surveillance

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After attending this presentation, attendees will appreciate how using additional sources of information to classify opioid overdose cases can result in increases in the number of heroin-related classified deaths and in the identification of non-heroin, injection-related opioid analgesic deaths.

This presentation will impact the forensic science community by offering new tools for the characterization of opioid overdose deaths and the identification of meaningful subgroups of opioid users who can be targeted by public health programming.

Opioid overdose is a leading cause of death in the United States. While opioid analgesics were responsible for the rapid increase in overdose mortality during the 2000s, deaths due to heroin and fentanyl have increased sharply in recent years. Surveillance of the national opioid overdose epidemic demonstrate that the types of opioids causing overdose are evolving. For example, fentanyl mixed with or sold as heroin or other prescription opioids increases the risk of overdose death and complicates interpretation of postmortem toxicology. Opioid overdose surveillance systems report rates and counts classified by the type of opioid involved. Opioid types are extracted from the International Classification of Diseases (ICD) codes on death certificates, which may result in under-estimation or misclassification of specific opioid types.[1] Up to one-quarter of death certificates with drug overdose listed as the cause of death do not include the specific drugs implicated. Failure to include opioid type can result in substantially underestimated counts of overdose deaths involving opioid analgesics. Current opioid overdose mortality surveillance methods do not capture the complexity of the overdose epidemic. Most rely on death certificates which may underestimate heroin overdose deaths. In addition, categorizing deaths using characteristics beyond the type of opioid implicated in the overdose, such as the route of administration, can provide information to design and evaluate targeted public health interventions.

Methods: This study reviewed California Electronic Death Reporting System designations of cause of death and San Francisco Office of the Chief Medical Examiner postmortem toxicology reports and investigative case narratives for all unintentional deaths attributed to opioids occurring in the county of San Francisco from 2006 to 2012. Using these data sources, this study created enhanced classification systems for heroin-related and injection-related opioid overdose deaths and compared demographic, death scene, and postmortem toxicology characteristics between these groups.

Results: This retrospective analysis resulted in the identification of 816 unintentional opioid overdose deaths during the time period of interest. This study classified 152 of these deaths (19%) as "standard" heroin deaths (designated by the case medical examiner or confirmed by the presence of 6–monoacetylmorphine). An "expanded" classification of heroin deaths using data from postmortem toxicology reports and case narratives added 20 additional heroin deaths (+13% increase), accounting for 21% of all opioid deaths. Based on case narratives, 205 deaths (25%) were injection-related, 60% of which were attributed to heroin. A combined classification of enhanced heroin and injection-related deaths accounted for 31% of opioid overdose deaths during this period.

Conclusions: Using additional sources of information to classify opioid overdose cases resulted in a modest increase in the count of heroin-related deaths, but identified a substantial number of non-heroin injection-related opioid analgesic deaths that would otherwise have gone amiss. This current study reveals that including the route of administration in the characterization of opioid overdose deaths will identify meaningful subgroups of opioid users who can be targeted by public health programming.

This study was supported by a National Institute on Drug Abuse (NIDA) grant.

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Opioid Overdose, Injection Drug Use, Heroin

K43 Prescription Drug Degradation in a Simulated Postmortem Blood Model

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The goal of this presentation is to demonstrate the stability of prescription medications in an environment of optimal microbial activity, such as may be encountered in decomposed postmortem specimens.

This presentation will impact the forensic science community by providing fundamental information in regard to the stability of two important classes of drugs during the postmortem interval and throughout all stages of the analytical process. This information will assist attendees in the interpretation of antidepressant and antipsychotic drug concentrations measured in postmortem specimens.

Hypothesis/Proposition: Degradation of xenobiotics by micro-organisms is a complication that must be accounted for by forensic toxicologists analyzing postmortem specimens. This phenomenon is especially of concern prior to specimen collection, as during the postmortem interval (between death and autopsy), environmental conditions may favor microbial activity. Prescription medications (e.g., antidepressants and antipsychotics) are commonly observed in casework. Therefore, it is important to establish whether medications can degrade during this period to ensure accurate quantitation and detection of degradation products in toxicology screening methods. This study utilizes a "simulated postmortem blood" model enriched with microorganisms to investigate prescription drug stability.

Methods: The "simulated postmortem blood" model was constructed by directly inoculating antemortem blood (sourced from the Australian Red Cross Blood Service) with microorganisms from pooled stool samples of nine healthy donors. Antipsychotics investigated were in the structural classes of phenothiazines, tricyclics, thioxanthenes, butyrophenones, phenylpiperazines, and benzo(iso)thiazolepiperazines. Antidepressants investigated were tricyclics, Norepinephrine Reuptake Inhibitors (NRIs), and Noradrenergic and Specific Serotonergic Antidepressants (NaSSAs). These drugs were spiked into the model samples and non-inoculated controls. Risperidone was included in all experiments as a known microbially labile "positive" control. Preserved samples with 2% weight by volume (w/v) sodium fluoride were also prepared concurrently for both the model and non-inoculated controls. An Agilent® 1100 Series LC-UV was used to quantitatively monitor drug degradation over the course of a week's incubation of the samples at 37°C and extended incubations at room temperature, 4°C, and -20°C. Microbial communities were profiled throughout the experiments to determine which species were present in the initial inoculations and how communities changed over time with respect to sample environment, drugs present, temperature, and the presence of preservatives.

Results: Successful inoculation of viable microorganisms from the pooled stool samples was confirmed by the degradation of risperidone to its established bacterial degradation product, 2-hydroxybenzoylrisperidone, in unpreserved "simulated postmortem blood" samples. No degradation of risperidone was observed in the non-inoculated controls, which was consistent with prior studies performed to assess its stability in blood. After a week at 37°C, minimal losses were reported for all other investigated antipsychotics with none exhibiting significantly enhanced degradation in the "simulated postmortem blood" samples compared to the non-inoculated antemortem blood controls. In non-inoculated unpreserved samples, losses of up to 50% were observed for the phenothiazine antipsychotics after 38 days at 37°C. Experiments are currently ongoing for antidepressant drugs and extended incubation samples. At the time of presentation at the AAFS 2018 Annual Scientific Meeting, antipsychotic drugs will have been incubated at room temperature, 4°C, and -20°C for seven months. Results and analysis of microbial communities for 37°C experiments will also be completed later in 2017.

Conclusion: The simulated postmortem blood model allowed for the investigation of drug degradation as caused by a wide variety of relevant microorganisms; however, microorganisms sourced from unhealthy individuals, those taking any drugs or medications, and invasive species that may enter the body after death were not targeted in this study. Therefore, the potential for the postmortem degradation of these drugs cannot be excluded in all cases.

Drug Degradation, Putrefaction, Prescription Drugs

K44 Strange Bedfellows: Fentanyl Mixed With the Antiquated Poison Strychnine

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After attending this presentation, attendees will be familiar with two overdose deaths in which toxicological analysis detected strychnine in addition to fentanyl. These cases highlight the diverse methods often employed by clandestine laboratories in the production of illicit drugs.

This presentation will impact the forensic science community by providing two examples of cases of fentanyl overdoses in which strychnine was also detected. Attendees will find it an interesting juxtaposition of a modern synthetic drug with an antiquated natural poison. These cases also emphasize the wide spectrum of drugs the modern toxicologist could encounter.

The potent synthetic opioid fentanyl was first developed in the 1960s, having been derived from the structurally related drug meperidine. After discovering its potent effects, fentanyl was and is used regularly for palliative purposes. Illicit use of fentanyl began in the 1970s and continues to grow. Like all opioids, overdose deaths related to fentanyl have also increased dramatically in recent years. This growing demand has, in turn, been met by an increased supply and has also spurred greater diversity in techniques employed by clandestine laboratories in the illicit preparation of fentanyl. Presented here are two unusual death cases in which it is proposed that the illicit fentanyl was prepared with strychnine.

A 59-year-old White male was complaining of heartburn and vomiting one evening and was later found dead in his bed. Autopsy revealed changes related to hypertension, but no specific anatomic cause for death. Toxicological analyses of iliac blood reported fentanyl (0.003mg/L), strychnine (<0.025mg/L) and heroin metabolites: 6-monoacetylmorphine (0.006mg/L), morphine (0.159mg/L), and codeine (0.011mg/L). Death was attributed to heroin and fentanyl toxicity. Four days later, a 29-year-old Black male in another city, but in the same county, was discovered dead on his living room floor. The decedent had recently been released from prison and had also been treated at a hospital for pneumonia. Autopsy did not detect any residual pneumonia in the lungs or other anatomic cause for death. Toxicological analyses of iliac blood detected fentanyl (0.008mg/L), olanzapine (<0.025mg/L), and strychnine (<0.025mg/L). Death was attributed to fentanyl toxicity.

Strychnine is a potent alkaloid classically derived from the seeds of the *Strychnos nux-vomica* tree, which grows in warm climates in southern Asia and Australia. Strychnine was first isolated from the *Strychnos* genus in 1753, though the toxic effects of the *nux-vomica* bean had been well known in India and China for centuries before that. It is primarily used as a pesticide to kill small vertebrate pests such as rodents and has restricted availability in the United States due to the potential for deaths of animals for which it is not intended. Consumption of strychnine causes generalized muscle spasms. At lower doses, this can be restricted to tachycardia, cramping, rigidity, and agitation. Higher doses can lead to seizures, hypertension, cyanosis, and opisthotonus (dramatic back spasms causing arching of the back and neck). Death can occur from resultant cardiac arrest, respiratory failure, or brain damage.

In neither of these two cases was strychnine determined to be at sufficient levels to have contributed to death, especially given the presence and concentrations of the opioids. Nonetheless, its presence in these two cases underscores the diverse methods employed by clandestine laboratories in the production of illicit opioids and also illustrates an unusual marriage of a historic natural poison with more modern, synthetic drugs such as fentanyl.

Fentanyl, Strychnine, Opioids

K45 An Investigation Into the Analysis of Fentanyl in Postmortem Blood Using Biocompatible Solid-Phase Microextraction (BioSPME).

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After attending this presentation, attendees will better understand how BioSPME can be an alternative extraction method for fentanyl in postmortem blood.

This presentation will impact the forensic science community by providing an extraction method that is faster than current analytical methods. BioSPME coupled with Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) allowed for minimal sample collection, preparation, and shorter analysis time in the analysis of postmortem blood samples from overdose victims.

The abuse of opioids, particularly fentanyl, has become a slow-motion mass disaster over recent years in the United States. Due to the frequent abuse of opioids, there has been an increase in drug-related deaths.[1] Forensic pathologists are responsible for collecting various postmortem samples that are then sent to a toxicology laboratory to be analyzed for drugs, such as fentanyl. This process can be time consuming and may result in a backlog, which could hinder a criminal investigation. A solution could be BioSPME using coated fibers that can be directly injected into a biological matrix and absorb any drug present without the interference of macromolecules, thus allowing for a faster analysis time.

A method has been developed to analyze fentanyl in postmortem blood using BioSPME followed by GC/MS and LC/MS/MS analysis. BioSPME fibers were conditioned, directly injected into blood, washed, filtered, desorbed into solution, dried down, and reconstituted. The extracted samples were screened by GC/MS and subsequently analyzed by LC/MS/MS. GC/MS was performed using splitless injection on a Rxi-5Sil MS column (30.0m x 0.25mm, 0.25μm) in the Selected Ion Monitoring (SIM) mode. Samples were confirmed using an AB SCIEX™ 3200 QTRAP® triple quadrupole MS with an Electrospray Ionization (ESI) source in the positive ion mode. LC was performed on a Shimadzu® LC system using an Ascentis® Express Biphenyl column (50mm x 2.1mm, 2.7µm) with the weak mobile phase of 0.1% (volume/volume (v/v)) formic acid in water and the strong mobile phase of 0.1% (v/v) formic acid in acetonitrile. The flow rate was 0.30mL/min, column temperature was 30ºC, injection volume was 1μL, and an analysis time of seven minutes per sample. This method was developed using bovine blood, then applied to 43 postmortem blood samples from overdose victims from the Lehigh County Coroner's Office in Allentown, PA.

In conclusion, the use of BioSPME as an extraction method allows for minimal sample preparation and collection for the detection of fentanyl in postmortem blood.

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1. U.S. Drug Enforcement Administration, Office of Diversion Control. National Forensic Laboratory Information System Special Report: Opiates and Related Drugs Reported in NFLIS, 2009-2014. Springfield (VA): U.S. Drug Enforcement Administration.

BioSPME, Forensic Toxicology, Fentanyl

K46 Fatal Hydromorphone Overdose in a Child: A Case Report

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The goal of this presentation is to describe the circumstances and postmortem toxicology results in a case of fatal hydromorphone toxicity in a child.

This presentation will impact the forensic science community by contributing to the forensic literature regarding hydromorphone blood concentrations in children. Furthermore, this case provides support for comprehensive drug screening in pediatric deaths.

Hydromorphone is a semi-synthetic opioid analgesic that is approximately eight times more potent when compared to morphine. It is prescribed for the treatment of post-operative or chronic pain; however, it may also be encountered in forensic casework as a drug that is used recreationally. Toxicity due to hydromorphone is dependent on an individual's tolerance, but can include stupor, hypotension, muscle flaccidity, coma, and respiratory depression.

The decedent in this case was a 13-month-old child found vital signs absent in her crib. Emergency medical services were notified, but she was pronounced dead upon arrival at the hospital. Prior to being put to bed the night before her death, she was described as "fussy" and lethargic. The autopsy described a well-developed, well-nourished child, with no significant injuries to head, neck, or torso, and no anatomic cause of death. Heart blood, femoral blood, liver, and stomach contents were submitted for toxicological analysis. Comprehensive drug screening was performed according to a pediatric death protocol used in the province of Ontario, Canada. This protocol comprised: Gas Chromatography/Nitrogen Phosphorous Detection (GC/NPD) and Gas Chromatography/Mass Spectrometry (GC/MS) screen for chemically basic drugs; immunoassay for acetaminophen, salicylates, barbiturates, benzodiazepines, cannabinoid metabolites, cocaine metabolite, opioids (morphine, hydromorphone, codeine, hydrocodone, levorphanol), oxycodone, and fentanyl; Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis for benzodiazepines; and headspace Gas Chromatography/Flame Ionization Detector (GC/FID) for ethanol and other volatiles.

The only toxicologically significant finding in the case was hydromorphone, which was tentatively identified in heart blood by immunoassay and confirmed in femoral blood by a quantitative LC/MS/MS method. The concentration of hydromorphone was determined to be 60ng/mL. Additional case information revealed a variety of prescription and over-the-counter medications used by adults in the home, including hydromorphone in both sustained-release (24mg) and immediate-release (1mg) formulations. The medication history for the deceased included infant acetaminophen drops and a mouthwash preparation comprised of diphenhydramine, aluminum hydroxide and magnesium hydroxide antacid, and lidocaine, prescribed for the treatment of mucositis, mouth pain, and/or oral ulcer.

The scientific literature is of limited assistance with respect to the toxicological interpretation of postmortem blood hydromorphone concentrations in children. Only one previously published case of pediatric overdose provides information on postmortem blood concentrations in children.[1] In that case, postmortem peripheral and heart blood concentrations of 30ng/mL and 60ng/mL, respectively, were measured in a 3-year-old who was determined to have accidentally ingested hydromorphone. Clinical studies are also rare, but provide information on plasma concentrations in children receiving hydromorphone therapeutically. For example, an average plasma concentration of 4.7ng/mL was reported for ten children receiving 2mg hydromorphone intravenously.[2] By comparison, oral administration of a 5mg sustained-release preparation to a 7-year-old child every 12 hours produced a plasma concentration of 1.48ng/mL.[3]

Based on the clinical history, autopsy results, and toxicology findings, the coroner determined the cause of death in this case to be hydromorphone toxicity. The manner of death was undetermined.

Reference(s):

1. Cantrell F.L. et al. A pediatric fatality due to accidental hydromorphone ingestion. *Clin Toxicol.* (Phila). 2017; 55(1): 60-62.

2. Collins J.J. et al. Patient-controlled analgesia for mucositis pain in children: A three-period crossover study comparing morphine and hydromorphone. *Pediatr*. 1996; 129(5): 722-8.

3. Babul N.B., Darke A.C., Hain R.H. Hydromorphone and metabolite pharmacokinetics in children. *J Pain Symptom Manage*. 1995; 10: 335-337.

Hydromorphone, Child, Overdose

K47 An Accidental Death Due to Paraquat Poisoning: An Unusual Case Requiring Toxicologist, Pathologist, and Investigator Collaboration

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After attending this presentation, attendees will better understand the toxicological and pathological findings that indicate death due to paraquat toxicity and how knowledge of the circumstances of death can aid in differentiating between a possible diquat toxicity case and a paraquat toxicity case.

This presentation will impact the forensic science community by demonstrating the necessity of a seamless working relationship between the forensic pathologists, forensic investigators, and toxicologists to understand unusual postmortem cases.

In December 2015, a 38-year-old White female presented to the hospital after accidently drinking from a water bottle that contained weed killer and vomiting twice. The decedent and her husband owned a cleaning business and had obtained the weed killer from the landscaping company at their apartment complex and stored it in a water bottle. The decedent and her husband advised the hospital staff that they believed the liquid to be diquat dibromide and were unsure how much the decedent had consumed before spitting out the liquid.

The hospital staff consulted the Poison Control Center who recommended that the decedent be observed for nine hours before she could be safely discharged. The decedent was treated and discharged later that day. Two days later, the decedent presented to a different hospital after continuing to vomit and complaining of nausea and a burning sensation. Despite medical intervention, her condition deteriorated, and she expired in January 2016, four weeks after the initial ingestion.

Autopsy findings were significant for consolidation of the lungs (right lung weight: 700 grams, left lung weight: 710 grams) with necrosis and purulent exudate. Microscopically, the section of lung exhibited dense pulmonary fibrosis with associated intraparenchymal and intra-alveolar hemorrhages. Delayed pulmonary fibrosis is a characteristic pathological finding in paraquat poisoning that is not seen in diquat poisoning.

A sample of the unknown liquid the decedent drank was submitted to the Miami Dade County Medical Examiner Department (MDME) Toxicology Laboratory for analysis in addition to antemortem samples taken during the decedent's second hospital visit. Toxicological screening of the unknown liquid by Gas Chromatography/Mass Spectrometry (GC/MS) indicated that 4,4-bipyridine was present in the sample. No other analytes were detected in the unknown liquid. 4,4-bipyridine is used as a precursor to paraquat. This finding caused the MDME Toxicology Laboratory to question if the decedent had consumed diquat, as she thought, or if she had consumed paraquat.

Due to the fact that paraquat and diquat could not be distinguished from one another by GC/MS, a fit-for-purpose method was developed by high-performance Liquid Chromatography/Ion Trap/Mass Spectrometry with MS^n capability (LC/Ion Trap/MS^n) to differentiate these two analytes. Analysis of an antemortem urine sample, dated five days after the initial ingestion, by LC/Ion Trap/MS^n indicated that paraquat was present in the sample. The detection of paraquat in a urine sample five days after ingestion is consistent with the literature, which indicates that paraquat can be detected in the urine for up to 26 days after an acute ingestion.

Based on the decedent's history, the sequence of terminal events, autopsy findings, and toxicology findings, the forensic pathologist determined that the cause of death was complications of paraquat toxicity and the manner of death was an accident. This case is a prime example of the necessary working relationship and the ability to share information between the forensic pathologists, forensic investigators, and toxicologists to allow the forensic pathologist to determine a cause of death.

Paraquat, Diquat, Postmortem

K48 Postmortem Tissue Distribution of Synthetic Cathinones

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After attending this presentation, attendees will better understand tissue distributions and the potential for some synthetic cathinones to exhibit significant Postmortem Redistribution (PMR).

This presentation will impact the forensic science community by increasing the fundamental understanding of synthetic cathinone distribution in postmortem toxicological samples and the influence of PMR.

Postmortem toxicology results can provide crucial information in death investigations regarding the cause and manner of death; however, postmortem drug concentrations may not always reflect antemortem concentrations. Drugs may also undergo PMR, resulting in significant differences between central and peripheral blood. To assess PMR, Central to Peripheral (C/P) blood ratios can be calculated. PMR can be somewhat predicted using drug properties, including volume of distribution, lipophilicity, and p*Ka*. Blood and tissue distributions have been studied for many common illicit drugs, but this information is still limited for synthetic cathinones, a class of designer drugs that has been increasing in popularity over the past decade. In this presentation, postmortem tissue distributions and C/P ratios for select synthetic cathinones from cathinone-positive fatalities will be presented.

Postmortem samples from 60 cathinone-positive cases were included in the study. A total of 210 specimens were evaluated, including liver, urine, vitreous humor, and blood collected from the aorta, inferior vena cava, iliac, subclavian, and femoral vessels. Quantitative analysis was performed using blood or urine calibrators with matrix-matched controls. Samples were analyzed using a previously published and validated procedure for the determination of 22 synthetic cathinones in urine and blood using Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). A total of nine isotopically labelled internal standards were used. The principal compounds of interest were methcathinone, 3-fFuoromethcathinone (3-FMC), 4-Fluoromethcathinone (4-FMC), ethcathinone, ethylone, methedrone, buphedrone, butylone, mephedrone, eutylone, 4-Methylethcathinone (4-MEC), 3,4-Methylenedioxy-α-Pyrrolidinobutyrophenone (MDPBP), pentedrone, pentylone, 3,4-Dimethylmethcathinone (3,4-DMMC), α-Pyrrolidinopentiophenone (α-PVP), 4-Ethylmethcathinone (4-EMC), 4-Methyl-α-Pyrrolidinobutiophenone (MPBP), Methylenedioxypyrovalerone (MDPV), pyrovalerone, and naphyrone.

Of the 22 cathinones in the assay, 9 were identified in at least one case: α-PVP (*n*=18), methylone (*n*=17), ethylone (*n*=15), MDPV (*n*=6), pentylone (*n*=3), methedrone (*n*=2), 4-MEC (*n*=1), butylone (*n*=1), and MDPBP (*n*=1). Concentration ranges in blood, urine, and liver, respectively, were <2 to 1,090ng/mL, 33 to 7,580ng/mL, and 14 to 663ng/g for α-PVP; <2 to 202 ng/mL, 2 to 38,064ng/mL, and 28 to 5,731ng/g for methylone; <2 to 2,743ng/mL, 32 to >20,000ng/mL, and 10 to 18,893ng/g for ethylone; 3 to 80ng/mL, 4 to 5,210ng/mL, and 64 to 840ng/g for MDPV; <5 to 322ng/mL in blood and 122 to >5,000 in urine for pentylone. Where possible, average C/P ratios were determined as follows: methylone (4.0, range 2.39-6.0, *n*=4), ethylone (2.9, range 0.5-9.2, *n*=6), pentylone (2.0, *n*=1), α-PVP (1.2, range 0.5-1.9, *n*=8), methedrone (1.1, *n*=1), MDPV (1.0, *n*=1), and butylone (0.7, *n*=1). Although C/P ratios were highly variable, some cathinones appeared to have significant potential for redistribution. Generally, the highest C/P ratios were observed in methyenedioxy-type cathinones bearing secondary amines. Although the pyrrolidine-type cathinones are less polar and subsequently more lipophilic, they are less basic than their secondary amine counterparts. Vitreous humor was only available in a small number of cases, but concentrations in vitreous were comparable to peripheral blood within this limited population. Although the concentration range of forensic interest was wide, the results also highlight the need for low limits of detection and quantification.

Tissue distributions and C/P ratios are presented and compared with existing literature. Variability of C/P ratios and the potential for cathinones to degrade *in situ* and during storage significantly complicates their interpretation. Of the 60 cases submitted, 50 cases had at least one specimen test positive for a synthetic cathinone. The results highlight the potential for some cathinones to exhibit PMR, the importance of collecting multiple specimens, and the interpretation of results within the full context of investigative information.

Synthetic Cathinones, Postmortem Redistribution, LC/qTOF/MS

K49 Acute Intoxications With Phenibut (ß-Phenyl- γ -Aminobutyric Acid), an Emergent Psychoactive γ -Aminobutyric Acid (GABA) Agonist

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After attending this presentation, attendees will be familiar with the behavioral and physiological effects of the psychoactive compound known as phenibut. Attendees will also understand the importance of updated screening libraries for the detection of Novel Psychoactive Substances (NPS).

This presentation will impact the forensic science community by raising awareness regarding phenibut abuse and by providing examples of screening and confirmation techniques for this analytically challenging substance.

Phenibut is an analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and the antispasmodic baclofen. Neuromodulation occurs when phenibut binds to GABA receptors, primarily at $GABA_B$. While not licensed for medical use in the United States, phenibut has been prescribed in Russia since the 1960s for the treatment of anxiety, alcohol withdrawal, and insomnia. It is currently available on the internet as a nutritional supplement and marketed as a nootropic, anxiolytic, and euphoriant, where typical doses range from 500mg to 2g. Phenibut tolerance develops rapidly, similar to other GABA receptor modulators. Tolerance may precipitate substantial dose increases. Additionally, co-administration with other GABA modulators, such as ethanol, may result in more pronounced pharmacological effects. In this submission, two unrelated cases involving phenibut intoxication are presented.

Case 1: A 21-year-old male was found unconscious in a dormitory hallway. The subject was minimally responsive, slurring his words, and walking into walls when emergency personnel arrived. The man vomited, his condition deteriorated, and he was transported to the hospital. The attending physician described his aggressive and combative behavior as excited delirium. He assaulted medical staff, was restrained, and remained in the hospital for two days.

Case 2: A 21-year-old male was unconscious and unresponsive in a dormitory stairwell the morning after a night of drinking with friends. Two individuals were sent to retrieve the man and found him staggering and slurring his speech. The man was taken to his dorm room, where he laid down and fell asleep. His roommate attempted to wake him 1h later, but he appeared incoherent and confused. Medical personnel applied multiple sternum rubs to revive the individual. He jolted awake, appeared disoriented, and his pupils were non-reactive to light stimulus. He was transported to the hospital where toxicology revealed no drugs or alcohol.

Urine from each case was submitted to the Armed Forces Medical Examiner System (AFMES) Division of Forensic Toxicology. Both specimens were negative for ethanol by gas chromatography/flame ionization detection and for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opioids, phencyclidine, and sympathomimetic amines by immunoassay. In addition, both cases screened negative for alkaline-extractable drugs by gas chromatography/full-scan mass spectrometry. After case histories were reviewed, a non-targeted liquid chromatography/quadrupole time of flight/mass spectrometry drug screen was added. In Case 1, phenibut and ondansetron were detected in the urine. Phenibut, naloxone, and ondansetron were detected in the urine from Case 2. Phenibut was confirmed in the urine from both cases by liquid chromatography/tandem mass spectrometry.

Unknown to the lab at time of analysis, the individual in Case 1 later admitted to self-medicating with an internet-purchased supplement for his social anxiety and attention-deficit hyperactivity disorder. On the date of the incident, he self-reported ingesting 20g of phenibut, causing him to vomit shortly thereafter. He then ingested an additional 10g in an attempt to account for the amount he regurgitated. The individual in Case 2 was interviewed after discharge and stated he drank wine, beer, and champagne the night prior to the incident. He did not recall any events 8h prior to medical intervention in his dormitory room. The individual also admitted to purchasing phenibut from the internet, but did not provide information concerning the amount ingested or if the dose was co-ingested with alcohol.

In summary, two acute intoxications with phenibut that emphasize the difficulties encountered during extraction and instrumental analysis are presented. If proper analytical techniques are not available, methods are not current, or history is incomplete, it is possible to overlook phenibut and other NPS. In these cases, history was a key factor in directing additional testing. Intoxication effects corroborated previous reports of somnolence/stupor, confusion, agitation, nausea, and vomiting. These cases underscore the need for vigilance when evaluating casework and promote the use of comprehensive screening techniques that may reveal rare, but significant, findings.

Phenibut, NPS, QTOF

K50 A Forensic Characterization of Bacterial and Fungal Organisms in Traditional Chinese Herbs

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After attending this presentation, attendees will be aware of the potential presence of toxic microorganisms in Chinese herbal products used for medicinal purposes.

This presentation will impact the forensic science community by potentially being used to identify unknown specimens of herbs, provide provenance of the herbs themselves, and help reconstruct toxicological episodes that result in medical emergencies or death.

Traditional Chinese Medicine (TCM) is one of the oldest healing methods used in Chinese culture, often referred to as "formula." TCM's herbal formulations are prescribed for a variety of illnesses, based on symptoms presented. Herbal remedies are selected based on Chinese ancient literature and individual experiences of patients or doctors. Use of TCM in the United States has increased because herbal remedies are believed to be less expensive and more effective with less adverse effects in comparison to traditional pharmaceutics. Therefore, sales have increased, despite articles and case studies that have demonstrated the dangers, such as injury and death, related to TCM, stemming from improper labelling, toxic contaminants, and, in some cases, the presence of pathogenic bacteria.

Objective: The purpose of this study was to conduct a molecular and biochemical survey of microorganisms of 11 Chinese herbal products purchased from a traditional medicine shop in Beijing, China. Bulk analysis of microbial/fungal lipids from the herbs was conducted using a rapid method for extraction of cellular fatty acids and derivatization into Fatty Acid Methyl Esters (FAMEs) prior to profiling with Gas Chromatography/Flame Ionization Detector (GC/FID).

Methods: Eleven over-the-counter Chinese herbs were purchased from Tong Ren Tang in Beijing, China. These herbs were chosen based on reported pharmacological activity: sedative and hypnotic (Suan Zao Ren, Fu Ling, Sha Yuan Zi, and Di Long); anticonvulsant (Gou Teng, Tian Ma, and Jiang Can); and analgesic (Yan Hu Suo, Chuan Duan, Wu Yao, and Mo Yao).

Approximately 100mg of the herbal product was placed in 1x Phosphate Buffered Saline (PBS). A 100µL aliquot of the solution was spread onto Tryptic Soy Agar (TSA) with and without blood supplements (50mg/L). Plates were then incubated overnight at 30°C. Colony growths were photographed, harvested, and subjected to FAME profiling.

FAME profiling of the herbal products was performed using GC/FID equipped with a series of analytical standards to detect and quantify fatty acids between 9 and 20 carbons in length.

Products were incubated with methanolic potassium hydroxide (5% KOH, 95% CH_3OH). The methyl esters were then extracted into hexane and analyzed. The individual fatty acids were identified by their retention time through comparison to reference standards.

Results: Strains within the *Bacillus* group were identified in nearly all 11 of the herbal samples. These included *B. subtilis* and *B. cereus*, as well as *B. megaterium*, *B. circulans*, and *B. atrophaeus.* Organisms belonging to the *Bacillus* ACT group (anthracis, cereus, thuringiensis) were identified in 5 out of 1 herb cultures as evidenced by the large ratio of 15:0 iso to 15:0 anteiso fatty acid biomarkers. A gram-positive, aerobic bacteria related to the *Bacillus* group*, Paenibacillus thiaminolyticus*, was also detected. This bacteria differs from *Bacillus* ACT and has been reported to cause bacteremic infections in humans. All of the herbal specimens also exhibited fungal biomarkers such as polyunsaturated 20:4 ω6,9,12,15c, and 18:3 ω6c (6,9,12). The presence of fungal biomarkers would be consistent with the origin of some herbal samples such as Jiang Can, silkworm larvae that are claimed to have been killed with the fungus *Beauveria bassiana*; however, in others, they could represent contamination of fungal spores.

Conclusion: The characterization of microorganisms present in these traditional Chinese herbs was successful through analysis of their FAME profiles and by the presence of particular and unique fatty acids. The bacterial and fungal identification can potentially be used to identify unknown specimens, provide provenance of the herbs themselves, and help to reconstruct toxicological episodes that result in medical emergencies or death.

Chinese Herbs, FAME Analysis, GC/FID

K51 The Role of Toxicology in Child Custody Disputes

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After attending this presentation, attendees will be able to discuss the importance of good laboratory practices, the pharmacology of drugs in analysis, and the interpretation of specimens in child custody cases.

This presentation will impact the forensic science community by describing the role of toxicology in child custody disputes.

Two cases are presented which highlight the importance of validated testing and interpretation of results in child custody cases. For both cases, a private toxicology lab (Lab A) was contacted and asked to review the analytical work performed at other laboratories, review the reported findings, and/or comment on opinions provided by other scientists.

Case 1: The mother of three children was accused of exposing them to Gamma Hydroxybutyrate (GHB), diazepam, and cocaine. Lab A performed hair testing; Lab B performed blood and segmented hair analysis on the mother and all the children; and Lab C tested urine, hair, and blood form both parents and the children. In total, more than 50 different tests were performed at Lab C. The following results will be discussed: diazepam in blood and urine specimens from both adults; diazepam in hair of all tested individuals at concentrations of 39.3ng/mg–215ng/mg; GHB in hair of all tested individuals at concentrations of 76.3ng/mg–225.5ng/mg; and methylecgonine in one hair segment from child 1 at a concentration of 39.3ng/mg. Despite testing hair specimens covering the same time range with reporting limits well below the results reported by Lab C, Labs A and B had no positive findings.

Despite the fact that analytical data was not available for review, the following inconsistencies between the known disposition of analytes in biological matrices and previously reported findings were identified: diazpeam was reported in blood and urine specimens in the absence of nordiazepam or temazepam though the reports indicated these analytes were in the scope of testing; diazepam concentrations in hair were approximately 1,000 to 10,000 times higher than the concentrations reported in women with known dosing regimens of the drug and at least five times higher than those reported in a drug abuser; nordiazepam was reported in the hair despite published studies that indicate that nordiazepam concentration typically exceeds those of diazepam in this matrix; GHB concentrations reported in this case were 76ng/mg–225ng/mg while one reported case of an individual given multiple doses had a maximum hair concentration of 1.66ng/mg; and, methylecgonine levels reported in the hair segments were at least 25x higher than what has been reported in patients who were administered cocaine, and neither cocaine nor benzoylecgonine were detected.

Case 2: A father was accused of exposing two children to phensuximide. Lab D performed testing on a powder found in the home and reported no drugs found. The data was forwarded to a chemical engineer (Dr. X, who concluded that the powder was 88% phensuximide. Subsequently, urine specimens were collected from the children and analyzed by the chemical engineer at Lab E, who indicated in a deposition that the urine samples contained succinimide and phenol, which he concluded were metabolites of phensuximide, proving exposure.

The data from Lab D and multiple reports, letters, and the deposition of Dr. X were provided to Lab A for review; no data was made available from Lab E. Based on the review of the available data and the deposition of Dr. X, it appeared as if Dr. X concluded that a large peak in Lab D's data was phensuximide based on the results of Lab D's in-house library search, which identified the peak as phensuximide with a match factor of 50; however, comparison of the spectrum of the unknown to the spectrum of phensuximide proved that this conclusion was not valid. Additionally, there is no literature or metabolic pathways that support the conclusion that succinimide or phenol are metabolites of phensuximide. A review of the data and comparison to known mass spectra led to the preliminary conclusion that the large unknown peak from the brown powder that smelled like cinnamon was cinnamaldehyde and none of the testing performed provided any evidence of phensuximide exposure.

Child Custody, Hair Testing, Jurisprudence

K52 A Segmental Analysis of Endogenous Gamma-Hydroxybutyric (GHB) Acid in Human Hair

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After attending this presentation, attendees will be informed about a validated Liquid-Liquid Extraction (LLE) and Liquid Chromatograph/Tandem Mass Spectrometric (LC/MS/MS) method for the detection of GHB in human head hair. The validated method uses multi-point calibration curves and quality control samples. The research presented also investigates segmental analysis of hair samples and inter- and intra-variation among individuals.

This presentation will impact the forensic science community by providing information regarding endogenous GHB concentrations in hair. The research presented will address baseline levels of GHB within and between individuals to determine if a background threshold can be established for an unexposed population. Segmental analysis of hair samples will provide information regarding the variation of endogenous concentrations within an individual over a period of time that corresponds to hair growth rates.

GHB has been used in drug-facilitated crimes and is also a popular recreational drug of abuse. Ingestion of this drug may induce euphoria, amnesia, dizziness, and unconsciousness, depending on dosage. Because GHB is a natural chemical found in humans, it can be difficult to separate naturally occurring levels from levels following ingestion. GHB poses an additional challenge to the forensic community in that it is rapidly excreted by the body. Hair analysis is a good alternative to blood and urine due to the longer detection window available to establish the involvement of drugs in reported crimes. The scientifically accepted mean growth rate of human head hair is 1cm per month, which can be used to estimate the time period of ingestion. This research utilizes segmental hair analysis to determine baseline GHB concentrations among non-GHB users and to evaluate variability along the length of the hair. Knowing how much variation is present within an individual will help determine if an individual can serve as their own control in cases of ingestion.

Before collecting hair samples, a full quantitative validation was performed for the extraction procedure. The parameters assessed were: accuracy, precision, calibration model, carryover, interferences, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), and processed sample stability. Due to the endogenous nature of GHB in hair, ionization suppression/enhancement experiments were not completed. Instead, the study relied on the deuterated GHB to compensate for any suppression or enhancement that may occur. Accuracy and precision were found to be within ±20% at low (1.2ng/mg), medium (4.0ng/mg), and high (9.6ng/mg) concentrations. A linear model was obtained from 0.4ng/mg to 12ng/mg and no carryover was observed in unspiked synthetic hair samples following injections of 12ng/mg or 24ng/mg GHB. No interfering signals (not including background GHB) were observed in hair extracts. The LOD and LOQ of the method were experimentally determined to be 0.4ng/mg. Lastly, extracts were observed to be stable after eight days while being stored at ≤14°C.

To evaluate the baseline GHB concentrations, hair collected from non-GHB users was segmented into 1cm increments based on proximity to the scalp. The segments were washed with organic solvents, cryogenically ground, and digested with sodium hydroxide. After digestion, the samples were neutralized with sulfuric acid and extracted via LLE with ethyl acetate. The extracts were then evaporated to dryness, reconstituted in mobile phase, and filtered for analysis by LC/MS/MS to determine the levels of GHB present. Initial results for 37 non-GHB users reveal an average baseline GHB concentration of 0.90ng/mg, with a minimum of 0.43ng/mg, maximum of 3.49ng/mg, and median of 0.84ng/mg. The average variation within individuals was 13%. Work continues on the processing of additional hair samples from other non-drug users.

GHB, Hair Analysis, LLE

K53 A Wastewater Analysis for Tobacco and Drug Detection in New York City

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After attending this presentation, attendees will understand the utility of wastewater analysis to monitor tobacco and drug exposure in a certain community and will know how to perform the analysis of these types of samples by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

This presentation will impact the forensic science community by demonstrating the applicability of utilizing wastewater analysis by LC/MS/MS to investigate tobacco and drug use in different communities in an urban location.

Recent changes in the drug scene, such as the current issues with prescription opiates and fentanyl, among other substances, have sparked an increased interest in new tools to monitor what drugs are coming onto the market in a faster, more efficient manner than conventional population surveys. Wastewater analysis is an innovative approach to testing the drug consumption in a geographical area by analyzing human excretion products (biomarkers) in wastewater, which is essentially a large urine pool. Wastewater can provide independent, low-cost, reliable, and nearly real-time information. This methodology has not been fully explored in the United States.

A method was developed to determine tobacco (nicotine and cotinine), cocaine (benzoylecognine, cocaethylene, and cocaine), amphetamines (methamphetamine, MDMA, MDA, and amphetamine), opiates (6-monoacetilmorphine, morphine, codeine, oxymorphone, oxycodone, hydromorphone, hydrocodone, fentanyl, norfentanyl, methadone, EDDP), and cannabis (delta-9-tetrahydrocannabinol, 11-nor-9-carboxy-tetrahydrocannabinol, and 11-nor-9-carboxy-tetrahydrocannabinol-glucuronide) biomarkers in 50mL of wastewater. Wastewater samples were filtered, extracted using mixed-mode cation cartridges, and analyzed by LC/MS/MS using positive Electrospray Ionization (ESI). All compounds were analyzed on a Kinetex® C18 column (with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phases) using two different gradients (one for cannabinoids and another for the remaining compounds). Each compound was monitored by two Multiple Reaction Monitoring (MRM) transitions. Method validation included linearity (5ng/L-1,000ng/L for all compounds, except 10-1,000ng/L for tobacco biomarkers), limit of detection (1ng/L-10ng/L) and quantification (5ng/L-10ng/L), imprecision (<20%), accuracy (80%-120%), matrix effect and extraction efficiency, interferences, and auto-sampler stability. This study applied this method to wastewater samples collected from wastewater treatment plants in New York City (The Bronx, Brooklyn, Queens, and Manhattan) throughout one year. Wastewater samples were collected into Environmental Protection Agency (EPA) -certified sample containers and stored at −20°C until analysis.

This study emphasizes the method of analysis, particularly the use of LC/MS/MS in terms of its sensitivity and selectivity, as a means to detect licit and illicit drugs in wastewater samples. It also provides a means by which new drug trends could be tracked by testing wastewater, thus providing real-time results in different boroughs within New York City.

Wastewater, Cannabis, Opiates

K54 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will gain an appreciation for the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 19th Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings will be discussed. As a relative dearth of interpretive information exists involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation or issue-specific concern, including pharmaco-toxicokinetic data and other relevant ancillary information, followed by audience participation to provide interpretive clarity around case-specific impacts of the toxicological findings. This session, attended by various sections of the Academy, allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause of death in children.

Four cases will be presented that highlight the difficulty in assessing the role of toxicants in each case or the lengths to which one must go, in some cases. Richard Harruff, MD, PhD, Carl Schmidt, MD, Thomas Rosano, PhD, and Robert Middleberg, PhD, will be reviewing cases from their years of experience as forensic pathologists and toxicologists, respectively, that highlight the issues and confounders in the pediatric population.

Dr. Harruff will discuss a case involving colchicine. This esoteric substance in relation to the pediatric population has a number of therapeutic uses based on being an inhibitor of mitosis, including one of the primary treatments for gouty arthritis. The case presentation will address potential means of exposure, post-exposure effects, and consequences of colchicine exposure in the pediatric population.

Dr. Schmidt will discuss the case of twins who were administered lidocaine-containing teething gel. The toxicokinetics and toxicodynamics of this commonly used local anesthetic and cardio-active substance will be reviewed in relation to adverse outcomes, especially in the pediatric population. The risks associated with use of teething gels, in particular, will be emphasized.

Dr. Rosano will present a case involving the death of an 18-month-old who came in contact with liquid nicotine intended to be used in electronic cigarettes. This case will highlight how the child came in contact with the material, surrounding factors leading to exposure, the signs and symptoms after exposure, and postmortem toxicological findings. Finally, the outcome of the investigations will be addressed.

Dr. Middleberg will discuss the difficulties associated with assigning manner of death in pediatric cases. Special emphasis will be placed on the issues surrounding child endangerment versus homicide and the difficulties associated with the distinction. An open discussion regarding whether there is ever a way to readily move from child endangerment to homicide in toxicologically related cases will take place.

Pediatric, Postmortem, Toxicology

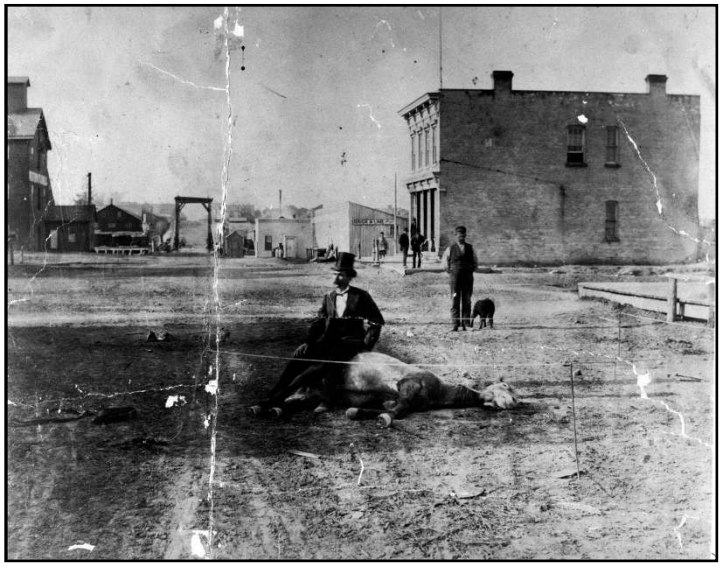
LW1 The Dead Horse Investigation — Forensic Photo Analysis Meets Genealogy

Colleen M. Fitzpatrick, PhD, 18198 Aztec Court, Fountain Valley, CA 92708*

After attending this presentation, attendees will better understand how forensic photo analysis, used in conjunction with genealogical research techniques, has been used to extract important investigative information from a historical "crime scene" photo about which very little prior information was available. Although photo-identification techniques from the forensic community were initially applied to the picture, by combining them with genealogical methods, it was possible to extract much more information from the photo, which could then be integrated into a cohesive narrative. Attendees will learn genealogical photo-identification techniques they may not have been aware of, but that can be used to enhance the investigative power of forensic analysis.

This presentation will impact the forensic science community by demonstrating the synergy that exists between forensic and genealogical investigations. This presentation will broaden understanding of how a seemingly irrelevant detail can be detected by one set of tools and analyzed by another, leading to an appreciation of the complimentary nature of the two disciplines.

The hat, the horse, the man, the scene … the mystery. Who was he and why was he photographed in a top hat and tails sitting on a dead horse in the middle of 8th Avenue in Sheboygan, WI?



When the Sheboygan Dead Horse Picture was published in 2007 by the *Sheboygan Daily Press* newspaper, numerous theories were advanced to explain the bizarre scene. Perhaps the image depicts the aftermath of the tornado that struck Sheboygan while a horse show was in town in 1901. Maybe the owner of a Sheboygan tannery is pictured staking his claim to the hide after someone's horse died in the street. No one seemed able to offer a satisfactory explanation.

Using a combination of forensic and genealogical photo-analysis techniques, much information concerning the time and place the photo was taken has been derived. Based on the type of lens used to take the picture and the presence or absence of certain shadows, it was possible to determine an earliest and latest year for the photo. The appearance of an unusual sundial in the picture provided the exact time of day and the day of the year. Assuming the streets were deserted because the photo was taken on a Sunday, the weather played a crucial role in further narrowing the year to only two possibilities.

Combined forensic and genealogical analysis indicates the picture was taken at 4:52 p.m. on August 10, 1873 or 1879.

Neither the man nor the horse have been identified. No arrests have been made in the case.

Forensic, Photograph, Analysis

LW2 Three Complementary Analyses of Ansel Adams' *Moonrise, Hernandez, New Mexico*

Roderick T. Kennedy, JD, PO Box 7041, Albuquerque, NM 87194-7041*

The goal of this presentation is to illustrate an interdisciplinary investigation of an iconic photograph to demonstrate a deeper context and meaning of the image.

This presentation will impact the forensic science community by demonstrating a greater appreciation for alternative methods of viewing photographic evidence, revealing layers of interpretation not previously contemplated, either by its creator or subsequent observers.

Ansel Adams created his most popular and iconic photograph with the single click of a shutter. *Moonrise Over Hernandez, New Mexico* was taken in late 1941, although it likely did not see the light of day until 1943 when it was published in the *U.S. Camera 1943* annual, preceding a 1944 show at the Museum of Modern Art, where it was exhibited. Ian Jeffrey, curator of the Phaidon Press' *The Photography Book*, said this about the work: "In this, one of the most epic of Adams' landscapes, humanity is signaled by a field of scattered crosses in the near foreground. The settlement itself makes an irregular diminishing rhythm from left to right, in contrast to the flowing horizontals of the mountain range and the swift, painterly markings in the sky. The whole of this musicality is related to the imperceptible slowness of the moon rising."[1]

This photograph is the subject of many stories, from Adams' jumping out of a car on Route 84 to capture the last moments of fading daylight, with or without his new-fangled light meter, or whether the photography was really captured at f32 with a one-second exposure.[2] At any rate, the hurried roadside process resulted in only a negative being created, and Adams did not record the exact date and time the picture was created.

While not discounting Jeffrey's poetic license about the musicality of the photograph's composition (Adams had studied piano), the legend of the image has created a miasma around the possible facts of its creation, all of which can be subject to further investigation and interpretation using different modalities, which will be discussed during this presentation.

First, the collecting of first-hand accounts, both near-contemporaneous and later, of how the photograph was captured by Ansel Adams will be presented. Hopefully, historical consensus can provide both depth and breadth to the circumstances of Adams' actions, including using the new technology of hand-held light meters.

Second, as the image gained in popularity, the question of just when it had been taken took on some importance. Two astronomers have pegged the date and time variously on October 31, 1941, and November 1, 1941. The calculations and celestial tables may track the moon; however, history suggests both the movement of the road after 1941 and the fact that Adams may have taken the photograph from the top of his station wagon.[3] Scientific measurements frequently depend on contextual information; perhaps the answers emanating from the High Altitude Observatory in Boulder, CO, and *Sky and Telescope* magazine will be found wanting for lack of an adequate background.

Finally, all of these discussions are divorced from the cultural aspects of the photograph, which contains the depth and breadth of human experience to fulfill the promise of the detailed image. The crosses and gravestones in the cemetery alit in the setting sun, the houses behind, the woods, the rivers, and the churches in the picture all tell their story without which a full understanding of *Moonrise Over Hernandez, New Mexico* may not be clear to the viewer.

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Photography, Anthropology, Astronomy

LW3 A Review of Changing Crime Patterns and the Development of Forensic Science in Ireland

Sheila Willis, PhD, Forensic Science Ireland, Garda HQ, Phoenix Park, Dublin, IRELAND*

After attending this presentation, attendees will better understand how various techniques became acceptable and which techniques suited particular cases.

This presentation will impact the forensic science community by presenting the changes in the use of explosives, the variation in drugs abused, and the increased capability developed due to the availability of DNA.

This presentation reviews some case histories and uses them to outline the changing patterns in crime and the developments in forensic science from 1979 to 2017.

Following this presentation, attendees will appreciate the manner in which forensic science developed in one Common Law country and will have received an overview of the way in which crime pattern changes prompted changes in the management and resource allocation of forensic science services.

The forensic science service consisted of 5 people in 1979 and had grown to more than 100 in 2017. During this period, the nature of organized crime changed from one in which subversive crime and ordinary crime were quite separate to one in which the boundaries are more difficult to decipher. The rapid increase in drug abuse in the early 1980s began with the abuse of heroin in the poorer sections of the inner city to the situation today in which the full range of drugs are abused across all sections of the population countrywide. The ease of availability of drugs caused a drop in the number of armed bank robberies, as did improvements in bank security. Until the 2000s, the challenge for forensic science was quantity, but this changed with the so-called "head shop" phenomenon. The analytical challenges of dealing with new psychoactive substances are particularly focused on the acquisition of suitable reference material.

The "Troubles" in Northern Ireland affected crime patterns in the Republic of Ireland, and this presentation will trace the changing use of explosives throughout the period. While the Good Friday Agreement put an end to most of the subversive crime, the effect continues to this day with a more widely spread use of explosives within the criminal world than previously experienced. The nature and type of explosives changed according to availability of materials and the challenge for the forensic science community was a lack of information in a pre-internet era.

Over time (from the mid-1990s on), as with most laboratories, resources were diverted from particulate trace evidence to DNA. The focus in the introduction of DNA tended to be on the technology; thus, the lessons from traditional trace evidence did not necessarily transfer to the DNA area. High successes using this technology masked this issue for many years.

Historical, Case Histories, Technique Development

LW4 The 2014 Killing of Five Babies in Oulu, Finland, and the Neonatal Line (NNL) Investigation as the Definitive Bottom Line

Vivian Visnapuu, University of Turku, Turku, WA 20520, FINLAND*

After attending this presentation, attendees will better understand the NNL investigative method, which can be used in newborn homicide cases.

This presentation will impact the forensic science community by informing attendees of the NNL investigative method that can be applied to newborn homicide cases.

Background: Police arrested a 35-year-old woman after discovering five decomposed human fetuses in a basement cabinet of her apartment in Oulu, Finland, in 2014. The woman said she thought all the babies had been born dead. The woman had put the remains of her five newborn infants in buckets and plastic bags between 2005 and 2013 and transported them from place to place. The police began a forensic investigation immediately. The remains were so damaged that a forensic pathological investigation was infeasible, so two independent experts conducted osteological investigations. A forensic odontological investigation was conducted, as was an NNL investigation, which was the crux of this case. Also, a psychiatric assessment of the woman was administered pursuant to an order of the District Court of Oulu.

Material and methods: The delivery-related NNL appears in the enamel of primary teeth and first permanent molars at birth and is a marker of the live birth process.[1-4] It varies in width and location, is different in each deciduous tooth type, and is indicative of gestation time.[5,6] It is unclear what triggers determine NNL at birth. NNL separates pre- and postnatal enamel and dentin and varies in location in different tooth types. Four primary teeth from each fetus were investigated by histological methods and light microscopy.[6,7] The teeth were preserved in 100% alcohol. Samples were run through a rising alcohol series (70%, 90%, and 100%), embedded in resin (Technovit1®/alcohol 50%/50%), and in 100% Technovit® twice. Sections of 20 microns were made by buccal-lingual/palatial and axial cutting and, when no wear appeared, through incisal middle and cusp tip/underlying dentin horn.[7,8]

Results: All the infants' gestational ages were 38-40 weeks per the results of odontological investigation and 38 weeks per the results of osteological investigation.[1,5] NNL was observable in all infants' cases and at least 1-4 days of enamel formation after NNL was observable in each investigated primary tooth.[5] This means that all the infants were born alive and all lived 1-4 days; however, the cause of death was not possible to investigate because the remains were so badly damaged.

The conclusion of the psychiatric assessment was that the woman was criminally responsible. After the psychiatric assessment, the judgment of the District Court of Oulu was a verdict of five counts of murder and five counts of violence of grave peace; a life sentence was imposed. The defendant was not content with the judgement and appealed to the Rovaniemi Court of Appeal, which commuted the sentence to five counts of manslaughter and a 12-year sentence. The Oulu District Prosecutor was not satisfied and appealed to the Supreme Court of Finland in August 2016.

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Neonatal Line, Primary Tooth, Killing Babies

LW5 A Forensic Examination of 19th-Century Archaeological Remains

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The goal of this presentation is to educate attendees on the importance of forensic information in determining the identity of archaeological remains.

This presentation will impact the forensic science community by demonstrating the power of forensic examinations by multiple disciplines in narrowing the field of possible identities of unknown individuals.

In March of 2012, construction workers accidently uncovered an unmarked grave during a renovation project at a private home in Deadwood, SD. The remains were contained within a wooden casket that was damaged by a backhoe during excavation of the reconstruction site. Because this site was part of Deadwood's first cemetery, state archivists and Deadwood Historic Preservation officials were on hand and exhumed the burial. After the exhumation of the remains, officials discovered the skeleton was nearly entirely intact. Subsequent examination of the dentition revealed many unusual features for someone from the 1870s. Preservation officials and archivists did not know the identity of this individual and decided to undertake a forensic examination of the remains in an attempt to identify this person.

Initially, the remains were sent to a forensic anthropologist, who determined that the individual was a White male who was in his late teens to early twenties at the time of death. Additionally, the forensic anthropologic analysis determined this individual was 5'6" to 5'11" in height and the skeleton revealed no indication of violent trauma. Subsequently, there were additional forensic examinations, including odontology, DNA, and elemental and isotopic analyses. Several of the forensic analyses supported and/or corroborated other forensic findings concerning the possible identity of the individual and where he lived during his lifetime.

Although this person has not been identified, the list of possible identities was initially narrowed using death records of individuals that fit the ancestry, age range, height range, and non-violent death circumstances. Subsequently, the list was narrowed based on the likely origin of distinctive, expensive dental restorations. Other dental evidence pointed to a different location later in life, but before he died in Deadwood. This information was corroborated by isotopic analyses. DNA analyses were able to extract a full profile from the skeleton and teeth. Additionally, Single Nucleotide Polymorphism (SNP) analysis was able to determine that this individual was not only a White male, but also that he was likely from Western Europe, specifically the British Isles, and had red hair and brown eyes. As a result of this inquiry, officials in South Dakota decided to produce a documentary that outlined the conclusions of the investigation. In May of 2017, South Dakota Public Broadcasting released the documentary *Deadwood Pioneer: A Face From the Past*. This presentation will discuss the forensic conclusions drawn from this investigation and what remains to be accomplished to identify this 1870's "Deadwood Pioneer."

Archaeological, Exhumation, Identification

LW6 The Dark Side of the Show: Investigating Mysterious Aspects of Traditional American Sideshows

Matteo Borrini, PhD, Liverpool John Moores University, School of Natural Science & Psychology, Byrom Street, Liverpool, AE L3 3AF, UNITED KINGDOM*

After attending this presentation, attendees will appreciate the possible contribution that forensic science can provide to understanding apparently mysterious aspects of traditional American sideshows.

The presentation will impact the forensic science community by providing examples of different disciplines applied to cold cases and old pieces of evidence. This presentation will also suggest a new method of presenting science to young generations of students based on traditional American sideshows, offering the opportunity for reflection on ethical issues related to the use of human samples.

In the American tradition, a sideshow is a secondary production associated with a circus or a fair. These attractions entertained the audience by exhibiting living "human oddities" (a "freak" show) or unusual animals, stuffed abnormal creatures, and exotic paraphernalia (a museum show).

Some of the most popular living performances (working acts) exhibited fire eating, sword swallowing, and lying on a bed of nails. These stunts can be used as interesting examples of physical and physiological principles that can be appreciated by modern generations of students. These attractive cases are not only appropriate for a better understanding of scientific aspects, but also help people preserve part of the American tradition.

An example of how it is possible to use one of the brightest stars of the vaudeville stage to understand physics concepts is represented by Annie May Abbott. During her performance, the small lady, known as the "Little Georgia Magnet," was able to resist the combined efforts of four men to move her while she stood on one foot. She also could lift all four men while they were on a chair by simply touching the chair. The application of balance, leverage, and force vectors to anatomical knowledge help solve the source of this apparently inexplicable strength.

If the living stunts can be only analyzed through the contemporary newspapers and pictures, some of the most unusual specimens from museum shows are still available in public and private collections. An example is the human mummy Sylvester (still present in the Ye Olde Curiosity Shop in Seattle, WA), who recently underwent a virtual autopsy to discover his origin and identity.

Furthermore, the history of sideshow abounds with real and fake mummies. Fiji mermaids and alleged human shrunken heads were a must-have for any impresario of a "freak" museum. The luckiest ones, such as Frank Hansen, a Minnesota showman who claimed ownership of a frozen Bigfoot-like creature, were able to present a giant hairy man preserved in ice.

Two mummies that have a strong link to the forensic sciences belonged to Elmer McCurdy and Julia Pastrana. Elmer, a thief, was killed in a shoot-out with police and his mummified body traveled for 40 years throughout the United States as a sideshow attraction. The body then vanished without a trace until it was discovered in The Pike amusement zone in California and identified via a forensic investigation.

Julia Pastrana, a woman affected by hypertrichosis and gingival hyperplasia, was employed as a living attraction in a "freak" show and was advertised as a hybrid between human and an ape. Married to her manager, Theodore Lent, she had a baby with the same pathological condition. During a European tour, they both died a few days after childbirth and their bodies were mummified by the impresario to be displayed in a glass cabinet. After the show ended, Julia's body went through several difficulties, was stolen and recovered, and then stored at the Oslo Forensic Institute. In 2013, it was finally buried at her birthplace in Mexico.

In conclusion, the story of Julia Pastrana, as well as the decline of the human oddities shows and the legal actions that forbid this attraction, offers the possibility of evaluating the modern approach to rare and disfiguring pathological conditions. These ethical considerations currently become more important, considering the recent revival of reality television shows on the topic.

This review of some of the most iconic and mysterious aspects of the traditional American sideshows is an opportunity to apply forensic science to cold cases and to teach science in a different and more attractive way, with a stronger ethical attention to human remains.

Cold Cases, Education, Forensic Anthropology

LW7 The Phoenix Canal Murders + Forensic Genealogy = Solved!

Colleen M. Fitzpatrick, PhD, 18198 Aztec Court, Fountain Valley, CA 92708*

After attending this presentation, attendees will understand how Y-chromosome DNA (Y-DNA) analysis methods borrowed from the genetic genealogy community led to an arrest in a 25-year-old serial killer case when all other investigative methods had failed. Although the techniques described have been used successfully by the genetic genealogy community for many years for surname studies, they are relatively new to law enforcement. Attendees will better understand a novel identification technique they may not yet be aware of, but that has the potential for immediate use by the forensic community for solving cold cases and identifying John Does in the absence of a Combined DNA Index System (CODIS) hit.

This presentation will impact the forensic science community by demonstrating the successful resolution of a cold case through the use of techniques borrowed from genetic genealogy. This presentation will broaden the understanding of various types of metadata that can be extracted about an unknown perpetrator from a match between his Y-DNA profile and public genetic genealogy databases, leading to an appreciation of genealogical Y-chromosomal Short Tandem Repeat (Y-STR) testing as an alternative source of generating investigative leads for cold cases.

One evening in 1991, Angela Brosso went for a bicycle ride along the Arizona Canal in Phoenix, AZ. She never returned home. Her body was found nearby in a vacant lot a few days later. Melanie Bernas went for a bicycle ride along the same canal in early 1992. She never returned either. Her body was found floating in the canal a few days later. The Canal Murders were apparently random killings in which the perpetrator had no connection to his victims, limiting the effectiveness of conventional investigative techniques. Over the years, numerous suspects were investigated, but with no success. There was no CODIS hit to the DNA from the crime scenes.

The Phoenix Canal Murders remained unsolved until 2014, when the Phoenix Police Department asked for the comparison of the Y-DNA collected from the crimes to genetic genealogy Y-STR profiles posted on public websites. Using in-house software to interrogate thousands of public genealogical databases, a Y-DNA match was found to a small group of Millers of Irish origin. This narrowed the list of suspects from perhaps 2,000 individuals to just 5. This led the authorities to arrest Brian Patrick Miller, who is to be tried for the murders of both women.

The Phoenix Canal Murders are only one example of how cold case investigations are benefitting from the use of genealogical resources. A match between a forensic Y-profile and a profile found in a genetic genealogy database can advance a cold case investigation in many ways. Perhaps the most interesting is that forensic genealogy, used in conjunction with DNA phenotyping, is making it possible to predict the appearance, ethno-ancestry, and last name of a killer, based on DNA alone.

Included in this presentation are results from other cases that have benefitted by comparisons to the ~300k Y-STR profiles posted online by the genealogy community; these results are based on surprising leads generated for ethnicity, nationality, and geographic origins, even in the absence of a surname match.

Phoenix, Canal, Genealogy

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C

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D

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Ruben Dario Diaz-Martin, PhD - E87
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Elizabeth A. DiGangi, PhD - A51
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Alessandro Di Luca, MD - H66, H134
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Aldo Di Nunzio - H43, H44
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Ciro Di Nunzio, MFS, PhD - H12, H43, H44, H45
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Giancarlo Di Vella, MD, PhD - E1, E2, E69, G1, I6
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Leslie Ethan Dodson, BA - I29
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Stephanie Domitrovich, JD, PhD - W24, D28, F3, F16, F19
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Meaghan C. Dougher, BA - B130
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Liotta N. Dowdy, MA - A153
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James Downs, MD - F4, W22
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Steven L. Downs, MFS - W03
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Derek M. Draft, DDS - G24
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Gwenola Drogou, DDS - G46
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Nicholas L. Drury, BSc - K19
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Aurora Dumitra, MS - J9
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Rhian Dunn, BA - A138
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R. Gregg Dwyer, MD, EdD - I7, I22
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E

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Discloses no financial relationships with commercial entities.
Melanie Eckberg, MSFS - K35
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Heather J.H. Edgar, PhD - E33
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Erwin Van Eijk, MS - W24
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Natasha K. Eklund, BA - B133
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Angeline Eliasson, MD - E32
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Kelly M. Elkins, PhD - B46, B47, B114
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Patrick A. Eller, MS - C3
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Synthesized SERS swabs (Discussion of Unlabeled/ Investigational Use of Product/Device) - B78
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F

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Pamela A. Ferreira, MD - H83
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Jillian C. Fesolovich - B143, B163
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G

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H

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L

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Discloses no financial relationships with commercial entities.
Caitlin E. Porterfield, MS - W08
Discloses no financial relationships with commercial entities.
Deborah L. Powers, BA - K25
Discloses no financial relationships with commercial entities.
Mark C. Pozzi, MS - D15, D19, D20, D22, D25
Discloses no financial relationships with commercial entities.
Joseph A. Prahlow, MD - H73, S2
Discloses no financial relationships with commercial entities.
Sebastien S. Prat, MD - I38
Discloses no financial relationships with commercial entities.
Dragan Primorac, MD, PhD - S2
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Mechthild K. Prinz, PhD - B145
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Q

Da Qin, PhD - J15
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R

Roberto Raffaele - C30, E41, H9, H10, H11, H13, H14
Discloses no financial relationships with commercial entities.
Mithun Rajshekar, MFSc - G25
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Kelli B. Raley, MSFS - B7
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Donald J. Ramsell, JD - F22
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Anjali A. Ranadive, JD - W22
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Sara Raponi, JD - F29
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Lionel Raymon, PhD - W20
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Linda Razzano, MS - W17
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Matthew F. Redle, JD - S1
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Sarah Davis Redman, PhD - E104
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Brittany N. Reed, BSc - H81
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Erin C. Reed, JD - W05
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John A. Reffner, PhD - B150
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Pamela Reid, PhD - W10
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Gary W. Reinecke, MA - E82
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Robin C. Reineke, PhD - A92
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Jenise Reyes-Rodriguez, BS - C7
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Agathe J.G. Ribereau-Gayon, MSc - A86
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Sarah M. Richer, PhD - A135
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Michelle Richmond, BA - E1
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Michael F. Rieders, PhD - BS5
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Paulina Riess, MD - I19
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Anders Rietz - H48
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Meaghan Ringel, BS - K32
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Leann G. Rizor, BS - B181
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Zackery Roberson - B170
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Graham J. Roberts, MDS - G26
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C. Andrew Robinson, Jr., PhD - K3
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Thomas Rodriguez, MD - I37
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Sandra E. Rodriguez-Cruz, PhD - B184
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Amanda L. Roe, PhD - E43
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Marcus Rogers, PhD - C25, S2
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Madeline G. Roman, BS - B49
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Jeri D. Ropero-Miller, PhD - BS5
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Roberto Rosa, PhD - B53
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Roberto Rosa, PhD - B52
Discloses no financial relationships with commercial entities.
Thomas G. Rosano, PhD - K54
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Karen B. Rosenbaum, MD - I36, S1
Discloses no financial relationships with commercial entities.
Ann H. Ross, PhD - A131
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Katie M. Rubin, MS - A32
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Norah Rudin, PhD - B103
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Katarina G. Ruehl - B56, B118
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Eric R. Ruiz Hernandez, MD - E66, E72
Discloses no financial relationships with commercial entities.
Catherine G. Rushton, EdD - E110, W08
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S

Kenneth J. Saczalski, PhD - D20, D22, D25
Discloses no financial relationships with commercial entities.
Lydie Samie - B34
Discloses no financial relationships with commercial entities.
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Jana Andrea D.S. Santos, BA - A5
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Tiffany B. Saul, PhD - A149
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Vincent J. Sava, MA - A79
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Madison R. Schackmuth, BS - K11
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Audrey D. Schaefer, BA - A4
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Carl J. Schmidt, MD - K54, W09
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Christopher W. Schmidt, PhD - W11
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Candace H. Schoppe, MD - W15
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Willem A. Schreuder, PhD - D29
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Jason L. Schroeder, MS, MBA - B194
Discloses no financial relationships with commercial entities.
Cassidy M. Schultheis - B196
Discloses no financial relationships with commercial entities.
Leah M. Schuppener, DO - H21
Discloses no financial relationships with commercial entities.
Andrew J. Schweighardt, PhD - B36
Discloses no financial relationships with commercial entities.
Wolf Schweitzer
Barwig, Conrad Electronic Sorin, Stoeckert Shiley (Discussion of Unlabeled/Investigational Use of Product/Device). - H61
Discloses no financial relationships with commercial entities. - H67
Piper Schwenke, MS - E28
Discloses no financial relationships with commercial entities.
Sarah Schwing, BA, BS - A61
Discloses no financial relationships with commercial entities.
Veronica Scotti, LLM - F8
Discloses no financial relationships with commercial entities.
Jan Seaman Kelly, BA - J11
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Ismail M. Sebetan, MD, PhD - E8, F28, I14
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Jagmahender Singh Sehrawat, PhD - A147
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Andrew C. Seidel, MA - A2
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Kathryn C. Seigfried-Spellar, PhD - C25
Discloses no financial relationships with commercial entities.
Francesco Sessa, MS - E13
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Puneet Setia, MD - H93
Discloses no financial relationships with commercial entities.
Amanda L. Setser, BS - B25
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Kelsey E. Seyfang, BSc - B61, B195
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Valerie Sgheiza, BS - A102
Discloses no financial relationships with commercial entities.
Nathan S. Shaller, MD - H38
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Brandy Shattuck, MD - H85
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Melvin Shaw, MS - J9
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Donald E. Shelton, JD, PhD - F30
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Kevin E. Sheridan, PhD - A51
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Iris L. Shields, DDS - G39
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Jessica Shiffert, BA - E88
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Shelly Y. Shih, MS - B73
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Michael E. Sigman, PhD - B154
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Ricardo H.A. Silva, PhD - E106
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Susan Sincerbox, BS - A19
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Rachel S. Singer, JD - L1
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Michael Singleton, PhD - D21
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Nadine Smit - E64
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Amber J. Smith, BA - B119
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Angeline Smith - A120
Discloses no financial relationships with commercial entities.
Jeff M. Smith, MS - C4
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Tore T. Solheim - G6
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Laure Spake, MA - A14
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Discloses no financial relationships with commercial entities.
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Michala K. Stock, MA - A65
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Kyra E. Stull, PhD - A73
Discloses no financial relationships with commercial entities.
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Nicole Sussman, MD - I20
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Edward M. Suzuki, PhD - W04
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.

T

Sabrina C. Ta'ala, MA - A7
Discloses no financial relationships with commercial entities.
Elham Tabassi, MS - E58
Discloses no financial relationships with commercial entities.
Mohammad A. Tahir, PhD - J1, J3
Discloses no financial relationships with commercial entities.
Chikako Takei - B65
Discloses no financial relationships with commercial entities.
Sean D. Tallman, PhD - A40
Discloses no financial relationships with commercial entities.
Tobin A. Tanaka, BS - J4
Discloses no financial relationships with commercial entities.
Braden E. Taylor - E86
Discloses no financial relationships with commercial entities.
Melissa K. Taylor, BA - L2
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Riya Thekdi, BA - B39
Discloses no financial relationships with commercial entities.
Jennifer Thomas, PhD - K52
Discloses no financial relationships with commercial entities.
Christopher R. Thompson, MD - S1
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Robert M. Thompson, MFS - B98
Discloses no financial relationships with commercial entities.
Drake Thrasher - H100
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Cristina Tica, MA - E36
Discloses no financial relationships with commercial entities.
Lauren Todd, BS - B64
Discloses no financial relationships with commercial entities.
Joshua M. Toman, LLM - W24, D31
Discloses no financial relationships with commercial entities.
Jeffery K. Tomberlin, PhD - E76, W24
Discloses no financial relationships with commercial entities.
Steve Toupenay, DDS - G12, G13, G42
Discloses no financial relationships with commercial entities.
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Brittany M. Trapp, BA - A137
Discloses no financial relationships with commercial entities.
Kristy Tredway - C31
Discloses no financial relationships with commercial entities.
An Truong, BS - B175
Discloses no financial relationships with commercial entities.
Nilesh K. Tumram, MD - H87, H88, H89, H90
Discloses no financial relationships with commercial entities.

U

Douglas H. Ubelaker, PhD - W11
Discloses no financial relationships with commercial entities.
Samantha Upton, BA - A106
Discloses no financial relationships with commercial entities.

Tugba Ünsal, PhD - E77
Discloses no financial relationships with commercial entities.

V

Michele Vaira, JD - F31
Discloses no financial relationships with commercial entities.
Lorena Valencia Caballero, PhD - E87
Discloses no financial relationships with commercial entities.
Julie L. Valentine, PhD - E67
Discloses no financial relationships with commercial entities.
Jesus R. Valenzuela, BA - C5
Discloses no financial relationships with commercial entities.
Anne Marie R. Valera, BA - A5
Discloses no financial relationships with commercial entities.
Joshua Vandeburgh - H75
Discloses no financial relationships with commercial entities.
Giswinne van de Wijdeven, MS - A5
Discloses no financial relationships with commercial entities.
Oluseyi A. Vanderpuye, PhD - E89
Discloses no financial relationships with commercial entities.
Wesley Vandiver - D16
Discloses no financial relationships with commercial entities.
Michael L. VanErp, JD - W09
Discloses no financial relationships with commercial entities.
Katrina Van Pelt, DO - H18
Discloses no financial relationships with commercial entities.
Eduard Van Zalen, MSc - W24
Discloses no financial relationships with commercial entities.
Thomas W. Vastrick, BS - J12, S2
Discloses no financial relationships with commercial entities.
Mark Vecellio, MFS - W03
Discloses no financial relationships with commercial entities.
Jessica Ann Veltri, MS - W18
Discloses no financial relationships with commercial entities.
Elvira Ventura Spagnolo, MD - F12, H2, K7
Discloses no financial relationships with commercial entities.
Nicole Marie C. Vesagas, BA - A5
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Vivian Visnapuu - LW4
Discloses no financial relationships with commercial entities.
Silvia D. Visona, MD - H97, H112
Discloses no financial relationships with commercial entities.
Eleanor B. Vo, MD - I11
Discloses no financial relationships with commercial entities.
Caitlin C.M. Vogelsberg, MS - A95
Discloses no financial relationships with commercial entities.
Laura Volpini, PhD - I32
Discloses no financial relationships with commercial entities.

W

Nickolas P. Walker - B142
Discloses no financial relationships with commercial entities.
Mackenzie Walls - A6
Discloses no financial relationships with commercial entities.
Brian J. Walsh, JD - F15
Discloses no financial relationships with commercial entities.
Erin E. Walsh, MS - K47
Discloses no financial relationships with commercial entities.
Heather E. Waltke, MS - E65
Discloses no financial relationships with commercial entities.
Ling Wang, MS - B26
Discloses no financial relationships with commercial entities.
Jane Wankmiller, PhD - A81
Discloses no financial relationships with commercial entities.
Parris Ward, JD - C10, E73
Discloses no financial relationships with commercial entities.
Eric T. Washington, DDS - G14
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Paul Wax, MD - W20
Discloses no financial relationships with commercial entities.
Lauren Weidner, PhD - H28
Discloses no financial relationships with commercial entities.
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Kurt D. Weiss, MS - D8, D14
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Joseph Levi White, MS - C3, C18, C21
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Mackenzie E. Whiting - B3
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Gary Whitman, BS - D27
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Timothy Wiegand, MD - W20
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Emily F. Wiegers, MA - E78
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Carl Wigren, MD - W02
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Emily J. Will, MA - L2
Discloses no financial relationships with commercial entities.
Andrew S. Williams, MD - H35, W20
Discloses no financial relationships with commercial entities.
John A. Williams, PhD - E109, W08
Discloses no financial relationships with commercial entities.
Karl E. Williams, MD - B81
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Mary R. Williams, MS - B128
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Sheila Willis, PhD - B82, F35, LW3, S2
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Lori J. Wilson, PhD - E75
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Teresa V. Wilson, PhD - A141
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Rebecca J. Wilson-Taylor, PhD - A50
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Melissa Wils-Owens, BS - H79
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Allysha P. Winburn, PhD - A72
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Mark Windschitl, PhD - W08
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Carl Johan Wingren, PhD - E32
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Emily Wolak, DO - H137
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David A. Wold, DDS - E99
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Matthew R. Wood, PhD - W08
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Robert E. Wood, DDS, PhD - G4, G5
Discloses no financial relationships with commercial entities.
Charlotte J. Word, PhD - F17
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Erin M. Worrell, BSc - B81, E49, W20
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Travis J. Worst, PhD - B27
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Thomas Wortman, BS - B95
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Jessica Wright - C1
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Alan H. Wu, PhD - W14
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Richard T. Wyant, MS - B99
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Timothy Wysozan, BS - H102
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Y

Nicole A. Yarid - H47, H86, H124, W20
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Hatice Yilmaz, BSc - F1, F2
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Jessica Yopak, BA - A125
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Carmen Young - B8
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John L. Young, MD - I9
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Jorn Chi-Chung Yu, PhD - B127
Discloses no financial relationships with commercial entities.
Nandar Yukyi, MA - A63
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Laura Yurka, MA - A78
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Z

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Xiang Zhang, MD - H22, H23
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Joel A. Zlotnick, MSFS - J7, J8, W06
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Walter F. Zoller, DMD - G38
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Key Word Index – 2018

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Key Word Index – 2018

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Presenting Author Index – 2018

The presenting author index can provide a quick reference to find when and in what section presenting authors are scheduled to present at the 2018 Annual Scientific Meeting. The reference table below assists you in finding the section in which the presentation will be given. Letters correspond to the scientific discipline/section in which the presentation is being made while the number corresponds to the numerical sequence of the presentation within the section.

A	Anthropology	J	Questioned Documents
B	Criminalistics	K	Toxicology
C	Digital & Multimedia Sciences	LW	Last Word Society
D	Engineering Sciences	BS	Breakfast Seminar
E	General	ES	Evening Session
F	Jurisprudence	L	Luncheon
G	Odontology	S	Special Session
H	Pathology/Biology	W	Workshop
I	Psychiatry & Behavioral Science		

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