META-ANALYSIS OF THE SECONDARY TRANSFER OF DNA

By

Tara DUNHILL

A thesis submitted in fulfilment of the requirements for the degree of

Master of Forensic Science (Professional Practice)

in

The School of Veterinary and Life Sciences

Murdoch University

Supervisor: Mr. Brendan Chapman

Semester 1, 2018

Murdoch
UNIVERSITY

DECLERATION

I declare that this manuscript does not contain any material submitted previously for the award of any other degree or diploma at any university or other tertiary institution. Furthermore, to the best of my knowledge, it does not contain any material previously published or written by another individual, except where due references has been made in the text. Finally, I declare that all reported experimentations performed in this research were carried out by myself, except that any contribution by others, with whom I have worked is explicitly acknowledged.

Signed: Tara Dunhill

ACKNOWLEDGEMENTS

I would like to acknowledge firstly Mr. Brendan Chapman for his ongoing support and mentorship for this dissertation. Secondly, I would like to acknowledge all my family and friends for their support and encouragement despite being over the other side of the country, in particular Jessica Georgakis and Ashley Symons.

TABLE OF CONTENTS

THIS PAGE HAS BEEN LEFT INTENTIONALLY BLANK

PART ONE

LITERATURE REVIEW

META-ANALYSIS OF THE SECONDARY TRANSFER OF DNA

ABSTRACT

Deoxyribonucleic acid (DNA) is complex molecule present in nearly every cell of the human body that contains all of an individual's genetic material. Trace DNA has allowed DNA to be detected on everyday objects such as door handles, tables and chairs. Currently, the idea of the secondary transfer of DNA has arisen, involving the transfer of genetic profiles from an individual through a vector and then to final individual or object. This has become a re-occurring term in the court system, as individuals standing trial use this argument for the presence of their DNA. It is therefore hypothesized that through the exploration of literature and completion of a meta-analysis that an appropriate and concise guide will be produced to determine the chances of a secondary transfer event occurring and what conditions are required, to aid Biologists in expert testimony. Database searches were conducted to gain resources in the topic, followed by a number of screens to determine the suitability of articles for the meta-analysis. With the resultant 38 articles, data extraction was additionally carried. However, due to the lack of quantitative variables and results, a meta-analysis was not able to be conducted. It is suggested that in the future, studies publish their results in a more scientifically rigorous manner or allow the access of raw results for external interpretation purposes.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

DNA = Deoxyribonucleic Acid

ng = Nanograms

ng/μL = Nanograms per microliter (concentration)

pg = Pictograms

STR = Short tandem repeat

μL = Microliter

UV = Ultraviolet

1. INTRODUCTION

Deoxyribonucleic acid (DNA) is complex molecule present in nearly every cell of the human body that possess all of an individual's genetic material.[1] Each individual has approximately three billion base pairs which is essential for the characterization of each individual.[1] Of these three billion base pairs, it is thought that approximately only 10% contains genetically relevant information.[1] The other 90% contain sequences that are highly repetitive and polymorphic, meaning that they are also highly variable.[1] One type of these polymorphic sequences is known as short tandem repeats (STRs), a repeated sequence of DNA that is usually around one to six base pairs in length.[2] STRs differ in terms of their sequences between sites although within a site the number of repeats of the unique sequence differs with series that can be up to 100 nucleotides in length.[2] These polymorphic areas and STRs are of great interest in the field of forensic science, as they are variable between individuals.

In Forensic Science, STR typing is used to infer individualized sequencing of DNA where today kits such as PowerPlex 21 and GlobalFiler are used within forensic laboratories in Australia. Previously kits such as ProfilerPlus have been used, a kit that tests for ten individual STR loci, although the more STR loci that are tested for, the greater the discrimination value.[1] The PowerPlex 21 kit, as the name suggests, tests for 21 STR loci and the GlobalFiler kit tests for 24 STR loci but currently is only used in one state of Australia. All STR loci chosen for testing within kits are mostly tetranucleotide repeats (four base pairs in length) and are codominant, meaning that they are inherited just like any other gene, one from the paternal side and another from the maternal side.[1] STRs are therefore able to be used for familial searching due to this codominant inheritance pattern.

Since its first use in the court systems in 1986, DNA has been a highly regarded piece of evidence, something that has been very hard to argue against. As DNA becomes more understood, scientists are able to gain a greater insight into who has been present at a crime scene. Increased sensitivity of kits allows forensic scientists to gain DNA profiles from the smallest of biological substances. Increased sensitivity of kits and a greater understanding overall, has allowed claims such as the secondary transfer of DNA. An increased sensitivity of kits allows multiple individuals to be within a mixture and a greater understanding allows the possibility of the secondary transfer of DNA. This means that no longer does an individual deposit their DNA onto an object, it is transferred through that object (vector) to a final object. This is becoming increasingly common within court and a need for a concise guide to determine the possibility of secondary transfer has now emerged. A proposed method to produce a concise guide to the secondary transfer of DNA is through a meta-analysis, an analysis that pools results from multiple studies together to increase its strength and sample size.[3] The aim of this literature review was to produce a combined review into the secondary transfer of DNA in addition to drawing multiple studies together to attempt to provide an analysis of the literature. It is therefore hypothesized that through the exploration of literature and completion of a meta-analysis that an appropriate and concise guide will be produced to determine the chances of a secondary transfer event occurring and what conditions are required, to aid Biologists in expert testimony.

1.1 THE TRANSFER OF DNA

Trace DNA has been regarded as a large phenomenon over the past couple of decades purely due to its significance in the field of forensic science. It has allowed the detection of

individuals DNA on everyday objects such as door handles, phones, tables and chairs just based on a single touch. Trace DNA is defined as cells off the skin surface that have been carried onto another surface through physical contact.[4] This meant that fingerprints were no longer the only way to identify an individual at a crime scene, collecting DNA provided a new avenue for identification between individuals. The concept of trace DNA was very limited when it was first discovered but as technology has developed, understanding of it has evolved. Trace DNA was first explored in great depth by van Oorschot and Jones in 1997, where they found that they could obtain DNA profiles from swabbing objects that were regularly handled by a particular individual and those objects that were pre-cleaned.[5]

Even though trace DNA is now relatively well understood, there are still some differing opinions as to where it originates from.[6-9] It was first thought that trace DNA originated from the outermost layer of the epidermis, but more recently studies have shown that there is actually little DNA left by the time the keratinocytes (epidermal cell) reach the epidermis.[9] Besides this, it is not known as to where touch DNA originates from but we have now well-understood the technologies used to test it. At the beginnings of touch DNA approximately 1ng of DNA was needed to gain a full profile but currently approximately only 100pg is needed.[10,11] Due to this increase in sensitivity of the technology, concepts such as secondary and tertiary transfer of DNA have now developed.

The secondary transfer of DNA involves the transfer of genetic profiles from an individual through a vector and then to another individual or object.[12] It is defined as the transfer of DNA from one person or object to another via an intermediate person or object, not involving a direct link between an individual and the target surface.[11,13,14] The first instance

of the secondary transfer of DNA occurred in a study conducted by van Oorschot and Jones in 1996 into the primary transfer of DNA, from an individual to an object.[5] They swabbed regularly used objects of individuals and all had provided genetic profiles that were consistent with the owner of the object, however one telephone displayed a genetic profile of a second user.[5] This was the first instance where the secondary transfer of DNA was seen to occur. Further, it occurred through an indirect manner as it was not specifically tested for, prompting additional research into the area.

With continued and more developed research into certain areas also comes with an increase in knowledge. Even though we are still trying to understand how secondary transfer occurs, the concepts of tertiary and even quaternary transfer have arisen. As like secondary transfer of DNA, tertiary transfer involves the transfer of genetic profiles from one person or object to another through two intermediary steps involving a vector.[15] Quaternary transfer involves an additional transfer events, resulting in four transfers of DNA in total. These phenomenon's have been indicated to occur in numerous published papers, but have not yet been conclusively shown to occur consistently.[16-20]

1.2 META-ANALYSIS

In order to combine studies that explore the secondary transfer of DNA, an appropriate and effective investigation must be carried out. The chosen method of carrying this out was a meta-analysis. A meta-analysis is a systematic review following certain criteria where results are pooled and analysed quantitatively.[3] They aim to summarise results of numerous studies, effectively increasing a sample size and thus the power of the study.[3,21] Rare outcomes of a study may also be overcome through the use of a meta-analysis as the

sample size of studies is increased and therefore a rare outcome becomes an outlier.[21] To carry out a correct meta-analysis, it should be done as a part of a systematic review, including features such as a comprehensive, reproducible search and transparent selection criteria.[21] There are two main types of reviews, systematic and narrative. Narrative reviews explore more broader topics and qualitatively summarise evidence, whereas systematic reviews focus more deeply on primary studies who explore clinical questions.[21]

Meta-analyses were first known back in the 17th century when astronomers thought that the combination of data might be preferred to individual choice.[22] In the medical field, the first combination of data from numerous studies occurred in 1904, but not as regularly used in the field as it was in education and psychology.[21,23] The term meta-analysis was first devised in 1976 by a psychologist named Gene Glass, and now has become one of the highly cited publication type and whose use is still increasing.[24,25]

Recently, meta-analyses are mainly used in the medical field purely due the large amount of information that is gathered through studies.[3] They aim to provide readers with the most current research published in a particular area.[3] In 2007, a search of "meta-analysis" would yield 1,473 articles.[3] Meta-analyses have also been used in the agriculture field with the first study carried out by Fisher in the early 1900's involving the probable and real concerns that fertilizer effects will vary by year and location.[26] They have also been used in other fields such as public policy, clinical practice, psychology and educational research.

Two of the main purposes as to why meta-analyses are carried out are to summarise results of several studies in addition to overcoming small sample sizes in studies.[21] By pooling

these results together, statistical power of these studies can be increased when otherwise impossible.[21] In the medical field in particular, performing a meta-analysis can often help to reduce "negative" results, helping in providing effective treatments without delay.[21] Although performed indirectly, meta-analysis may also even indicate of lack of evidence in particular areas, highlighting the need to perform more studies.[21]

Although meta-analyses have shown to be a powerful tool, misleading results can be created when conditions are not met correctly.[3] As stated by Walker et al., there is believed to be four critical issues that need to be addressed when carrying out a meta-analysis.[3] These include: heterogeneity of results, availability of information, identification & selection of studies and the analysis of data.[3] For example, during the selection and identification of studies, it must be ensured that bias in any way does not come into play. In regard to the availability of information, some studies tend to only include summaries of their results, not raw data.[3] This can limit the type of analyses and conclusions that can be made with a meta-analysis.[3]

There are two types of models that are used to accomplish meta-analyses, random effect or fixed effect models.[21] The fixed effect model assumes that the 'true' effects are fixed whereas a random effect model assumes that only a random sample is included.[21] The random effect model is more preferred for a meta-analysis as it better reflects reality as studies barely ever mimic each other.[21] This is therefore why a random effect model will be used for the meta-analysis carried out in this review where it will be attempted to summarise results from studies of the secondary transfer of DNA.

2. LITERATURE REVIEW

Regarding any transfer event of DNA, the way in which it is performed is relatively similar across the board. There must always be a primary transfer event involving the movement of genetic profiles most commonly from an individual to either an object or another individual. Then there must be another transfer event, hence the term secondary transfer, where that same genetic profile will be transferred either to an individual (coming from an object) or an object (coming from an individual). It cannot be said defiantly as to which type of method would be more common in real-life situations as both are as feasible as each other. In terms of an individual being a vector, this may occur when two individuals come into contact with each other, either through a handshake or more intimate contact, and then the second individual deposits both their own DNA in addition to the first individuals on an object perhaps at the scene of a crime. The other method may mean that the two individuals never come into contact with each other. In this case it may be more feasible to call the second individual a victim to a physical crime. In this case the first individual may have touched an object in a public place and the victim had also touched this object afterwards. DNA tests on the victim may show the DNA profile of this first individual placing them at the scene of the crime.

In this case, it could be hypothesized that the more common scenario that would occur would be that of an individual as the vector. Often in the course of an investigation, individuals will be linked with the crime based on their DNA being at the scene. In this case, it could be argued that secondary transfer was the cause for their DNA being present at the crime scene especially if they had never visited that area. Below studies using both

instances as vectors will be explored and reviewed to gain a greater understanding as to how this mechanism occurs and its likelihood.

2.1 OBJECT AS VECTOR

When the secondary transfer of DNA is put forth as a possible explanation, it is more sensible to have occurred through an object rather than an individual. This scenario at least is believed to be more reasonable in how it occurs, as the chances of the two individuals coming into direct contact with each other is highly unlikely. One of the first study that performed secondary DNA transfer through an object was performed in 2012. The author's used a case scenario to base their experiments off. The first experiment followed hands coming in contact with a kid's toy or a singlet and then that object coming in contact with a laboratory coat, both actions occurring for one minute and immediately after each other.[27] The results showed that in 19 out of 20 repeats, DNA from the donor was observed.[27] This study however doesn't mimic real-life nearly as much as the case study would have. Actions were performed immediately after each other with no time delay and the individual who participated in the second transfer event was wearing gloves, therefore not creating the issue of additional DNA present in the transfer event. This increase the transfer percentage likelihood as the donor's DNA is the only cellular material present.

The findings in Goray et al. are also in accordance with a study conducted by Lehmann et al. the following year where they additionally observed secondary transfer occurring.[27,28] Lehmann et al. additionally uses glass as a vector object, however underwent a transfer event onto the same type of material as the vector.[28] Similar to the study conducted by Goray et al, this study does not take into consideration of background DNA that may be

present through transfer events therefore increasing the percentage of cellular material transferred. In contrast, Verdon et al. conducted an experiment the same year that used seven different types of substrates as the vector or secondary substrate and used either plastic or cotton as the opposite substrate.[29] The differing types of substrates paired against one another yielded the same result of that in Lehmann et al., where secondary transfer had shown to occur. However, within the study there were differences seen between the porosity of the substrates. When the transfer went from a non-porous to a porous substrate, the transfer of DNA was higher.[29]

A unique approach was used by French et al. where Ultraviolet (UV) powder was used through the transfer experiment. This approach can be used for multiple types of material that may be transferred through an individual's hand. Three participants sat around a table drinking from a bottle with their own three glasses for 30 minutes where only one individual started with the UV powder.[30] There was no direct contact between individuals but the study showed the indirect transfer of the UV powder to all individuals.[30] Obviously, UV powder does not mimic the actions of DNA precisely but it may be used as a beginning point in order to understand the mechanisms behind the transfer especially within networks of individuals.

Goray and van Oorschot performed a very similar experiment to French et al. the following year where the same "uncontrolled" conditions were used. Here however they did not use UV powder but tested for the DNA itself. In the area immediately surrounding participants from the chairs and table, no other DNA than that of the relevant participant was detected.[31] In 27% of the table samples and then 33% of the chair samples had a DNA

profiles from participants that did not come into contact with the surface indicating that these profiles might have been transferred from the jug.[31] A further 58% of table samples and 42% of chair samples had DNA profiles from unknown individuals, possibly due to the secondary transfer of DNA.[31] These results support the findings of French et al. where secondary transfer does occur through a seemingly normal social situation. One downside to the study conducted by Goray and van Oorschot was that they had cleaned most areas involved in the experiment beforehand meaning that background DNA would not be present. In this case transfer percentages may be untruly inflated which is the same of previous studies.[28-30]

In 2015, researches seemed to realise the idea that secondary DNA transfer may occur within the laboratory so there seems to be an influx of the studies conducted with objects frequently used in laboratories acting as the vector. Margiotta et al. used gloves as the vector for transfer where 16 used gloves were examined for DNA present. In half the cases tested for, they found a mixture of DNA relating to both the test samples and alleles belonging to an unknown individual different to that of the volunteer.[32] In addition, in another 15% of the cases just alleles from another unknown individual were found, unable to be attributed to the volunteer.[32] This could be attributed to secondary transfer by the volunteer onto their gloves from an unknown source although authors did point out some of their negative controls from supposedly “clean” gloves did yield alleles present.[32]

Szkuta et al. continued on from other studies that focused on the amount of DNA that is left on vectors after the transfer event has occurred. They did this by the conducting a secondary transfer event of DNA between two “exhibits” using tools such as forceps,

scissors and gloves as the vector.[33] It was shown that secondary transfer could occur from exhibit to exhibit by DNA-free vectors but the main finding was still having a sufficient quantity of DNA present on the vector to continue to produce secondary transfer to other objects.[33] These findings are particularly more prevalent for blood rather than touch DNA during the transfer event which seems to be due to the higher concentration of DNA present, additionally shown in numerous other studies.[28,34-36] Here though this study used DNA-free vectors which again in real-life scenarios is very unlikely to occur. As mentioned previously, this may increase the transfer percentage hence giving false indications of the secondary transfer of DNA.

Fonneløp et al. investigated the transfer of DNA from an object to individual and then back to an object, with this idea coming from Meredith Kercher murder case which will be further explored below. They used three volunteers who were known to be good shedders, those that deposit a full DNA profile 15 minutes after hand-washing.[17] The first individual picked up an object and handled it for 30 seconds then placed it back.[17] A second individual then handled this same object and then subsequently put pressure on a piece of bench paper or fabric with pressure causing a tertiary transfer event.[17] Substrates included in this study were plastic conical tubes, metal door handle, disposable gloves, fabric and bench papers.[17] Results showed for the first transfer event, 83% of the DNA was high enough quality for the detection of a full profile and for the second transfer event, that figure dropped to 53% which saw those profiles up to the standard of case reporting and database searches.[17]

In 2017, Buckingham et al. followed on from their study in the previous year and used cotton covered glass plates (porous) rather than just glass plates themselves (non-porous) to investigate the resulting yield and profile of DNA. They again used the same method of the knife as the transfer vector with the four individuals all touching it at one stage then placing their hand on the cotton plate.[37] 40 handprints were collected with some results producing evidence of secondary and tertiary transfer of DNA although the proportion of this to the depositor's DNA was less than 10%.[37] There were additionally some unknown sources of DNA found in swabs from cotton plates.[37] In comparison between both of Buckingham et al.'s studies show that the transfer of DNA both directly and indirectly appears to be dependent on the surface in which the DNA is recovered from as well as how the DNA is recovered.[37]

2.1.1 *LAUNDERING*

A laundry machine for the transfer of DNA is a relatively well covered topic in terms of spermatozoa but this study conducted by Kamphausen et al. in 2015 was one of the first testing for touch DNA. 15 individuals rubbed necks against a cloth (primary transfer) for 5 seconds with medium pressure.[35] With a total of 46 independent washes completed by hand or machine with/without detergent secondary transfer of touch DNA was deemed almost impossible for reliable STR analysis.[35]

In 2018, another study was conducted into the mechanisms of the transfer of DNA via laundry. Eight unworn socks were washed in a normal load of household laundry of four families (2 per wash) and then subsequently air-dried.[20] Results showed that out of the 32 samples that were collected after laundering only 7 of them (22%) yielded a result higher

than the minimal threshold for casework (0.06ng/μL).[20] Four of those sample matched a profile of a female from the household which was either a single source or major profile indicative of the transfer of DNA occurring but unknown as to either direct or indirect transfer.[20]

2.1.2 *FINGERPRINT BRUSHES*

Most studies conducted exploring the secondary transfer of DNA involve a vector that is most likely an object such as a glass beaker or tube, but there are objects that are used in the processing of crime scenes that may cause for exploration. Fingerprint brushes are used by Forensic Scientists and Police Officers to develop latent fingerprints at the scene of a crime. Often these individuals do not use disposable brushes but more commonly reusable brushes without thinking of biological contamination issues that may arise. Van Oorschot et al. was the first to test the extent to which DNA transfer may occur using squirrel-hair fingerprint brushes.[38] Out of 26 samples from pre-used brushes brushed on paper, only 2 resulted in profiles which were partial, both brushes were used in casework.[38] Another 73 squirrel-hair fingerprint brushes were in current use by staff and were brushed over 5 sheets of plastic with 4 being clean and 1 (the 3rd in sequence) presenting a fresh deposited handprint.[38] The results showed that very limited pick up and transfer of DNA occurred by the brushes however when changes were made to the DNA analysis, significant pickup and transfer of DNA was seen.[38]

The previous study had confirmed that the transfer of DNA can occur through fingerprint brushes at crime scenes but not sufficiently to show that it occurs consistently. A second study was conducted by Proff et al. where they determined that DNA contamination with

fingerprint brushes was quite common, where 85% of the brushes they tested resulted in either full or partial profiles present.[39] In testing brushes for the potential for secondary transfer, the authors used 16 brushes against an acetate sheet with carbon black powder with 25% of surfaces had DNA detected with one full profile.[39] As with the previous study, this one also found a limited risk of the secondary transfer of DNA through fingerprint brushes.[38,39]

Even though these are the only two studies that have been explored in the secondary transfer of touch DNA, there have been studies performed with other biological substances. These will be review in later sections. From just these two studies, it shows the need to be more aware of contamination and transfer events occurring. One set of authors event suggest that decontamination procedures should be put into place regarding the continuous use of fingerprint brushes between crime scenes.[39] In today's crime scene examination, this has already been put into place where either disposable fingerprint brushes or de-contaminated fingerprint brushes are used.

2.2 INDIVIDUAL AS VECTOR

Although this scenario seems like the more common scenario to occur in a "real-life" situation, it seems to be the least researched of them all. The first study that did use an individual as the vector for the transfer of DNA was conducted by Lowe et al. in 2003. The authors used pairings of good and poor shedders who shook hands for one-minute and then the poor shedder gripped an object for 10 seconds.[40] One pairing in particular consistently found that the good shedder more than often had their full profile transferred and the poor shedder, the vector, could not be detected.[40] By putting in place a 30 second

time delay between transfer events, mixed profiles were discovered where 70% were from the original source.[40] Although this study did confirm events of secondary DNA transfer occurring, it is performed under controlled conditions where pairings were created to optimize the desired outcome and typically there may be hours or days between transfer events occurring.

Farmen et al. additionally conducted a study where two individuals undertook a handshake for 30 seconds followed by the gripping of beakers for 30 seconds.[14] A total of 60 swabs were taken and all resulted in a DNA profile of some form, either partial, full or mixed.[14] Although not specified, the authors suggest that all samples were mixtures containing both profiles of the known contributors, indicating that secondary DNA transfer had successfully occurred.[14] This is in agreeance with the previous study conducted by Lowe et al. where they also saw secondary transfer occur, with both studies seeing that mixtures can have higher contribution of individuals who were not the vector.[14,40] Typically, in the scenario of secondary DNA transfer, the individual who deposits the DNA at the last touch will leave a higher amount of their DNA as that transfer is primary. Those DNA profiles that have gone through multiple transfer before the final deposit will typically decrease significantly in concentration.

A study conducted by Meakin et al in 2015 also showed instances of the secondary transfer of DNA occurring through a handshake as the vector and a knife as the final transfer event. A “regularly-used” was artificially created over a period of two days and after that time period two volunteers shook hands then immediately stabbed their knife into foam occurring for the next three days with three different knives.[41] The study found that for

three of the four sets of knives mixtures were found containing three different profiles.[41] The first two could be attributed to the regular user of the knife and the hand shaker, but the other was an unknown profile, possibly the cause of secondary transfer from the regular user through other means or possibly tertiary transfer from the hand shaker.[41] As with the study conducted by Lowe et al., this study has tested the persistence of the secondarily-transferred DNA and found that the best chance of recovering that DNA would be to sample as soon as possible after the final transfer event had occurred which is consistent with Lowe et al.'s findings.[40,41]

With a new-found gap in the literature, Cale et al. performed a standard study into the secondary transfer of DNA with a kit of increased sensitivity. The authors aimed to study how the presence of secondary DNA transfer would affect the interpretation of DNA typing results.[11] In addition, the effects between surface texture was also explored.[11] Participants washed hands before wearing glove for 1.5 hours before shaking hands with another participant for 2 minutes and then handling a pre-cleaned knife for a further 2 minutes.[11] Significant results of this study concluded that texture did not appear to have a significant effect in addition to the data obtained from five of the 24 samples that suggest that individuals can have their DNA deposited on objects in sufficient quantities that they can be identified as the only or major contributor, suggesting that they had come in direct contact with the object.[11] These results seem to be a trend through numerous studies where if good shedders are the donor DNA profile, they will typically overpower the DNA of that of the vector.[9,42-44] All these papers discussed within this section are highly unrealistic and lack scientific rigour. In an everyday scenario, individuals would barely ever

shake hands for 30 seconds and further handle another object immediately after when committing a crime.

2.3 INDIRECT FINDINGS OF SECONDARY TRANSFER

There are also instances in where primary transfer or persistence of DNA may have been experimented on during a study although unknown alleles may be detected on the object or individual. Here all the studies that have detected secondary transfer of DNA during another experiment have been collated. However, in these studies, unless the authors have specifically stated we do not know where the secondary transfer has occurred and who the unknown alleles are from. Additionally, the unknown alleles may be part of the background DNA present on an object unless the object of individual has been pre-cleaned beforehand.

The first instance of accidental occurrence of secondary DNA transfer was during a study conducted by Lowe et al. in 2002 where the authors tested shedder statuses of individuals. Secondary transfer was detected when participants held a tube for 10 seconds but the extent was dependent on the subject pairings.[42] In an additional two experiments, secondary transfer was not conclusively shown as DNA recovered varied considerably between replicates.[42] Overall, the authors conclude that the secondary transfer of DNA was shown to occur under ideal conditions.[42] A few years later, Phipps et al. conducted a study in great similarity where they found that the occurrence of secondary transfer was low and not likely but possible.[43]

A study conducted by Rutty found an instance of possible secondary DNA transfer occurring where male-female pairs were used in order to simulate a strangulation event.[45] Men

placed two finger pads on the women's neck, an action that was repeated a total of 29 times creating 116 samples.[45] Two swabs of the male's fingers (controls) yielded a profile of a female in the study although these two individuals had never been paired or come into contact with one another.[45] Partial profiles were also found from one or more individuals in pairs of controls up to 10 days after the experiment.[45] Although in a different circumstance to that of Lowe et al., both studies show the presence of additional alleles that may be attributed to secondary transfer.

Using another different example, Petricevic et al. used five volunteers who slept in their own bed but with a new lower bed sheet.[46] Another scenario saw that individuals slept in a bed where they never had previously.[46] When individuals slept in their own beds, a second individual's DNA profile was found in at least one sample from 3 out of the 5 volunteers.[46] The authors were able to attribute these from the volunteer's partners, possibly transferrin from other bedding used or from the volunteer themselves.[46] When individuals slept in an unknown bed, all unknown samples could be attributed to the regular user's profile which did not indicate secondary DNA transfer.

Another similar experiment to that conducted by Lowe et al. and Phipps et al., Djuric et al. had volunteers hold plastic tubes and individual's ankles for 10 seconds.[47] When volunteers held tubes, out of the seven profiles obtains, one resulted in a partial profile not matching the volunteer's, indicating the occurrence of secondary transfer.[47] When ankles were used as deposition sites, no occurrence of the secondary transfer of DNA was noted.[47] Goray et al. conducted a very similar experiment again but tested the deposition onto glass plates rather than plastic tubes. A total of 40 samples were taken and non-self DNA (unexpected

alleles) was seen in 79% of the samples.[9] Surprisingly, 7 samples had the primary contributor profile excluded from the mixture.[9] Goray et al. hypothesized that the most common explanation for the presence of non-self DNA in deposits were due to the transfer of non-self DNA picked up through every-day activities.[9] The authors also ran the mixtures through staff elimination databases and other workers who were known to be in close proximity to participants matched to the non-self DNA deposited.[9]

A paper published by Oldoni et al. applied the constant unknown from contact stains at break-ins to conducted an experiment. Here, volunteers would handle an object regularly over 8-10 days then a second user would handle the same object for a pre-determine period of time.[48] A total of 231 samples were created where approximately 2-10% contained unknown alleles, possible suggesting secondary transfer.[48] Additionally, in two of the simulations, there were full DNA profiles present that did not correlate to individuals directly in those simulations but rather other volunteers from the study.[48]

Many of these studies conducted in the secondary transfer of DNA are carried out at a forensic laboratory and involve volunteers who work in an office/laboratory based environment. As these individuals may be at high risk for transferring DNA to forensic exhibits if working with them, an exploration into the ways in which volunteers may transfer DNA between themselves was conducted by Fonneløp in 2015. Here four volunteers had their keyboards and mouse swapped with another's with swabs taken from both objects and volunteers at the beginning and throughout the study.[49] Swabs taken before the study had begun showed DNA present from unknown individuals, but unfortunately it cannot be concluded if this DNA was secondarily transferred or through

direct contact. The author's attributed this occurrence to background DNA being present on everyday objects.[49] In addition, the study also showed that secondary transfer of DNA occurred through the presence of the original user's DNA on the hands of the second user up to 8 days after receiving the computer equipment.[49]

One of the simplest of ways to determine the possibility of secondary DNA transfer or background DNA present would be to directly swab an individual's hand which was carried out by Lacerenza et al. 120 samples were directly collected from 60 individual's hands where 56 samples had one or more unknown alleles and 36 of those could be classified as mixtures (64.3%).[50] As these DNA samples present in the mixtures were foreign to that of the volunteer, it could be concluded that either secondary DNA transfer occurred or that these profiles were a part of the background DNA present on that individual's hand.[50]

A new substrate was then used by van den Berge et al. where individuals dragged each other mimicking an activity-related scenario. Samples were taken from the knee-area of the trousers where the dragger did not touch resulting in 26 samples, 3 of which did not result in over 7 alleles.[51] Donor alleles were present in 100% of the samples and non-donor in 71%.[51] The authors concluded that more care must be taken when interpreting mixtures as alleles matching a perpetrator may not be distinguishable from background signals.[51]

In a very recent study, Magee et al. used collars and cuffs of upper garments to determine the presence of DNA.[52] Out of 55 samples taken, non-wearer DNA was recovered, an average of 1.3ng from the collar and 2.7ng from the cuffs.[52] From one particular samples, a non-wear contributed approximately 57% of DNA to a mixture, followed by the wear

contributing 37% and a second non-wear, 7%.[52] Three other non-wearer samples were able to be attributed to spouses of the volunteers.[52]

2.4 SHEDDER STATUS

Shedder status is one of the more recent concepts under the umbrella of the transfer of DNA and one that will continue to grow as more research is conducted into the topic. The first study to directly carry out an experiment on individual's shedder status' was conducted by Lowe et al. in 2002 and is considered the backbone study to this topic. The authors got participants to grip a sterile tube, 15 minutes, 2 hours and 6 hour periods after hand-washing.[42] They determined that there were differences between individuals in regard to how much DNA that they deposited on a surface.[42] Good shedders were then classed if the individual left an entire profile after 15 minutes of hand-washing.[42] All other individuals were classed as poor shedders.

A fair few years later, Phipps et al. conducted a second study that investigated shedder status and used almost identical experiments to that of Lowe et al. Plastic tubes were used for deposits and hand washing occurred 15 minutes prior to the experiment.[43] Unexpectedly, the study had shown that individuals do not produce consistent quantities of DNA over time, varying as much within themselves than compared to other people.[43] Finally, using the criteria laid out in Lowe et al., this study discovered no "good" shedders and all were classified as "poor" shedders suggesting this level of classification may not be all as simple as once thought.[42,43]

The following year Farmen et al. conducted a study on shedder status in conjunction with an experiment on secondary DNA transfer. Authors measured individuals' shedder status just based on swabs from their hands.[44] This unfortunately doesn't test the quantity of DNA that the individual's actually deposit on a surface, not classing as a true representation of shedder status. Out of the nine individuals who participated in the study, two individuals had a low DNA yield and seven individuals produced profiles that were able to be analysed.[44]

More recently in 2016, Goray et al. carried out an experiment that explored the effect of the variables hand dominance, hand size and gender on individual's shedder statuses. 240 samples were collected from handprints on glass plates where only four of the samples had no detectable quantities of DNA.[9] Two of the ten participants could be classed as good shedders but this was done under different criteria and experimental criteria as to the base study conducted by Lowe et al.[9] The only correlation or significance that the study found with the variables tested was that of gender, where a significant difference was seen between genders in the amount of DNA that they deposited.[9]

2.5 OTHER FORMS OF DNA INVOLVED IN SECONDARY TRANSFER

2.5.1 *TRANSFER OF SPERMATOZA*

One of the first studies carried out in the broader realm of the transfer of DNA was by Kafarowski et al. in 1996. This was conducted before the first occurrence of the secondary transfer of touch DNA and was one of the first of its kind. It aimed to determine the likelihood of the transfer of spermatozoa between articles of clothing during a machine-cycle wash.[53] Although the retention of spermatozoa during machine wash had previously

been explored, the transfer of it to other articles of clothing hadn't.[54,55] The authors used underpants as a means for the primary deposit of spermatozoa, reflecting real-life situations that may depict a sexual assault. This pair of underpants was washed with three additional clean pairs and then machine dried.[53] In all three trials, trace quantities of spermatozoa were found where a minimum of one sperm head and maximum of eight per sample.[53] As this was one of the first studies performed in the secondary transfer of DNA, there are many downfalls to it that have improved over time with knowledge and technology.

2.5.2 *TRANSFER OF SALIVA*

Saliva is an additional biological substance that contains human DNA that has the possibility to be transferred through objects and individuals to cause a secondary transfer. The first that explored into this secondary transfer of saliva was conducted by van Oorschot et al. where they explored the transfer of saliva through a squirrel-hair fingerprint brush. In one experiment a single brush was swept over 19 sheets of paper with dried-saliva present then brushed over another 20 clean sheets of paper.[38] Out of the 80 samples generated, 13 resulted in partial profiles, 1 in no profile and the remaining 66 resulted in full profiles.[38] The second experiment saw both the powder type and DNA extraction tested in the transfer of saliva from brush to six sheets of plastic.[38] For the comparison on extraction techniques, out of 6 samples, 3 were full profiles, 2 partial profiles and 1 no result.[38] Any presence of a profile in this instance confirms the secondary transfer of DNA has occurred from the paper, to fingerprint brush and then back to the paper.

Next researches decided to carry out experiment very similar in nature to those conducted with touch DNA but with saliva instead. Wiegand et al. transferred saliva onto either paper, cotton or plastic surfaces, left them to air dry then rubbed a thumb onto the strain.[34] The thumb was then placed onto another piece of paper and then the area was swabbed.[34] When paper was the primary surface, only about 50% of the transferred saliva produced DNA profiles although they were in very low concentrations (max. 1 pg/μL).[34] In total only 3 out of 96 gave complete profiles, all from plastic as the original source but only one being from saliva.[34]

The secondary transfer of saliva was additionally tested through a vector of pens and plastic tubes for the overall aim of determining if saliva would be a more prevalent biological material during transfer events.[16] Their study concluded that secondary transfer of saliva did occur, in greater levels of retention than touch DNA and that moist surfaces may facilitate DNA transfer to a greater degree than a dry surface as previously discovered in Goray et al. 2010.[16,56] The authors additionally stated a finding of background DNA present on an individual's hand when dealing with the transfer of DNA.[16]

In 2015, the transfer of saliva through laundering was studied, something in which touch DNA had been explored multiple times. Individuals deposited saliva onto textile cloths and then these were subsequently washed with clean textile cloth.[35] Between the experiment additionally conducted with blood and touch DNA, a total of 46 independent washes were completed both by hand and machine with/without detergent.[35] Saliva was seen to undergo transfer events which supports other studies performed.[16,34,35,38,56]

The most recent study conducted in relation to the transfer of saliva was through individual's hands as vectors for the transfer. 14 areas of the individuals palm were swabbed in addition to the glass plate which the saliva was transferred to.[57] The study showed a significant decrease in the quantity of DNA through the final transfer events, where previously an average of 9.16ng was seen and post transfer there was an average of 0.225ng.[57] The study also yielded a number of unknown alleles on the final plate where the authors contributed this to prior interaction with individuals, supporting the finding that secondary transfer of DNA had occurred.[57]

2.5.3 TRANSFER OF BLOOD

Following on from the secondary transfer of saliva, Wiegand et al. additionally conducted a study into the risk of the transfer of bloodstains from surfaces such as plastic, cotton and paper. The bloodstains were left to air dry then an individual placed their thumb on the stain then onto another piece of paper.[34] From the cotton surface, DNA concentrations after secondary transfer occurred were remarkably similar to those following the primary transfer event.[34] When gloves were used instead of bare hands, the DNA concentration following DNA transfer increased.[34] Both these results supported the findings of secondary transfer of DNA through blood occurring. Lehmann et al. conducted a very similar experiment but all transfer events were conducted with substrates only and no human interaction. The experiment supported the existence of the secondary transfer of DNA through blood and additionally found that if the blood were wet, it would transfer further and would end in a higher concentration as more substance was transferred.[28]

A study investigated secondary transfer of DNA through laundering in a washing machine or by hand by Kamphausen et al. in 2015. Artificial bloodstains were also created on textile cloth, then dried and these were then washed with clean textile cloths.[35] In total 46 independent washes were completed both by hand and machine in addition to with/without detergent.[35] Transfer of blood cells were seen in this study in addition to numerous previous ones.[28,34,35,36]

A gap was then identified in the literature, where none of these previous studies had really thoroughly tested the effect the aridity of the biological substances, here specifically blood. van Oorschot et al. researched into this using cotton as the secondary substrate.[36] Drying time, temperature and humidity all acted as dependent variables in this study.[36] The secondary transfer of blood was seen to have occurred with significant differences in the transfer percentage of blood between 5 and 60 minutes.[36]

2.6 TERTIARY TRANSFER OF DNA AND BEYOND

Although the secondary transfer of DNA is still in the process of gaining a greater understanding of its mechanisms, through these studies the discovery of transfer events occurring after a second transfer have occurred. These studies are still in their very preliminary stages and like secondary DNA transfer, still require a greater understanding into the mechanisms surrounding the process. The first study that explored the occurrence of the tertiary transfer of DNA was conducted by Warshauer et al. where an object was the original vector and was further transferred from an individual to another individual or object as the tertiary transfer event.[16] They were able to prove that tertiary transfer of DNA could occur but were unable to recover enough DNA to estimate the rate of DNA lost during

the additional transfer step.[16] Out of the additional transfer steps conducted, 87.5% displayed less than half of the expected alleles.[16] These results correlated with those produced by Fonneløp et al. where after the final transfer step only 17% of samples were high quality that could be reported and go through database searching (5 out of 30 transfer events).[17]

In the study that was conducted by Fonneløp et al. they also tested the instance of quaternary transfer occurring, which was a first. They found that once again, it occurred but as with most of the issues with the studies explored in this review, all surfaces and objects were cleaned thoroughly to begin with to eliminate the background DNA present. A total of 17 out of the 108 analysed samples contain unknown alleles and these unknown alleles could not be attributed to the presence of background DNA as it was eradicated.[17] There was additionally one transfer sequence that the authors were able to determine the donor of the DNA present in the tertiary transfer event.[17] The source of the DNA was from the girlfriend of the volunteer, a sample found in all transfer events.[17] Previously, these two individuals had not been in contact with each other for more than 10 hours and the volunteer had additionally washed his hands prior to the experiment beginning.[17]

In 2016, Helmus et al. performed the first study singly testing the tertiary transfer of DNA due to the gap identified within the literature. Three pairs of individuals were used with a cotton cloth as the vector and final depository where a total of 180 samples was generated.[19] Out of those 180 samples generated, only 72 had detectable DNA that identified that tertiary transfer had occurred (40%).[19] In a study completed the year earlier they had additionally shown the occurrence of the tertiary transfer of DNA but the authors

noted that they were unable to distinguish between tertiary transfer or interference from a low-level of non-donor DNA, something that may have to do with type of system used, the Promega ESI-17 Fast PCR.[18]

In a more recent study, tertiary transfer of DNA evolved to a more complex mechanism than that of individual-object involved transfer. Six new unworn socks and a t-shirt were laundered together with no additional items in a machine that had been previously used.[20] Results showed that there were no DNA results recovered from quantity (above threshold) through to profiling therefore not suggesting that tertiary transfer could occur.[20] In this case, either the kits with improved sensitivity need to be created or this is the point at which the extent of tertiary transfer will occur. This is however unknown without additional studies conducted in this area.

2.7 CASE STUDIES

In 2002, a paper was published by Ansell exploring a case study involving the secondary transfer of seminal constituents. A rape occurred in Sweden whereby a woman was allegedly raped by a man where mixed DNA profiles were found in both the vaginal swabs, underwear swabs and penile samples.[58] Both vaginal and underwear swabs excluded the man as a suspect as his DNA was not found (had a vasectomy), although the woman's boyfriend DNA was additionally found due to consensual intercourse the previous day.[58] The penile swabs also showed a mixed profile but that of the women's DNA in addition to the women's boyfriends DNA.[58] The alleged rape of this women was able to be proved in court by the secondary transfer of the boyfriend's DNA to the suspect.[58]

More recently, in 2017, an article was created following the incidence of contamination by Police Officer's in Austria which without elimination databases, many instances of indirect or secondary transfer of DNA may go undetected.[59] During the period of 2000 to 2016, a total of 46,000 trace samples were submitted through DNA and out of those a total of 347 contamination incidents were detected through the use of the elimination database (0.75%) with a majority from the cause of indirect transfer.[59] The authors further went into detail regarding 3 separate incidents that there were able to pinpoint where the transfer events had occurred. The first occurrence happened through shared camera equipment that had been used by a Police Officer completely unrelated to the case.[59] The second shows the indirect transfer by a Police Officer through a shared vehicle to a volume crime case and the third through packaging of evidence material on an unrelated Police Officer's desk.[59]

3. PERFORMANCE OF META-ANALYSIS

3.1 METHOD

A thorough review of the literature was conducted through the use of online databases to gather articles in the secondary transfer of DNA. Databases were chosen based on their relevance to the topic of Forensic Science. Databases chosen were Scopus, Web of Science Core Collection, ProQuest, Medline, Science Direct and Research Gate. Some databases were more specific to Forensic Science but general science databases were included, such as Science Direct, in order to broaden the search results to ensure greater coverage of the entire literature into the secondary transfer of DNA.

The key terms used for searching the databases included the following:

- "Secondary transfer" AND "DNA" AND "Forensics"
- "DNA transfer events" AND "Forensics"
- "Tertiary transfer" AND "DNA" AND "Forensics"
- "Direct DNA transfer" AND "Forensics"
- "Shedder status" AND "Forensics"
- "Indirect transfer" AND "DNA" AND "Forensics"

No additional search terms were excluded due to the specificity of these databases and their smaller range of journal articles available.

The only restrictions that were made on the database searches were that of eliminating types of articles, e.g. book chapters, encyclopedias etc. No additional restrictions were placed on the database searches. All databases already had restrictions such as language and dates applied to searches, where English was selected as the language and beginning dates of searches went to the beginning of the database. This date was most commonly in the 1990's and search results went to April 2018.

3.1.1 *SCREENING OF ARTICLES*

After the completion of the database searches, all articles were then screened for their appropriateness to the topic of the secondary transfer of DNA. A total of 307 articles were identified through the database search. These articles were compiled of all yielded search results from all six search terms through all six databases. These 307 articles then had their reference lists screened yielding a further 103 eligible articles, creating a total of 410 articles through the literature search.

The next step of the process was to screen all 410 articles found as a result of the literature search based on their titles and abstracts. For this, inclusion and exclusion criteria were used to narrow down the possible articles. The main exclusion criteria that was used was if the article did not involve transfer event of DNA in any form (biological substance such as touch, spermatozoa, blood and saliva) it would be excluded from the meta-analysis. Other exclusion criteria included studies that involves a case study, those that are a response to a journal article and a form of writing that was not a journal article or theses (e.g. book or a book chapter). From this screening process, a total of 141 articles were selected based on their title and abstracts.

These 141 articles then went through a further screening process that involved the inclusion of the entire body of text. Articles were again excluded based on inclusion and exclusion criteria, where if an article had a recorded or measured occurrence of the secondary transfer of DNA in any form, it was able to be encompassed in the meta-analysis. Articles that also found an occurrence of the secondary transfer of DNA but indirectly (was not the main variable testing for) were also excluded. The second screening resulted in a total of 38 articles that were able to be continued through to the meta-analysis. This resulted in 103 studies excluded from this screening process.

3.1.2 *DATA EXTRACTION*

Data was extracted from all 38 articles discovered through the screening process and collated into a table using Microsoft Excel. This data included the following:

- Author;
- Year;

- Conditions of the experiment (Controlled, semi-controlled, uncontrolled);
- Number of Participants;
- Number of samples taken;
- Type of vector;
- Final object of the DNA deposition;
- Time between the primary and secondary transfer event;
- Time handling the vector;
- Time handling the final object;
- Type and pressure of contact of vector;
- Average total DNA recovered (ng);
- Total DNA concentration (ng/μL);
- Total number of unknown alleles detected (indicative of secondary transfer);
- Number of mixtures seen;
- If a reference sample of volunteers had been taken; and
- If secondary transfer of DNA in any form was detected.

3.2 RESULTS AND DISCUSSION

It was originally hypothesized that through the exploration of literature and completion of a meta-analysis that an appropriate and concise guide will be produced to determine the chances of a secondary transfer event occurring and what conditions are required, to aid Biologists in expert testimony. This hypothesis was rejected on the basis that the meta-analysis was found not be an adequate model for the data that was available. The screening process of articles exploring the secondary transfer of DNA was sufficient for this field of

science, as articles were able to be easily included and excluded based on a set of criteria. Data extraction was additionally able to be carried out to some extent as most journal articles give accurate representations of the study and how it was performed. However, through the creation of a table, incorporating all of the included studies for the meta-analysis and the conduction of the meta-analysis, it was determined that the meta-analysis would not be adequate for the data that was available.

For this meta-analysis, it was hoped to use average amount of total DNA recovered (ng) as one of the quantitative variables. Articles however did not all give results that would be sufficient to calculate an average from. Some gave all values of total DNA recovered from the final objects after secondary DNA transfer, where an average was able to be calculated. Conversely, some articles would state a range of the total amount of DNA recovered which an average could not be calculated from as the raw results were not present. Therefore, there was not a consistent set of values that would be used to represent the total amount of DNA recovered through all included studies.

As a second quantitative variable was required, out of the data available to extract, time handling the vector, final object or time between handling the vector and final object, were the only two options available. Although this value differed between studies, within a study it was highly likely that only a single time value was utilized. For example, Goray et al. used one minute for the time handling the vector and the final object for all volunteers.[27] Two different vector types were used within the study with two different times between handling the vector and final object (immediately and 24 hours after).[27] Both touch DNA and blood was used as the biological substances, creating four possible scenarios.[27] As

these quantitative variables did not vary substantially within the study, it would not be possible to compare the variables within the study. Other studies additionally only had one vector type and biological substance, and therefore only one quantitative value of time handling vectors or objects.[28,29,35,41,45]

Reflecting back onto the hypothesis, it was stated that it was anticipated that "an appropriate and concise guide will be produced to determine the chances of a secondary transfer event occurring and what conditions are required". As mentioned previously, the framework that a meta-analysis works on is the comparison of two quantitative variables in the hope to perform statistical tests such as Cohen's D or Pearson's coefficient. In order to determine the conditions required for the secondary transfer of DNA to occur, quantitative values would need to be gathered. The condition that secondary DNA transfer are conducted under would include things such as the vector type, final object type, conditions of the experiment, background DNA present and type of pressure and contact of the vector or final object. These variables unfortunately are qualitative and do not have an option to measure them quantitatively. In that case, performance of comparisons between these variables and quantitative variables such as total DNA, DNA concentration or time handling objects cannot be carried out.

4. LIMITATIONS OF THE SECONDARY TRANFER OF DNA

4.1 CONTROLLED NATURE OF THE STUDIES

With a vast number of papers explored in this literature review, none of them tested the occurrence of secondary DNA transfer happening with completely uncontrolled scenarios.

In my opinion, the closest study that was to an uncontrolled scenario was a study conducted by Goray & van Oorschot in 2013. They used a small group of individuals with a small number of objects (glasses, jugs, tables, chairs) participating in social interaction for 20 minutes.[31] Prior to the experiment occurring the authors did pre-clean all surfaces and items and the majority of controls produced a negative result as expected. As close as this study was to being uncontrolled, the occurrence of pre-cleaning diminishes all background DNA present on those items, not reflecting what were to occur in a "real-life" situation.

4.2 TIME BETWEEN TAKING SAMPLES

There seems to be a common theme between studies published that most samples from transfer events are taken almost immediately after the event occurs. As stated in one of the studies, "the best chance of recovering the DNA would be to sample as soon as possible after the final transfer event has occurred".[41] This unfortunately is not possible in real-life situations as often a crime scene may not be discovered for hours or days after it has occurred. This same principle can be applied for the time between transfer events occurring. Lowe et al. had found when placing a 30 second delay between transfer events the final sample went from containing a full profile with all the first individuals' DNA present to only have 70% of that same individuals present.[42] Even with that 30 second time delay, the situation is still not indicative of real-life as individuals would not shake hands immediately prior to committing a crime. Judging off these results, it could be said that unless these situations were to have occurred and the addition of some other variables it would be highly unlikely that DNA found at a crime scene would be anyone else's other than that individual carrying out a primary transfer.

4.3 REFERENCE SAMPLES

In nearly all of the studies explored throughout this review, reference samples were taken from the volunteers. This could be considered normal for studies in this field, although when trying to best represent what occurs in the real-world, this action does not correctly reflect that. When interpreting mixtures or just complex single source profiles, forensic scientists will more likely than not never have a reference sample for comparison purposes. Here it may then be more difficult to pull out multiple contributors of a mixture without knowledge of the major contributor, or any contributor. By using a reference sample of the volunteer during these studies, essentially most of the work forensic scientists conduct in regard to profile interpretation is void.

5. LIMITATIONS OF META-ANALYSIS

5.1 NEW APPLICATION TO THIS FIELD

Meta-analyses are a very common method applied to that of the medical field currently and as previously mentioned, it has not yet previously been applied to the field of Forensic Science. Because of this it was not known if the method could be applied in the same way as it has done before in the medical field. As discovered through attempting a meta-analysis on the secondary transfer of DNA, it was not an appropriate method on these types of studies published.

5.2 TYPE OF RESULTS PUBLISHED

Through conducting a meta-analysis of the secondary transfer of DNA, it was determined that the studies conducted and published were not in a form that data extraction could

occur efficiently. Not all studies published their results in the same way and it was difficult to obtain two quantitative variables for comparison. Close to none of the 38 studies published in the secondary transfer of DNA and included in the meta-analysis, published their raw results of variables such as total amount of DNA recovered. Due to this, averages, means or standard deviations were not able to be computed for results included in these studies, and therefore a meta-analysis was not able to be carried out.

6. THE FUTURE FOR THE SECONDARY TRANSFER OF DNA

The main point that should be taken out of this review is that in this field we need more studies that examine the occurrence of the secondary transfer of DNA in more life-like scenarios. Until then, only so much can be speculated about if this transfer event occurs enough to be a valid argument in court for why an individuals' DNA may be present. Although performing these uncontrolled studies may not produce high instances of secondary DNA transfer occurring, through an application of a meta-analysis results from each study can be combined to produce a higher statistical power.

7. CONCLUSION

At this stage, it can be concluded that the hypothesis, through the exploration of literature and completion of a meta-analysis that an appropriate and concise guide will be produced to determine the chances of a secondary transfer event occurring and what conditions are required, to aid Biologists in expert testimony, can be rejected. Data published in the 38 studies included for the meta-analysis was not adequate enough to perform quantitative comparisons between variables in order to combine results to strengthen the power of

each study. In future, it should be considered that articles published in the field of Forensic Science display their results in different ways or give access to readers of their raw results for further interpretation purposes.

8. REFERENCES

1. Panneerchelvam S, Norazmi MN. Forensic DNA Profiling and Database. The Malaysian Journal of Medical Sciences. 2003;10(2):20-26.
2. Fan H, Chu J-Y. A Brief Review of Short Tandem Repeat Mutation. Genomics, Proteomics and Bioinformatics. 2007;5(1):7-14.
3. Walker E, Hernandez AV, Kattan MW. Meta-analysis: Its strengths and limitations. Cleveland Clinical Journal of Medicine. 2008;75:431-439.
4. Bright J, Petricevic SF. Recovery of trace DNA and its application to DNA profiling of shoe insoles. Forensic Science International. 2004;145:7-12.
5. van Oorschot RAH, Jones MK. DNA fingerprints from fingerprints. Nature. 1997;387:767.
6. Quinones I, Daniel B. Cell free DNA as a component of forensic evidence recovered from touched surface. Forensic Science International: Genetics. 2012;6:26-30.
7. Sijen T. Molecular approaches for forensic cell type identification: On mRNA, miRNA, DNA methylation and microbial markers. Forensic Science International: Genetics. 2015;18:21-32.
8. Zoppis S, Muciaccia B, D'Alessio A, Ziparo E, Vecchiotti C, Filippini A. DNA fingerprinting secondary transfer from different skin areas: Morphological and genetic studies. Forensic Science International: Genetics. 2014;11:137-143.
9. Goray M, Fowler S, Szkuta B, van Oorschot RAH. Shedder status – An analysis of self and non-self DNA in multiple handprints deposited by the same individuals over time. Forensic Science International: Genetics. 2016;23:190-196.
10. Wickenheiser RA. Trace DNA: A Review, Discussion of Theory and Applications of the Transfer of Trace Quantities of DNA Through Skin Contact. Journal of Forensic Science. 2002;47(3):442-450.
11. Cale CM, Earll ME, Latham KE, Bush GL. Could Secondary DNA Transfer Falsely Place Someone at the Scene of a Crime? Journal of Forensic Science. 2016;61(1):196-203.
12. Ladd C, Adamowicz MS, Bourke MT, Scherczinger CA, Lee HC. A Systematic Analysis of Secondary DNA Transfer. Journal of Forensic Science. 1999;44(6):1270-1272.
13. Meakin GE, Butcher EV, van Oorschot RAH, Morgan RM. Trace DNA evidence dynamics: An investigation into the deposition and persistence of directly- and indirectly-

transferred DNA on regularly-used knives. Forensic Science International: Genetics. 2017;29:38-47.

14. Farmen RK, Jaghø R, Cortez P, Frøyland ES. Assessment of individual shedder status and implication for secondary DNA transfer. Forensic Science International: Genetics Supplement Series. 2008;1:415-417.
15. Meakin G, Jamieson A. DNA transfer: A review and implications for casework. Forensic Science International: Genetics. 2013;7:434-443.
16. Warshauer DH, Marshall P, Kelley S, King J, Budowle B. An evaluation of the transfer of saliva-derived DNA. International Journal of Legal Medicine. 2012. doi: 10.1007/s00414-012-0743-1.
17. Fonneløp AE, Egeland T, Gill P. Secondary and subsequent DNA transfer during criminal investigation. Forensic Science International: Genetics. 2015;17:155-162.
18. Davies C, Thomson J, Kennedy F. Assessing primary, secondary and tertiary DNA transfer using Promega ESI-17 Fast PCR chemistry. Forensic Science International: Genetics Supplement Series. 2015;5:e55-e57.
19. Helmus J, Bajanowski T, Poetsch M. DNA transfer – a never ending story. A study on scenarios involving a second person as carrier. International Journal of Legal Medicine. 2016;130:121-125.
20. Voskoboinik L, Amiel M, Reshef A, Gafny R, Barash M. Laundry in a washing machine as a mediator of secondary and tertiary DNA transfer. International Journal of Legal Medicine. 2018;132:373-378.
21. Nordmann AJ, Kasenda B, Briel M. Meta-analyses: what they can and cannot do. Swiss Medical Weekly. 2012;142. doi:10.4414/smw.2012.13518.
22. Plackett RL. Studies in the history of probability and statistics: VII. The principle of arithmetic mean. Biometrika. 1958;45:130-135.
23. Pearson K. Report on certain enteric fever inoculation statistics. British Medical Journal. 1904;3:1243-1246.
24. Glass GV. Primary, secondary and meta-analysis of research. Educational Researcher. 1976;5:3-8.
25. Patsopoulos NA, Analatos AA, Ioannidis JP. Relative citation impact of various study designs in the health sciences. The Journal of the American Medical Association. 2005;293:2362-2366.

26. Fisher RA. Statistical Methods and Scientific Inference. Oxford, England: Hafner Publishing Co.; 1956.
27. Goray M, Mitchell JR, van Oorschot RAH. Evaluation of Multiple Transfer of DNA using mock case scenarios. Legal Medicine. 2012;14:40-46.
28. Lehmann VJ, Mitchell RJ, Ballantyne KN, van Oorschot RAH. Following the transfer of DNA: How far can it go? Forensic Science International: Genetic Supplement Series. 2013;4:e53-54.
29. Verdon TJ, Mitchell RJ, van Oorschot RAH. The influence of substrate on DNA transfer and extraction efficiency. Forensic Science International: Genetics. 2013;7:167-175.
30. French JC, Morgan RM, Baxendall P, Bull PA. Multiple transfers of particulates and their dissemination within contact networks. Science and Justice. 2012;52:33-41.
31. Goray M, van Oorschot RAH. DNA transfer during social interactions. Forensic Science International: Genetic Supplement Series. 2013;4:e101-e102.
32. Margiotta G, Tasselli G, Tommolini F, Lancia M, Massetti S, Carnevali E. Risk of dna transfer by gloves in forensic casework. Forensic Science International: Genetic Supplement Series. 2015;3:e527-e529.
33. Szkuta B, Harvey ML, Ballantyne KN, van Oorschot RAH. Residual DNA on examination tools following use. Forensic Science International: Genetic Supplement Series. 2015;5:e495-e497.
34. Wiegand P, Heimbold C, Kelin R, Immel U, Stiller D, Klintschar M. Transfer of biological stains from different surfaces. International Journal of Legal Medicine. 2011;125:727-731.
35. Kamphausen T, Fandel SB, Gutmann JS, Bajanowski T, Poetsch M. Everything clean? Transfer of DNA traces between textiles in the washtub. International Journal of Legal Medicine. 2015;129:709-714.
36. van Oorschot RAH, McArdle R, Goodwin WH, Ballantyne KN. DNA transfer: The role of temperature and drying time. Legal Medicine. 2014;16:161-163.
37. Buckingham AK, Harvey ML, van Oorschot RAH. Transfer of picked-up DNA to cotton plates. Forensic Science International: Genetic Supplement Series. 2017;6:e6-e8.
38. van Oorschot RAH, Treadwell S, Beaurepaire J, Holding NL, Mitchell RJ. Beware of the Possibility of Fingerprinting Techniques Transferring DNA. Journal of Forensic Science. 2005;50(6):1-6.

39. Proff C, Schmitt C, Schneider PM, Foerster G, Rothschild MA. Experiments on the DNA contamination risk via latent fingerprint brushes. International Congress Series. 2006;1288:601-603.
40. Lowe A, Murray C, Richardson P, Wivell R, Gill P, Tully G, Whitaker J. Use of low copy number DNA in forensic inference. International Congress Series. 2003;1239:799-801.
41. Meakin GE, Butcher EV, van Oorschot RAH, Morgan RM. The deposition and persistence of indirectly-transferred DNA on regularly-used knives. Forensic Science International: Genetic Supplement Series. 2015;5:e498-e500.
42. Lowe A, Murray C, Whitaker J, Tully G, Gill P. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. Forensic Science International. 2002;129:25-44.
43. Phipps M, Petricevic S. The tendency of individuals to transfer DNA to handled items. Forensic Science International. 2007;168:162-168.
44. Farmen RK, Jaghø R, Cortez P, Frøyland ES. Assessment of individual shedder status and implication for secondary DNA transfer. Forensic Science International: Genetic Supplement Series. 2008;1:415-417.
45. Rutty GN. An investigation into the transference and survivability of human DNA following simulated manual strangulation with consideration of the problem of third party contamination. International Journal of Legal Medicine. 2002;116:170-173.
46. Petricevic SF, Bright J, Cockerton SL. DNA profiling of trace DNA recovered from bedding. Forensic Science International. 2006;159:21-26.
47. Djuric M, Varljen T, Stanojevic A, Stojkovic O. DNA typing from handled items. Forensic Science International: Genetic Supplement Series. 2008;1:411-412.
48. Oldoni F, Castella V, Hall D. Exploring the relative DNA contribution of first and second object's users on mock touch DNA mixtures. Forensic Science International: Genetic Supplement Series. 2015;5:e300-e301.
49. Fonneløp AE, Johannessen H, Gill P. Persistence and secondary transfer of DNA from previous users of equipment. Forensic Science International: Genetic Supplement Series. 2015;5:e191-e192.
50. Lacerenza D, Aneli S, Omedei M, Gino S, Pasino S, Berchialla P, Robino C. A molecular exploration of human DNA/RNA co-extracted from the palmar surface of the hands and fingers. Forensic Science International: Genetics. 2016;22:44-53.

51. van den Berge M, van de Merwe L, Sijen T. DNA transfer and cell type inference to assist activity level reporting: Post-activity background samples as a control in dragging scenario. Forensic Science International: Genetic Supplement Series. 2017;6:e591-e592.
52. Magee AM, Breathnach M, Doak S, Thornton F, Noone C, McKenna LG. Wearer and non-wearer DNA on the collars and cuffs of upper garments of worn clothing. Forensic Science International: Genetics. 2018;34:152-161.
53. Kafarowski E, Lyon AM, Sloan MM. The retention and transfer of spermatozoa in clothing by machine washing. The Canadian Society of Forensic Science Journal. 1996;29(1):7-11.
54. Brayley-Morris H, Sorrell A, Revoir AP, Meakin GE, Court DS, Morgan RM. Persistence of DNA from laundered semen stains: Implications for child sex trafficking cases. Forensic Science International: Genetics. 2015;19:165-171.
55. Farmen RK, Cortez P, Frøyland ES. Spermatozoa recovered on laundered clothing. Forensic Science International: Genetics Supplement Series. 2008;1(1):418-420.
56. Goray M, Eken E, Mitchell RJ, van Oorschot RAH. Secondary DNA transfer of biological substances under varying test conditions. Forensic Science International: Genetics. 2010;4:62-67.
57. McColl DJ, Harvey ML, van Oorschot RAH. DNA transfer by different parts of a hand. Forensic Science International: Genetic Supplement Series. 2017;6:e29-e31.
58. Ansell R. Secondary transfer of seminal constituents. Canadian Society of Forensic Science Journal. 2002;35(1):23-27.
59. Pickhrahn I, Kriendl G, Muller E, Dunkelmann B, Zahrer W, Cemper-Kiesslich J, Neuhuber F. Contaminations incidents in the pre-analytical phase of forensic DNA analysis in Austria – Statistics of 17 years. Forensic Science International: Genetics. 2017;31:12-18.

THIS PAGE HAS BEEN LEFT INTENTIONALLY BLANK

PART TWO

MANUSCRIPT

META-ANALYSIS OF THE SECONDARY TRANSFER OF DNA

ABSTRACT

Deoxyribonucleic acid (DNA) is complex molecule present in nearly every cell of the human body that contains all of an individual's genetic material. Trace DNA has allowed DNA to be detected on everyday objects such as door handles, tables and chairs. Currently, the idea of the secondary transfer of DNA has arisen, involving the transfer of genetic profiles from an individual through a vector and then to final individual or object. This has become a re-occurring term in the court system, as individuals standing trial use this argument for the presence of their DNA. It is therefore hypothesized that through the exploration of literature and completion of a meta-analysis that an appropriate and concise guide will be produced to determine the chances of a secondary transfer event occurring and what conditions are required, to aid Biologists in expert testimony. Database searches were conducted to gain resources in the topic, followed by a number of screens to determine the suitability of articles for the meta-analysis. With the resultant 38 articles, data extraction was additionally carried. However, due to the lack of quantitative variables and results, a meta-analysis was not able to be conducted. It is suggested that in the future, studies publish their results in a more scientifically rigorous manner or allow the access of raw results for external interpretation purposes.

Keywords: forensic science, secondary transfer, DNA transfer, indirect transfer, touch DNA, DNA

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

DNA = Deoxyribonucleic Acid

ng = Nanograms

ng/μL = Nanograms per microliter (concentration)

pg = Pictograms

STR = Short tandem repeat

μL = Microliter

UV = Ultraviolet

INTRODUCTION

Deoxyribonucleic acid (DNA) is molecule present in nearly every cell of the human body that possess all of an individual's genetic material.[1] Each cell has approximately three billion base pairs which is essential for the characterization of each individual.[1] These base pairs can create polymorphic sequences, for example a short tandem repeat (STR), a repeated sequence of DNA that is usually around one to six base pairs in length.[2] These areas are of great interest in the field of forensic science, as their combinations are variable between individuals. In Forensic Science, STR typing is used to infer the number of repeats of DNA where today kits such as PowerPlex 21® and GlobalFiler® are used within forensic laboratories in Australia.

Trace DNA has become more established over the past couple of decades in the field of forensic science. It is defined as cells off the skin surface that have been carried onto another surface through physical contact, allowing detection of DNA on everyday objects.[3] Trace DNA was first explored in great depth by van Oorschot and Jones in 1997, where they found that they could obtain DNA profiles from swabbing objects that were regularly handled by a particular individual.[4] Previously approximately 1ng of DNA was needed to gain a full profile but now approximately only 100pg is needed.[5,6] Due to this increase in sensitivity of the technology, concepts such as secondary and tertiary transfer of DNA have now developed.

The secondary transfer of DNA is defined as the transfer of DNA from one person or object to another via an intermediate person or object, not involving a direct link between an individual and the target surface.[6-9] With continued research can result in an increase of

knowledge, where now concepts such as tertiary and quaternary transfer has arisen though secondary transfer is still being explored. Similar to secondary transfer of DNA, tertiary transfer involves the transfer of DNA from one person or object to another through two intermediary vectors.[10] Quaternary transfer requires an additional transfer events, resulting in a total of four transfers of DNA. All transfer events have been shown to occur in numerous published papers, but not consistently.[11-15]

Since its first use in the court systems in 1986, DNA has been a highly regarded item of evidence, something that has been very hard to argue against. As DNA becomes more understood, scientists are able to gain a greater insight into who has been present at a crime scene and increased sensitivity of kits allows forensic scientists to gain DNA profiles from the smallest of biological substances. Claims of the secondary transfer of DNA can now occur due to this increased knowledge of DNA and sensitivity of kits, meaning that now an individual may not directly deposit their DNA onto an object, but rather transferred through a vector. This is becoming increasingly common within the court system and a need for a concise guide to determine the possibility of secondary transfer has now emerged.

A proposed solution is through a meta-analysis, an analysis that pools results from multiple studies together to increase its strength and sample size, thus the power of the studies.[3,16] To carry out a correct meta-analysis, it should be done as a part of a systematic review and include features such as a comprehensive, reproducible search and transparent selection criteria.[16] There are two main types of reviews, systematic and narrative. Narrative reviews explore more broader topics and qualitatively summarise evidence, whereas systematic

reviews focus more deeply on primary studies who explore clinical questions.[16] There are two types of models that are used to for a meta-analysis, random effect or fixed effect models.[16] The fixed effect model assumes that the 'true' effects are fixed whereas a random effect model assumes that only a random sample is included.[16] The random effect model is more preferred for a meta-analysis as it better reflects reality as studies barely ever mimic each other.[16] This is therefore why a random effect model will be used for this meta-analysis.

The aim of this literature review was to produce a combined review into the secondary transfer of DNA in addition to drawing multiple studies together to attempt to provide an analysis of the literature. It is therefore hypothesized that through the exploration of literature and completion of a meta-analysis that an appropriate and concise guide will be produced to determine the chances of a secondary transfer event occurring and what conditions are required, to aid Biologists in expert testimony.

MATERIALS AND METHODS

DATABASE SEARCHES

A literature review was conducted in conjunction with the meta-analysis where resources were gathered using online databases into the secondary transfer of DNA. Databases were chosen based on their relevance to the field of Forensic Science. Chosen databases included Scopus, Web of Science Core Collection, ProQuest, Medline, ScienceDirect and Research Gate. These included both specific and general databases to ensure high coverage of all

published literature in the secondary transfer of DNA. Key terms used for searching the databases included the following:

- "Secondary transfer" AND "DNA" AND "Forensics";
- "DNA transfer events" AND "Forensics";
- "Tertiary transfer" AND "DNA" AND "Forensics";
- "Direct DNA transfer" AND "Forensics";
- "Shedder status" AND "Forensics" and
- "Indirect transfer" AND "DNA" AND "Forensics".

No additional search term restrictions were applied due to the specificity of the databases and the lack of function provided to do so. The only restriction that was applied to the database searches were those that eliminated specific types of resources, e.g. book chapters & encyclopedias. All databases already had restrictions applied to searches such as language and dates, where English was the language and dates ranged from the 1990's to April 2018.

SCREENING OF ARTICLES

A total of 307 articles were identified through the database search, compiled of all yielded search results. All articles then had their reference lists screened resulting in a further 103 eligible articles, creating a total of 410 articles identified through the literature search. The next step was to screen all 410 articles using their titles and abstracts, based on inclusion and exclusion criteria. The main exclusion criteria that was applied was if the article did not involve the transfer event of DNA in any form (e.g. touch DNA, spermatozoa, blood and saliva) it would be excluded from the meta-analysis. Other exclusion criteria included papers that involves a case study, those that are a response to a journal article and a form

of writing that was not a journal article or theses (e.g. book or a book chapter). From this screening process, a total of 141 articles were deemed suitable. These 141 articles further had the entire body of text screened, again using inclusion and exclusion criteria. If an article had a recorded or measured occurrence of the secondary transfer of DNA in any form, it was able to be encompassed in the meta-analysis. Articles that discovered an occurrence of the secondary transfer of DNA but indirectly (was not the main variable testing for) were also excluded. This screen resulted in a total of 38 articles that were able to be continued through to the meta-analysis, and 103 studies excluded.

DATA EXTRACTION

Data was extracted from all 38 articles discovered through the screening process and collated into a table using Microsoft Excel. This data included the following:

- Author;
- Year;
- Conditions of the experiment (Controlled, semi-controlled, uncontrolled);
- Number of Participants;
- Number of samples taken;
- Type of vector;
- Final object of the DNA deposition;
- Time between the primary and secondary transfer event;
- Time handling the vector;
- Time handling the final object;
- Type and pressure of contact of vector;

- Average total DNA recovered (ng);
- Total DNA concentration (ng/μL);
- Total number of unknown alleles detected (indicative of secondary transfer);
- Number of mixtures seen;
- If a reference sample of volunteers had been taken; and
- If secondary transfer of DNA in any form was detected.

RESULTS AND DISUCSSION

LITERATURE REVIEW

Regarding any transfer event of DNA, the way in which it occurs is relatively similar across the board. There must always be a primary transfer event involving the movement of DNA from an individual to either an object or another individual. There then must be a subsequent transfer event, where that same genetic profile will be transferred either to an individual or an object. In this case, it could be hypothesized that the more common scenario would be that of an individual as the vector. Below studies using both instances as vectors will be explored and reviewed to gain a greater understanding as to how this mechanism occurs and its likelihood.

OBJECT AS VECTOR

When the secondary transfer of DNA is put forth as a possible explanation, it is more sensible to have occurred through an object rather than an individual. One of the first study that performed secondary DNA transfer through an object was performed in 2012. The first

experiment saw hands come in contact with a toy or a singlet and then that object coming in contact with a laboratory coat.[17] Results showed that in 19 out of 20 repeats, DNA from the donor was observed.[17] A unique approach was used by French et al. where Ultraviolet (UV) powder was used through the transfer experiment. Three participants sat around a table drinking from a bottle with their own three glasses for 30 minutes where only one individual started with the UV powder.[18] There was no direct contact between individuals but the study showed the indirect transfer of the UV powder to all individuals.[18]

Goray and van Oorschot performed a very similar experiment to French et al. the following year where the same "uncontrolled" conditions were used. Here however they did not use UV powder but tested for the DNA itself. In 27% of the table samples and then 33% of the chair samples had a DNA profiles from participants that did not come into contact with the surface indicating that these profiles might have been transferred from the jug.[19] A further 58% of table samples and 42% of chair samples had DNA profiles from unknown individuals.[19]

Margiotta et al. used gloves as the vector for transfer where in half the cases tested for, they found a mixture of DNA containing alleles belonging to an unknown individual different to that of the volunteer.[20] In an additional 15% of the cases just alleles from another unknown individual were recovered not attributed to the volunteer.[20] Here secondary transfer has occurred, although authors did point out some of their negative controls from supposedly "clean" gloves did yield alleles.[20] Szkuta et al. furthered work of other studies that focused on the quantity of DNA that is left on vectors after a transfer event has occurred. Secondary transfer events were conducted between two "exhibits"

using tools such as forceps, scissors and gloves as the vector.[21] It was shown that secondary transfer could occur from exhibit to exhibit by DNA-free vectors but the main finding was the presence of a sufficient quantity of DNA on the vector for further transfer events.[21]

Fonneløp et al. investigated the transfer of DNA based on a case study, using three volunteers who were known to be good shedders.[12] The first individual handled an object for 30 seconds followed by a second individual.[12] Results showed for the first transfer event, 83% of the DNA was high enough quality for profile detection and for the second transfer event, that figure dropped to 53%.[12] In 2017, Buckingham et al. furthered a previous study where porous were used rather than non-porous surfaces to investigate the resulting yield and profile of DNA. Some results produced evidence of secondary and tertiary transfer of DNA although the proportion to the depositor's DNA was less than 10%.[22]

The use of a laundry machine for the transfer of spermatozoa is a relatively well covered topic. A study conducted by Kamphausen et al. in 2015 was one of the first testing for the transfer of touch DNA however the secondary transfer of touch DNA was deemed almost impossible for reliable STR analysis.[23] A second study conducted in 2018 saw unworn socks were washed in a normal load of household laundry.[15] 22% of samples yielded a result higher than the minimal threshold for casework (0.06ng/μL).[15] Four samples matched a profile of a female from the household indicative of the transfer of DNA occurring but unknown as to either direct or indirect transfer.[15]

Van Oorschot et al. was the first to test the extent to which DNA transfer may occur using fingerprint brushes.[24] 73 currently-used fingerprint brushed were brushed over 5 sheets of plastic, including one containing a fresh deposited handprint.[24] The results showed that very limited pick up and transfer of DNA occurred by the brushes however when changes were made to the DNA analysis, significant pickup and transfer of DNA was seen.[24] A second study was conducted by Proff et al. where they determined that DNA contamination with fingerprint brushes was quite common (85%) and for the secondary transfer, 25% of surfaces had DNA detected with one full profile.[25] As with the previous study, they also found a limited risk of the secondary transfer of DNA through fingerprint brushes.[24,25]

INDIVIDUAL AS VECTOR

This scenario appears to be the more common scenario to occur although is the least researched. The first study that used an individual as a vector for the transfer of DNA was conducted by Lowe et al. in 2003. The study found that when poor shedders were the vector, more often than not, the original individual had their full profile transferred.[26] Additionally, with a 30 second time delay between transfer events, mixed profiles were discovered where 70% were from the original source.[26] Farmen et al. additionally conducted a study where two individuals undertook a handshake followed by the gripping of beakers.[9] Although not specified, the authors suggest that all samples were mixtures containing both profiles of the known contributors, indicating that secondary DNA transfer had successfully occurred.[9]

A study conducted by Meakin et al in 2015 also showed the secondary transfer of DNA through a handshake and then handling a knife. The study found that three of the four sets

of knives, mixtures were found containing three different profiles.[27] Two profiles could be attributed to the two volunteers, but the other was an unknown profile, possibly the cause of secondary or tertiary transfer.[27] Cale et al. performed a standard study into the secondary transfer of DNA with a new kit with increased sensitivity due to a gap found in the literature. The authors aimed to study how the presence of secondary DNA transfer would affect the interpretation of DNA typing results.[6] Data obtained from five of the 24 samples suggest that individuals can have their DNA deposited on objects in sufficient quantities that they can be identified as the only or major contributor, suggesting that they had come in direct contact with the object.[6]

INDIRECT FINDINGS OF SECONDARY TRANSFER

There are also instances that occur in where primary transfer or the persistence of DNA that have been explored during a study although unknown alleles are detected. Here, these studies that have detected secondary transfer of DNA during another experiment have been collated. However, in these studies, unless the authors have specifically stated where the secondary transfer has occurred and who the unknown alleles belong to, we do not know the outcome. Additionally, unknown alleles attributed to background DNA present on a regularly-used object.

The first instance of accidental occurrence of secondary DNA transfer was during a study conducted by Lowe et al. in 2002 where the authors tested shedder status of individuals. Secondary transfer was detected when participants held a tube for 10 seconds but the extent was dependent on the subject pairings.[28] A few years later, Phipps et al. conducted a study in great similarity where they found that the occurrence of secondary transfer was

low and not likely but possible.[29] Only 14 alleles out of 581 could not be attributed to the volunteers equating to 0.02%.[29] A study conducted by Rutty found an instance of possible secondary DNA transfer occurring where male-female pairs were used in order to simulate a strangulation event.[30] Two swabs of the male's fingers (controls) yielded a profile of a female in the study although these two individuals had never been paired or come into contact with one another.[30]

Petricevic et al. used five volunteers who slept in their own bed but with a new lower bed sheet or individuals slept in a bed where they never had previously.[31] When individuals slept in their own beds, a second individual's DNA profile was found in at least one sample from 3 out of the 5 volunteers.[31] When individuals slept in an unknown bed, all unknown samples could be attributed to the regular user's profile which did not indicate secondary DNA transfer. Djuric et al. had volunteers hold plastic tubes and individual's ankles for 10 seconds.[32] When volunteers held tubes, out of the seven profiles obtains, one resulted in a partial profile not matching the volunteer's, indicating the occurrence of secondary transfer.[32] When ankles were used as deposition sites, no occurrence of the secondary transfer of DNA was noted.[32] Goray et al. conducted a very similar experiment again but tested the deposition onto glass plates rather than plastic tubes. A total of 40 samples were taken and non-self DNA (unexpected alleles) was seen in 79% of the samples.[33]

A paper published by Oldoni et al. applied the constant unknown from contact stains at break-ins to conducted an experiment. A total of 231 samples were created where approximately 2-10% contained unknown alleles, possible suggesting secondary transfer.[34] In 2015, Fonneløp showed that secondary transfer of DNA occurred through the presence

of the original user's DNA on the hands of the second user up to 8 days after receiving the computer equipment.[35] Lacerenza et al. directly swabbed individual's hand's where out of 120 samples, 56 had one or more unknown alleles and 36 of those could be classified as mixtures (64.3%).[37] In a study conducted by van den Berge et al., individuals dragged each other mimicking an activity-related scenario. Out of 26 samples, 3 of which did not result in over 7 alleles.[37] Donor alleles were present in 100% of the samples and non-donor in 71%.[36] More recently, Magee et al. used collars and cuffs of upper garments to determine the presence of DNA.[38] Out of 55 samples taken, non-wearer DNA was recovered, an average of 1.3ng from the collar and 2.7ng from the cuffs.[38]

SHEDDER STATUS

Shedder status is one of the more recent concepts under the umbrella of the transfer of DNA and one that will continue to grow as more research is conducted into the topic. The first study to directly carry out an experiment on individual's shedder status' was conducted by Lowe et al. in 2002 and is considered the backbone study to this topic. The authors got participants to grip a sterile tube, 15 minutes, 2 hours and 6 hour periods after hand-washing.[29] They determined that there were differences between individuals in regard to how many alleles that they deposited on a surface.[29] Good shedders were then classed if the individual left an entire profile after 15 minutes of hand-washing.[29] All other individuals were classed as poor shedders.

Phipps et al. conducted a second study that investigated shedder status and used almost identical experiments to that of Lowe et al. Unexpectedly, the study had shown that individuals do not produce consistent quantities of DNA over time, varying as much within

themselves than compared to other people.[30] Using the criteria laid out in Lowe et al., this study discovered no "good" shedders and all were classified as "poor" shedders suggesting this level of classification may not be all as simple as thought.[29,30] Farmen et al. measured individuals' shedder status just based on swabs from their hands.[39] It did not test the quantity of DNA that the individual's actually deposit on a surface, so it may not class as a true representation of shedder status. Out of the nine individuals who participated in the study, two individuals had a low DNA yield and seven individuals produced profiles that were able to be analysed.[39] More recently, Goray et al. collected 240 samples from handprints on glass plates where only four of the samples had no detectable quantities of DNA.[33] Two of the ten participants could be classed as good shedders but this was done under different criteria and experimental criteria as to the base study conducted by Lowe et al.[33]

OTHER FORMS OF DNA INVOLVED IN SECONDARY TRANSFER

One of the first studies carried out in the broader realm of the transfer of DNA was by Kafarowski et al. in 1996. Conducted before the first occurrence of the secondary transfer of touch DNA, it aimed to determine the likelihood of the transfer of spermatozoa between articles of clothing during a machine-cycle wash.[40] Although the retention of spermatozoa during machine wash had previously been explored, the transfer of it to other articles of clothing hadn't.[41,42] The authors used underpants as a means for the primary deposit of spermatozoa, washing them with three additional clean pairs and then machine dried.[40] In all three trials, trace quantities of spermatozoa were found where a minimum of one sperm head and maximum of eight per sample.[40]

Saliva is an additional biological substance that contains human DNA that has the possibility to be transferred through objects and individuals to cause a secondary transfer. The first that explored into this secondary transfer of saliva was conducted by Wiegand et al. where saliva was transferred onto either paper, cotton or plastic surfaces, left them to air dry then a thumb rubbed onto the stain.[43] The thumb was then placed onto another piece of paper and then the area was swabbed.[43] In total only 1 out of 96 gave complete profiles, all from plastic as the original source.[43] Vectors of pens and plastic tubes were used in an additional study. Their study concluded that secondary transfer of saliva did occur, in greater levels of retention than touch DNA and that moist surfaces may facilitate DNA transfer to a greater degree than a dry surface as previously discovered in Goray et al. 2010.[11,43]

In 2015, the transfer of saliva through laundering was studied where individuals deposited saliva onto textile cloths and then these were subsequently washed with clean textile cloth.[23] A total of 46 independent washes were completed both by hand and machine with/without detergent for saliva, blood and touch DNA.[23] Saliva was seen to undergo transfer events which supports other studies performed.[11,23,24,43,44] The most recent study conducted was using individual's hands as vectors for the transfer. 14 areas of the individuals palm were swabbed in addition to the glass plate which the saliva was transferred to.[45] The study yielded a number of unknown alleles on the final plate where the authors contributed this to prior interaction with individuals, supporting the finding that secondary transfer of DNA had occurred.[45]

Wiegand et al. conducted a study into the risk of the transfer of bloodstains from surfaces such as plastic, cotton and paper. The bloodstains were air dried then an individual placed

their thumb on the stain then onto another piece of paper.[43] Secondary transfer of DNA was found to occur on the cotton surface, both with and without gloves.[43] Lehmann et al. conducted a very similar experiment but all transfer events were conducted with substrates only and no human interaction. The experiment supported the existence of the secondary transfer of DNA through blood and additionally found that if the blood were wet, it would transfer further and would end in a higher concentration as more substance was transferred.[46]

Kamphausen et al. investigated the secondary transfer of DNA through laundering in a washing machine or by hand. Artificial bloodstains were created on textile cloth, dried and then washed with clean textile cloths.[23] Through 46 independent washes, transfer of blood cells was seen supporting previous studies.[23,43,46,47] A gap had then been identified in the literature, where none of the previous studies had thoroughly tested the effect of aridity of blood. van Oorschot et al. used drying time, temperature and humidity as dependent variables and cotton as the secondary substrate.[47] The secondary transfer of blood was seen to have occurred with significant differences in the transfer percentage of blood between 5 and 60 minutes.[47]

TERTIARY TRANSFER OF DNA AND BEYOND

Although the secondary transfer of DNA is still in the process of gaining a greater understanding of its mechanisms, through these studies the discovery of transfer events occurring after a second transfer have occurred. These studies are still in their very preliminary stages and like secondary DNA transfer, still require a greater understanding into the mechanisms surrounding the process. The first study that explored the occurrence

of the tertiary transfer of DNA was conducted by Warshauer et al. where an object was the original vector and was further transferred from an individual to another individual or object as the tertiary transfer event.[11] They were able to prove that tertiary transfer of DNA could occur, where 87.5% displayed less than half of the expected alleles.[11] These results correlated with those produced by Fonneløp et al. where after the final transfer step only 17% of samples were high quality that could be reported and go through database searching (5 out of 30 transfer events).[12]

In the study that was conducted by Fonneløp et al. they also tested the instance of quaternary transfer occurring. They found that once again, it occurred where a total of 17 out of the 108 analysed samples contain unknown alleles.[12] In 2016, Helmus et al. performed the first study solely testing the tertiary transfer of DNA, a gap identified within the literature. Out of 180 samples generated, only 72 had detectable DNA that identified that tertiary transfer had occurred (40%).[12] In a study completed the year earlier they had additionally shown the occurrence of the tertiary transfer of DNA but the authors noted that they were unable to distinguish between tertiary transfer or interference from a low-level of non-donor DNA.[13] In a more recent study, tertiary transfer of DNA evolved to a more complex mechanism than that of individual-object involved transfer. Six new unworn socks and a t-shirt were laundered together with no additional items in a machine that had been previously used.[15] Results showed that there were no DNA results recovered from quantity (above threshold) through to profiling, suggesting that tertiary transfer didn't occur.[15]

CASE STUDIES

In 2017, an article was published following the incidence of contamination by Police Officer's in Austria which without elimination databases, many instances of indirect or secondary transfer of DNA may go undetected.[48] During the period of 2000 to 2016, a total of 46,000 trace samples were submitted through DNA and out of those a total of 347 contamination incidents were detected through the use of the elimination database (0.75%) with a majority from indirect transfer.[48] The authors further went into detail regarding 3 separate incidents that they were able to pinpoint where the transfer events had occurred. The first incident occurred through shared camera equipment that had been used by a Police Officer completely unrelated to the case.[48] The second shows the indirect transfer by a Police Officer through a shared vehicle to a volume crime case and the third through packaging of evidence material on an unrelated Police Officer's desk.[48]

LIMITATIONS

Limitations of the conduction of this literature review included the level of control of studies, time between taking samples and the acquisition of reference samples. No studies presented in this literature review had tested for the secondary transfer of DNA under completely uncontrolled scenarios. The closest study to having a completely uncontrolled study was conducted by Goray & van Oorschot in 2013. They used a small group of individuals with a small number of objects (glasses, jugs, tables, chairs) participating in social interaction for 20 minutes.[19] Prior to the experiment occurring the authors did pre-clean all surfaces and items. As close as this study was to being uncontrolled, the occurrence of pre-cleaning diminishes all background DNA present on those items, not reflecting what were to occur in a "real-life" situation.

A common theme between studies saw samples from objects or individuals being taken almost immediately after the transfer event occurring. As stated in one of the studies, "the best chance of recovering the DNA would be to sample as soon as possible after the final transfer event has occurred".[27] This unfortunately is not possible in real-life situations as often a crime scene may not be discovered for hours or days after it has occurred. This same principle can be applied for the time between transfer events occurring. Lowe et al. had found when placing a 30 second delay between transfer events the final sample went from containing a full profile with all the first individuals' DNA present to only have 70% of that same individuals present.[28] Even with that 30 second time delay, the situation is still not indicative of "real-life" as individuals would not shake hands immediately prior to committing a crime. Based off these results, it could be said that unless these situations were to have occurred and the addition of some other variables it would be highly unlikely that DNA found at a crime scene would be anyone else's other than that individual carrying out a primary transfer.

Another variable which does not mimic "real-life" situations is the acquisition of reference samples of volunteers during studies. For interpreting any DNA profile, forensic scientists will most likely not have access to a reference sample for comparison purposes unless a known suspect had been previously profiled. Here it may be more difficult to pull out multiple contributors of a mixture without knowledge of the major contributor, or any other contributor. By using a reference sample of the volunteer during these studies, essentially most of the work forensic scientists conduct in regard to profile interpretation is void.

META-ANALYSIS

It was originally hypothesized that through the exploration of literature and completion of a meta-analysis that an appropriate and concise guide will be produced to determine the chances of a secondary transfer event occurring and what conditions are required, to aid Biologists in expert testimony. This hypothesis was rejected on the basis that the meta-analysis was found not be an adequate model for the data that was available. The screening process of articles exploring the secondary transfer of DNA was sufficient for this field of science, as articles were able to be easily included and excluded based on a set of criteria. Data extraction was additionally able to be carried out to some extent as most journal articles give accurate representations of the study and how it was performed. However, through the creation of a table, incorporating all of the included studies for the meta-analysis and the conduction of the meta-analysis, it was determined that the meta-analysis would not be adequate for the data that was available.

For this meta-analysis, it was hoped to use average amount of total DNA recovered (ng) as one of the quantitative variables. Articles however did not all give results that would be sufficient to calculate an average from. Some gave all values of total DNA recovered from the final objects after secondary DNA transfer, where an average was able to be calculated. Conversely, some articles would state a range of the total amount of DNA recovered which an average could not be calculated from as the raw results were not present. Therefore, there was not a consistent set of values that would be used to represent the total amount of DNA recovered through all included studies.

As a second quantitative variable was required, out of the data available to extract, time handling the vector, final object or time between handling the vector and final object, were the only two options available. Although this value differed between studies, within a study it was highly likely that only a single time value was utilized. For example, Goray et al. used one minute for the time handling the vector and the final object for all volunteers.[17] Two different vector types were used within the study with two different times between handling the vector and final object (immediately and 24 hours after).[17] Both touch DNA and blood was used as the biological substances, creating four possible scenarios.[17] As these quantitative variables did not vary substantially within the study, it would not be possible to compare the variables within the study. Other studies additionally only had one vector type and biological substance, and therefore only one quantitative value of time handling vectors or objects.[23,28,29,27,30]

Reflecting back onto the hypothesis, it was stated that it was anticipated that "an appropriate and concise guide will be produced to determine the chances of a secondary transfer event occurring and what conditions are required". As mentioned previously, the framework that a meta-analysis works on is the comparison of two quantitative variables in the hope to perform statistical tests such as Cohen's D or Pearson's coefficient. In order to determine the conditions required for the secondary transfer of DNA to occur, quantitative values would need to be gathered. The condition that secondary DNA transfer are conducted under would include things such as the vector type, final object type, conditions of the experiment, background DNA present and type of pressure and contact of the vector or final object. These variables unfortunately are qualitative and do not have an option to measure them quantitatively. In that case, performance of comparisons

between these variables and quantitative variables such as total DNA, DNA concentration or time handling objects cannot be carried out.

Other limitations of the meta-analysis included the new application to the field of forensic science, types of results published and the source of the DNA. As mentioned previously, meta-analyses are most commonly used within the medical field and has not yet been used within the field of Forensic Science. Therefore, it was not known if the application of this method could be applied the same way that is has done previously in the medical field. As discovered through this paper, it was deemed an inappropriate analysis for the type of studies that are published on the secondary transfer of DNA. This is mainly due to the type of results that are published. Results were not published in a form that data extraction could occur efficiently, additionally making it difficult to obtain two quantitative variables for comparison. Close to none of the 38 studies published in the secondary transfer of DNA and included in the meta-analysis, published their raw results of variables such as total amount of DNA recovered. Due to this, averages, means or standard deviations were not able to be computed for results included in these studies, and therefore a meta-analysis was not able to be carried out.

It was also difficult to pinpoint as to where the DNA had come from, as the presence of DNA on an object or individual could either be a result of secondary DNA transfer or background DNA. In this case, only studies who can specifically tested for the secondary transfer of DNA could be included in the meta-analysis, as it was certain that the non-donor DNA had come from that specific event. However, this than decreased the experiment's

applicability to the real world, where now background DNA was not present, possibly causing an unrealistic result.

CONCLUSION

At this stage, it can be concluded that the hypothesis, through the exploration of literature and completion of a meta-analysis that an appropriate and concise guide will be produced to determine the chances of a secondary transfer event occurring and what conditions are required, to aid Biologists in expert testimony, can be rejected. Data published in the 38 studies included for the meta-analysis was not adequate enough to perform quantitative comparisons between variables in order to combine results to strengthen the power of each study. In future, it should be considered that articles published in the field of Forensic Science display their results in different ways or give access to readers of their raw results for further interpretation purposes.

REFERENCES

1. Panneerchelvam S, Norazmi MN. Forensic DNA Profiling and Database. The Malaysian Journal of Medical Sciences. 2003;10(2):20-26.
2. Fan H, Chu J-Y. A Brief Review of Short Tandem Repeat Mutation. Genomics, Proteomics and Bioinformatics. 2007;5(1):7-14.
3. Bright J, Petricevic SF. Recovery of trace DNA and its application to DNA profiling of shoe insoles. Forensic Science International. 2004;145:7-12.
4. van Oorschot RAH, Jones MK. DNA fingerprints from fingerprints. Nature. 1997;387:767.
5. Wickenheiser RA. Trace DNA: A Review, Discussion of Theory and Applications of the Transfer of Trace Quantities of DNA Through Skin Contact. Journal of Forensic Science. 2002;47(3):442-450.
6. Cale CM, Earll ME, Latham KE, Bush GL. Could Secondary DNA Transfer Falsely Place Someone at the Scene of a Crime? Journal of Forensic Science. 2016;61(1):196-203.
7. Ladd C, Adamowicz MS, Bourke MT, Scherczinger CA, Lee HC. A Systematic Analysis of Secondary DNA Transfer. Journal of Forensic Science. 1999;44(6):1270-1272.
8. Meakin GE, Butcher EV, van Oorschot RAH, Morgan RM. Trace DNA evidence dynamics: An investigation into the deposition and persistence of directly- and indirectly-transferred DNA on regularly-used knives. Forensic Science International: Genetics. 2017;29:38-47.
9. Farmen RK, Jaghø R, Cortez P, Frøyland ES. Assessment of individual shedder status and implication for secondary DNA transfer. Forensic Science International: Genetics Supplement Series. 2008;1:415-417.
10. Meakin G, Jamieson A. DNA transfer: A review and implications for casework. Forensic Science International: Genetics. 2013;7:434-443.
11. Warshauer DH, Marshall P, Kelley S, King J, Budowle B. An evaluation of the transfer of saliva-derived DNA. International Journal of Legal Medicine. 2012. doi: 10.1007/s00414-012-0743-1.
12. Fonneløp AE, Egeland T, Gill P. Secondary and subsequent DNA transfer during criminal investigation. Forensic Science International: Genetics. 2015;17:155-162.

13. Davies C, Thomson J, Kennedy F. Assessing primary, secondary and tertiary DNA transfer using Promega ESI-17 Fast PCR chemistry. Forensic Science International: Genetics Supplement Series. 2015;5:e55-e57.
14. Helmus J, Bajanowski T, Poetsch M. DNA transfer – a never ending story. A study on scenarios involving a second person as carrier. International Journal of Legal Medicine. 2016;130:121-125.
15. Voskoboinik L, Amiel M, Reshef A, Gafny R, Barash M. Laundry in a washing machine as a mediator of secondary and tertiary DNA transfer. International Journal of Legal Medicine. 2018;132:373-378.
16. Nordmann AJ, Kasenda B, Briel M. Meta-analyses: what they can and cannot do. Swiss Medical Weekly. 2012;142. doi:10.4414/smw.2012.13518.
17. Goray M, Mitchell JR, van Oorschot RAH. Evaluation of Multiple Transfer of DNA using mock case scenarios. Legal Medicine. 2012;14:40-46.
18. French JC, Morgan RM, Baxendall P, Bull PA. Multiple transfers of particulates and their dissemination within contact networks. Science and Justice. 2012;52:33-41.
19. Goray M, van Oorschot RAH. DNA transfer during social interactions. Forensic Science International: Genetic Supplement Series. 2013;4:e101-e102.
20. Margiotta G, Tasselli G, Tommolini F, Lancia M, Massetti S, Carnevali E. Risk of dna transfer by gloves in forensic casework. Forensic Science International: Genetic Supplement Series. 2015;3:e527-e529.
21. Szkuta B, Harvey ML, Ballantyne KN, van Oorschot RAH. Residual DNA on examination tools following use. Forensic Science International: Genetic Supplement Series. 2015;5:e495-e497.
22. Buckingham AK, Harvey ML, van Oorschot RAH. Transfer of picked-up DNA to cotton plates. Forensic Science International: Genetic Supplement Series. 2017;6:e6-e8.
23. Kamphausen T, Fandel SB, Gutmann JS, Bajanowski T, Poetsch M. Everything clean? Transfer of DNA traces between textiles in the washtub. International Journal of Legal Medicine. 2015;129:709-714.
24. van Oorschot RAH, Treadwell S, Beaurepaire J, Holding NL, Mitchell RJ. Beware of the Possibility of Fingerprinting Techniques Transferring DNA. Journal of Forensic Science. 2005;50(6):1-6.

25. Proff C, Schmitt C, Schneider PM, Foerster G, Rothschild MA. Experiments on the DNA contamination risk via latent fingerprint brushes. International Congress Series. 2006;1288:601-603.
26. Lowe A, Murray C, Richardson P, Wivell R, Gill P, Tully G, Whitaker J. Use of low copy number DNA in forensic inference. International Congress Series. 2003;1239:799-801.
27. Meakin GE, Butcher EV, van Oorschot RAH, Morgan RM. The deposition and persistence of indirectly-transferred DNA on regularly-used knives. Forensic Science International: Genetic Supplement Series. 2015;5:e498-e500.
28. Lowe A, Murray C, Whitaker J, Tully G, Gill P. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. Forensic Science International. 2002;129:25-44.
29. Phipps M, Petricevic S. The tendency of individuals to transfer DNA to handled items. Forensic Science International. 2007;168:162-168.
30. Rutty GN. An investigation into the transference and survivability of human DNA following simulated manual strangulation with consideration of the problem of third party contamination. International Journal of Legal Medicine. 2002;116:170-173.
31. Petricevic SF, Bright J, Cockerton SL. DNA profiling of trace DNA recovered from bedding. Forensic Science International. 2006;159:21-26.
32. Djuric M, Varljen T, Stanojevic A, Stojkovic O. DNA typing from handled items. Forensic Science International: Genetic Supplement Series. 2008;1:411-412.
33. Goray M, Fowler S, Szkuta B, van Oorschot RAH. Shedder status – An analysis of self and non-self DNA in multiple handprints deposited by the same individuals over time. Forensic Science International: Genetics. 2016;23:190-196.
34. Oldoni F, Castella V, Hall D. Exploring the relative DNA contribution of first and second object's users on mock touch DNA mixtures. Forensic Science International: Genetic Supplement Series. 2015;5:e300-e301.
35. Fonneløp AE, Johannessen H, Gill P. Persistence and secondary transfer of DNA from previous users of equipment. Forensic Science International: Genetic Supplement Series. 2015;5:e191-e192.
36. Lacerenza D, Aneli S, Omedei M, Gino S, Pasino S, Berchialla P, Robino C. A molecular exploration of human DNA/RNA co-extracted from the palmar surface of the hands and fingers. Forensic Science International: Genetics. 2016;22:44-53.

37. van den Berge M, van de Merwe L, Sijen T. DNA transfer and cell type inference to assist activity level reporting: Post-activity background samples as a control in dragging scenario. Forensic Science International: Genetic Supplement Series. 2017;6:e591-e592.
38. Magee AM, Breathnach M, Doak S, Thornton F, Noone C, McKenna LG. Wearer and non-wearer DNA on the collars and cuffs of upper garments of worn clothing. Forensic Science International: Genetics. 2018;34:152-161.
39. Farmen RK, Jaghø R, Cortez P, Frøyland ES. Assessment of individual shedder status and implication for secondary DNA transfer. Forensic Science International: Genetic Supplement Series. 2008;1:415-417.
40. Kafarowski E, Lyon AM, Sloan MM. The retention and transfer of spermatozoa in clothing by machine washing. The Canadian Society of Forensic Science Journal. 1996;29(1):7-11.
41. Brayley-Morris H, Sorrell A, Revoir AP, Meakin GE, Court DS, Morgan RM. Persistence of DNA from laundered semen stains: Implications for child sex trafficking cases. Forensic Science International: Genetics. 2015;19:165-171.
42. Farmen RK, Cortez P, Frøyland ES. Spermatozoa recovered on laundered clothing. Forensic Science International: Genetics Supplement Series. 2008;1(1):418-420.
43. Wiegand P, Heimbold C, Kelin R, Immel U, Stiller D, Klintschar M. Transfer of biological stains from different surfaces. International Journal of Legal Medicine. 2011;125:727-731.
44. Goray M, Eken E, Mitchell RJ, van Oorschot RAH. Secondary DNA transfer of biological substances under varying test conditions. Forensic Science International: Genetics. 2010;4:62-67.
45. McColl DJ, Harvey ML, van Oorschot RAH. DNA transfer by different parts of a hand. Forensic Science International: Genetic Supplement Series. 2017;6:e29-e31.
46. Lehmann VJ, Mitchell RJ, Ballantyne KN, van Oorschot RAH. Following the transfer of DNA: How far can it go? Forensic Science International: Genetic Supplement Series. 2013;4:e53-54.
47. van Oorschot RAH, McArdle R, Goodwin WH, Ballantyne KN. DNA transfer: The role of temperature and drying time. Legal Medicine. 2014;16:161-163.

48. Pickhrahn I, Kriendl G, Muller E, Dunkelmann B, Zahrer W, Cemper-Kiesslich J, Neuhuber F. Contaminations incidents in the pre-analytical phase of forensic DNA analysis in Austria – Statistics of 17 years. Forensic Science International: Genetics. 2017;31:12-18.