

Accepted Manuscript

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PII: S1872-4973(18)30395-8
DOI: <https://doi.org/10.1016/j.fsigen.2018.10.014>
Reference: FSIGEN 1986

To appear in: *Forensic Science International: Genetics*

Received date: 13-7-2018
Revised date: 19-10-2018
Accepted date: 22-10-2018



Please cite this article as: van Oorschot RAH, Szkuta B, Meakin GE, Kokshoorn B, Goray M, DNA transfer in forensic science: a review, *Forensic Science International: Genetics* (2018), <https://doi.org/10.1016/j.fsigen.2018.10.014>

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DNA transfer in forensic science: a review

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Highlights:

- Understanding the variables impacting DNA transfer is highly relevant
- DNA transfer awareness is required to limit contamination risk
- Dedicated training is required for experts providing opinion on DNA transfer
- More research is required to generate probability estimates for more situations

Abstract:

Understanding the variables impacting DNA transfer, persistence, prevalence and recovery (DNA-TPPR) has become increasingly relevant in investigations of criminal activities to provide opinion on how the DNA of a person of interest became present within the sample collected. This review considers our current knowledge regarding DNA-TPPR to assist casework investigations of criminal activities. There is a growing amount of information available on DNA-TPPR to inform the relative probabilities of the evidence given alternative scenarios relating to the presence or absence of DNA from a specific person in a collected sample of interest. This information should be used where relevant. However, far more research is still required to better understand the variables impacting DNA-TPPR and to generate more accurate probability estimates of generating particular types of profiles in more casework relevant situations. This review explores means of achieving this. It also notes the need for all those interacting with an item of interest to have an awareness of DNA transfer possibilities post criminal activity, to limit the risk of contamination or loss of DNA.

Appropriately trained forensic practitioners are best placed to provide opinion and guidance on the interpretation of profiles at the activity level. However, those requested to provide expert opinion on DNA-related activity level issues are often insufficiently trained to do so. We advocate recognition of DNA activity associated expertise to be distinct from expertise associated with the identification of individuals. This is to be supported by dedicated training, competency testing, authorisation, and regular fit for purpose proficiency testing.

The possibilities for experts to report on activity-related issues will increase as our knowledge increases through further research, access to relevant data is enhanced, and tools to assist interpretations are better exploited. Improvement opportunities will be achieved sooner, if more laboratories and agencies accept the need to invest in these aspects as well as the training of practitioners.

Key words: DNA, transfer, persistence, prevalence, recovery, trace, activity level

1. Introduction

1.1 Scope of review

This review will traverse what we know about DNA transfer and the associated elements of DNA persistence, prevalence, and recovery, sometimes collectively referred to as DNA-TPPR. It will consider the factors impacting transfer during different types of contact and the likelihood of detecting DNA from a sample of interest following a particular sequence of events. It will not, however, collate all the data available to provide probability estimates for specific observations in certain circumstances. Furthermore, it is not our intention to analyse the abundance of cases where indirect transfer has been a major issue, or the different types of scenarios that have been presented in court deliberations. This is therefore not a definitive account of all matters and information relating to the transfer of DNA within the forensic context. Instead, this review provides a snap-shot of the current knowledge, along with pointers to areas requiring improvements and a brief discussion on our readiness to utilise the available data to help address activity level inquiries. Activity level refers to the generally accepted hierarchy of propositions [1] for evaluation of evidence in forensic science. We refer to this as ‘activity level reporting’ or ‘activity level assessments’ for legibility. We wish to stress that whenever we refer to ‘activity level reporting’ in this review we discuss the evaluation of evidence *given* activity level propositions. By no means do we wish to endorse the practice of commenting by experts on activities given their findings (e.g. ‘primary transfer is more likely given the results’). See Taylor et al. [2] for more information on formal methods for evaluation of evidence given activity level propositions.

This review will reference several papers that have significantly impacted this field. Although the review intends to capture the bulk of such papers, it is not exhaustive of all related publications, and since hands can be a major and highly contested source and/or vector for transfer of (often invisible) DNA containing material detected at crime scenes, the transfer of ‘touch’ DNA will at times have preferential focus. Those papers not included are by no means irrelevant, and we urge readers to consider these too when seeking information relevant to their specific needs. As the research activity in this field is ramping up, there is also a good chance that this review will be remiss of material published after the submission of this manuscript. Furthermore, this review does not intend to summarise all the details within the many references listed, rather, we urge readers to extract these from the relevant publications as deemed relevant to their needs.

We advocate the need for forensic scientists to equip themselves well, by constantly staying abreast of the available DNA-TPPR related knowledge, to facilitate activity level assessments. However, whilst we will be discussing the available data, we will not be providing details on how best to conduct activity level assessments, as this is the focus of another paper within this series by Taylor et al. [2]. We also hope this review will entice greater commitment and investment (from stakeholders as well as government and other funding bodies) towards further research to improve our knowledge of DNA-TPPR and its application to casework and legal deliberations addressing activity level questions. Note, the

views expressed in this paper are our own and do not necessarily reflect those of our host institutes.

1.2 Why it is important to understand DNA transfer

The demonstrated ability to attribute a DNA profile to a specific person, and the increased sensitivity of the profiling systems to generate these profiles from decreasing quantities of DNA, has seen an increasing reliance on trace biological samples, especially from touched objects, to assist investigations of criminal activity. The increased sensitivity and the types of objects from which samples are collected, however, also means that many of the profiles generated are mixed profiles, that is, DNA from multiple contributing individuals represented together in the one profile. Consequently, the number of potential scenarios that may have led to the transfer and deposition of detected DNA have increased substantially. There is, thus, apart from the need to determine the identity of individuals whose DNA has become part of the evidence, an increasing need to understand how the DNA within a trace got to where it was collected from.

1.3 Brief history

The notion that, during a criminal act, an offender will both leave trace evidence at a scene and take it away on their person or clothes was raised by Locard almost 100 years ago [3], and further discussed by Inman and Rudin [4]. The pursuits of detecting these traces have spawned many forensic disciplines. Since the initial discovery of the ability to generate unique genetic profiles from biological materials and its application to address questions of identify to assist investigations of criminal activity by Sir Alec Jeffreys and his colleagues [5, 6], the subsequent technological advances allowing relatively quick and affordable generation of extremely discriminating profiles from many sources of biological material [7-9], along with uniformity across jurisdictions and the construction of offender DNA databases with associated legislations [10-13], have seen DNA playing an ever increasing role in the identification of those who have committed criminal offences and exonerating the innocent [14-21]. The same methodologies have also played equally increasing roles in the identification of victims of disasters and missing persons [22-25]. The discovery that DNA can be detected from non-visible biological material left on a surface merely through touching it by hand [26], and the extrapolation of this observation to contact with skin in general, drastically broadened the types of items that could be targeted to obtain DNA profiles and the variety of situations in which DNA profiling could be applied [7, 14, 18, 27, 28]. This discovery of the ability to generate profiles from touched objects was initially met with disbelief by many within the forensic community, but once verified, became a welcome tool for law enforcement agencies. Within several jurisdictions, samples collected from touched objects now represent more than half the total number of samples processed for DNA profiling [16, 29].

The notion that DNA could also be picked-up and transferred to somewhere else, and the potential implications thereof, was presented in the same *Nature* paper reporting the discovery of touch DNA [26]. The relevance of this aspect took longer to appreciate and propagate into forensic investigations and legal deliberation. Over the last several years an increasing number of cases no longer question 'whose DNA it is' but wish to know 'how or when it got there' [30]. Cases thus hinge on the relative likelihoods of the DNA of a certain person being deposited directly by that person or by someone, or something, else. Whilst very few studies were published in the first fifteen years post-discovery of indirect transfer of DNA by hands to help understand the variables associated with the indirect transfer of DNA [18, 31-38], it has only very recently become more widely recognised that much more needs to be done to resolve the current paucity of empirical data on the variables that may or may not impact DNA-TPPR [39-43] and assist those tasked with addressing questions at the activity level [44-47]. It is thus timely to take stock of what we currently know about the transfer of DNA and consider the direction of required further efforts.

1.4 What is DNA transfer and the meaning of associated terms?

1.4.1 *Direct and indirect transfer*

Direct and indirect transfer relates to the routes by which DNA may be transferred (Fig 1A-D). The terms 'direct' and 'primary' transfer are, and can be, used interchangeably. The terms 'indirect' and 'secondary' transfer are also used interchangeably, however, as a specific source of DNA may have been transferred multiple times, i.e. secondary, tertiary, quaternary etc. (multi-step transfer pathway), one needs to be clear on what is meant by secondary transfer within the context of the scenario at hand. For some, secondary transfer means any transfer event after the primary transfer; for others it only refers to the singular transfer step after the initial deposit. In addition, when referring to a specific contact event within a long sequence of multiple contacts, one may refer to the primary and secondary substrates involved in a specific contact even though they may not be the first or second substrates within the sequence of contacts. As in most case scenarios, when contemplating the possibility of direct versus indirect transfer, the number of indirect steps are unknown, therefore, we prefer using the term 'indirect transfer' rather than 'secondary transfer', unless the scenarios put forward by prosecution or defence, or known facts in the case, establish that the indirect transfer is based on only a single step after initial deposit.

A person's DNA can be directly deposited onto an object/surface or hand/skin of another person just by contacting it. Some examples of direct/primary transfer are:

- Person drops their blood after sustaining an injury, spits their saliva or ejaculates their semen onto a surface or someone.
- Person's DNA transferred when touching an object or surface with their bare hand.
- Person's DNA transferred when touching another person's bare hand with their bare hand.
- Person's DNA transferred to their clothing and jewellery contacting their skin whilst wearing it.

If the DNA deposited during direct transfer by any of the means outlined above is transferred again, an indirect transfer event has taken place. During indirect transfer, there is no direct contact of the original source of the DNA with the location/surface on which it is located. Some examples of indirect transfer are:

- Blood or saliva from an individual (person A) deposited on surface 1 is transferred to surface 2 upon the two surfaces coming into contact. DNA from person A is detected on surface 2 [38, 48, 49].
- DNA deposited on surface 1 by an individual handling it with bare hands (person A) is transferred to surface 2 upon the two surfaces coming into contact. DNA from person A is detected on surface 2 [37].
- DNA from one individual (person A) is deposited on a knife handle (object 1) when handling it with bare hands. DNA from person A is then acquired by a second individual (person B) when they handle the knife (object 1) with bare hands [50]. DNA from person A residing on the hand of person B is then transferred to object 2 when contacted by person B with bare hands. DNA from person A is detected on object 2 [50].
- DNA originating from, and residing on, the hand of one individual (person A) is transferred to the hand of a second individual (person B) during handshaking. DNA from person A residing on the hand of person B is then transferred to object 1 when contacted by person B with bare hands. DNA from person A is detected on object 1 [51-54].
- DNA residing on a specific area of a collected item (object 1), that has been packaged along with a second item (object 2) in the same packaging, is transferred to another area of object 1, to another item (object 2), or to the inside of the packaging [55]. DNA residing on the external packaging is transferred to an exhibit during examination [56].
- DNA picked-up by gloves or tools coming into contact with various items and surfaces at a crime scene during the investigation, or an exhibit during examination, is transferred to another item or surface if gloves and/or tools are not cleaned or replaced in between use [57-62].
- During a social setting where multiple individuals and items are present, DNA from one individual (person A) is transferred to other individuals and items without person A having touched them [63].
- Within a work environment, DNA from co-workers is transferred to items and surfaces within a shared space during use. DNA from co-workers is transferred to the clothing worn by an individual (person A) through contact with items and surfaces within the shared space [64].

(Additional examples of indirect transfer are presented in later sections.)

1.4.2 DNA transfer vs. contamination

DNA transfer, as explained above, and DNA contamination refer to the same physical phenomenon of DNA movement from one surface/location to another. However, it is the timing of this movement that defines whether DNA transfer is associated with a crime-

related activity prior to securing a crime scene (be it pre-, during, or post-criminal activity), or a non-crime related contamination event during, or post-, securing of a crime scene (see Rudin and Inman [65] for further exploration of this concept). In a forensic setting, non-crime related contamination can come in many forms and via different vectors. For example, a police officer at the scene, a scientist examining the evidence, a dirty examination tool, or a non DNA-free reagent used during sample analysis. Conversely, crime-associated DNA transfer refers to the movement of DNA from a source that may, or may not, be involved in the criminal activity, such as a perpetrator acting as a vector for the transfer of someone else's DNA to the crime scene while performing a specific activity relevant to the crime (e.g. handling an alleged weapon). This someone else's DNA could be that of an innocent individual (otherwise not associated with the offender, crime or crime scene) picked-up by the perpetrator during an interaction directly with that person, or an object that person had previously touched, just prior to the criminal activity taking place. While crime-associated DNA transfer occurs only before the crime scene is established by the authorities, contamination can only occur afterwards. See section 9 for more on contamination.

1.4.3 Simultaneous direct and indirect transfer

One contact event can simultaneously include both direct/primary and indirect/secondary transfer events (Fig 1D). Transfer of DNA during a hand contact can result in deposits of DNA of the person making the contact as well as any other DNA that may have been present on that person's hand. For example, following contact with a DNA-free surface, most of the self DNA deposited within the handprint may be considered a direct deposit, but the non-self component will have been indirectly deposited.

In some situations, the self component deposited by a hand may not have been generated by the hand itself, but acquired (through touching) from other parts of the person's body from where it was generated (section 2.2.2) or from personal objects previously touched by the individual. One must be mindful of the various means a particular source of biological material could be deposited onto an item. For example, saliva on an item could have been deposited directly through contact with the mouth, or via a hand that had been placed in the mouth (e.g. to remove something, bite their fingernails, or wet a finger to flip a page of a book).

1.4.4 Bi-directional transfer

Transfer upon contact can be bi-directional (Fig 1E). When two surfaces (be it an inanimate object or a person) that both have DNA on them come into contact, there can be an exchange of DNA material between the two. For example, when a hand of person A touches the hand of person B, then not only can the DNA on the hand of person A be transferred to the hand of person B, but DNA from the hand of person B can transfer to the hand of person A [52, 53]. The same can be applicable for DNA, of different origins, on contacting objects.

During bidirectional transfer events, the total amount of DNA on the hands of persons A and B, or their objects, may increase, stay similar or be reduced. This would be dependent on the relative amounts of DNA on the contacting surfaces and the impacts of the variables

pertinent during the contact event (including substrate types and manner of contact). The amount of DNA of the source originally present on the hand of person B, or the object, however, will have been diminished. (Unless, one happened to be dealing with a unique situation where the source of interest on the hand of person B, or their object, happened to have been the same as on the hand of person A, or their object.)

1.4.5 Multiple contacts and multi-step transfer

When multiple different contacts are made with an originating surface upon which a finite DNA resides (Fig 1F), the amount of DNA remaining on the originating surface will diminish after each contact. The level of reduction will be dependent on the substrates and manner of contacts involved. Depending on the magnitude of the loss of this DNA, the amount remaining on the surface may become undetectable. Further, the same original amount could also become undetectable within a mixture during a bi-directional transfer, if the amount of DNA transferred from any of the contacting surfaces is sufficient enough to overwhelm the DNA on the original surface [66]. Alternatively, transfer from multiple different sources to the originating surface can result in a mixture of DNA of such complexity that renders it uninterpretable.

Conceptually, 0 to 100% of the DNA present on a substrate could be transferred to another substrate upon contact. Usually a proportion of the DNA will be transferred; how much will depend on a range of variables to be discussed in the following sections. During a multi-step transfer event (Fig 1C), each subsequent step will involve diminished DNA amounts (a proportion of the initial deposit) and the amount of DNA recovered from an ultimate substrate will be dependent on the number and type of the sequential contacts in the transfer pathway [49]. If DNA from one or more different biological sources are present on multiple substrates contacted along a multi-step pathway, this “other” DNA may also be picked up and transferred further, complicating the interpretation of the DNA profile obtained from the ultimate surface/location, especially if these other sources are more prevalent [66].

If during any contact event within a series of contacts along a direct pathway, the portion of transferred DNA is very limited, then this becomes a limiting factor for what will be detected on the last substrate (within the sequence) from which a sample is collected. This is irrespective of the variables associated with the subsequent contact events within the transfer pathway, even if these variables are conducive to high transfer rates. The amount of DNA transferred during initial and subsequent transfer steps may be influenced by: the type of substrates contacting (section 2.1) and/or manner of contact (section 2.3), alignment of areas making contact (relative to where the sample of interest is located on the item), and the time and/or activities performed between contacts of interest (section 5.3).

1.4.6 Pathway interruption

Any surface/object involved in the multi-step transfer pathway may come in contact with other surfaces/items not related to the direct pathway in question, resulting in multiple divergent transfer pathways. If the item within the pathway of interest, after receiving the

biological material of interest, has been contacted multiple times, or has been cleaned or washed, prior to the item contacting the following item within the pathway, then the amount of DNA that can be transferred from that substrate further along the pathway of interest will have diminished. Therefore, the amount of DNA from the original deposit retrievable from an ultimate substrate at the end of the transfer pathway of interest is reduced relative to the amount transferred if no intersecting pathways were involved (Fig 1G) (see also section 5.3).

1.4.7 DNA parking

The term 'DNA parking' was first coined, and the potential thereof considered, in a study by Szkuta et al. [52]. 'DNA parking' refers to secondarily transferred DNA that is temporarily deposited on an object and recollected again prior to being deposited on the surface of interest (Fig 1H). For example: DNA acquired by person A from an acquaintance (person B) during handshaking may be transferred to an object (object 1) (such as a cup, tap, phone) immediately after the handshake. Object 1 could remain unused for an extended period of time (possibly days) whilst person A continues to undertake a myriad of activities, consequently losing any remaining DNA of person B from their hand. At a later point in time, person A may contact object 1 and pick-up some of the DNA from person B that was deposited during the previous contact. DNA from person B may then be transferred to another item (object 2), that could potentially be involved in a criminal activity, via the hands of person A. Further studies by a subset of co-authors is currently being undertaken to further demonstrate the legitimacy of the phenomenon. This phenomenon should be closely considered together with aspects of persistence (see section 4).

1.5. Terminology: trace, skin, touch, wearer, handler, shedder, background DNA

From a forensic perspective, 'trace' can be, and often is, referred to as any substance collected for testing to assist investigations [67]. From a DNA perspective, this includes biological material from which DNA profiles may be generated. The term used this way does not imply anything regarding the quantity of the material collected or used for testing. Some have defined 'trace' more from a quantitative perspective, i.e. when the quantity of DNA available for testing is below a certain threshold at which the chance of obtaining a full DNA profile is low [7, 18]. Such low amounts of DNA have been described as 'low template' and 'low copy number DNA' [10, 68, 69]. DNA, of otherwise sufficient quantity, that does not provide a full profile due to the presence of inhibitors affecting part of the profiling process (e.g. amplification), and/or degradation due to exposure of the DNA to adverse environmental conditions and/or time, is also often referred to as 'trace'. van Oorschot et al. [7] defined it more holistically as: 'any sample which may fall below the recommended thresholds at any stage of the process – detection, collection, extraction, amplification and interpretation'. Gill [42] redefined this to: 'any sample where there is uncertainty that it may be associated with the crime event itself—so that it is possible that the transfer may have occurred before the crime event (innocent transfer) or after the crime event (investigator mediated)'. Gill wrote that the definition is deliberately vague as it hinges upon an

assessment of the relevance of a 'trace DNA' profile to the crime event. We feel that such a definition is inadequate for intended uses of the term.

The context in which 'trace' is used tends to make its meaning implicit, but where it may be ambiguous and relevant the intended meaning should be clarified.

'Trace' or 'trace DNA' does not say anything about the source of the sample/DNA, the action of how it got to where it was collected from, or if it was deposited during a criminal act. Meakin and Jamison [39] defined 'trace DNA' as 'DNA that cannot be attributed to an identifiable body fluid'. This could be refined to: DNA that has not been attributed to an identifiable biological material. Perhaps because a) no tests have been conducted, b) some were conducted and provided negative results, or c) only a positive presumptive test result is available which is not accompanied by a second more accurate test result, or the latter is negative.

'Skin DNA' is sometimes used to describe DNA collected from surfaces assumed to have been touched, thus trying to imply the source of DNA and avoiding any context associated with the action of how it got there. However, as discussed in later sections, the source of DNA on skin, especially hands, can be from multiple sources. So referring to 'skin DNA', without having performed confirmatory tests to establish the source, could be misleading.

'Touch DNA' is a term commonly used to describe DNA collected from a range of item types that are assumed to have been handled. The broader use of the term can include samples and/or DNA from marks made by contact with other areas of the skins surface (e.g. forehead, ears, feet, arm, breast, back etc.) and/or include the terms 'wearer DNA' and 'handler DNA' (see below). Usually, it relates to DNA from a biological source that is invisible and has not been tested for a source. Sometimes the source is unknown, but has been shown not to be semen, blood and/or saliva, making it more likely to be from skin cells or skin associated glands. Where the sample has been taken from an area verified, by the application of fingerprinting methodologies, to have been touched, the utilisation of the term 'touch DNA' may appear to be more applicable. However, as the DNA subsequently collected and profiled may include 'background DNA' (see below) that was present on the surface prior to it being touched during the action of interest, which may have been deposited by other means (originating from the same and/or other individuals), as well as indirectly transferred DNA during the touching action, implying that all the DNA collected is 'touch DNA' could be misleading. Furthermore, the action of touch can take many forms, so where the specific details of the 'touch' is known, this should be made clear. Alternatively, limitations of the knowledge should also be conveyed.

The term 'touch DNA' is often used to describe trace samples of unknown cellular source, and/or mode of action associated with its deposition. Describing such a sample as 'touch DNA' can be misleading, as it implies a specific mode of action and, to some degree, also a type of biological source. Using a less descriptive term such as 'trace DNA' is a more appropriate term to be used in casework when the source and mode of deposition are unknown. If the source and/or action associated with the trace DNA are known (such as in research projects or mock simulations) then it is acceptable to use a term reflecting this.

Similarly, use of the term '*wearer DNA*' could be misleading, as it implies the action of wearing, associating a specific person as the person who wore the clothing from which the sample was collected. In casework, this is sometimes assumed rather than being verifiable (e.g. if the sample was of a shirt removed from an individual by investigators, and a reference sample of the individual matches the one collected from the shirt). Furthermore, DNA collected from clothing is often from multiple individuals that have been deposited by various direct and indirect means and, as such, could be of different sources (section 3.5, 5.2). The same issues are applicable in relation to the use of the term '*handler DNA*' used to describe DNA from handled items (e.g. knives, firearms, tools etc.) such that using this term could also be misleading.

'*Shedder*', and associated terms such as '*shedder status*', '*shedder type*', '*good shedder*' and '*poor shedder*', are terms used to categorise an individual with respect to the degree of DNA deposited on a surface when touched, usually relating to hands. However, there is currently no standard quantity of DNA left on a specific area when touched in a standardised manner, to help categorise an individual as poor, intermediate or good shedder, or to score them based on some kind of scale. It is currently just a term applied based on relative quantities among a cohort of individuals. Further, the relative origins of DNA sources (shed skin vs. cells/DNA deposited in a medium, self vs. non-self, or directly vs. indirectly acquired DNA) within a deposit are not always known, or considered, when referring to someone's shedder status. As the term '*shedder status*' (and its derivatives) are in common use, we will for now continue referring to this within the manuscript. However, as we advocate refraining from using terms that imply an action or source without knowing the ground truth, a more neutral term should be considered for use in the future. A more appropriate term may be '*prevalence status/index*' with a prefix identifying the relevant surface being sampled, or from which the sample was derived, e.g. '*hand prevalence status*'. Further, words being used to indicate quantity such as '*good*' and '*poor*' could be replaced with '*high*' and '*low*', preferable according to a still to be determined standardised scale. See section 2.2.3 for more information on shedder status.

'*Background DNA*' can have different meanings [42, 70-73]: a) The DNA present on the surface prior to the deposit of interest being placed on the surface during the action of interest; b) The DNA of sources present within a sample other than the person of interest (POI); c) The DNA present within the sample derived from unknown individuals. Meanings 'b' and 'c' of background DNA may be inclusive of DNA from other sources that may have been deposited during the same action of interest, and/or pre- and/or post- action of interest. When we use the term '*background DNA*' in this review, this relates to meaning 'a'.

Gill [42] has used the terms '*active*' and '*passive*' transfer. '*Active*' is used to distinguish DNA originating from the perpetrator being transferred during a criminal event, from DNA that is present due to events unrelated to the crime event. However, as perpetrator DNA could be on a surface of interest, not just because of direct transfer during the criminal activity, but also due to direct transfer while using the item prior to use during the criminal activity or indirectly transferred there by a vector before or during the criminal activity, the use of this term could be misleading. Further, '*passive*' could be read to mean '*background DNA*' on the

item prior to the offence taking place and/or indirectly transferred DNA of innocent others that happen to be on the hands of the perpetrator. We therefore refrain from using these terms when discussing DNA transfer.

Many trace samples produce mixed profiles where the sources and actions of the contributors are different from one another. Using a term relating to the source and/or action of interest (which could relate to a major or minor component of a profile), such as the aforementioned terms, without acknowledgement of the other sources and/or actions could be misleading. In casework, when reporting DNA findings, it is important that the terminology used is neutral to the source and/or action when they are unknown or questioned. If terminology is used that does imply a source and/or action, then the supporting evidence should be made available. When the supporting evidence is not based on validated test results or verifiable action, but assumptions based on common uses, applications, and/or histories, then the potentially misleading terminology should be avoided or at least the limitations and/or assumptions made explicit to the trier of fact.

Throughout this paper, we will frequently be referring to 'biological material' (i.e. undefined source) 'deposited' on or 'transferred' (i.e. undefined action of contact) to an item/substrate by placing a hand on or handling it (i.e. defining the action of contact). We also use the term 'touch DNA', but only when referring to data from research experiments where the ground truth is known, i.e. that the DNA recovered is from touch.

2. Core factors impacting transfer

The core factors currently known to impact DNA transfer include the substrate of the contacting surfaces, the nature of the biological material on the contacting surfaces, the manner of contact between the contacting surfaces, and shedder status (for DNA transferred through contact with hands or other areas of the skin). Further, these variables do not affect DNA transfer in isolation and depending on the different variable combinations, different transfer rates will be achieved. This section will explore each of these factors.

2.1 Substrates

Goray et al. [37, 38] demonstrated that the type of substrates/surfaces on which a biological material resides (primary substrate) and the type of substrates they come into contact with (secondary substrate) will impact how much is transferred. The authors demonstrated that less DNA will be transferred from a porous substrate than a non-porous primary substrate, while a porous secondary substrate will facilitate transfer from the primary substrate. For example, Goray et al. [38] found that <1% of DNA from dried blood transferred from a porous cotton substrate to another cotton substrate or to a non-porous flat plastic substrate, when pressure with friction was applied. In contrast, far more of the deposit was transferred under the same conditions when on the non-porous plastic primary substrate and contacting another plastic substrate (~45%) or cotton (~16%). In all these situations, far

less (0-3.4%) was transferred when no friction was applied between substrates (i.e. contact was 'passive' or 'pressure without friction').

The same study found that when the blood was wet on the plastic substrate, approximately half (44-64%) was transferred to another plastic substrate, and more (90-98%) to a cotton substrate, irrespective of the type of contact (passive, pressure, friction). When both substrates are non-porous, during contact, liquid biological material can equalise between the two surfaces; while with porous secondary substrates liquid will continually absorb into the surface until saturation is reached, resulting in an increased transfer rate. In contrast, when the wet blood was deposited on a porous cotton substrate, far less ($\leq 3\%$) transfer was observed to the secondary substrate, irrespective of substrate type (plastic, cotton) or manner of contact (passive, pressure, friction) due to biological material absorbing into the primary substrate.

In similar experiments to the study above, Goray et al. [37] found that deposits of biological material from hands through the action of touching appeared to be impacted by moisture; however, in most instances these were insignificant. In this study, less DNA appeared to be transferred when touch deposits were left for 24 h prior to transfer than when the material was freshly deposited, in most of the pairwise combinations of substrate types contacting each other, during passive and pressure contacts. In contrast, more of the dried ($\sim 13\text{-}49\%$) than the fresh ($\sim 8\text{-}33\%$) appeared to be transferred when friction was applied.

When similar substrates were applied in secondary transfer experiments conducted by Verdon et al. [74] and Fonnelløp et al. [60], results were consistent with those of Goray et al. [37, 38]. Furthermore, studies by Goray et al. [37], Daly et al. [75], Fonnelløp et al. [60] and Helmus et al. [76] also found differences in the quantities of DNA directly transferred by hands depending on the type of substrate, observing that more DNA was transferred to wood and fabric than glass, metal or plastic.

The initial studies by Goray et al. [37, 38] used a single type of cotton fabric to represent a porous substrate and hard flat plastic to represent a non-porous substrate. Verdon et al. [74] explored a wider range of fabrics, with different compositions and types of weaves, and found that these impacted the transfer significantly. In transfer experiments by Buckingham et al. [50, 77], DNA picked up by a hand, from a previously touched knife handle, that subsequently contacted a glass plate or a cotton plate was far less retrievable from the cotton than from the glass. The substrate difference influenced the transfer of the DNA to the secondary plates and/or the ability to retrieve DNA from the substrate given the DNA collection method applied.

Physical and chemical differences in contacting surfaces, including those relating to their topography, chemical compositions, fibre type, weave, thickness, electrical charge etc., are likely to impact transfer, persistence and recovery of biological materials and DNA from substrates to varying degrees. Further research into the impacts of variables within such factors would be welcomed.

See section 7 for a discussion regarding the impact of the interaction between substrate, collection method and extraction method.

2.2 Biological material

2.2.1 *Source and freshness /moisture content*

Biological material such as blood, saliva and semen are often transferred during altercations, whether while fresh or at some time after it has dried. Blood and saliva transfer at a similar rate [38], with wet/liquid biological materials being transferred more readily than dried deposits. When all other variables (substrates and manners of contact) are kept constant, wet/liquid biological sources had significantly higher transfer rates than when the same biological materials were dry [38]. Results of a study by Warshauer et al. [78] are consistent with these findings. Given the large difference in the relative quantity of DNA transferred between wet and dry substances (~44 – 100% and <1% respectively, depending on substrate and manner of contact [38]), and because altercations can occur over extended periods, it becomes important to have an awareness of how quickly the material dries, and the duration between its initial deposition and contact with another surface. The moisture level of a sample at the time of contact will potentially impact the likelihood of its detection given alternate scenarios.

Blood dries relatively quickly depending on the temperature, humidity and presence/absence of wind [79-81]. A study by van Oorschot et al. [82] showed that blood (15 and 30 μ l) on a hard non-porous substrate will dry within 30-60 min (quicker in warmer temperatures), and that the transfer rates of blood, 5 min after deposition, declines exponentially until the deposit is dry. Furthermore, drying rates did not differ among the different conditions tested (primary substrate is plastic; secondary substrate is cotton; contact manner is pressure; at temperatures of 4 to 40°C). Lesser and greater volumes of blood (such as a small blood spatter stain created during a forceful event or a large pool of blood created when a body with a bleeding injury remains in the same position for a while) may have different reaction characteristics.

Drying of blood can induce flaking, and has been shown to impact transfer [74]. The extent to which biological substances flake and/or powderise over time is dependent on substrates [74]; its potential impact on transfer is poorly understood and requires more research.

DNA deposits by hand transfer at a different rate to blood and saliva [37]. Goray et al. [37] found that such DNA deposits transfer at significantly lower rates than blood and saliva; however, drying has little impact on touch (at least 24 h post deposit) and therefore increased transfer rates, but not necessarily total amounts of DNA, were noted for 'touch DNA' compared to dried biological liquids [37]. 'Touch DNA' is not present in a liquid medium and therefore a time delay post-deposit does not appear to play a significant role when considering transfer for this biological material. However, these experiments only investigated the transfer of DNA through touching ~24 h after deposit when blood and saliva deposits are dry but skin deposits are not. Further assessment of transfer of primary touch deposits after longer time periods may be useful. It would also be of interest to determine the drying patterns and transfer rates of other relevant biological materials, such as semen, and the impact different substrates may have.

2.2.2 Source of DNA within touch deposit

2.2.2.1 Skin derived DNA: epithelial contained DNA + cell free DNA

Following its initial discovery, skin-derived DNA was believed to originate from epithelial cells sloughed from the outermost layer of the skin's surface [83-86]. Morphological analyses of fingerprints established that the majority of deposited cells were nuclei-free corneocytes, with only a limited number of nucleated cells and stripped nuclei observed [87, 88], suggesting earlier views regarding the transfer of shed epithelial cells may have been oversimplified.

Immunological staining of human skin from the neck revealed the granular layer of the epidermis comprised flattened cells with condensed nuclei that had lost their shape [89]. As described by Kita et al. [89], the nucleus disintegrates as epidermal cells (keratinocytes) move through the outer, cornified layer of the skin. Also in their study, Kita et al. reported the finding of fragmented DNA in samples taken directly from the skin's surface. They suggested that keratinocytes containing residual DNA are sloughed from the cornified layers and transferred onto a secondary surface by sweat. Others described the possibility of extracellular bodily secretions, such as sweat or sebum, as vectors for the transfer of cell-free DNA through the layers of the skin [90, 91]. This latter point fits with an earlier suggestion by Linacre et al. [92] who noted an increase in the number of alleles in profiles obtained following omission of the extraction process, whereby free DNA acted as a template for direct amplification by PCR.

Excluding the palms of the hands and soles of the feet, sebum secreting glands, or sebaceous glands, are dispersed over the entire body within the dermis. In their study on human skin samples from autoptic subjects, Zoppis et al. [90] described the finding of fragmented single stranded DNA in the cells forming the sebaceous gland, but not in the epidermis layers. They concluded that secretions from sebaceous glands in the skin, which are abundant in the scalp, face and around apertures of ear, nose, mouth and anus, may act as a vector for DNA residing in the fluid, part of what is transferred upon contact with skin areas. The study implies that the variable activity of sebaceous glands, which are under hormonal control, and the different parts of their own body an individual touches with their hands, will influence how much DNA is transferred by hands (see also section 2.2.3 regarding shedder status).

In contrast to sebaceous glands, sweat glands are located on all areas of the body, with the highest density in palms and soles. Quinones and Daniel [91] evaluated whether cell free nucleic acids (CNAs) could be detected in sweat and whether these contributed to the DNA recovered from a touched surface. They noted that the overall quantity of DNA retrieved within samples taken from sweaty hands increased when CNAs were present as opposed to those where CNAs were eliminated during processing.

The findings from these studies suggest that a combination of sources and factors influence what is deposition of skin-derived DNA through touch. While it is likely that sweat and

sebum act as vectors for the transfer of sloughed-off nucleated (and non-nucleated) cells, stripped nuclei and CNAs, the transfer of nucleated cells to the hands from other parts of the body is also a possibility, as is discussed in the following section.

2.2.2.2 Source identification

Any biological material can be transferred. Material such as blood when transferred tends to provide a stain that can be identified as 'what appears to be blood'. However, stains or smears may be of a minute level that is not obvious to the eye and/or present on a surface type and/or colour that makes visualisation difficult. Stains of saliva and/or semen are less visible to the eye. Methods are available to visualise these stains, and to some extent provide an indication of the likely source of the material [93, 94]. Various tests are available to determine the source of biological materials [95, 96] .

The source identification of skin is possible using mRNA techniques [97-101] or through microbiome analyses [102, 103]. However, as suggested [18, 95] and later demonstrated using RNA-based methodologies [104, 105], touch samples may consist of more than just skin cells. Biological material from other parts of the body, and from within the general environment [106], can be transferred to the hands, thus complicating the characterisation of material and increasing the quantity of DNA residing on the hands. This has an impact on source identification, as well as shedder status (section 2.2.3) and further DNA transfer.

Further, as the yield of DNA from a small volume of semen, blood and saliva tends to be greater than DNA collected from touched objects [27, 107], knowledge of the origin source of the DNA may assist with the interpretation of the profiles generated from a collected sample.

Source identification of the main biological substances can be highly relevant to the activity level assessment, either because the type of cellular material (1) will inform the probability of the material transferring, persisting or being recovered, or (2) may be informative regarding the alleged activity. Examples of the first are given earlier in this section (e.g. touch deposit by hand transfer at a different rate to blood and saliva). An example of the second could be taken from an alleged sexual assault case. Assume that a victim claims that the suspect has touched her inappropriately and has penetrated her vagina with his fingers. The suspect states that this did not happen, but that they have been dancing intimately in a club that evening. Determining whether any DNA from the victim found under the fingernails of the suspect was derived from vaginal epithelial cells [95] or another type of cellular material may be very informative towards assessing these findings given activity level propositions [2]. Associating a specific cell type to a specific contributor in a mixed stain may be very complex, particularly if there are no sex-specific cell types (e.g. semen or vaginal epithelial cells) or if there are multiple contributors of the same gender present in the mixture. Probabilistic assessment at source level, that is, assigning an evidential weight to source testing results, has been described by Taylor et al. [108], Taylor [109], De Wolff et al. [110] and de Zoete et al. [111, 112].

2.2.3 *Shedder status*

The study by van Oorschot and Jones [26] indicated that the amount of DNA deposited by hands was dependent on the individual. Several studies since have observed that there is variation in the propensity of individuals depositing their DNA upon contacting an item [32-34, 51, 52, 64, 71, 87, 113-115].

Lowe et al. [32] and Wickenheiser [18] suggested that there are good and poor shedders. It appears that some individuals consistently deposit more or less DNA than others [113]; these individuals are often referred to as 'good shedders' and 'poor shedders' respectively. From the studies by Goray et al. [113] and Kanokwongnuwat et al. [115] that investigated a number of individuals for their shedder propensity, it appeared that most individuals landed in the middle of the range, with relatively few individuals consistently being either very good or very bad shedders. This phenomenon requires much more investigation.

A study by Szkuta et al. [52], in which different pairs of individuals shook hands prior to depositing a handprint on DNA-free glass plates, demonstrated the impact that the relative shedding ability of the persons involved can have on the type of profiles generated from the post-handshake deposits. Good shedders (whether depositor or contributor) could swamp poor to intermediate shedders, while the pairing of two good, two intermediate or two poor shedders could result in the detection of both individuals.

The shedder status of individuals associated with an item of interest will impact how much of their DNA is detected on the item and its relative contribution to a mixture profile generated from a sample of interest. This impact has been demonstrated by Fonnelløp et al. [64], whose study also showed that this will depend on the type of the item and its use history (i.e. frequency and type of use).

However, it is yet unclear how best to categorise an individual into a shedder class, or how to allocate a shedder score on a sliding scale. It is also uncertain how one could best determine the shedder status of a POI at some point of time after the event of interest. Fonnelløp et al. [64] described a simple, binary shedder test which distinguishes low shedders from high shedders, whereby, individuals held plastic conical tubes in their dominant hand for 10 s to deposit their DNA. Associated factors were also investigated including the effect of time since hand wash and of wearing gloves (both of which were not controlled), and gender. High shedders were assigned when the DNA quantities obtained were above the average concentration in deposits made by all participants and at least two profiles were of high quality, while all other participants were defined as low shedders. They also provided an example of incorporating shedder status in casework considerations by means of the Bayesian approach, which considers the evidence in relation to the probability of secondary and/or direct transfer given shedder status. Counting the number of cells (within a specified area) deposited by a finger on a glass slide visualised by Diamond™ Nucleic Acid Dye [115] is an avenue worth considering further. Further studies are required to gain appreciation of the frequency of different shedder categories within specific populations and how this knowledge can be applied in casework deliberations.

While there is evidence that some people shed more than others, the factors influencing this are somewhat uncertain. Warshauer et al. [78] and Oleiwi et al. [114] demonstrated that the quantity of DNA shed from the palm surface is significantly less than from fingers, which, as previously suggested, may be linked to the prominence of sweat and sebum secreting glands in different areas of the body [90, 91]. Skin conditions that increase the proliferation rate of the skin cells, such as dermatitis and psoriasis, may also result in the deposition of higher quantities of DNA through touch [116]. Further, studies have shown that younger individuals tend to deposit greater quantities of DNA compared to those that are older [117, 118], while males may be more likely to be classified as heavy shedders compared to females [105, 117, 119]. Determining the extent to which genetic factors and various non-genetic factors (e.g. behavioural traits, health situation, and/or environmental conditions), and their potential interactions, impact shedder status, as well as identifying and understanding their underlying properties, warrants further investigation.

As indicated in the previous section, other sources of DNA may be present on the hand and contribute to what is deposited. The extent to which this occurs would be dependent on an individual's behaviour and habits, and would likely impact one's apparent shedder status. Warshauer et al [78] demonstrates this in their examination of transfer of saliva.

As shedder status may be a relevant factor when interpreting profiles in activity level assessments, further research focussed on shedder status is highly desirable.

2.3 Manner of contact / handling

van Oorschot and Jones [26] observed that substantial transfer of DNA material occurs during initial contact. Findings of others [18, 60, 120] concur with these observations. One may predict that there will be some accumulation of DNA on an object due to increased duration or frequency of contact. However, little is known with respect to DNA quantity accumulation profiles for objects of different types and substrates that may be dependent on duration and/or frequency of use; this requires further research.

How contact is made with an object will impact the level of transfer. As indicated in section 2.1, Goray et al. [37, 38] demonstrated that in most situations, when two objects come into contact with each other, more DNA tends to be transferred when pressure with friction is applied compared to passive contact or pressure contact without friction. Tobias et al. [121] confirmed that when surfaces were contacted by fingertips, increasing the pressure significantly increased the amount of DNA deposited, which resulted in the detection of more alleles from both the donor and unknown sources.

Different parts of a hand will contact different items in different ways. In some circumstances, it may be relevant to have an awareness of the impact of these differences on the amount of DNA deposited and the profiles generated. Oleiwi et al. [114] observed that less DNA was deposited by, or collected directly from, the entire palm area than by the combined total of the distal phalanges of the middle and ring fingers, despite the differences in surface area. The results of McColl et al. [122] concur with these observations.

They observed that different parts of a hand appear to have proportionally more DNA, more non-self DNA, and may be more likely to pick-up and transfer non-self DNA, than others (especially fingertips relative to other hand parts).

Different objects are handled differently depending on their shape, size and use. Furthermore, identical objects may be used differently by different people. Therefore, different parts of an object may possess different levels of DNA, possibly from different sources. For example:

- Handles of most tools and weapons are used relatively similarly by different users, but objects such as a long pipe or stick, or a stretch of rope or tape, can be handled in different locations depending on use.
- The handle of a knife is handled by the stabber, and if done so by bare hands, tends to contain DNA of the offender, whereas the blade inserted into a victim will typically contain DNA from the victim.
- A chair seat and armrest are contacted as a matter of course when using a chair to sit on, but may be grabbed by its back legs (an area normally very rarely touched by hands or exposed to human legs), when the chair is used as a weapon of opportunity.
- A door may be pushed at different locations depending on the height of the person pushing the door.
- An adjustable lamp or instrument with handles on the right and left side, may be grabbed solely from one side depending on the handedness of the user.
- The switch or plug of a standing lamp may be targeted for the normal user, but other areas not frequently touched, like the lamp's stem, may be targeted for DNA from the intruder who is suspected of having grabbed it there to use as a weapon of opportunity, or the lamp's base targeted for DNA from the victim whose injuries are suspected to have been caused by contact with it during an assault.
- Inner collar and wrist areas of an upper garment are targeted preferentially to detect DNA from a potential wearer, whereas an external area of the upper arm or shoulder may be targeted to detect DNA from the person who grabbed them during an alleged assault.

Furthermore, the level of interaction (from a single static contact of a specific sub-area of an object, to multiple, frequent, varied manners and locations of contact) of a handled object with another object will also vary widely. A study by Pfeifer and Wiegand [123] demonstrated the impact of different intensities of handling a tool (intense mock break-in to a premises versus normal use of a tool according to its designed purpose) on the types of profiles subsequently collected.

An awareness of the common means of use/handling of items of relevance, in combination with knowledge of a) the specific histories of the item of interest, b) the contacts with the item relevant to the alleged criminal activity, and c) the differences in transfer rates by different parts of a hand (or other body part), may become useful when a choice needs to be made regarding which area of an object, or print, to prioritise when sampling.

3. Prevalence of DNA / Background DNA

3.1 General considerations

It is reasonable to assume that most surfaces and items, unless new or cleaned, will have some DNA on them that has been acquired from previous use. This DNA is often termed 'background DNA'. This is especially so for personal items and shared items used regularly. Surfaces that are not regularly contacted may have less DNA on them. If items possessing background DNA are then used by others (i.e. the offender) during the course of an alleged criminal activity, mixtures from the usual user and offender can be expected [27, 82, 104, 123-128]. For example, a study of background levels of DNA on common burglary entry points from 20 different premises (windows, doors) found 26% (n=150) produced a DNA profile [129]. Furthermore, the authors observed the level of DNA on common entry points to be relatively low and suggested that, as windows are a common entry point for burglars, but have a low baseline level of DNA compared to other surfaces, these may therefore be a good target area for evidence recovery if a window was used to gain entry [129].

Background DNA may be derived from one person (mainly personal objects/environments) or from several individuals (mainly shared objects/environments, but also personal objects). When the sample of interest contains a substantial quantity of semen, blood or saliva that has been deposited on an item with a background of 'touch', or minute levels of other sources of DNA, then an appropriately collected sample of interest (the semen, blood or saliva) may provide a single source profile, as the quantity of DNA from this source can overwhelm that of the background. However, many such samples still provide mixed profiles. If the stain of semen, blood or saliva is itself at minute levels, one is more likely to generate a mixture profile that is inclusive of DNA from the semen/blood/saliva, as well as one or more individuals from the background DNA. This is illustrated in a study by Peel and Gill [130] who found that when dilute blood/saliva samples were deposited on a glass slide that had been handled before or after the deposit, DNA was recovered from both the body fluid and the handler; the respective ratios of which were dependent on the volume and concentration of the body fluid and the shedder status of the handler. If both the background DNA and the deposit of interest are 'touch' sources, then a mixed profile inclusive of owner and the second user is likely. However, the proportional contributions will be dependent on the levels of background DNA due to history of use, how the second user interacted with the surface, the duration of this interaction, and the shedder status of both users.

When dealing with 'touch' DNA as a background or a deposit of interest, the presence of non-self DNA on the hand of the individuals involved may contribute to a more complex mixed DNA profile. Furthermore, from a casework perspective, it may be relevant to assess whether the same unknown contributor has contributed to multiple traces. This is often difficult due to limitations in mixture interpretation. Developments in probabilistic models, including mixture deconvolution, may assist in identifying the same unknown in multiple traces [131].

3.2 Distinguishing background from deposit

Deciphering what was already on the object and what was added during the action of interest can become a contentious issue. Therefore, awareness of general levels of background DNA quantity, origin and quality may be useful. Further complicating factors are a person's shedder status and frequency of item use. This type of data is however very sparse and needs addressing.

Researchers are investigating means of distinguishing recent deposits from old deposits (background) and/or time since deposition. These include exploiting time dependent changes relating to nucleic acid degradation, mRNA, proteins, biomarkers and cell morphology [132-135].

Whilst not a prescribed policy in most jurisdictions, and often not possible, in some situations it may be useful to collect a sample from an area immediately adjacent to the area targeted for an evidentiary sample, thus revealing a profile that is possibly very similar to the background component of the target evidentiary sample [136]. The similarity, however, may have been compromised depending on the nature of the action depositing the biological substance of interest in the target area, as some of the background may have been lost from the primary surface to the contacting secondary surface (bi-directional transfer). van den Berge et al. [137] present data from a small example study considering the potential value of collecting post-activity background samples as a control to assist activity level reporting.

There was a time when such background samples, known as 'substrate controls' were taken alongside areas of body fluid staining, for example in the UK (and possibly other jurisdictions). However, these were for the purpose of checking for any potential interference from the substrate when using blood grouping tests on a specific area of body fluid staining. When DNA profiling superseded blood grouping, the value of such substrate controls for the purpose of detecting interference was brought into question [138] and ultimately the taking of substrate controls ceased. Interestingly, although critical of the use of substrate controls for their original purpose, Gill also commented that the taking of such substrate controls could help with certain types of DNA interpretation [138].

Within the broader context, when the prevalence of a POI within a crime scene is relevant, one may benefit from collecting a range of samples, depending on the case, including: (1) an item for controls (for instance immediately adjacent to targeted area), (2) similar items (like knives from a kitchen), (3) items in the immediate vicinity of the item of evidence (like the pillow under which the gun was found), and/or (4) from the wider crime scene. Such samples would provide case specific probabilities for prevalence/background, which would be better than generalised probabilities based on experimental studies. From a cost/benefit perspective, such samples could be collected, but only analysed if they become relevant within the context of the case.

The availability of reference samples of known regular users/wearers of an object may also assist the interpretation of the profile, irrespective of them being the victim, POI, or an incidental non-associated individual.

Further research is required to understand the influence of any background DNA on the interpretation of mixed profiles.

3.3 Prevalence and origin of non-self DNA on hands

Several studies have detected the presence of foreign DNA on the hands of individuals [33, 51, 52, 54, 60, 75, 82, 104, 113, 139-141]. For example:

- van den Berge et al. [104] found non-self alleles present in 83% (n=20 profiles) of samples taken from the neck and 88% (n=40) from hands, originating from up to 3 and 4 contributors respectively. The donor was present in all samples and was the major contributor in 100% and 93% of the samples, with an average peak height donor to background ratio of 7.6 and 8.8, respectively, in these sample sets.
- Goray et al. [113] found that 79% of samples (n=236) generated from touch deposits of left and right hands (of 10 individuals three times a day on four separate days, without any prescribed action prior to placing deposit) onto DNA-free glass plates contained non-self DNA.
- Samie et al. [139] found non-self alleles present in 97% (n=64) of samples of direct deposits by hands (after carrying out normal daily activities in their office environment) on DNA-free plastic knife handles (16 from each of 4 individuals). Three percent of samples were single source DNA profiles of the donor. The donor was clearly the major contributor in 83% of samples; 12% of the profiles were mixtures with no clear major/minor contributor; and 5% were deemed of insufficient quality.
- Meakin et al. [51] found non-self alleles present in 100% of samples of direct deposits by hands onto DNA-free plastic knife handles (3 from each of 4 individuals). The donor was also present in all samples as the major component. The non-self component contributed 1-3% of the total DNA on knives from three of the participants, but ~25% of the total DNA from the hands of the fourth participant.

Few of these studies tried to identify the origin of the non-self DNA, except for the following:

- The study by Samie et al. [139] tested for the presence of colleagues, of the donors of hand prints on glass plates, encountered during the day of the experiment, and did not observe their presence within the profiles generated from the plates.
- The prominent minor component reoccurring in the samples of one participant in the study by Meakin et al. [51], upon further investigation, was attributed to the romantic partner of the donor.

The extent to which non-self DNA is present on a person's hand, or is deposited upon contact with items, is dependent on the actions of the hands prior to sampling or depositing (see also sections 5.1, 5.3). For example:

- Cale et al. [54] maximised the likelihood of transfer of DNA from person A to person B and then from person B to a knife handle by having the persons A and B wash and dry their hands, then wear a glove for 1.5 h prior to shaking hands and to shake hands vigorously for 2 min prior to handling a knife handle for 2 min. They found that the detectable DNA of person A transferred to the knife in 85% of occasions, and was observed as either the only or major contribution in 25% of occasions (n=25).
- Szkuta et al. [52] also used handshakes between persons A and B (with bare hands and without any prior washing protocol) as a means of transferring DNA; however, they used a more realistic time of 10 s, followed by the subsequent immediate placement of a handprint for 10 s with pressure on a glass plate. They found DNA of person A was transferred to the glass plate by person B in 58% of occasions (n = 12). Person A was never observed as the major component in these profiles, nor were they observed as the sole contributor.
- Meakin et al. [51], also employed a 10 s handshake between persons A and B, but instead of person B touching a DNA-free object, they immediately picked up a knife handle they had regularly used previously and proceeded to stab a stabbing apparatus repeatedly for 60 s. In their study, person A was observed as a minor contributor (~10%) to the mixture profiles retrieved from the knives of three pairings, but not on knives of the fourth pairing.

In most circumstances within these studies, when person A was observed as a major contributor, the DNA of person B was still present as a co-major or minor.

However, in other studies where the circumstances were less controlled and/or optimised, the non-self component of the transferred profile was the major or only contributor in no more than 3% of samples [63, 75, 123, 139, 142, 143].

These studies focused on elements of transfer that incorporated variables within their experimental design, such as specific actions prior to DNA sampling, use of pre-cleaned objects, reuse of the same cohort of participants in different combinations, and/or use of optimised conditions, which limit their relevance for casework investigative/evaluative purposes [144, 145]. The overall numbers of samples are also low. To acquire more accurate probabilities of finding non-self DNA, and of the different relative mixture proportions within a deposit made by a hand, we need to collect more samples from random individuals in a wide range of situations. For many research laboratories, this will require more extensive ethical considerations.

3.4 Prevalence and origin of non-self DNA on body areas other than hands

Graham and Ritty [71] found non-self DNA present on the neck of 14 of 24 volunteers, with most non-self DNA being detected on the necks of volunteers who were married or lived with partners. In all instances the number of non-self alleles was fewer than the number of self alleles detected.

In a separate study by Graham et al. [72], surface swab samples collected from 12 face/neck sites and 20 body sites of 50 children less than 5 yr of age showed that overall, extremely small amounts of non-self DNA was present, with foreign DNA observed on 35% of swabs tested (most showing only a few alleles). Furthermore, their study found a high degree of variation between children and between areas tested.

Evaluation of findings, particularly in cases of sexual assault, often requires information on the prevalence of DNA on other body parts than the hands. Especially on the more intimate locations of the bodies of both men and women. We do encourage, where possible, more research effort on the prevalence of non-self DNA on the bodies of both children and adults.

3.4.1 Fingernail samples

It is well established that foreign DNA can accumulate under an individual's fingernails [70, 146-159], and this knowledge has been considered further in respect to its use in investigating crimes [39, 42]. This DNA is relatively protected compared to other parts of the body that are exposed to physical contacts and actions. When evaluating DNA profiles obtained from under the fingernails in a forensic context, direct versus indirect contacts and normal everyday versus criminal activities are often assessed against each other when presenting this evidence in court. This assessment is further complicated when the victim and offender are known to each other, are in a relationship or live together. Understanding of the prevalence, persistence and type of foreign DNA profiles under the fingernails after different sets of circumstances can assist profile interpretation.

The incidence of direct versus indirect DNA transfer under the fingernails is difficult to assess as, to our knowledge, no studies to date have specifically investigated indirect transfer to the fingernails (for instance, by occupying another person's space for a certain amount of time). Currently, inferences regarding indirect transfer can only be made, to a degree, from the results showing the detection of foreign DNA in direct contact studies. Initial results show that there is a higher probability of finding full, good quality DNA profiles from simulated direct contact studies, than from indirect transfer of DNA in these persistence studies. However, one could also argue that fingernails are a site where non-self DNA could accumulate over time, and from several different types of actions. The potential quantity and quality of this relative to the quantity and quality of DNA from the same other individual, but from a single direct contact activity, needs to be researched.

Examples of various studies include:

- *General population:* Various studies have found foreign DNA in fingernail samples, with occurrence ranging from 5 to 41% of samples, and higher quality mixtures (as defined by the respective authors) detected in 6-14% of these [70, 148, 150, 155]. Of

the over 300 people tested within these studies, only one sample had foreign DNA detected as a major contributor, however this sample was taken from a participant who self-reported previous intimate activity. When these studies investigated factors affecting foreign DNA detection, time since last human contact, incidence of intimate contacts, participants' sex (increased detection in males), and in some studies, the length of the fingernails, were found to be significant. Notably, fingernail scrubbing/washing did not guarantee complete removal of foreign DNA [147, 148].

- *Co-habitation*: A study by Kettner et al. [151] of the male quantification results in a scratching study showed highly significant differences in the amount of DNA between scratching and non-scratching fingers of females that scratched their partners, indicating that even if females are living with male partners, the amounts of male DNA can provide an indication of different types of contact taking place. Studies that look at cohabitating individuals, both in intimate and non-intimate relationships, found mixed DNA profiles in 14 to 37% of the samples (collectively n= 45 people), however only 6 to 17% were higher quality mixtures [146-148]. Interestingly, with non-intimate cohabitation, in one study, household members were excluded as the source of foreign DNA in all samples (n=25 people) [148]. Most of the mixtures obtained in these studies originated from two people indicating that more complex mixtures are rare. Within these studies, there was only one reported instance of the mixture inversion, where the foreign DNA was detected as a major component, highlighting its rarity (<1%) in fingernails sampled after everyday activities
- *Intimate activity and digital penetration*: A study by Flanagan and McAlister [147] observed single source female profiles and mixtures with the major female contributor in all male fingernail samples immediately after digital penetration. The female DNA was shown to decline over time; however, informative female profiles (more than 4 alleles) were still detected within a mixture with the male donor in 75% and 63% of the samples at 12 and 18 hours post penetration, respectively.
- *Physical altercation/scratching*: A study by Wiegand et al. [152] of evaluating the impact of superficial scratching of arms, necks and scalps, detected more instances of foreign DNA in fingernail samples after scratching of necks and scalps than arms. However, fingernail samples from the scalp/neck scratching were collected with the less invasive sampling procedure (light sample removal avoiding abrasions to the fingernails versus extensive cleaning leading to abrasion of the fingernail and skin), which the authors suggest can possibly explain the result. A study by Matte et al. [148] of fingernails of 30 male/female pairs that scratched each other on the underside of the forearm detected foreign DNA in 37% of the samples. Increased vigour and number (x30) of scratches resulted in better quality foreign DNA detection. The foreign DNA under the fingernails diminished with time. The length of time that foreign DNA can still be detected after scratching was shorter than after digital penetration [147]. A study by Kettner et al. [151] that included vigorous

scratching of the flank of the 34 participating males by their female partners showed that scratching fingers had significantly higher concentration of male DNA. However, in approximately 31% of all couples, no male DNA was detected after scratching, thus highlighting that a negative result does not necessarily indicate that scratching did not take place.

- *Casework fingernail samples*: A number of casework sample studies (including homicides and sex offences) reported mixed DNA profiles in the range of 13 to 35% of samples (n=over 300 cases); however, 'higher quality' mixtures were detected in 7-21% of these [148, 149, 153, 156-158]. In several instances, the foreign component was detected as the major contributor. Note, as the ground truth knowledge within all these cases may not be fully known, these limitations should be made clear to the court when used in casework practice.

The above studies assess the impact of different activities performed prior to sample collection, and utilise different collection strategies and methodologies, which can complicate comparisons and use (sections 7.4, 8.4.1, 8.4.2). However, in instances where the study differences relate to differences in methodologies post collection, the mixture proportions of donor to foreign DNA will in many cases likely hold true, as both will be lost and gained at a similar rate.

Samples taken from fingernail scrapings may be informative when evaluating findings given scenarios on the manner or timing of contact. There are differences found in both the persistence of DNA and the composition and quality of the DNA profiles after different contact situations. However, we also note a crucial gap in our knowledge. Specifically: a) acquisition of non-self DNA during regular social interaction (e.g. the defence position involving dancing in the case example in section 2.2.2.2.); b) indirect transfer by occupying another person's space for a certain amount of time. Hence we encourage further investigations into these aspects. These should include further investigations of the effects of personal habits, and an individual's shedder status, on detection of donor and foreign DNA under fingernails. Availability of more data would benefit assessments of findings given alternative propositions relating to innocent versus criminal contact.

3.5 Prevalence and origin of non-self DNA on personal items

Personal items and clothing are often collected as evidentiary or reference material. It is usually assumed that these will provide the profile of the regular user/wearer, which they usually do; however, they often also contain DNA from other sources [64, 82, 104, 123, 137, 160-164]. For example:

- van den Berge et al. [104] found non-self alleles present in all samples collected from exterior surfaces of trouser legs (n=48) and armpits of shirts (n=16) originating from up to 5 and 3 contributors respectively. The donor/wearer was present in all of these samples and was the major contributor in 60% and 100%, with an average peak height donor to background ratio of 4.4 and 5.3 in these sample sets respectively.

- Noël et al. [161] found that of 168 samples taken from underwear (n=24) of children that had been regularly worn and washed with the rest of the family's laundry, 52% yielded interpretable mixtures of DNA corresponding to multiple family members (including fathers, brothers, sisters and mothers) post-washing and drying. DNA corresponding to the mother was detected in 51% of samples contributing 5 to 90% of the total genetic mixture.
- Stouder et al. [163] found that, for all 11 items of clothing (T-shirts and hosiery) that were worn for a day following laundering, DNA from the wearer was recovered as either the major or co-major profile. In the latter case, the other co-major profile was attributed to the wearer's spouse. Minor profiles on the other items were attributed to the respective wearers' spouses, but also to their children and to unknown sources.
- Magee et al. [164] observed the spouse of the wearer as a minor contributor in DNA profiles obtained from three of four garments sampled (from the collar or cuff) that had non-wearer DNA components, and for which reference profiles were available from the spouse.

One of the likely sources of non-self DNA on personal objects is transfer from the hands of the owner/user acquired by handling items belonging to and used by others. It is of interest to become informed on not only the extent to which non-self DNA resides on the hands/skin of an individual and their personal objects, but also of its origin.

Very few studies have focused on establishing the origin of the non-self DNA on personal objects. Awareness of likely contributions of various known associates of an owner/user of an item/space may assist understanding of contributions to background DNA and the interpretation of mixture profiles. We encourage further studies be conducted to improve our awareness of contributors to the non-self component of DNA retrieved from personal objects and occupied spaces. Such studies will require appropriate consent from relevant associates of primary volunteers, as well as adherence to relevant ethics protocols. Improved awareness of the probability of detecting specific known close associates (e.g. a family member, live-in partner, co-resident, friend and/or colleague) of the owner/handler/wearer/occupant of an item or space, given the history of the item or space, may identify common associates regularly found on specific types of items. This may highlight the need for reference samples to be collected from such close associates during casework to assist profile interpretation. During casework investigations, comparisons to reference/elimination samples from known potential contributors may assist interpretation of mixture profiles.

4. Persistence of transferred DNA

4.1 Persistence of deposit over time without further use

The DNA of biological samples, including those assumed be deposited by handling, can retain sufficient quantity and quality to generate full STR profiles for several years, even decades, which is being successfully exploited to assist cold case investigations [36, 124,

165-168]. The persistence of any biological material will be dependent on a range of environmental factors, including: temperature, exposure to UV, rain, wind, humidity and presence of micro-organisms on the surface.

Caseworkers have anecdotally collected trace samples, of unknown source assumed to be deposited by handling, from many items after various exposures. However, Raymond et al. [129] showed that the recovery percentages of naked DNA and buffy coat cells placed onto external painted wooden window frames of a residential building and collected at different times after deposit (1 day to 6 wk) declined more rapidly than the replicate samples on glass slides kept in a dark laboratory cupboard, with the naked DNA declining more rapidly than the buffy coat.

Wigand & Klieber [83] reported detecting DNA derived from the suspect in a sample taken from an area of the neck of a strangulation victim ~48 hr after death.

A study by Meakin et al. [51], in which participants shook hands and then immediately stabbed one of their previously handled knives into a foam block, observed that indirectly-transferred DNA from the opposing 'handshaker' could be recovered from the knife handles for up to at least one week after the stabbing. Whilst the peak heights of the unique alleles attributed to the opposing handshaker significantly decreased over the course of the week, only minor partial DNA profiles were obtained at any time point [51].

4.2 Persistence of deposit after continued use

A study by Szkuta et al. [52] aimed to determine the probability of detecting DNA from person B on five glass surfaces contacted sequentially by person A either immediately or 15 min after shaking hands with Person B. Following immediate contact post-handshake, Person B was observed as a minor contributor, or as a contributor when no major was assigned, in 27% (n = 60) of profiles obtained. This decreased to 15% (n = 60) when contact was delayed by 15 min. Of these profiles, person A (the individual contacting the surfaces) was excluded as a contributor from three profiles (5%) obtained from the surfaces following immediate contact post-handshake and a single profile (2%) when contact was 15 min post-handshake. Regarding the latter, both bi-directional transfer and DNA parking were postulated as the mechanisms for transfer of person B. In a separate study, where the deposit after handshaking was onto a wood handle (firmly grasped while rotating the hand to create friction) 40 min, 5 h and 8 h after the handshake, person B was observed as a contributor (minor in all cases) were 25% (n = 12), 8% (n = 12) and 0% (n = 12) respectively [53]. Person A (the individual contacting the handle) was observed as a contributor on all occasions. Thus, in these studies, person B was observed as the only contributor in a single profile obtained following immediate contact post-handshake and was never observed as the major contributor in mixed profiles [52, 53].

In a study by Graham and Ritty [71], saliva deposited on the neck of another person by licking appeared to still be detected in 3 of 5 samples of necks collected after a day of normal activity.

The quantity of biological source of interest present on any one item will likely diminish after each contact. One can expect this is also the case when considering self and non-self DNA on hands after touching multiple objects. This has been demonstrated in studies by van Oorschot et al. [35], Buckingham et al. [50, 77], and Szkuta et al. [52].

4.3 Persistence of original user after use by another

As demonstrated by a number of studies, it is possible for the DNA of an original handler/wearer of an item to persist for lengthy durations after being used by a second person. For example:

- van Oorschot et al. [82] showed that DNA contributions from the original user (rubbed vigorously for a total of 210 s) and second user of a pen were approximately in equal proportions after 1-5 min through to 61-80 min use by the second person. The detectability of the original user gradually declined as a pen was used more by a second person, but was still detectable at 15% of profile contribution after 90 min. In a separate experiment, the original wearer (worn for a total of 34 h) of elastic armbands also declined as they were worn longer by a second person, but was still a major contributor to the profile retrieved up to 28 h after wearing by the second person. It gradually declined to ~10% after 96 h [82]. A full profile of the original wearer was present in 57 of 60 armbands sampled after being worn by a second person. The remaining three, worn by the second person for 8 or 16 days, provided a partial profile of the original wearer. In another experiment, most of the profiles generated from 108 samples taken from a wide range of previously used personal objects (owned for different periods of time) from known individuals that had been temporarily used for various durations by known second persons, were mixture profiles inclusive of the original owner and second user [82]. The profile of the owner was present in 80% of samples. This included samples such as a fabric lanyard worn by a second person for 1 month (work days). Items where the profile of the second user completely replaced that of the owner included a lighter, eye shadow case, USB stick, hair clip, lip stick holder, lip gloss container and lid, pen and pen lid, sunglasses nose and ear bridge, and perfume bottle lid. Some of these had been used by the second user for relatively long and short periods. Full profiles of the owner were recovered from 39% of the non-porous substrate samples and 79% of porous substrate samples.
- Oldoni et al. [141] examined profiles generated from a wide range of items (including computer mouse, key, pen, watch, internal and external part of disposable glove, cap, bracelet, necklace) after sequential contact by two different users for different time periods. They found large variability in relative profile contributions depending on the duration of use by a second person, substrates of the items, and the individuals handling the items. Contributions of the second individual progressively increased relative to the first user with prolonged use. The second user was observed as the major contributor in ~15%, 33% and 55% of samples taken from a variety of items after 5, 30 and 120 min of use respectively. The first user was no

- longer detectable after use by the second person in 2 of 234 (0.9%) of samples (both were bracelets, one worn for 30 min, the other for 120 min by a second person).
- Fonnelløp et al. [140] showed that DNA of an initial user of a personal regularly-used computer keyboard and mouse, can persist and detectable level transferred to hands of a second user, up to 8 days after 30 min of use per day. Differences in the ability to detect the initial user's DNA on the hands of the second user among participating pairs over time, was reasoned to be due to differences in shedder status.
 - In simulation robbery tests conducted by Raymond et al. [124], wallets used for 1 wk by one person and then used by a second person for either 1 min, 1 h or 1 wk (n=5 per handling time of second person, at normal amplification) all showed mixture profiles representing both persons, except for one sample (1 wk first person, followed by 1 min handling by second person) which contained DNA of the first handler only, and another sample (1 wk first person, followed by 1 h second person B) which contained DNA of the second person only.
 - Pfeifer and Wiegand [123] studied the persistence of DNA on burglary-related tools (including screwdrivers, crowbars and hammers) that had been owned, or mock owned (i.e. used for 30 s in a manner the tool was designed for, 30 min after washing hands with soap, with the amount deposited deemed comparable to the amount found on real tools tested), by one person and then used by a second person. Tools were either used intensely in a mock burglary action (~30 s) (n=30), or moderately as per normal use (30 s) (n=30), with bare hands and without any prior handwashing protocol. They found the second user to be more frequently present than the first user (80% and 3.3%, respectively) in the profiles from the samples of the tools after the mock burglary situation, whereas, the second and first users were present in a similar percentage (64.7% and 52%, respectively) of profiles after moderate handling. It is to be noted that, apart from the manner of handling, the types of tools and the substrates of the contacted areas within the two sample sets were different; this may have contributed to the differences in profile compositions observed.
 - In a study by van den Berge et al. [104], trouser ankles (n=48) and armpits of shirts (n=16) of known wearers were sampled after being contacted during dragging events by known individuals. The profiles contained 2 to 5 contributors and all indicated the presence of both the wearer and grabber. The peak height ratios of victim to background ranged from 0.7 to 29.8, and of grabber to background from 0.6 to 14.7.
 - Poetsch et al. [120] found that of 84 sweat bands, sampled separately from the inside and outside, worn for 4 h to 3 days by a first wearer, followed by 10 min to 3 days by a second wearer, 92.9% provided a mixture profile containing both wearers. A single source profile of the first wearer was observed in only one sample. This sample was from the outside after being worn for the shortest period by a second wearer (1st wearer 4 h + 2nd wearer 10 min). A single source profile of the second wearer was observed in 6 outside samples and 5 inside samples. In each of these

cases the sweatband had been worn for a far longer period by the second wearer than by the first wearer.

- In a study by Breathnach et al. [125] underpants that had been worn for ≥ 12 h by a male, were gripped at a specific location of the waist band (while unworn), with two hands for 15 s by a female (n=63), then sampled from the whole waist band. Based on their suite of applied methodologies and internal interpretation guidelines they found that the probability of observing reportable DNA profiles to be 61.9%. In addition, the wearer was detected as a single source profile or part of a mixture in 50.8% of samples; the wearer when present in a mixture was always observed as the major contributor; the gripper was detected in 11.1% of samples; and the background DNA (non-wearer and non-gripper) was present in 87.3% of samples, with a reportable unknown profile observed in 14.3% of samples (as a major in one sample and as a minor in eight samples).

In respect to the study by Breathnach et al. [125], the authors themselves indicate that their observed rate of detecting the wearer, and presence as a major contributor, was lower than in another study, may be attributable to differences in sampling methods, RFU thresholds/interpretation standards and/or kit/instrument sensitivities.

Apart from the impact of these suggested potential contributing factors, the experimental designs of the studies discussed have features that will have impacted the outcomes and would thus also need to be considered when contemplating utilisation of data for interpretation of casework related profiles and events [169].

Overall, the above examples, whilst demonstrating that DNA on an item derived from an original user can persist after the use of the item by a second person, show differences in outcomes dependent on items/substrates, activities/action, and durations involved, as well as the interpretation methods utilised, thus illustrating the need to apply data from circumstances most closely aligned with the conditions of the scenario of interest.

4.4 Persistence of temporary user after original user resumes using the object

Within some casework scenarios an object of interest that belonged to, and was originally used by, one person may be temporarily used by a second person. This second person may be the POI, but rather than the object not being used again after an action of interest, it was used again by the original user, prior to securing the item for examination. For example, an accused may claim that they:

- Had been an incidental driver in a vehicle at some time in the past rather than at the time of the offence; i.e. that after they drove the vehicle, it was used again by the regular driver/owner.
- Borrowed and wore a balaclava at a fancy dress party once, and that after the party, it was returned to its owner and subsequently worn by a perpetrator during a robbery.

- Belonged to a group of individuals that commit burglaries, for which the accused was convicted in the past. The group shared tools like crowbars among themselves whenever they committed a burglary. Although the accused was active in the past, they claimed they had not committed any offence (including the one they are charged with) recently, and that one or more others must have used the item after the accused last handled it.

To our knowledge, no major study has been conducted to determine the persistence of the temporary user of an item after use by the original owner has resumed. Whilst one may extrapolate from the data presented in the studies mentioned in the previous section, and those mentioned in the following sub-section, further research on this aspect may be warranted.

4.5 Persistence after multiple users on non-personal items

When an item has been touched by multiple individuals in similar fashions one is likely to obtain a mixed DNA profile. For communal non-personal items, the last user is not necessarily going to be the major contributor. The mixture proportions generated from the item will depend on the shedder status of the individuals in question and the type and frequency of contacts by all users. This was observed in the initial study on DNA transfer by van Oorschot and Jones [26], and in subsequent studies under controlled [63] and non-controlled conditions [127].

A study by van den Berge et al. [104] of DNA profiles generated from public items (n=51) such as railings at train stations, door handles and flush buttons of public toilets, handles of shopping carts and baskets, library books, coins and indoor handle bars, found that useful quantities of DNA were retrieved from the majority of samples with most profiles having multiple contributors. A major contributor was detectable in 17% of profiles. Of the banknotes (n=51) and coins (n=6) where the last user was known, the last user was not necessarily the major contributor to the profile obtained; they were the major contributor in 5 of 9 samples where a major contributor was detectable.

To determine which of multiple last known handlers of an item is detectable, Buckingham et al. [50] had sets of four individuals each consecutively handle a knife by simulating a stabbing action. They found that in each situation (n=6) all four individuals were detectable, with the more recent handlers of a knife being the most prominent within the DNA profile generated from the handle. However, the last handler was not always the major contributor.

Greater knowledge would be welcomed of probabilities of detection, and relative contribution, of individuals to profiles retrieved from a wider array of shared objects and surfaces within confined shared spaces (e.g. homes, offices, cars) and public spaces, given known histories.

4.6 Persistence of sperm (and other foreign DNA sources) in the vaginal cavity

Sperm persistence in vaginal samples decreases over time, and is believed to result from vaginal lavage and drainage, menstruation, and general time-dependent cellular degradation. However, studies have suggested that phase of menstrual cycle is not a crucial factor [170, 171]. Further factors considered during sperm survival assessment are the use of oral contraceptives and presence of vaginal flora that are known to affect detection frequency. The cellular sperm degradation in the vagina, assisted by the immune system, is also associated with increased structural fragility that can cause premature lysis of sperm cells during differential extraction and detection in the non-sperm fraction. In such instances, male DNA may not be detected with autosomal STRs due to high female to male DNA ratios.

Various studies have contributed to our knowledge of the persistence of sperm in the vagina post intercourse. This knowledge includes, but is not limited to:

- Morrison [172] detected spermatozoa 12 and 9 d after intercourse in the cervical and vaginal samples respectively, but noted that detection rate decreased progressively after 48 h.
- A study by Casey et al. [173] of the results of 1450 cases observed the longest sperm persistence in the vagina to be 96 h after intercourse with significant decline in detection after 18 h and again after 48 h. The same study noted that, on rare occasions, spermatozoa were detected in the anal and oral samples 48 h after the alleged offence, however rapid decline in detection was reported after 6 and 15 h respectively.
- Hellerud et al. [174] found very few sperms in samples taken at 96 h and no acid phosphatase (AP) positive reactions past 24 h.
- An analysis of 900 alleged vaginal penetration cases detected sperm in 29%, 12% and 4% of vaginal, anal and oral swabs with the longest recorded detection time of 83 h, 29 h and 12 h respectively [175].
- Another study based on casework samples showed that while most positive samples were taken within 6 h of the alleged offence, on occasion spermatozoa can persist for 24 h and on one occasion was detected in a sample from a deceased person taken 48-96 h after the offence [176].
- Willott et al. [177] detected spermatozoa in vaginal, rectal, anal and oral swabs 120 h, 65 h, 46 h and 6 h after the alleged offence, respectively.
- A study by Astrup et al. [178] of three different sites (external genitalia, posterior fornix and cervical canal) of 60 women post intercourse (all samples taken within 48 h) detected spermatozoa in 88% of women who reported ejaculation into the vagina and in 14% of women who reported that no ejaculation occurred. They detected significantly higher numbers of spermatozoa in the posterior fornix sites. The same study also reported observing significant inverse correlation between time since intercourse and the number of spermatozoa detected and observed a negative effect of the use of lubricants on sperm detection.

Some of the above data are from collated casework findings. For further studies, see also DiFrancesco & Richards [179]. As noted previously, limitations should be stated when interpreting collated casework data to assign probabilities as the ground truth is very rarely known. However, it is to be noted that the ground truth has many aspects. We tend to focus on those that are deemed relevant to the factors of interest in the case. Whilst overwhelmingly the ground truth of the full circumstances of a violent crime is unknown, there are different types of crime and different elements to a crime (biological and non-biological). There are circumstances where the ground truth of a relevant element is known. For example: a sexual assault case where the debate is about whether or not the act was consensual. The time of intercourse is not questioned, and may be used to infer persistence of semen in the case file study.

Olofsson et al. [180] showed that the detection and persistence of both sperm and trace male DNA in the vagina increased when using Y-STR DNA profiling. Further, a study by Hall and Ballantyne [181] showed that sperm persistence in vaginal samples taken at 0-6 d post intercourse analysed using an autosomal DNA profiling test detected male donors up to 12 h post-coitus, whereas Y-STR testing increased partial male DNA detection to up to 4 d.

Benschop et al. [182] compared post-coital vaginal sampling success using cotton and nylon flocked swabs and found improvements in total male DNA yields and cell release during elution when using the latter. The authors suggested that nylon swabs may retain sperm cells more efficiently or vaginal cells less efficiently. Positive presumptive tests (PSA and RSID- semen) in this study were reported up to 60 h after intercourse; however, over 50% of the samples that were negative during presumptive testing produced male DNA profiles during autosomal typing, and Y-STR profiles were detected up to 84 h post intercourse.

Male DNA found in the vagina post intercourse could be derived from sperm within ejaculate and/or biological material (e.g. skin derived or saliva derived DNA) on the penis, fingers, tongue or object that entered the vagina [183-186]. McDonald et al. [183] in their study of persistence of male DNA, with no detected spermatozoa after penile and/or digital penetration, obtained Y-STR positive results up to 48 h post alleged offence, however as no samples were taken past the 48 h cut off the authors suggested that male DNA may be detected even later if tested. An analysis by Albani et al. [187] of 259 vaginal samples analysed for the background levels of Y-STR profiles showed that without intercourse, no profiles with three or more alleles were detected in the tested samples. However, 14% of these samples had between 1-2 non-reproducible alleles that did not correspond to the expected males and were likely introduced during sampling. In contrast, 93% of samples taken after unprotected intercourse produced partial and full Y-STR profiles up to six days later.

5. Other factors impacting transfer

5.1 Washing of hands

Lowe et al. [32] first suggested that handwashing may impact DNA transfer. They showed that the proportion of donor DNA, deposited on a clean DNA-free tube during a hold of 10 s, increased with increasing time since handwashing. At 6 h post-handwashing, all eight participants deposited full DNA profiles (detected using low copy number profiling of 34 PCR cycles); non-donor DNA was also recovered. Similarly, when comparing DNA recovered from clean tubes handled for 10 s at 15 min post-handwashing versus without controlling for handwashing, Phipps & Petricevic [33] observed a general increase in numbers of alleles when handwashing was not controlled.

Further effects of handwashing have been observed more recently. In particular, Zoppis et al. [90] only observed recovery of self and non-self DNA from fingermarks on glass slides that had been deposited prior to handwashing, as no DNA was recovered when participants touched the slides 10 min after either conventional handwashing with regular hand soap or deep handwashing with antiseptic soap. This study also suggested that handwashing may impact secondary DNA transfer. When participants rubbed their finger on a sebaceous skin area (e.g. back of the hand) of another individual and then touched a glass slide, mixed DNA profiles were recovered when hands were not washed first, while in the majority of cases (5 of 8) post conventional handwashing, only DNA from the other individual was recovered and not from the participant who touched the slide [90]. Addressing more than just DNA recovery, Stanciu et al. 2015 [188] considered the contribution of whole cells versus extracellular DNA to the DNA deposited via touch before and after handwashing with soap and water. They found that a greater quantity of extracellular DNA was transferred from unwashed hands (0-4.646 ng) than from washed hands (0-0.242 ng) on to clean tube held for 5 min. Interestingly, although they observed a greater transfer of whole cells from washed hands than from unwashed hands, these cell pellets resulted in very low DNA yields.

In contrast, a number of transfer-related studies that recorded when participants last washed their hands prior to placing a deposit did not observe an impact from handwashing. For example, Goray et al. [113] found no significant difference in DNA deposits recovered from handprints on glass plates between those left by individuals who had washed their hands less than an hour prior to the deposition (64 of 240) and those left by individuals who had washed their hands more than an hour prior (176 of 240). Similarly, Szkuta et al. [52] observed no connection between the time since handwashing and the contribution of donor DNA to the samples recovered from handprints on glass plates deposited after a handshake, even though time since handwashing ranged from as short as 5 min to as long as 6 h.

Given these mixed results, a greater understanding of the impact of handwashing on the amount and quality of DNA deposited from hands is required, particularly through further systematic study. Factors, such as different methods of washing hands, the natural accumulation of DNA on hands post-handwashing, and the different personal habits of individuals, and their effect on the accumulation of self and non-self DNA on hands need further attention.

5.2 Washing of clothes

Research has been conducted to examine the persistence of DNA on clothing after washing and the extent of DNA transfer to clothing during washing. Initial studies investigated the persistence and transfer of DNA from body fluids, such as semen, blood and saliva. For example, DNA profiles from semen stains were found to still be retrievable after washing and also retrievable from other co-washed clothing as well as the washing machine drum [161, 189]. Similarly, reportable and/or informative DNA profiles from blood tended to be obtained from washed blood-stained cloths and from co-washed clean cloths [190-192]. In contrast, van den Berge et al. [104] found that the average persistence rates of DNA from both blood and saliva-stained cloths after washing were less than 0.001%, and the transfer of DNA from these stains onto the co-washed items was found to be extremely limited with only a very few alleles, if any, being detected. Whilst Kulstein and Wiegand [191] also obtained no reportable or informative DNA profiles from clean cloths washed with saliva-stained cloths, they found 52% of saliva-stained cloths gave reportable DNA profiles after washing. The reason for such different results between the studies is unclear, especially given that the starting volumes of blood and saliva in Kulstein and Wiegand's study were smaller than those used by van den Berge et al. (20/100 μ l versus 500/1000 μ l).

Further studies have started to address the transfer and persistence of skin derived biological material on washed clothing. DNA deposited onto cotton cloths via rubbing on the neck for 5 s gave full DNA profiles from 40% of cloths held under a running tap (with cold or hot water at different time points up to 10 min) and 56% of cloths submerged in water in a bathtub (with or without soap and at different time points up to one week) [193], but gave only partial profiles from 13% of cloths that were machine washed [192]. Kamphausen et al. [192] observed few alleles, if any, on the remaining cloths that were machine washed and similarly few alleles were recovered from co-washed clean cloths.

Although both van den Berge [104] and Kamphausen et al. [192] concluded that DNA persistence and transfer in a washing machine is very unlikely, further studies have found different results. Of unworn items washed in household washing machines with normal loads of dirty laundry, Voskoboinik et al. [194] found that 19% gave single-source or major DNA profiles matching that of a member of the household and Ruan et al. [160] found 76% gave single-source or mixed DNA profiles. As suggested by Ruan et al. [160], this increase in detection of DNA transfer could be due to the use of a profiling kit with increased sensitivity (PowerPlex21[®] as opposed to SGM Plus[™]). Voskoboinik et al. [194] also found that no detectable DNA was recovered from unworn socks washed alone or from swabs taken directly from the interiors of several washing/drying machines, suggesting that DNA transfer occurs via the dirty clothes within a washing machine rather than via the machine itself.

These studies demonstrate that background DNA can be acquired on clothing via various means, including washing. Ruan et al. [160] raise the valuable point that whilst we assume that the observed DNA transfer occurs within the washing machine itself, there are other opportunities for DNA transfer during the whole process of washing and drying clothes, such as via the mixing of clothing in a laundry basket prior or after washing and the varied modes of drying clothes. A wide range of possibilities may therefore need to be considered during the DNA evaluation process, depending on the scenarios under consideration.

5.3 Activities between activities

When considering the likelihood of transfer from one area to another, with a hand as the vector, one tends to focus on the variables potentially impacting transfer associated with the original deposition of the sample of interest onto a primary substrate and its pick-up by the hand, then consider those associated with the contact of that hand with the item of interest from which a sample was collected. However, it would be deficient if one did not also consider the known facts and/or probabilities of that hand contacting things in the period of time between those two actions of interest, given:

- A) Knowledge that loss of DNA, and the potential gain of DNA, can occur upon every contact. Thus loss of DNA, and/or acquisition of more own and/or other DNA, from intermediary contacts, will impact the likelihood, quantity and relative proportions of the DNA of interest detected from the final surface of interest.
- B) Studies have shown that a person's hands will contact many surfaces (including themselves, objects they own, objects they share, and/or non-personal objects) within a very short period of time, in a range of everyday general activities [195] as well as during criminal activity [196].

Any contacts by a hand in the interim period will impact what may be found in the collected sample. Similarly, an object that acquires biological material of a POI during one activity, and sometime later biological material from a second POI, could have had nothing contacting it and not be exposed to any detrimental environmental factors in the interim period, or could have been contacted by one or more individuals or other objects with or without other DNA on them, to different degrees, and/or exposed to detrimental environments, causing loss of existing DNA and/or gain of other DNA in the intervening period.

5.4 Flies

Transfer of human DNA associated with criminal activities will usually be through direct contact between individuals, an individual and an object, or between objects. However, in some settings it may be relevant to consider the possibility of transfer via animals. One example is the possibility of transfer of human DNA via flies. Flies will be attracted to blood, semen, and dead bodies and feed from them. Their artefacts (defecation and regurgitation) deposited later, at the same site or elsewhere, can contain sufficient human DNA to generate full profiles [197-199]. Sampling of fly artefacts in the absence of a body and/or stains due to removal/cleaning, as the only remaining potential source of DNA associated with a POI, may thus be of assistance. However, as artefacts can be deposited on surfaces some distance away from the feeding site, and because their detection and distinguishing features can be difficult [197, 200, 201], they could also be collected as an incorrectly assumed stain source, misdirecting profile interpretations and relevance to the crime event.

Human DNA can also be profiled from the excreta of adult human crab louse [202] and from mosquitos after feeding from humans [203-205].

6. Complexities of trace DNA dynamics

When assessing the probability of detecting DNA of interest (section 11) within a scenario incorporating multiple contact events where transfer may have occurred, one first needs to determine the order of contact events within the pathway of interest and then to consider the impact of the variables present at each event. All of the variables mentioned in the earlier sections need to be considered for each contact event within a sequence, as well as any potential inter-variable effects. Even factors which at first may not appear to be relevant in the process of committing a crime may still have a very significant impact on the ability to identify the POI associated with the criminal activity. This includes consideration of what might have been happening to the sample of interest in the interim period between assumed key contact events.

It is relevant to consider available knowledge of DNA quantities and profile types at the starting point, intermediate points, and final point of transfer pathways. This is assisted by having access to data on: how much DNA is typically present on a wide range of common surfaces/items (when handled in common ways, and in manners associated with specific criminal activities); and the profile types and/or whose DNA is present on surfaces/items given the use, shedder status of users, and environment within which the item is used and stored.

One can assume that the amount of DNA present on a final substrate that is derived from an original deposit on a preceding substrate at the beginning of a transfer pathway will, after having been transferred multiple times before landing on the final substrate, be less than was originally deposited on the primary substrate at the beginning of the transfer pathway. The total amount of DNA collected from the final substrate could, however, be similar or greater than what was on the primary substrate due to background DNA presence on the final substrate, additional DNA being added along with the source DNA of interest derived from the original deposit by the contacting vector, and/or a larger sampling area.

Factors that may contribute to the reduced transfer from one substrate to the next, other than the type of substrate and the persistence of the biological material, may include the relative areas and manner of contact (i.e. the extent to which the secondary substrate overlaps the whole, or only a portion of, the area of the primary substrate where the relevant biological substance is located).

Goray et al. [48] performed a few mock case scenarios involving multiple transfer steps and compared the expected transfer percentages, given the then available knowledge of the impacts of substrate, manner of contact and biological material on transfer, with what was observed. In some instances, they were similar; however, major differences were also recorded between the observed and expected transfer percentages, as well as among repeats of the same scenario, implying that other variables were having an impact. Some of these relate to the need for a better understanding of the elements of the core variables impacting the transfer of DNA, and may also relate to the interactions among these variables. Other factors discussed in earlier sections may also have influenced the

differences detected, as may other not yet identified factors. This all points to the need for further research to understand the variables influencing transfer.

DNA, through various combinations of direct, indirect, bi-directional, multiple contacts and pathways, can end up in unexpected places. Some of these pathways and contacts are difficult to decipher without ground truth knowledge of all the actions, and associated relevant details, involved. For example:

- Goray and van Oorschot [63] in their study where three individuals participated in a social interaction of sitting at the table and drinking from a communal jug of juice, found DNA of an individual who did not directly contact a surface and postulated several multi-step transfer pathways to explain the finding.
- Taylor et al. [127] observed the DNA of individuals in areas of a laboratory they did not frequent.
- Forensic scientists deal with case scenarios on a daily basis that require consideration of the various pathways and contacts that could have led to the DNA evidence observed, even with limited scenario information and in the absence of ground truth knowledge. This is illustrated by cases such as that of Meredith Kercher [43], Daniel Fitzgerald [206], Farah Jama [207], Phantom of Heilbronn [208], Dirk Greineder [209], and Steven Wayne Hillier [210].

7. Recovery

7.1 Impact of different recovery methodologies

Different laboratories use different methods to collect DNA from similar items. The main methodologies applied are swabbing, tape lifting or direct extraction via excision [7, 211]. There are however many types of swabs and tape-lifts, and means of their application, with significant differences in retrieval rates [7, 212-221]. Some other methods are available such as a wet-vacuum system [222] or sampling of individual skin flakes [167], but these are less commonly utilised. In some special situations, DNA recovery is aided by soaking the item in solutions, for example fired cartridge cases [223]. Furthermore, there are a wide range of methodologies applied to extract DNA from collection devices or directly from the substrate the sample is on, with varying degrees of efficiency [9, 224]. A number of studies have also shown that direct amplification of trace quantities of sample from swabs, small items and fabrics can provide profiles as good as or better than using traditional methods [92, 225-229].

Section 2.1 showed that the transfer rates at interactions between two surfaces are dependent on the substrates involved. However, the observed transfer rates can also be impacted by the efficiency of the sampling methods applied and the efficiency of the extraction methods applied to extract the DNA from the collection device.

Verdon et al. [74] showed that the amount of DNA retrieved from different amounts of biological material on the same and different substrates, using the same collection and extraction methodology, can differ significantly. They indicated that these differences

should not be ignored when analysing and interpreting results from transfer perspectives. They demonstrate the application of a correction factor for extraction efficiency when comparing results of samples collected from different substrates after similar transfer events, and go on to suggest consideration of applying appropriate correction factors where relevant. The generation of correction factors, for a range of suites of conditions, could conceptually be an avenue to allow fairer comparisons of data, and their application, to address a range of situations. Utilisation of correction factors has also been advocated by others [47, 143, 164].

7.2 Targeting

Targeting the sample of interest to gain the best possible profile to assist investigations is not always an easy task, especially when that sample is of a minute nature. Poor targeting can affect the quality of results obtained. For example, sampling beyond the boundaries of the sample of interest heightens the risk of collecting more of the background DNA, thus increasing the likelihood of mixed profiles and reducing the proportional profile contribution of the POI. Furthermore, if one collects within the boundaries of the sample of interest, but only collects a small proportion of it, then this could lead to a partial or less informative profile.

Some touched surfaces and objects sampled for DNA have initially been examined for fingerprints using methods to visualise the contacted areas. Many other objects sampled for DNA are sampled from target areas, where the object is logically assumed to have been contacted (e.g. weapon and tool handles). However, with some objects, assumptions of areas contacted are less straightforward (e.g. grab impressions on clothing [230]). In these latter instances, it may be beneficial to apply visualisation techniques, e.g. gold or silver vacuum metal deposition, fluorescent in-situ detection [231-234], to try to locate the areas of contact for increased sampling accuracy.

7.3 Differential sampling

As it is often the last deposit that one is interested in and not the background DNA that is present on the substrate, Verdon et al. [212] hypothesised that the last/fresher deposit may be somewhat layered on top of the background DNA and, thus, may be able to be differentially collected using subtle taping techniques. Their findings, however, did not demonstrate that this was readily possible, probably because of the extensive mingling of the different contributions to the biological mixture on the substrate.

More promising means of separating different cellular contributions to mixtures prior to DNA extraction are available to assist obtaining clearer profiles of the separate contributions. These include: differential lyses methods where the DNA from the non-sperm cell fraction can be separated from the sperm cell fractions [235], laser microdissection [236-242] and cell sorting [243-246]. Where cells of the same type are within a mixture, but derived from individuals of different sex, these too can be separately isolated [247, 248].

Where cells of the same type within a mixture are from individuals of the same sex, use of fluorescently-labelled human leukocyte antigens may assist separation of cells [246].

7.4 Impact of different suites of methodology from collection through to profiling

A general comparison of success rates of similar samples collected from similar items demonstrate the impact different suites of methodologies may have on the success rates [27, 29, 249, 250]. There is unfortunately a paucity of meaningful success rate data available for comparison. Several authors have advocated that more success rate data be collected and disseminated, and accompanied with all the relevant details in relation to the methods, processes and thresholds applied, to allow proper comparisons of the impacts of different suites of methodologies [47, 251-253]. Such information allows for: assessments of impacts over time as internal methods/processes/training change; comparisons with performances of other laboratories for like sample types; identification of continuous improvement opportunities; improvement of sample targeting strategies and prioritisation; better work flow management; and equipping staff to better manage expectations of stakeholders (including crime scene officers, informants, legal fraternity). Reports by Mapes et al. [14] and Baechler [16] begin to demonstrate the value of success rate data collection and comparisons.

It is to be acknowledged, and considered, that as new methodologies, from collection through to profiling, are developed and applied, they may impact DNA-TPPR detection outcomes and thus influence probability assessments.

8. Data to be used in a case

8.1 General considerations

The primary source of information to rely on should be experimental/research data, where the ground truths are known, and where data on relevant variables and propositions are also well known. If there is ambiguity surrounding severity of impact of a specific factor within a sequence of events, then available research data on the variables potentially impacting this factor should be taken into consideration. Where such data are not available, consideration should be given to undertaking the research to acquire the necessary data. However, since time and resources devoted to a case may be limited, other sources of information may be used to assign probabilities to DNA TPPR [2]. We will discuss these sources in more detail below. See also guidelines from various agencies relating to evaluative reporting [44, 254-256].

8.2 Knowledge of case specific information

For any proper activity level assessment, there should be an understanding of all the potential factors that may impact DNA-TPPR, and as much task-relevant case-specific

information/details regarding these factors should be collected as possible and made readily available to the scientist conducting the evaluation of the findings. This includes those relating to:

A) Event details

- a. Pathway of alleged transfer events (including activities between and after those in focus – see also ‘g’)
- b. Time line of events
- c. Details of the items involved in each contact, including:
 - i. Type, size, substrate
 - ii. Areas of items making contact
- d. History of each item prior to the action of interest within the pathway, including:
 - i. Amount of use, by whom, when
 - ii. If cleaned, and if so, how and when
 - iii. Environment (storage) when not in use
- e. Details of the manner of contacts
- f. Location of events, including:
 - i. History of location (occupation, cleaning regime)
 - ii. Environmental details (indoors or outdoors, weather conditions)
- g. Details of what happened with the items of interest post criminal activity and prior to packaging of item or sampling of item, including:
 - i. Ongoing use by POIs or others
 - ii. Examinations conducted prior to packaging or sampling for DNA, including other types of examination (e.g. latent fingerprint enhancement techniques)

B) Relationships

- a. Suspect with the items of interest and the locations of interest
- b. Victim with the items of interest and the location of interest
- c. Suspect with the victim
- d. Known others not directly involved in the criminal activity, but associated with the suspect, victim, location or items of interest

C) Packaging, storage and transport of items examined

- a. Condition of item and biological material at scene (including if wet or dry, and whether dried, if wet prior to packaging)
- b. Sampled at scene or packaged, transported and stored
- c. Type of packaging
- d. Transport conditions
- e. Storage conditions

D) Recovery of sample through to profiling

- a. When recovery of the sample occurred relative to alleged criminal activity and exhibit creation
- b. Area sampled
- c. Method of sampling

- d. Method of extraction
- e. Method of profiling
- f. Method of profile interpretation
- g. Knowledge of biological materials from which the DNA was derived
- h. Availability of reference samples of suspect, victim, known associates, background of area sampled

Not all details will be equally relevant, and not all may be relevant in a particular case. Those relating to C and D should be readily available from the examiners; B will be indicated by the informant, instructing parties or the court; whilst much of A will come from the crime scene attending and investigating officers, victim and/or witnesses. Alternative information may be indicated by other parties. Limited information may be available for elements associated with these points, especially A, due to practicalities and/or legal framework. We encourage efforts towards understanding the impact of the absence/presence of the types of information one relies on that may be gathered by the crime scene attending officer, and to consider means of improving the gathering of relevant information in an efficient and consistent manner.

8.3 Re-enactments and mock simulation

Scenario re-enactment or mock simulation testing, incorporating all the relevant factors and potential variables within each as accurately as possible, relevant to the scenarios being investigated, is highly desirable. Care must be taken that any simulations truly address the questioned issues in the case given two or more competing scenarios. However, these case specific experiments can be very time consuming and costly, and the design/execution and interpretation of results of these require their own expertise.

8.4 Research data

8.4.1 Same or comparable suites of methodologies

In the absence of data from case-specific re-enactments, the use of empirically collected research data on the impact of a wide range of variables, for a breadth of factors potentially influencing transfer, can be appropriate. Ideally, this is data produced using the same suite of methodologies as applied in the case under investigation. However, most experts trying to address activity level questions use data from studies that have used suites of methodologies dissimilar to the ones used in the case being considered. Differences in the suite of methodologies applied can make it difficult to compare the data from one source with that of another. For some questions, the dissimilarities in suites of methodologies applied are not that relevant, but for others they may be. For instance, if interested in the relative proportion of known contributors to a profile, then in certain circumstances differences in collection, extraction and amplification methodologies applied may not be relevant, yet differences in sensitivity, number of loci and discrimination power of the kit used, and the thresholds applied to allele RFUs during interpretation, may impact the results

and interpretation, and subsequently an opinion on the potential for transfer. Alternatively, if the question relates to how much DNA from a particular source was collected from the area sampled, then differences in the methods applied to collecting the sample from the area, and how the DNA was extracted from the collection device, would be relevant, yet differences in amplification kit and profile interpretation would not. Further, some methodologies within each phase of the collection to profiling process may be very distinct from each other, and reliant on different technologies, but have been demonstrated to provide similar outcomes (e.g. different extraction methodologies providing the same quantity and quality of DNA from a specific type of sample and/or collection device). Alternatively, similar technologies may have different efficiencies (e.g. if both use a swabbing technique to collect the sample, but use different types of swabs and/or wetting solutions that impact directly the quantity and/or quality of what was retrieved, or interacts differently with the extraction method to affect the same). It is thus important to consider (and incorporate) the potential impacts of these differences during interpretation.

Apart from the impact of using different sample collection devices and strategies, and DNA extraction methodologies raised in section 7, the quality of the profiles obtained can be dependent on the amplification/profiling systems applied (especially with respect to their sensitivity and discrimination power). A further complicating factor is the highly divergent interpretation and statistical methodologies used.

To illustrate these difficulties, Steensma et al. [143] examined the effect of laboratory procedure on the outcome of a DNA transfer experiment. Five sets of 20 cable ties bound by different volunteers were distributed to four participating laboratories. These laboratories then sampled the cable ties, extracted the DNA, amplified and profiled the extracted DNA, and interpreted the resultant profiles, all according to their standard operating procedures. The results of the study showed that there were statistically significant differences between amounts of DNA recovered by the four laboratories, as well as different success rates in DNA profiling. The reportable profiles further showed differences in the number of mixtures versus single source profiles that were obtained. Steensma et al. [143] also demonstrated that packaging, transport and/or time delay before sampling of the items impacted the quantity of DNA retrieved and profiles generated, and thus potentially the interpretation of experimental results.

Furthermore, an inter- and intra-laboratory exercise on the assessment of complex autosomal DNA profiles showed variation within and between laboratories indicating that interpretation outcomes are impacted by differences between internal guidelines and methods available, as well as the need for improved guidelines and training within laboratories [257]. These findings are corroborated by Butler et al. [258], although it is likely that probabilistic genotyping in time will reduce the impact of the human factor in DNA profile interpretation [259]. Assessing the impact of interpretation guidelines may be difficult when relevant details of the suites of methodologies applied in relevant studies are not available or clear. It is thus desirable for future publications of DNA-TPPR related data to be inclusive of relevant details of the methods, protocols and thresholds applied to generate the data presented [73].

8.4.2 Experimental designs generating transfer data

Much of the available research data on DNA transfer incorporate experimental designs to ascertain if a particular factor impacts transfer and the general direction of that impact. These experiments did not aim to generate probability estimates of specific quantities of DNA transferred, or of the various profile types that may be encountered, in given situations. Furthermore, the circumstances being investigated in many of the publications thus far, have been elementary and not focussed on commonly encountered crime-related situations, or lack consideration of aspects of background DNA, prevalence or recovery methodologies, making translation of the data into real life situations less applicable/straightforward. This even extends to simple things; the samples in many reported research studies are collected relatively soon after the action of interest, which is quite different from casework samples that have been packaged, transported and stored for a period of time. The impact of these simple actions could be multiple and potentially profound [55, 143]. Whilst some recent studies are starting to address the need for such data, there remains a substantial void to be filled.

When considering the use of published data, one must, apart from considering the impact of the suite of methodologies used, also consider the experimental design and the potential implications/limitations they may have on their utilisation when interpreting the case scenarios at hand [144, 145]. For example, if assessing the probability of detecting DNA of person B from a full handprint on an object left by person A immediately after a handshake with person B, the factors requiring consideration should include: what persons A and B did with their hands prior to shaking hands; shedder status of persons A and B; manner and duration of handshake; object shape and substrate on which a handprint was placed; and the manner and duration of handprint deposit. Each of the available studies focussed on this type of scenario [51, 52, 54] incorporate different variables of the factors and combinations thereof (see section 3.3 for some details). Similarly, each of the studies focused on determining whose DNA is on a knife handle [50, 51, 54, 139] apply different scenario conditions within their experimental design. Hence, the outcomes of each of these studies may not be as directly relevant as the others to inform probabilities on DNA-TPPR in the case in question.

The interpretation of results in any specific casework scenario will depend on the subset of available data used during interpretation. One needs to utilise the data that are most fit for purpose. The choice of data used (and the associated limitations), plus the available relevant data not used accompanied with the reasons why, needs to be transparent to the trier of fact.

The different factors influencing DNA-TPPR will also impact each other to varying degrees depending on the set of circumstances. Contemplation and weighing the potential impacts of the multitude of potential interrelated factors within alternative scenarios/propositions can be very difficult. The use of Bayesian networks can facilitate such analyses (section 11).

A number of recent papers have collated the best available information to determine a specific probability and have applied it to specific scenarios, e.g. [53, 64, 108, 125, 139, 143].

There is a need to utilise probability estimates that closely relate to the factor of relevance and are as accurate as currently available data permit. Even if the variation is large then this needs to, and can, be incorporated within the Bayesian networks through modelling distributions and/or performing sensitivity analyses. Wide variation signals that other factors are influencing outcomes. Identification of these other factors can lead to creation of additional/separate nodes within Bayesian networks. However, any data analysis is only as good as the quality and quantity of the data it relies on; thus, unless sufficient data is utilised, results generated could be less accurate than desired and potentially result in an uninformative opinion.

Separately, the sourcing of the experiment participants is not always representative of the general population. Furthermore, the total number of participants or replicates within reported studies is often limited. Those relying on this type of data for activity level assessments would benefit from more expansive studies to help establish more accurate probability distributions for relevant factors impacting transfer.

8.5 Collating casework data

Laboratories examine many cases involving similar items in similar situations. For some, the ground truth is not contested and can be reasonably assumed. Where probabilities of obtaining a particular type of profile within a given situation is required, there is value in collating in-house casework information to determine the frequency of particular profile types from similar objects in similar situations, especially if there are a reasonable number of relevant cases and where the suites of methodologies applied are the same. Any use of this type of data should be transparent and the relevant data made available to other relevant parties in the case if and when appropriate.

8.6 Casework experience

DNA transfer expertise requires an understanding of the effects of different combinations and single variables affecting DNA transfer that comes from experimental results with known ground truths. In day to day casework practice, it may be found that no, or very limited, current data are available that align closely enough with the case at hand to be of any use. In these circumstances, the expert may refrain from providing an opinion, or, whilst acknowledging the caveats, resort to informing the court based on their expertise with forensic analysis of biological traces and DNA. Experience with casework DNA profiles which results from the interplay of many different real life variables is of value when determining someone's expertise in the topic of DNA transfer. Experimental data generated from transfer experiments allow researchers to see the impact of known variables, including what the profiles are likely to look like given the known variables. In casework, the ground truth knowledge is usually unavailable and competing hypotheses are often provided to explain

the DNA evidence. There can be great differences between the DNA profiles generated from experimental samples and real casework data. Effects of background DNA, environmental conditions, presence of inhibitors and interpretational complications, such as assumptions of known contributors, allow casework scientists to accumulate knowledge of the complexities of real life casework profiles. With experience, DNA interpretational case managers are usually exposed to thousands of profiles generated from a wide variety of biological materials and item types, accompanied with some knowledge of scenario components, established through casework relevant information. A case manager may also elicit accumulated experiences from other case managers. If different methodologies are applied within a laboratory, any impacts of these differences may become obvious to an experienced caseworker. In such situations the ground truth regarding the application of different methodologies is known and therefore the elicited knowledge regarding the impact may be considered slightly more reliable. When using elicitation as a source of information, proper procedures should be adhered to, to avoid confirmation or observation bias [260]. Utilisation of elicited casework experience must, like all data utilised, be transparent to the court.

Thus, both casework and experimental experience and understanding have their benefits and limitations, and in order for a court going biologist to be in the best position to assess a complicated issue such as DNA transfer, exposure and experience in both is beneficial when addressing the subject.

However, the fact remains that in most casework circumstances the ground truth is not known. Furthermore, human cognitive abilities to effectively accumulate, and weigh, all the relevant data without unconscious biases, are limited. We thus reiterate that elicitation of probabilities of DNA-TPPR based on general casework experience is a poor substitute to probabilities informed by structured analyses of collated casework data, when no experimental data are available.

Jamieson and Bader [169] noted that experience as a source of reliable scientific opinion on the probability of DNA transfer has been challenged, and question the validity of this approach. However, they support the conduct of research to determine the accuracy of 'experiential' assessment relative to 'experimental' assessment. We concur that such research would be useful. See Taylor et al. [2] for further discussion of the merits and limitations of expert elicitation.

8.7 Awareness and transparency of limitations of the data utilised

As noted above, one needs to use the best currently available data and be transparent about the potential limitations of these data (and potentially counter these limitations with other supporting evidence/data). Reporting guidelines should require disclosure of the data on which an evaluative opinion is based upon request, especially in cases where that particular opinion is in dispute. This has been noted previously when considering both case-specific data [261, 262] and unpublished data that may be shared among scientists working for one 'side', but not with those working on the other [41]. The limitations of the data

and/or methodologies used to provide probabilities of alternative scenarios should be known and taken into consideration when utilising the data, and also need to be clear and understood by the trier of fact [263, 264].

8.8 Accessibility and sharing of data

The limited published DNA-TPPR research data are presented in different formats, not all of which are readily accessible. Studies generally aim at presenting data to inform on the research question, and additional information (e.g. on number of contributors, DNA quantity etc.) may not be fully explored and/or presented. The data from unpublished in-house research and/or casework data collections are even less accessible to others. The paucity of available data limits the accuracy of probabilities and opinions provided.

There is thus a need to consider the potential value of having a quality controlled open access depository of relevant DNA-TPPR information, which can be easily mined for the various purposes that it could accommodate. Furthermore, if deemed of potential value to an array of stakeholders, then consideration of the potential means of establishing such a depository/database/portal is required [73].

9. Transfer as a contamination risk

Addition of DNA to a sample post-criminal offence activity (by investigation personnel, tools, equipment, etc) can complicate the interpretation of a profile and/or misdirect investigations. The contaminating source is usually from individuals who are not a POI (e.g. investigator or other person attending the scene or examining laboratory). However, depending on the what, when and how the contact causing the contamination occurred, the contaminating source could potentially be a POI within the case under investigation, or a POI in an unrelated case. Distinguishing the post-criminal activity contribution of DNA from background DNA, originally present prior to the sample deposited during the criminal activity, further complicates the interpretation of the generated profiles.

Contamination of DNA profiles through transfer events post crime scene establishment can occur through various means, including:

- Direct transfer by handling an item to be sampled without wearing gloves.
- Direct transfer through the air whilst talking or coughing over an item, when not wearing a mouth mask [265, 266].
- Direct transfer through the air, from a person moving when not wearing any protective clothing [266].
- Indirect transfer from item to item when packed together within the same packaging, or from one area of an item to another area of the item when inappropriately packaged [55].
- Indirect transfer from the external packaging to the internal packaging and/or exhibit during handling and transport [56].

- Indirect transfer via gloves, because: gloves were not DNA free when purchased [267]; the box of gloves was not kept DNA free during use; gloves were not replaced after picking-up DNA by touching something with DNA on it prior to touching the exhibit (especially an area from which is to be sampled) or an area that was designated to be and remain DNA free (such as a bag of DNA-free tools to be used later) [58, 60].
- Indirect transfer via dirty tools or equipment. For example, scissors or forceps not appropriately cleaned between uses [58, 59]; fingerprint brushes reused to powder different sites in the search for fingerprints within and over multiple scenes without cleaning the brush in between [57, 62]; having multiple items being fumigated simultaneously within a superglue chamber and/or the chamber not being appropriately cleaned after each use [268]; handling of dirty equipment such as a magnifying lamp, camera, torch whilst also handling the exhibit with the same gloved hand [269].
- Indirect transfer due to sharing of equipment among colleagues, such as a camera, without appropriate handling and/or protocols adopted [270]
- Indirect transfer due to placement of exhibits on unclean surfaces [270].
- Indirect transfer by handling of non-exhibit items during examinations without awareness that they contain DNA, such as the packaging and casefile notes [56, 127].

Such events may occur due to: absence of proper procedures and/or poor compliance to them; poor training; ineffective cleaning procedures and/or compliance to such; and absence of environmental monitoring procedures [57, 127, 128, 271-276].

Contamination events can be mitigated by: having proper procedures relating to crime scene access, examination laboratories, and exhibits; wearing, appropriate use and replacement of personal protective clothing; effective training and competency assessments; use of validated effective cleaning procedures and regimes; application of effective contamination monitoring procedures [168, 211, 266, 270, 271, 277-285] and use of certified DNA free consumables where possible [286, 287]. However, with respect to use of certified DNA free consumables one needs to be wary of the tests and standards applied by the manufacturer, relative to your use of the product [272, 288] and where possible conduct control tests using in-house suite of profiling methodologies.

Furthermore, procedures should be in place that allow detection of contamination events if they occur, so that the profiles can be interpreted accordingly. This should include controls and checks for item to item transfer during examinations and sample processing. This must also include having an elimination database, against which each evidentiary profile is compared to assess if any of the profiles on this database is clearly present within the profile generated from the evidentiary sample. The elimination database should be inclusive of all those who: attend a crime scene; handle an exhibit from which a DNA sample may be collected, either during examinations or just handling packages containing the exhibit; work in areas where the exhibit is stored and/or examined, including those entering these areas for the non-scientific reasons such as general cleaning, maintenance or repair [270, 271, 277-286, 289].

In order to allow the laboratory to improve their work practices and the confidence stakeholders have in the DNA evidence presented, appropriate contamination minimisation procedures must be used and monitored, with all contamination events being fully and transparently investigated [277, 279].

Contacts with an exhibit, especially the areas to be sampled, are not just risking contamination, but are also potentially reducing its probative value through the reduction of the amount of DNA material remaining for collection. When dealing with trace quantities of sample, any loss could reduce the ability to generate a full profile, thus lessening the probative value of the collected sample.

There are several high profile case examples of the impact of lack of, or inappropriate, contamination mitigation and detection procedures that emphasise the relevance of maintaining proper contamination minimisation procedures. These include the 'Phantom of Heilbronn' [208] and 'Farah Jama' [207] cases. See Taylor et al. [2] for some further cases.

10. Readiness of those addressing transfer related questions

Most laboratories conducting forensic examinations and reporting evidence do so under accreditation rules and guidelines, which require those performing these tasks to be deemed competent in doing so having undertaken appropriate training and be deemed knowledgeable of the relevant information/methodology and able to apply it competently. It is expected that those working within the same crime laboratory receive the same level of training according to established training units, demonstrate equal levels of competency, and apply them in the same manner as each other when confronted with the same set of circumstances.

It is often the expert interpreting the DNA profiles obtained from collected samples with respect to the identity of the person(s) from whom the sample was derived (i.e. sub-source level issue), who is then also asked about the relative likelihood of their findings given alternative means of how it may have gotten to where it was collected from (activity level issue). It is incumbent on those addressing such questions to be willing [290] and competent in doing so. This requires contemporary awareness of the available relevant knowledge and how to apply this knowledge in an appropriate unbiased transparent manner. In order to do so, training is required according to approved training modules, which are aligned with relevant procedures/protocols/methodologies and include appropriate competency assessments.

Where possible, opinions should be based on empirically collected and analysed data (section 8) using appropriate methodologies and tools (section 11). Training of scientists should include becoming familiar with a multitude of profiles where the ground truth is known, generated using different multi step transfer scenarios and a variety of different variable conditions. Furthermore, there should be regular refresher training and regular proficiency testing of authorised individuals.

Testing of some DNA reporting officers, including many with experience in reporting on DNA transfer, on their general understanding of DNA-TPPR and ability to identify key factors that

could impact transfer probabilities, has demonstrated differences between individuals (from between and within laboratories) in their levels of comprehension of DNA-TPPR [291].

Anecdotally, it appears that the training of those required to address DNA transfer activity level issues in legal settings is limited and ad-hoc. Further, some accrediting bodies do not identify the expertise required as distinct from other aspects of DNA collection, profiling and interpretation at sub-source level. Specific requirements are thus limited. There is also an absence of readily available proficiency tests to assist checking of the ongoing competencies of authorised individuals.

There is a need for the forensic community to acknowledge that addressing activity level issues requires separate skill sets and thus separate training programs, competency testing, authorisations and ongoing proficiency testing. Such programs could be composed of different levels to cater for different needs (e.g. from basic awareness, through intermediate, to expert). Standardisation within and among jurisdictions should be a major goal. Useful guidelines on evaluative reporting, including in relation to activity level assessments, have been published [44, 254, 255]. Some jurisdictions are already progressing well down this path. For example, the UK's Forensic Science Regulator, with guidance from statisticians, forensic scientists and judiciary representatives among others, is currently developing an evaluative interpretation standard to standardise activity level interpretations of trace evidence [292].

11. Tools to assist activity level assessments

There are many aspects to consider when evaluating forensic genetics findings at the activity level, including those alluded to above. The different aspects can also impact each other and the overall outcomes, to varying degrees. For example, the relative presence of sources of DNA other than the source of interest may inform the overall interpretation. Guidance and tools are becoming available to assist in these endeavours.

Some guidance has been published on evaluative reporting [44, 254]. These guidelines stress that reports providing opinions at the activity level should conform to four basic quality criteria. The evaluation should be;

- (1) *balanced* (e.g. address at least two competing propositions),
- (2) *logically correct* (e.g. logical fallacies, like transposing the conditional, should be avoided),
- (3) *robust* (the interpretation should not be overly sensitive to small variations in the parameter values) (Note, we have substituted the meaning stated by The Association of Forensic Science Providers, i.e.: '*should withstand scrutiny by the court or other experts*' [254], with this meaning as we feel it is more appropriate),
- (4) *transparent* (the line of reasoning and supporting data should be clearly stated).

Bayesian networks are becoming the *de facto* standard tool used in activity level evaluations in forensic science [293] and can assist experts with their compliance to these four criteria. Bayesian networks allow the expert to model all relevant parameters and their dependencies. The graphical visualisation of complex Bayesian formulations enhances the

transparency of the expert's reasoning. The model also explicitly requires experts to assign probabilities to all modelled parameters, thereby avoiding implicit assumptions. Sensitivity analyses on relevant parameters can further provide clarity on the robustness of the evaluation. The structure of the Bayesian network, particularly if based on existing template structures (e.g. Evett et al. [294]; Taylor et al. [45]), will also guard against logical fallacies, and will enforce balance in the evaluation since at least two propositions need to be specified.

Bayesian networks are flexible and can be used for different aspects relevant to assessment of DNA TPPR. In casework, they can be used to perform a proper case assessment [295] or to evaluate forensic genetic findings given propositions at activity level [53, 296, 297]. They may also be used to direct research efforts through clarifying, by sensitivity analysis, which parameters require more data [47]. See Taylor et al. [2] for more information on the use of Bayesian networks, and evaluation of forensic genetics findings given activity level propositions in general.

12. Where to from here

As noted by others [40, 41, 44, 46, 144, 145, 298], activity level questions concerning DNA need to be addressed. Forensic scientists with expertise in DNA-TPPR are therefore in a responsible position to provide guidance on the probabilities of specific evidence given specific scenarios using the information that is available at the time.

This review demonstrates that over the last few years we have become aware of several factors affecting DNA-TPPR, but much more research needs to be undertaken to understand the impact of the many variables, build the data necessary to determine probabilities of different profile type occurrences in different situations, and to improve the accuracy of the profile interpretation given the uniqueness of each case scenario to be considered. As the number of potential scenarios in which DNA-TPPR are to be contemplated is infinite, there will be reliance on extrapolating from research findings. The research thus needs to be of high quality, broad scope, and sufficient quantity. Sensitivity studies to identify the factors and variables most likely to impact likelihood ratios within transfer related scenarios will help prioritise the focus of further research.

We encourage:

- pursuit of the various suggested means of building our knowledge and data sets;
- efforts towards making generated data readily accessible to improve awareness and utilisation by stakeholders;
- greater use of available methods and tools to evaluate likelihood of forensic genetics findings given case specific scenarios using available DNA-TPPR data and further development of user friendly interactive tools to assist their adoption; and
- harmonisation and, where possible, standardisation of methodologies.

Recognition of DNA activity level as a defined expertise requiring dedicated training and competency testing towards authorisation will assist the provision of sound opinion and

guidance to the triers of fact. Apart from improvements in training of experts, further general education of all stakeholders in relation to DNA-TPPR would be beneficial.

We implore stakeholders to invest time, resources, funding and commitment towards enabling forensic scientists to provide high quality expert opinion and guidance to courts to accommodate the provision of a fair justice system.

Acknowledgements

The authors would like to acknowledge that the preparation of this manuscript benefited from addressing the comments of the anonymous reviewers.

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Figure 1. Diagrammatic illustration of various modes of transfer

