Title

Effective Use of Mass Spectrometry in the Clinical Laboratory.

Journal

Clinical chemistry, 62(1)

ISSN

0009-9147

Authors

Jannetto, Paul J

Fitzgerald, Robert L

Publication Date

2016

DOI

10.1373/clinchem.2015.248146

Peer reviewed

Clinical Chemistry 62:1
92–98 (2016)

Reviews

Effective Use of Mass Spectrometry in the Clinical Laboratory

Paul J. Jannetto[1*] and Robert L. Fitzgerald[2]

BACKGROUND: Historically the success of mass spectrometry in the clinical laboratory has focused on drugs of abuse confirmations, newborn screening, and steroid analysis. Clinical applications of mass spectrometry continue to expand, and mass spectrometry is now being used in almost all areas of laboratory medicine.

CONTENT: A brief background of the evolution of mass spectrometry in the clinical laboratory is provided with a discussion of future applications. Prominent examples of mass spectrometry are covered to illustrate how it has improved the practice of medicine and enabled physicians to provide better patient care. With increasing economic pressures and decreasing laboratory test reimbursement, mass spectrometry testing has been shown to provide cost-effective solutions. In addition to pointing out the numerous benefits, the challenges of implementing mass spectrometry in the clinical laboratory are also covered.

SUMMARY: Mass spectrometry continues to play a prominent role in the field of laboratory medicine. The advancement of this technology along with the development of new applications will only accelerate the incorporation of mass spectrometry into more areas of medicine.

Mass spectrometry (MS)[3] provides unique capabilities in the clinical laboratory and is rapidly transitioning from specialized testing to being broadly applied. Historically, major impacts of MS include confirmation of immunoassay-positive drug screens *(1)*, identification of inborn errors of metabolism *(2)*, and analysis of steroid hormones *(3)*. More recently, MS has dramatically improved the time required for microbial identifications *(4)*. This evolution, driven by continuous improvements in analytical platforms, is anchored by the analytical specificity of MS. Conclusive identification of molecules that range in size from tens of daltons (small molecules) to hundreds of thousands of daltons (biomolecules) is based on different principles. For example, small molecules are currently identified using LC-MS/MS. LC-MS/MS identifications are based on several unique characteristics, including retention time, parent ion, and ratios of fragment ions. In the case of newborn screening, samples are directly infused into the mass spectrometer with identifications based on specific transitions of precursor and product ions. In the microbiology laboratory, identifications are based on patterns of ions generated from microbial proteins ionized by laser ablation. In all cases, the analytical specificity of the analysis is based on the ability of a mass spectrometer to "weigh on the molecular scale" by determining the mass-to-charge ratio (*m/z*) of the ions of interest. In the simplest form, MS provides some type of a molecular fingerprint of the analyte of interest. This minireview provides a brief background on the evolution of MS in the clinical laboratory (Fig. 1) and summarizes how MS is being used to improve patient care in a cost-effective manner.

A major impetus that moved MS from the research laboratory to the clinical laboratory was the accident on the aircraft carrier Nimitz. On May 26, 1981, an aircraft crashed while landing on the Nimitz, killing 14 and injuring 45 *(5)*. Subsequent immunoassay tests demonstrated that a large percentage of urine samples from servicemen were positive for marijuana metabolites. This prompted President Reagan to develop a zero tolerance for drugs of abuse in the military *(6)*. Due to a large number of false-positive immunoassay results, antibody-based drug screens began to be considered "presumptive" until confirmed by GC-MS *(7)*. It was clear that the early use of drug testing was effective at reducing the number of positive employee drug test results, because positive rates dropped from 18% to 8% over a 10-year time span *(8)*. Several studies also demonstrated that urine drug testing was cost-effective *(8, 9)*. The requirement for GC-MS confirmation drove the development of MS in toxicology laboratories, where it also began to be used for therapeutic drug monitoring.

As the clinical laboratory became more familiar with GC-MS, the limitation of immunoassays for steroids became evident, especially when measuring low

[1] Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [2] Department of Pathology, University of California-San Diego, San Diego, CA.
* Address correspondence to this author at: Mayo Clinic, Laboratory Medicine and Pathology, 200 First St. SW, Rochester, MN 55905. E-mail jannetto.paul@mayo.edu.
Received August 19, 2015; accepted September 21, 2015.
Previously published online at DOI: 10.1373/clinchem.2015.248146

[3] Nonstandard abbreviations: MS, mass spectrometry; ESI, electrospray ionization; FDA, US Food and Drug Administration; LDT, laboratory-developed test; LIS, laboratory information system.

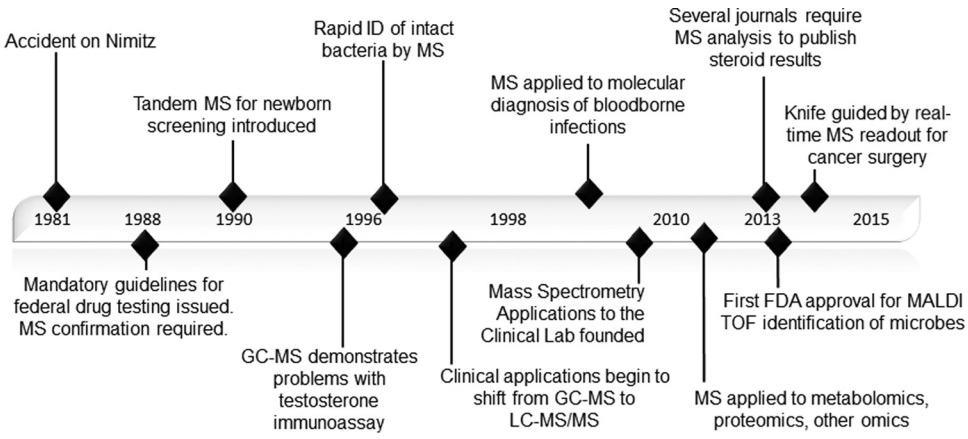

Fig. 1. Approximate timeline of some important events in the evolution of clinical MS.

concentrations of testosterone in women and children *(3, 10)*. A primary limitation of GC-MS was that analytes needed to be volatile and thus most clinical assays required multiple extraction/purification steps along with a chemical derivatization to render the analytes sufficiently volatile for analysis. The extensive sample preparation schemes required for GC-MS analysis limited the widespread application of MS in the clinical laboratory because of low throughput and high cost. Atmospheric pressure ionization techniques such as electrospray ionization (ESI) combined with high-performance LC-MS/MS were the next major analytical improvements that enabled MS as a viable platform for routine clinical laboratories.

ESI LC-MS/MS eliminated the need for volatile analytes and thus helped simplify sample preparation schemes. Simplified sample preparation equates to improved sample throughput and lower costs. GC-MS assays that took a technologist 8 h to prepare 50 samples could now be done by the same technologist at much higher throughput in a couple of hours when using LC-MS/MS. The rate-limiting step for GC-MS analysis has been the sample preparation time, whereas the rate-limiting step in LC-MS/MS typically is the LC run time of the assay. By simplifying sample preparation schemes LC-MS/MS has enabled MS to be a cost-effective analytical tool in the clinical laboratory.

A particularly effective tool for improving throughput of MS has been the development of multiplex assays *(11)*. From an initial capital outlay perspective, the mass spectrometer is the highest-cost component (typically $200 000 to $500 000). By interfacing several (commonly 2–4) LC systems (which cost $50 000 each) into a single MS, the cost-effectiveness of the entire process can be improved several fold. Multiplex LC works best for single-analyte assays for which the injections of the different LC systems are staggered so that the mass spectrometer is always measuring peaks of interest (Fig. 2). It can also be used to analyze different compounds, potentially enabling the technique to be applied in a random access mode as opposed to more traditional batch analysis. The basic principle that makes multiplexing effective is that peaks of interest typically elute over a several second time period during a chromatographic run, which can last several minutes. Without multiplexing, most of the time the mass spectrometer is "waiting" for peaks to elute. By staggering injections, a multiplex with 4 LC systems can increase the productivity of the MS several fold.

In the microbiology laboratory, the development of MALDI combined with TOF mass analyzers allowed for the rapid identification analysis of microbes *(12)*. Before implementation of MALDI-TOF, microbiology laboratories depended on gram stain, culture, biochemical tests, and susceptibility testing. US Food and Drug Administration (FDA)-approved approaches using MALDI-TOF have decreased the mean time to identification by 1.45 days compared with conventional techniques *(13)*. Tan et al. estimated that implementing MALDI-TOF would save more than 50% of the costs of reagents and labor compared with standard culture techniques *(13)*. These authors reported on the laboratory savings, but did not comment on the overall healthcare savings associated with rapid pathogen identification, which likely are substantial. The commercialization of MALDI-TOF continues for clinical microbiology and systems consisting of the MS, software, and databases of microorganisms by some manufacturers (bioMerieux Inc. and Bruker Daltonics Inc.) have been FDA cleared. Although the FDA-cleared list of organisms is not exhaustive and is primarily limited to gram-negative and gram-positive bacteria and yeast, research use–only libraries are also available *(14)*.

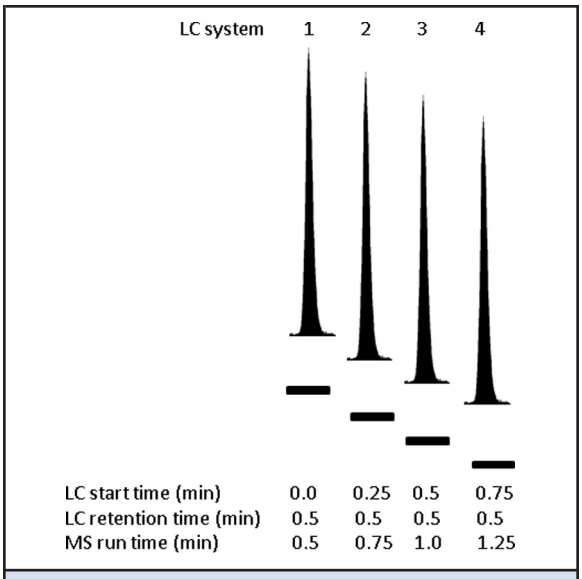

Fig. 2. Multiplex LC-MS system.

In this example, 4 LC systems are connected to one mass spectrometer via a multiposition switching valve. Peaks indicate the compound of interest. Solid bars indicate which LC system is interfaced with the MS in time. For LC 1 the peak of interest elutes at 0.5 min, yet the total LC run is 1.25 min due to factors such as column re-equilibration or injector cycle time. The injection of the LC systems is set so that LC 2 injects 0.25 min after the first sample. After the peak from LC 1 elutes, the valve switches so that LC 2 is now in line with the mass spectrometer. This is repeated for all 4 LC systems. In this fashion, the mass spectrometer is always collecting data when peaks of interest are eluting, increasing the efficiency of the system.

As a result, users may need to build their own mass spectral databases using locally isolated and relevant strains that can then be searched along with the commercial databases. For example, the Mayo Clinic has built a custom MALDI-TOF library that contains 1599 spectral images not covered by available databases *(14)*. However, MALDI-TOF may not be the best technology for all identifications because other technologies, like 16S rRNA gene sequencing, may be better at rapidly identifying tiny or mucoid colonies *(14)*. Furthermore, newer MS methods may also be superior at separating organisms down to subspecies taxonomic levels *(15)*. In addition to MALDI-TOF, other MS-based platforms for the rapid identification of microbes directly from biological samples have been developed. These platforms use ESI to identify PCR products amplified using generic primers *(16)*. While not currently FDA approved, this approach combines molecular specificity with an 8-h time to identification.

Other novel applications of MALDI MS include clinical imaging applications. In pathology research and practice, MALDI imaging has been used to identify proteins, peptides, drugs or metabolites, lipids, and other analytes in tissue *(17)*. Specifically, MALDI imaging has allowed the label-free, multiplex measurement of a wide variety of molecules in tissue sections while preserving the sample for conventional histology staining. This combined approach keeps the spatial localization of the resolved mass spectra, and the software can be used to provide a virtual microdissection of the tissue while visualizing individual molecules or groups of molecules. MALDI imaging studies have already been performed using fresh-frozen, paraffin-embedded, and formalin-fixed tissues *(18)*. A recent MALDI imaging study was able to show *de novo* identification and characterization of phenotypic tumor subpopulations that correlated to survival prognosis without any prior molecular knowledge about the tumor *(17, 19)*. Identification of spatially resolved molecules by direct mass measurement and alignment to databases can be done by high-mass accuracy MALDI imaging technologies. This information may help generate a more complete understanding of diseases and pathophysiology *(20)*.

Metabolomic, lipidomic, and proteomic, as well as other omic analyses of clinical samples using MS, is another exciting area of research that may have broad implications for the clinical laboratory. The goal of these techniques is to capture information on several biomolecules using a targeted approach *(21, 22)* to hundreds or thousands of compounds using an untargeted approach *(23)*. The untargeted approaches are generally used in the discovery phase to compare omic samples from 2 different populations (e.g., healthy and diseased). Once molecules that differentiate the 2 populations have been identified, a targeted approach can be used to further characterize the utility of monitoring changes in the putative biomarkers. One particularly novel approach to metabolomics is the use of stable isotopes to trace metabolic fate of energy sources in diseased and healthy cells *(24)*.

Advantages of MS

MS is slowly transforming the practice of laboratory medicine and is being driven by several factors, including improved analytical specificity and sensitivity. Measurements of testosterone and other sex steroids have been used as examples for which MS is the preferred method. Professional society guidelines (e.g., originally endocrinology and currently urology) requiring the use of MS help propagate this transition *(25, 26)*. The measurement of testosterone in children and adults of both sexes is important in the diagnosis and management of numerous conditions (e.g., infertility, certain cancers, virilization, and polycystic ovary syndrome) *(27, 28)*. Although

manual RIAs were originally used owing to their analytical sensitivity, automated immunoassays soon replaced them for improved throughput and the desire to eliminate radioactivity in the laboratory. However, these immunoassays suffered from analytical specificity problems and a limited dynamic measuring range *(26)*. Traditional immunoassays were shown to significantly overestimate testosterone concentrations in women and slightly underestimate testosterone in men *(3, 10)*. On the other hand, MS-based methods have been shown to have superior analytical specificity and the ability to measure testosterone over a wide concentration range required for children and adults of both sexes *(27)*.

The measurement of thyroglobulin is another clinical example for which MS has been shown to offer superior assay quality compared to traditional immunoassays. Thyroglobulin is used to evaluate the effectiveness of treatment and the reoccurrence of thyroid cancer. However, immunoassays can be affected by antithyroglobulin autoantibodies, resulting in falsely low thyroglobulin measurements *(29)*. Using tryptic digestion with peptide-specific immunocapture, LC-MS/MS quantification of the thyroglobulin-specific peptides is able to overcome the interference of antithyroglobulin autoantibodies *(29, 30)*. The MS-based method is also thought to overcome interferences due to heterophilic antibodies that can cause falsely increased thyroglobulin measurements in immunoassays *(31)*.

Cost reduction is another pressure driving the adoption of MS. Although the initial capital cost of the equipment is high and laboratory expertise in the development, validation, and maintenance of MS-based assays may be limited, it still can be cost-effective for laboratories to develop MS tests to avoid send-out costs on higher-volume tests. In addition, the ability to develop multianalyte panels using a single MS method offers additional time, labor, and expense savings, for which immunosuppressant assays are a great example. Clinical laboratories started to use LC-MS/MS to simultaneously measure cyclosporine A, tacrolimus, sirolimus, and everolimus. The simplified and standardized sample processing of a panel of drugs saves time, reagents, and labor expenses. The analytical specificity of MS-based immunosuppressant assays is also superior to that of existing immunoassays, which can overestimate the concentration of these medications because of the variable cross-reactivity with immunosuppressant metabolites *(32–34)*. Lastly, there is now one FDA-approved MS tacrolimus assay (Waters-MassTrak®) and several commercially available MS kits with IVD (in vitro diagnostic)-CE certification for the measurement of immunosuppressants (Chromsystems-MassTox®, Recipe-ClinMass®, and Waters-MassTrak®), which make MS-based assays more attractive for smaller laboratories with less expertise *(35)*.

Challenges with MS

Although MS continues to make significant contributions to patient care, there are substantial challenges that need to be understood before implementing an MS-based service. These challenges include the high capital cost of equipment, requirements for a skilled labor force, lack of automation, and regulatory uncertainty.

The high capital cost of equipment is straightforward to deal with using routine return on investment calculations. When considering MS, laboratories need to account for instrument costs, labor and training costs, proficiency testing, reagents, supplies, service contracts, and construction/renovation costs that may be required for installation of this type of instrumentation. In addition, the time and expense to support assay development and validation need to be accounted for. Instruments typically deployed in clinical laboratories cost between $200 000 and $500 000 but can be justified by incorporating expensive or high-volume send-out tests into the laboratories' offerings. Send-out tests are a financial loss for laboratories because Medicare prevents laboratories from billing more than what the reference laboratories charge. These send-out tests create a large deficit because most laboratories recoup only about 25% of the billed cost. For example, if your laboratory (laboratory A) sends a test to reference laboratory B, and laboratory B bills you $100, laboratory A is obligated to pay the full fee. Laboratory A then passes this $100 fee on to the patient's insurance company, which has an agreement with laboratory A's hospital that it pays only 25% of fees. This simple transaction costs the hospital $75 each time the test is sent out (not including costs associated with the send-out process). If you offer this same test in house you can bill appropriately and turn this loss into a potential profit. MS also provides the ability to expand the test menu more quickly for novel biomarkers instead of waiting for FDA-approved kits/assays.

Justification of MS can also be achieved by looking at existing in-house tests. Laboratories are always looking for ways to reduce or remove radioactivity (i.e., RIAs) from the laboratory due to safety and regulatory issues. MS offers a cost-effective alternative to measure traditional RIA-measured analytes like free testosterone, insulin, prolactin, and 1,25-dihydroxyvitamin D. Alternatively, the cost of some traditional, lower-volume immunoassays like C-peptide or β-hydroxybutyrate, which may exceed $20/test on commercial platforms, can be done for substantially less by MS while providing superior results. Recently, the cross-reactivity of 10 different immunoassays with 15 exogenous insulins was examined and the authors concluded that, due to the poor cross-reactivity of the immunoassays, MS should be used to diagnose hypoglycemia secondary to exogenous insulin *(36)*. Although any single assay may not be enough to

justify the costs of a mass spectrometer and all the required laboratory renovations, development, validation, training, and labor, combinations of multiple in-house and/or send-out tests can be used to successfully justify the acquisition of this technology.

Another challenge with setting up an MS-based laboratory service is deciding what instrumentation to purchase. The choice of instrumentation depends on the analytes of interest. For most small-molecule quantitative methods (e.g., testosterone, vitamin D, drugs of abuse) a triple-quadrupole mass spectrometer with LC is the instrument of choice. Triple-quadrupole mass spectrometers are also used for newborn screening and for quantification of peptides and proteins. For a clinical microbiology laboratory interested in qualitative identification of a variety of microbes, a MALDI-TOF mass spectrometer is optimal. Although new developments such as high-resolution MS offer tantalizing potential for improvement, existing instrumentation that has a proven track record in the clinical laboratory is a reasonable place to start. As has been pointed out, users of MS tend to think that the next analytical advance is a cure all, when most improvements are incremental *(37)*.

Traditionally, MS analysis is also more efficiently used when run in batch mode analysis. Although assays can be developed that use similar columns/mobile phases, the systems aren't completely random access and users can face challenges when needing to shift between positive/negative mode, column temperature, and mobile phases. In addition, one of the main factors that currently drive batch mode analysis is that the sample preparation (extraction/purification/processing) required before MS analysis is most efficiently performed in batches.

The requirement for skilled labor is especially acute for MS applications based on laboratory-developed tests (LDTs). LDTs require considerable expertise in method development and validation. These talents are difficult to acquire in a training environment and generally require several years of practical experience in a functional MS laboratory to become proficient. It is interesting to note that for the microbiology laboratory, MS has been sufficiently simplified so that MS expertise is not required to run and maintain the instrumentation. For clinical microbiology, MS is simply a read-out device. For MS to become broadly used in the clinical laboratory, more FDA-approved MS-based platforms and NIST-traceable assay kits, calibrators, QCs, and infusion standards need to be commercially available. Nowadays, many clinical laboratories have shifted to commercially prepared reagents, QCs, and calibrators, so laboratory technologists have become less familiar and adept at calculating, weighing out, and/or preparing these items. Lastly, arguments can be made that most of the technologists running sophisticated high-throughput chemistry analyzers don't understand how a select wavelength is used to monitor an endpoint reaction. Likewise, until MS becomes "just another detector" on an automated FDA approved platform, the full benefit of the technology will not be realized.

Another challenge facing laboratories is the lack of a seamless automated solution incorporating sample handling, processing, and preparation through analysis with direct bidirectional interfacing with the instrumentation to the laboratory information system (LIS). Currently, stand-alone components exist that can automate sample preparation/processing, but they operate independently of the LC-MS/MS system. There is a lack of a commercial middleware or other software solution to coordinate and control all the equipment and to bidirectionally communicate between the LIS and the MS. One module to track sample location, tests required, control sample processing, test analysis, and transfer of the results directly back to the LIS like other fully automated chemistry solutions is needed. Often laboratories have to export results from the MS and use Excel macros to perform calculations or reporting rules before getting the results into the LIS. Manual result entry is also not practical on high-volume tests and is prone to high error rates and labor costs.

The widespread clinical use of MS for various omics technologies faces several challenges as the technology moves from research applications comparing different populations to the diagnosis and treatment of individual patients. Earlier we listed the proteomic analysis of thyroglobulin as an area where MS can offer clinical benefit compared with immunoassay, due to the interference of autoantibodies. Unfortunately, MS does not provide all the needed answers in these cases because there are reports of image-confirmed disease recurrence that had undetectable concentrations of thyroglobulin by MS *(38)*. A closer examination of the technology reveals that many variables need to be controlled to provide an accurate quantitative result. These analytical challenges are substantial and similar to those with other omic approaches. As we move these applications from research laboratories into clinical practice, a host of infrastructure also needs to be developed, including reference materials, reference methods, and proficiency testing programs. In addition, laboratories will need regulatory guidance to design appropriate quality assurance and QC programs. New laboratory inspection protocols will also need to be developed, vetted, and implemented.

Lastly, the FDA released draft guidelines for regulation of LDTs on Oct 3, 2014, but it is unclear when the final guidelines will be issued and how long they will take to implement *(39)*. The draft document makes clear that the FDA will pursue a risk-based management strategy and that the prototypical MS-based LDT developed and offered within a single hospital setting will be low or

moderate risk (class 1 or 2 medical devices). The FDA will clarify classification of risk 24 months after the final guidance documents are published, but at present the time frame for release of the final guidance is unclear. Six months after the final LDT regulatory guidance is published all laboratories that perform LDTs will be required to register with the FDA. However, class 1 and class 2 medical devices (traditional MS based LDTs) will not have to apply for premarket approval until 5 years after the final guidance is issued, and the registration process will be phased in over the subsequent 4 years. From a near-term practical aspect, laboratories investing in MS should know if the instrument they are considering is FDA approved as a diagnostic device, but it appears that formal regulation of traditional MS based LDTs is at least 5 years in the future.

Future Applications

New horizons and trends indicate a bright future for MS in laboratory medicine. The miniaturization of MS systems being pursued at places such as Purdue University could allow a transportable device that minimizes the specialized skill set required for operators and allows for rapid and accurate MS analysis in a point-of-care format. These devices could be used in various settings (e.g., physician offices), but this appears to be some time from implementation *(40)*. Presently, MS is already being used outside of the clinical laboratory in the operating rooms at the Imperial College in London, where the surgeon's knife is connected to a mass spectrometer to differentiate between normal and cancerous tissue *(41)*. Additional areas where MS is expanding include the field of proteomics and protein panels. Immunoglobulins can now be measured by MS and are viewed as important biomarkers for immunity, autoimmunity, cancer detection, and immune system function *(42)*. Monoclonal antibodies are also being used as therapeutic agents for a wide variety of diseases. For example, infliximab is used to treat Crohn disease and ulcerative colitis, and therapeutic concentrations are associated with clinical response and improved prognosis. MS-based methods could overcome the interference seen with endogenous autoantibodies directed against infliximab that is present in the existing immunoassays used to measure these compounds.

Laboratories are also looking to connect MS-based systems to fully automated chemistry lines and integrate them directly to the LIS. In addition, MS vendors are working on more ready-to-use "FDA-approved" reagent kits for MS to help diminish barriers for clinical laboratories to adopt this technology. This trend will ultimately result in the introduction of a fully automated clinical chemistry analyzer that incorporates a mass spectrometer as the detector. Further improvements in analytical sensitivity and specificity, automated sample preparation, and throughput will allow more clinical applications to adapt this technology. MS-based methods are an essential component of diagnostic medicine and will continue to grow in scope as regulatory, analytical, and personnel challenges are solved.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

References

1. Irving J. Drug testing in the military–technical and legal problems. Clin Chem 1988;34:637–40.
2. Millington DS, Kodo N, Norwood DL, Roe CR. Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. J Inherit Metab Dis 1990;13:321–4.
3. Fitzgerald RL, Herold DA. Serum total testosterone: immunoassay compared with negative chemical ionization gas chromatography-mass spectrometry. Clin Chem 1996;42:749–55.
4. Holland RD, Wilkes JG, Rafii F, Sutherland JB, Persons CC, Voorhees KJ, et al. Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 1996;10:1227–32.
5. Gero D. Military aviation disasters: significant losses since 1908. Yeovil Somerset: Haynes Publications; 1999.
6. Reagan R. Executive Order 12564 – drug-free federal workplace; 1986. p 224. http://www.archives.gov/federal-register/codification/executive-order/12564.html (Accessed October 2015).
7. US Government Publishing Office. Mandatory guidelines for federal workplace drug testing. Federal Register 1998;63(219):11970. http://www.gpo.gov/fdsys/granule/FR-1998–11-13/98–30403 (Accessed October 2015).
8. Peat MA. Financial viability of screening for drugs of abuse. Clin Chem 1995;41:805–8.
9. Miller TR, Zaloshnja E, Spicer RS. Effectiveness and benefit-cost of peer-based workplace substance abuse prevention coupled with random testing. Accid Anal Prev 2007;39:565–73.
10. Taieb J, Mathian B, Millot F, Patricot MC, Mathieu E, Queyrel N, et al. Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography-mass spectrometry in sera from 116 men, women, and children. Clin Chem 2003;49:1381–95.
11. Bayliss MK, Little D, Mallett DN, Plumb RS. Parallel ultra-high flow rate liquid chromatography with mass spectrometric detection using a multiplex electrospray source for direct, sensitive determination of pharmaceuticals in plasma at extremely high throughput. Rapid Commun Mass Spectrom 2000;14:2039–45.
12. Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev 2013;26:547–603.
13. Tan KE, Ellis BC, Lee R, Stamper PD, Zhang SX, Carroll KC. Prospective evaluation of a matrix-assisted laser desorption ionization-time of flight mass spectrometry system in a hospital clinical microbiology laboratory for identification of bacteria and yeasts: a bench-by-bench study for assessing the impact on time to identification and cost-effectiveness. J Clin Microbiol 2012;50:3301–8.
14. Patel R. MALDI-TOF MS for the diagnosis of infectious diseases. Clin Chem 2015;61:100–11.
15. Gekenidis MT, Studer P, Wuthrich S, Brunisholz R,

Drissner D. Beyond the matrix-assisted laser desorption ionization (MALDI) biotyping workflow: in search of microorganism-specific tryptic peptides enabling discrimination of subspecies. Appl Environ Microbiol 2014;80:4234–41.
16. Ecker DJ, Sampath R, Li H, Massire C, Matthews HE, Toleno D, et al. New technology for rapid molecular diagnosis of bloodstream infections. Expert Rev Mol Diagn 2010;10:399–415.
17. Aichler M, Walch A. MALDI Imaging mass spectrometry: current frontiers and perspectives in pathology research and practice. Lab Invest 2015;95: 422–31.
18. Norris JL, Caprioli RM. Analysis of tissue specimens by matrix-assisted laser desorption/ionization imaging mass spectrometry in biological and clinical research. Chem Rev 2013;113:2309–42.
19. Balluff B, Frese CK, Maier SK, Schone C, Kuster B, Schmitt M, et al. De novo discovery of phenotypic intratumour heterogeneity using imaging mass spectrometry. J Pathol 2015;235:3–13.
20. Sun N, Ly A, Meding S, Witting M, Hauck SM, Ueffing M, et al. High-resolution metabolite imaging of light and dark treated retina using MALDI-FTICR mass spectrometry. Proteomics 2014;14:913–23.
21. Roberts LD, Souza AL, Gerszten RE, Clish CB. Targeted metabolomics. Curr Protoc Mol Biol. 2012; Chapter 30: Unit 30 2 1–24.
22. Gillette MA, Carr SA. Quantitative analysis of peptides and proteins in biomedicine by targeted mass spectrometry. Nat Methods 2013;10:28–34.
23. Nikolskiy I, Mahieu NG, Chen YJ, Tautenhahn R, Patti GJ. An untargeted metabolomic workflow to improve structural characterization of metabolites. Anal Chem 2013;85:7713–9.
24. Huang X, Chen YJ, Cho K, Nikolskiy I, Crawford PA, Patti GJ. ×13CMS: global tracking of isotopic labels in untargeted metabolomics. Anal Chem 2014;86:1632–9.
25. Handelsman DJ, Wartofsky L. Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. J Clin Endocrinol Metab 2013;98:3971–3.
26. Ketha H, Kaur S, Grebe SK, Singh RJ. Clinical applications of LC-MS sex steroid assays: evolution of methodologies in the 21st century. Curr Opin Endocrinol Diabetes Obes 2014;21:217–26.
27. Cawood ML, Field HP, Ford CG, Gillingwater S, Kicman A, Cowan D, et al. Testosterone measurement by isotope-dilution liquid chromatography-tandem mass spectrometry: validation of a method for routine clinical practice. Clin Chem 2005;51:1472–9.
28. Wang Y, Gay GD, Botelho JC, Caudill SP, Vesper HW. Total testosterone quantitative measurement in serum by LC-MS/MS. Clin Chim Acta 2014;436:263–7.
29. Kushnir MM, Rockwood AL, Roberts WL, Abraham D, Hoofnagle AN, Meikle AW. Measurement of thyroglobulin by liquid chromatography-tandem mass spectrometry in serum and plasma in the presence of antithyroglobulin autoantibodies. Clin Chem 2013;59:982–90.
30. Clarke NJ, Zhang Y, Reitz RE. A novel mass spectrometry-based assay for the accurate measurement of thyroglobulin from patient samples containing antithyroglobulin autoantibodies. J Investig Med 2012;60:1157–63.
31. Netzel BC, Grebe SK, Algeciras-Schimnich A. Usefulness of a thyroglobulin liquid chromatography-tandem mass spectrometry assay for evaluation of suspected heterophile interference. Clin Chem 2014;60:1016–8.
32. Hermida J, Tutor JC. Falsely increased blood tacrolimus concentrations using the ACMIA assay due to circulating endogenous antibodies in a liver transplant recipient: a tentative approach to obtaining reliable results. Ther Drug Monit 2009;31:269–72.
33. Rostaing L, Cointault O, Marquet P, Josse AG, Lavit M, Saint-Marcoux F, et al. Falsely elevated whole-blood tacrolimus concentrations in a kidney-transplant patient: potential hazards. Transpl Int 2010;23:227–30.
34. Moscato D, Nonnato A, Adamo R, Vancheri M, Caropreso A. Therapeutic monitoring of tacrolimus: aberrant results by an immunoassay with automated pretreatment. Clin Chim Acta 2010;411:77–80.
35. Adaway JE, Keevil BG. Therapeutic drug monitoring and LC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 2012;883–884: 33–49.
36. Parfitt C, Church D, Armston A, Couchman L, Evans C, Wark G, McDonald TJ. Commercial insulin immunoassays fail to detect commonly prescribed insulin analogues. Clin Biochem 2015;48:1354–7.
37. Fitzgerald RL, Herold DA, Yergey AL. Trade-offs in mass spectrometry. Clin Chem 1997;43:915.
38. Netzel BC, Grebe SK, Carranza Leon BG, Castro MR, Clark PM, Hoofnagle AN, et al. Thyroglobulin (Tg) Testing Revisited: Tg Assays, TgAb Assays, and Correlation of Results With Clinical Outcomes. J Clin Endocrinol Metab 2015;100:E1074–83.
39. FDA. Guidance, compliance and regulatory information (biologics). www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/default.htm (Accessed April 2015).
40. Xu W, Manicke NE, Cooks GR, Ouyang Z. Miniaturization of mass spectrometry analysis systems. JALA Charlottesv Va 2010;15:433–9.
41. Adaway JE, Keevil BG, Owen LJ. Liquid chromatography tandem mass spectrometry in the clinical laboratory. Ann Clin Biochem 2015;52:18–38.
42. Murray D, Barnidge D. Characterization of immunoglobulin by mass spectrometry with applications for the clinical laboratory. Crit Rev Clin Lab Sci 2013;50:91–102.