THE PRESENCE OF ENVIRONMENTAL HUMAN DNA WITHIN FORENSIC VEHICLES

By

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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

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Semester 2, 2016

Declaration

I declare that this thesis 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 reference 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:

Acknowledgments

Many thanks to Brendan Chapman for supervising this project and helping with the lab work and write-ups along the way. Thank you also to James Speers for coordinating the unit and giving us an opportunity to work with the various external institutes. Lastly, I am grateful to Gavin and PathWest for the gift of the Quantifiler and PowerPlex 21 System for use in this project.

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Part One

Literature Review

The Presence of Environmental Human DNA Within Forensic Vehicles

Abstract

DNA profiling is an extremely useful tool in criminal investigations for the identifications of persons of interest or victims. Although initially a tedious process, it had developed to become rapid and automatable with the implementation of PCR and STR kits. STR kits have also become increasingly sensitive, which is useful for the analysis of trace DNA but increases the possibility of detecting low levels of contaminant DNA. This could result in the generation of mixed and/or unrelated profiles which would be difficult to interpret and could negatively affect the investigation outcome. DNA deposition is said to be dependent upon an individual's shedding ability, although several authors had suggested that there are multiple other contributing factors such as diseases, increased sweating, and secondary transfer of DNA. Secondary transfer occurs when DNA is deposited onto a surface indirectly via vectors. The amount of DNA transferred is shown to be influenced by various characteristics such as the type, porosity, and manner and frequency of contact between the DNA-containing material, the vector(s) and the deposition surface. This literature review aims to assess the potential for contamination of evidence samples by background DNA. DNA contamination could have disastrous impacts on investigations, so it is imperative that measures are taken to ensure that cleaning regimes and standard operating procedures are followed to minimise DNA contamination. A new forensic standard ISO18385 was also produced to reduce the risk of manufacturer-related contamination in consumables and reagents used for DNA analysis.

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List of Abbreviations

bp	Base pairs
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
STR	Short tandem repeat

Introduction

DNA profiling is an invaluable tool that has revolutionised criminal investigations due to its ability to discriminate between individuals to identify persons of interest or disaster victims. First developed in 1984 by Dr Alec Jeffreys (McKie 2009), the technique has evolved from a process that required weeks to one that could be completed in a few days at most. This was largely due to: 1) the shift from the use of minisatellites to short tandem repeats (STRs) as genetic markers, 2) the use of the polymerase chain reaction (PCR), and 3) the manufacture of STR kits. This change was necessary for forensic casework, as the rapid reaction times, high-volume sample processing, and increased sensitivity resulted in more efficient and effective analysis of evidentiary samples (Butler 2010; Primorac and Schanfield 2014). In addition, STR kits had also been evolving, with each one targeting a greater number of loci and being more sensitive than the previous ones (Cotton et al. 2000; Evans 1996).

However, this ability to detect increasingly lower levels of DNA meant that trace amounts of environmental DNA could also be detected, which would then be considered as contamination. Contamination of exhibits results in the generation of mixed profiles or profiles that are unrelated to the crime being investigated (Fonneløp et al. 2016), which can be difficult to interpret and thus hinder investigations.

It has been argued that the amount of background DNA present is dependent on an individual's tendency to shed DNA, with some individuals being better shedders than others. Yet, the evidence for the validity of shedding status is conflicting, with some researchers observing consistent amounts of shedding (Lowe et al. 2002; van Oorschot et al. 2003; Goray et al. 2016) while others noted that that the amount of DNA shed by an

individual fluctuates (Phipps and Petricevic 2007; Bright and Petricevic 2004). Nevertheless, most studies suggested that other factors also likely affected shedding, such as medical, physiological or psychological conditions, physical activities and/or behavioural habits (Kamphausen et al. 2012; Kwok, Gralton, and McLaws 2015; Quinones and Daniel 2012).

Secondary transfer of DNA occurs when DNA are transferred to items of interest indirectly via vectors. Studies have shown that properties such as the type (Szkuta et al. 2015), porosity (Goray et al. 2010; Goray, van Oorschot, and Mitchell 2012; Wickenheiser 2002; Fonneløp, Egeland, and Gill 2015), moisture content (van Oorschot et al. 2003; Goray et al. 2010), the number of transfers (Poy and van Oorschot 2006a; 2006b) and the manner (Goray, Mitchell, and van Oorschot 2010) and frequency of contact (Poy and van Oorschot 2006b) between surfaces could affect the amount of DNA that is transferred. However, these studies were conducted in ideal laboratory conditions which vastly differ from crime scene settings, and might therefore produce overestimated results. Even so, secondary transfer has been proven to exist and could be source of contamination, as demonstrated in the Farah Jama case (discussed further in subsequent sections), which stresses the importance of minimising and containing contamination within a forensic environment.

The purpose of this literature review is to discuss some of the above points mentioned and provide insight as to the forensic value of DNA, the importance of determining DNA levels in the environment and its potential for contamination of crime scene evidence.

History of DNA Profiling

The DNA fingerprinting technique was first developed in 1984 by Dr Alec Jeffreys when he discovered that variations in the DNA between individuals could be visualised (Butler 2010). These variations were from regions of DNA that contained repetitive sequences, known as variable number of tandem repeats (VNTRs), whereby the number of repeated units differed from individual to individual.

Jeffreys' technique utilised the analysis of minisatellites, a type of VNTR with a core sequence of typically 10 to 60 base pairs. These units may be repeated two to several hundred times at each locus, differing between individuals. The process, known as restriction fragment length polymorphism (RFLP), involved cleaving DNA with restriction enzymes to obtain DNA fragments which were then separated by size using gel electrophoresis, and subsequently transferred onto a nitrocellulose or nylon membrane via Southern blotting. Finally, a radioactive or chemiluminescent hybridisation probe containing the target repeat motif sequence would be used to simultaneously detect multiple minisatellite loci. This could then be visualised on X-ray films (Jeffreys, Wilson, and Thein 1985a; 1985b; Committee on DNA Technology in Forensic Science 1996).

In 1986, DNA profiling was first used in an investigation of the rape and murder of two women in Leicestershire, England to convict Colin Pitchfork, and exonerate Richard Buckland who had confessed to one of the murders and was charged with it. Investigators had wanted to prove that Buckland had committed both crimes, but the analysis result

showed that his DNA did not match that of the semen sample from the victims. Pitchfork was only caught after it was reported that a friend had provided a DNA sample in his place, and his DNA was found to be a match (Evans 1996; McKie 2009; Butler 2010). Without DNA profiling, an innocent man would likely have been wrongly incarcerated for a crime.

The Evolution of STR Kits

Since then, DNA profiling has been widely applied in criminal investigations, as well as paternity and genealogy testing. Coupled with the incorporation of the PCR, discovered in 1983 by Kary Mullis (Mullis 2003), and the discovery STRs, DNA profiling has evolved to become much more efficient and sensitive. STRs, also known as microsatellites, have much shorter repeat units of about 2 to 10 base pairs; although the majority of markers used in forensic investigations are tetranucleotide repeats of 4 base pairs (Goodwin, Linacre, and Hadi 2011). By the late 1990s to early 2000s, various multiplex STR kits had become commercially available and most laboratories had converted to employing STR amplification using PCR for DNA analyses in forensic casework (Butler 2010).

While RFLPs vary greatly between individuals which allow for discrimination, the method was tedious and time-consuming, requiring days to weeks, as shown in Table 1 (Butler 2010). Multiplexed STRs on the other hand, although slightly less discriminatory, could be automated and was much more rapid. Furthermore, while RFLP analysis required intact double-stranded high-molecular-weight (>12 kb) DNA of 20-500 ng, PCR analysis enabled the use of degraded DNA in much smaller amounts of approximately 0.1-1 ng (Butler 2010; Primorac and Schanfield 2014). The ability to amplify degraded DNA is crucial as

most samples recovered from casework are usually highly degraded. Interpretation of STR results was also much easier as it analysed the number of repeating units, whereas RFLP analysed the lengths of the fragment sequences.

Table 1. Comparison of RFLP vs PCR-based Methods.

Characteristic	RFLP Methods	PCR Methods
Time required to obtain result	6-8 weeks with radioactive probes; ~1 week with chemiluminescent probes	1-2 days
Amount of DNA needed	20-500ng	0.1-1ng
Condition of DNA needed	High-molecular-weight, intact DNA	May be highly degraded
Capable of handling sample mixtures	Yes (single-locus probes)	Yes
Allele identification	Binning required since distribution of sizes are observed	Discrete alleles obtained
Form used in analysis	DNA must be double stranded for restriction enzymes to work	DNA can be either single stranded or double stranded
Power of discrimination	~1 in 1 billion with 6 loci	~1 in 1 billion with 8 to 13 loci (requires more loci)
Automatable and capable of high-volume sample processing	No	Yes

Adapted from (Butler 2010)

STR kits have also been evolving, starting with the British Home Office Quadruplex (Forensic Science Service, UK) which could only analyse 4 loci (Butler 2010), to AMPF/STR® SGM Plus™ (Applied Biosystems, Foster City, CA) which analysed 11 loci and could detect DNA at low levels of 1 ng (Cotton et al. 2000). Today, the kit used by Australian forensic

investigators is the PowerPlex® 21 (Promega, Madison, WI), which analyses 21 loci and is able to detect DNA in as minute amounts as 50 pg (Ensenberger et al. 2014).

Background/Trace DNA

Although the increase in DNA sensitivity has been extremely useful in forensic investigations where DNA is likely present in trace amounts, it also increases the possibility of detecting contaminating background DNA that would not have otherwise been detected with older techniques (Fonneløp, Egeland, and Gill 2015). Trace DNA generally refers to either invisible or very limited samples, or minute amounts of DNA of less than 100 (Gill 2001) to 200 pg (Budowle, Eisenberg, and van Daal 2009), which would be detectable by STR kits such as the PowerPlex® 21.

Environmental DNA may originate from laboratory equipment, consumables, staffs, manufacturers, or other exhibits that were previously examined in the laboratory. While the presence of environmental DNA in a laboratory setting is deemed to be low (Poy and van Oorschot 2006b), there is still a potential risk of DNA transfer and contamination to evidentiary samples (Szkuta et al. 2015). This then poses the issue of the generation of mixed profiles or profiles that are unrelated to the crime being investigated (Fonneløp et al. 2016).

A study conducted by Toothman et al. (2008) investigated whether the amount of human DNA present in indoor dust samples from 24 different locations were sufficient enough for amplification. They found that human DNA could be detected 97% of the time at an

average amount of 0.2-1.1 pg/cm2. Although degradation was evident, they concluded that the level of background DNA present could still be a significant source of contamination during DNA examination.

Factors Affecting DNA Deposition

Over the last two decades, several studies had been conducted to investigate the tendency of individuals to deposit DNA through contact with a surface. However, the results obtained were conflicting; some researchers discovered differences in the amount of DNA deposited between individuals from touch (Lowe et al. 2002; Bright and Petricevic 2004; Goray et al. 2016), while others reported that no significant differences were observed (Phipps and Petricevic 2007; van Oorschot et al. 2003).

Lowe et al. (2002) defined individuals who deposited a full DNA profile 15 minutes after hand washing as “good shedders”, while those who left only a partial profile as “poor shedders”. Their study revealed that poor shedders only started depositing a full DNA profile at 6 or more hours after hand washing. A recent study by Goray et al. (2016) showed that several individuals were found to consistently produce either greater or lower DNA deposits, which further demonstrates the ability of classifying people as good or poor shedders. However, the sample sizes used in these studies were small and therefore, may be skewed and inapplicable to the general population.

On the other hand, a study conducted by Phipps and Petricevic (2007) following similar test conditions as in Lowe et al. (2002), revealed that the amount of DNA shed by the same individual was not consistent over time. Individual shedding status was found to

vary from day to day and from hand to hand, implying that one could be either a good or bad shedder depending on situation. They also suggested a correlation between the shedding status of dominant and non-dominant hands, and the cleanliness of the hands (duration after hand washing). More DNA was deposited by the non-dominant hand when hands were recently washed, whereas the dominant hand tended to deposit more DNA when the hands were dirtier. In contrast, a study done by (van Oorschot et al. 2003) indicated no significant differences between the left and right hands of an individual; rather, there was greater variation between individuals (inter-individual) than between the left and right hands of an individual (intra-individual).

To date, no consensus has been reached regarding shedding status categorisation. These conflicting results appear to emphasise the unpredictability of shedding and question the validity of such categorisation which, as suggested by Phipps and Petricevic (2007), might be too simplistic as it ignores the possibility of other factors that could also influence the deposition of DNA.

One such contributing factor, as investigated by Kamphausen et al. (2012), could be skin diseases such as atopic dermatitis, psoriasis and dermal ulcers, which cause an increase in the proliferation rate of the skin. Their study suggested that such skin conditions result in increased number of scaled cells and also cells containing less degraded DNA, thus increased DNA shedding. The results showed that healthy individuals were usually bad shedders since healthy skin did not regularly leave enough DNA to generate a full profile. This is further confirmed by the low number of full profiles obtained from patients with dermal ulcers localised at the lower legs, and significantly greater profiles obtained from patients with atopic dermatitis and psoriasis localised on the hands.

Another possible factor could be the presence of circulating (cell free) nucleic acids (CNA) in sweat or sebaceous fluids (Quinones and Daniel 2012). Physical activity, or physiological or psychological states would influence the amount of sweat secreted and therefore, the amount of DNA that is eventually deposited (Goray et al. 2016). Although the mechanism for diffusion of CNA into sweat is currently unknown, it is believed to be similar to the excretion of metabolites in sweat through sweat ducts.

Lastly, individual behavioural traits, such as frequent face touching, resulting in the transfer of DNA from other parts of the body (such as saliva) to the hand, had also been suggested as a possible factor affecting DNA deposition (Kwok, Gralton, and McLaws 2015). Thus, there has been increasing evidence to imply that the shedder status of an individual is influenced by a multitude of factors, of which further studies would be required to determine the factors.

DNA Contamination

Contamination may appear as major or minor contributors in generated profiles, or completely flood the target DNA all together; any DNA contribution that is not directly associated with the crime being investigated could be considered as contaminants (Gill 2001). In this case, contamination could arise from any time before a crime was committed to the time when swab samples are taken (van Oorschot, Ballantyne, and Mitchell 2010).

Thus, it is imperative that measures are taken to minimise contamination for exhibits undergoing DNA analysis. This would include ensuring that all personnel who come into

contact with the exhibits are wearing the correct protective equipment, operating procedures are adhered to, and that laboratories follow a strict and effective cleaning protocol (Szkuta et al. 2015). Laboratories should also have dedicated DNA-free rooms or facilities for DNA analysis purposes (Henry, McGowan, and Brown 2015).

However, even with cleaning procedures in place, DNA had been found on items that may have been overlooked or deemed low risk to contamination, such as glove boxes, door handles, exhibit dying rails or stationery (Henry, McGowan, and Brown 2015) in the laboratories which may have come into contact with gloved or ungloved hands in between examination of evidentiary samples (Poy and van Oorschot 2006b). Some items may not have come into direct contact with the examined exhibit itself, but still contain DNA due to secondary transfer, usually via gloves worn by the examiner (Fonneløp, Egeland, and Gill 2015; Poy and van Oorschot 2006a). DNA had also been discovered on gloves from unopened boxes, therefore highlighting the importance of using certified DNA-free consumables whenever possible for DNA analysis (Daniel and van Oorschot 2011). In a survey conducted by Henry, McGowan and Brown (2015) of environmental DNA levels in South Australian police facilities, cameras were found to pose the highest risk of DNA contamination, yielding highly mixed DNA profiles in considerable quantities. This could be due to cameras being overlooked as a source of contamination and/or also because they are difficult to clean and decontaminate of DNA.

A well-publicised case of DNA contamination dubbed the “Phantom of Heilbronn”, or the “Woman Without a Face” (Paterson 2009; BBC News 2009; Himmelreich 2009) details the recovery of a woman’s DNA from 40 crime scenes in Germany over a span of 15 years, ranging from common burglaries to homicides. However, suspects who had been

convicted of committing some of those crimes denied the existence of a female accomplice. It was only when her DNA was recovered from a male asylum seeker, but disappeared with a second attempt, that investigators started suspecting contamination of the swabs used for DNA collection. It was later determined that the swabs had been contaminated with the DNA of a woman working in a cotton swab factory in Bavaria. Although the swabs were sterilised before use, they were not certified for DNA use, and cells might still survive the sterilisation process. Thus, this demonstrates the necessity of using only consumables that have been certified for DNA analysis.

In 2016, ISO 18385 was established as the first forensic standard intended to minimise the risk of human DNA contamination during the manufacturing process of products used for forensic DNA purposes. The consumables and reagents covered by the ISO include those used in the collection (sampling kits), storage (such as swabs, containers, and packaging), and analysis (such as tubes and other plastic ware, disposable laboratory coats, gloves) of evidentiary DNA samples (ISO18385:2016). With this new standard in place, there would be less risk of manufacturer-related DNA contamination as occurred in the Heilbronn case discussed above.

However, even if extraneous DNA was picked up during exhibit examination, it usually only becomes significant to the investigation if it is of a detectable amount and is approximately 10% or more of the total amount of DNA extracted from the sample (Henry, McGowan, and Brown 2015).

Secondary/Tertiary Transfer

Apart from manufacturer-related issues, secondary or tertiary transfer of DNA could also be a source of contamination, whereby DNA is indirectly deposited onto a surface via a vector. Although crime scene samples usually arise from direct transfer, secondary transfer have been shown to occur. Factors that possibly contribute to the risk of secondary transfer of DNA include, but are not limited to:

- the type, and nature of the substrates involved (Szkuta et al. 2015)
- moisture content of the DNA-containing sample (van Oorschot et al. 2003; Goray et al. 2010)
- the number of transfer steps between DNA picked up from primary source to transference onto final deposition surface (Poy and van Oorschot 2006a; 2006b)
- the manner of contact between surfaces (Goray, Mitchell, and van Oorschot 2010)
- the frequency of contact between surfaces (Poy and van Oorschot 2006b)

Several studies have also established that more DNA would be picked up by porous substrates than non-porous substrates, but that less DNA would then be subsequently transferred and deposited from these porous surfaces than from non-porous surfaces (Goray et al. 2010; Goray, van Oorschot, and Mitchell 2012). Epithelial cells have also been observed to adhere more readily to porous than non-porous surfaces (Wickenheiser 2002). A recent study conducted by Fonneløp, Egeland and Gill (2015) found metal to be an excellent vector for secondary transfer; the greatest amount of DNA was recovered from gloves that had come into contact with these metal surfaces as compared to wood

or plastic. This is possible since DNA would adhere more readily to fibrous and/or absorbent substrates and is therefore less easily transferred subsequently onto secondary substrates.

However, all these studies were conducted in idealised experimental conditions whereby the area for transfer was small and predetermined, which is different from casework settings where trace DNA are swabbed for "blindly" in the hopes of obtaining a profile. Moreover, the duration for DNA deposition and the manner of contact (i.e. passive, friction or pressure) would be highly varied and less exaggerated in an actual scenario. For instance, in the experiment by Goray et al. (2010), different types of contact were applied for 60s to each sample, but it is impossible to know which type or combination of contact was used and the duration that it was applied for in a real situation. Therefore, secondary transfer might not be as relevant in a forensic investigation as implied by these tests, which was noted by some authors who found little evidence for its existence (Ladd et al. 1999).

On the other hand, secondary transfer has been proven to occur as seen in the case of Mr Farah Abdulkadir Jama, who had been wrongly convicted of rape and imprisoned for about 15 months (Hagan 2009). It was eventually discovered that the lack of effective DNA decontamination procedures had resulted in contamination of the swab used for the victim. The day before the victim had her swab taken, Mr Jama had also had a DNA sample taken in the same hospital by the same officer for an unrelated investigation. Mr Jama was acquitted and it was concluded that almost certainly no rape had actually occurred. This incident illustrates that contamination of exhibits could result in serious

miscarriages of justice and that although rare, it is possible for secondary transfer to occur. Therefore, precautions should be taken to ensure that all contamination is minimised. In the report by Hon Frank Vincent (2010), he found that the level of cleaning of the examination rooms were ineffective against the removal of DNA and prevention of its spread. He also proposed the adoption of packaged examination kits over the pre-stocked examination trolley to reduce the possibility of contamination from tools and consumables.

Another precaution that should be taken is to frequently change gloves before and after handling of items so as to prevent transfer of DNA via the gloves (Fonneløp, Egeland, and Gill 2015). Disposable gloves have been shown to be efficient transfer vectors of DNA (Fonneløp, Egeland, and Gill 2015; Szkuta et al. 2015), whereby contaminating DNA may be introduced to the gloves or the glove boxes which could then subsequently contaminate exhibits being examined (Vogelsberg et al. 2016).

Conclusion

The development of DNA profiling had certainly been a breakthrough in both the scientific and investigation communities. Without the ability to distinguish between individuals and identify persons, many crimes would likely have taken much longer to solve, or have remained unsolved. With such a tool, a great number of miscarriages of justice would likely have been prevented in situations where an innocent suspect might have otherwise been convicted, as seen in the Colin Pitchfork case. With the advancements in technology, DNA profiling has progressed from the tedious RFLP method of digesting DNA with restriction enzymes, attaching the fragments to a membrane and

then hybridising them with microsatellite probes, to the much more rapid process of amplifying STR regions using PCR that can be completed within a matter of days. STR analysis can also be performed on highly degraded DNA which is the quality of DNA usually recovered from crime scenes. In addition, STR kits have evolved to become increasingly sensitive and also target a greater number of loci, as demonstrated by Promega's PowerPlex® 21, which analyses 21 loci and has an extremely low detection limit of 50pg.

An increased sensitivity and lower detection limit, however, could have the adverse effect of detecting trace amounts of environmental DNA that might not have previously been detected. The presence of background DNA is dependent on multiple factors, including medical conditions such as skin diseases, physical activities, or physiological or psychological conditions that result in excessive sweating, and behavioural habits (face-touching) that facilitate the secondary transfer of DNA to other areas. An individual's shedding status has also been said to be a huge factor although there are conflicting views as to the feasibility of such a categorisation. Some studies have shown that individuals have consistent DNA shedding capabilities and can therefore be classified as good or bad shedders. Conversely, other studies found the amount of DNA deposition from individuals to be significantly variable that they could not be clearly defined by their shedding tendencies.

If crime scene exhibits are contaminated with extraneous DNA, this could result in the generation of mixed profiles. Such profiles are difficult to interpret and might even negatively affect the outcome of investigations, as revealed in the Farah Jama case. Hence, there are standard practices in place to safeguard against contamination of

evidentiary items, which include ensuring that forensic laboratories follow strict cleaning procedures, and that any staff who comes into contact with exhibits are properly geared. However, secondary transfer of DNA has been found to occur through gloves when they are not changed in between handling of different exhibits. In addition, DNA has also been found on gloves from unopened boxes, which underlines the importance of using DNA-free consumables certified for DNA analysis use. To combat such manufacturer-related contamination issues, a new forensic standard ISO18385 was established which specify requirements for consumables and reagents used for forensic DNA examination. Therefore, the combination of these measures would ensure that DNA contamination is kept to the minimum and is not likely to jeopardise investigation outcomes.

Final Comments

In order to ascertain the effects of secondary transfer of DNA in real-life forensic situations, a research project will be conducted to investigate the presence of environmental DNA within vehicles. Although a survey of South Australian facilities had previously been done (Henry, McGowan, and Brown 2015), no such survey has been done in Western Australia as of yet. Furthermore, little research has been done regarding the presence and persistence of trace DNA within forensic vehicles, which may pose risk of contamination when consumables within the vehicles are brought onto crime scenes. Considering that there are no standard cleaning procedures in place for vehicles, it might be reasonable to suggest that DNA contamination of evidentiary materials could very possibly occur via secondary transfer.

The purpose of this project would be to determine the amount of background DNA present in vehicles and to highlight high-risk areas where most DNA might be found to persist. Thus, this will provide a guideline as to whether improvements need to be made to current practices regarding contamination from vehicles.

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Part Two

Manuscript

The Presence of Environmental Human DNA Within Forensic Vehicles

Abstract

With the advancement in DNA profiling techniques, STR kits have become increasingly more sensitive with lower DNA detection limits. However, this result in greater possibility of detecting background or contaminating DNA, which may generate mixed profiles that are difficult to interpret. This study investigates the background DNA level of six areas within 3 vehicles to determine whether the background levels in these areas were significant enough to contaminate exhibit samples during forensic investigations. The areas consisted of the driver-side inner door handles, buttons on driver-side door, steering wheel, gear, handbrake and seat belt buckle. Swab samples were taken from these areas, then extracted and quantified with qPCR. Amplification and profiling were also performed using PowerPlex® 21 System. The highest amount of DNA was recovered from the steering wheel and gear, which also generated profiles with the highest mean number of alleles per locus. The door buttons and handles however yielded little DNA and very partial profiles were generated. Therefore, areas with high background DNA levels may affect data interpretation in casework samples, but areas with low background levels are not likely to do so.

Keywords: DNA, Forensic, Environmental DNA, Background DNA, DNA profiling, Transfer, Contamination

Introduction

The development of DNA typing had certainly been a breakthrough in both the scientific and investigation communities due to its ability to discriminate between individuals to identify persons of interest or disaster victims. First developed in 1984 by Dr Alec Jeffreys (McKie 2009), the technique had progressed from the tedious RFLP method of digesting DNA with restriction enzymes, attaching the fragments to a membrane and then hybridising them with microsatellite probes, to the much more rapid process of amplifying STR regions using PCR, that can be completed within a matter of days (Butler 2010; Primorac and Schanfield 2014). STR analysis is preferred as it can be performed on highly degraded DNA, which is the quality of DNA usually recovered from crime scenes.

Modern STR kits have increased sensitivity and lower detection limits, such as Promega's PowerPlex® 21 System which can detect as low as 50 pg of DNA (Ensenberger et al. 2014). However, this could also have the adverse effect of detecting trace amounts of environmental DNA that would not have previously been detected. Environmental DNA may originate from laboratory equipment, consumables, exhibits that were previously examined in the laboratory, or deposited by staffs or consumable manufacturers. DNA deposition is dependent on multiple factors, including medical conditions such as skin diseases (Kamphausen et al. 2012), physical activities, or physiological or psychological conditions that result in excessive sweating (Quinones and Daniel 2012), and behavioural habits such as face-touching that facilitate the secondary transfer of DNA to other areas (Kwok, Gralton, and McLaws 2015). An individual's shedding status has also been said to be a huge factor although there are conflicting views as to the feasibility of such a

categorisation (Lowe et al. 2002; van Oorschot et al. 2003; Goray et al. 2016; Phipps and Petricevic 2007; Bright and Petricevic 2004).

If crime scene exhibits are contaminated with extraneous DNA, this could result in the generation of mixed profiles. Such profiles are difficult to interpret and might even negatively affect the outcome of investigations. Hence, it is important to have standard practices in place to safeguard against contamination of evidentiary items, which include ensuring that forensic laboratories follow strict cleaning procedures and that personnel who come into contact with exhibits are properly geared.

However, even with cleaning procedures in place, secondary transfer of DNA may still occur between exhibits if gloves are not changed in between handling of different exhibits (Poy and van Oorschot 2006). In addition, DNA was also found on gloves from unopened boxes (Daniel and van Oorschot 2011), which underlines the importance of using DNA-free consumables certified for DNA analysis use. To combat such manufacturer-related contamination issues, a new forensic standard ISO18385 was established in 2016 which specify requirements for consumables and reagents used for forensic DNA examination (ISO18385 2016).

In order to examine the potential for secondary transfer of DNA in real-life forensic situations, this study aimed to investigate the level of background DNA present in vehicles, highlight high-risk areas where most DNA might be found to persist, and determine whether these levels are significant enough to interfere with interpretation of DNA analysis results of crime scene evidence. This could provide a guideline as to whether improvements need to be made to current practices regarding contamination from vehicles. Although a survey of South Australian facilities had previously been done

(Henry, McGowan, and Brown 2015), no such survey has been done in Western Australia as of yet. Furthermore, little research had been done regarding the presence and persistence of trace DNA within forensic vehicles, which may pose risk of contamination when consumables within the vehicles are brought onto crime scenes. Considering that there are no standard cleaning procedures in place for vehicles, it might be reasonable to suggest that DNA contamination of evidentiary materials could very possibly occur via secondary transfer.

Materials and Methods

DNA Samples

18 trace DNA samples were taken with BD BBL™ CultureSwab™ EZ (BD, Franklin Lakes, NJ) moistened with sterile water. The areas sampled consisted of the inner handle of the driver-side door, buttons on the driver-side door, steering wheel, gear, handbrake, and seat belt buckle from three different Murdoch University vehicles (RAV4, Corolla, Falcon). A sterile moistened swab and reference buccal swab sample acted as the negative and positive control respectively. The swab samples were then stored at 4°C until ready for processing.

DNA Extraction

The swab foam tips were removed from the shaft and cut into smaller pieces, which were then placed into separate 1.5 mL Eppendorf Safe-Lock tubes. DNA was extracted using the QIAamp® DNA Investigator Kit (Qiagen, Hilden, Germany) as per the manufacturer's

protocol for swab samples with a final elution volume of 40 µL. The extracted DNA were then stored in the freezer for quantification analysis.

DNA Quantification

DNA quantification was performed using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Foster City, CA) as described in the manufacturer's manual. Quantification was performed on the ViiA™ 7 Real-Time PCR System (Applied Biosystems) with 1 µL of DNA in a total reaction volume of 12.5 µL.

DNA Amplification, Profiling and Analysis

The samples with quantifiable amounts of DNA were then amplified for profiling using the PowerPlex® 21 System (Promega, Madison, WI). 15 µL of template DNA was used for amplification, but in instances where the concentration of samples was above 0.5 ng/µL (positive control buccal swab), the sample was diluted to 0.5 ng/µL, where 1 µL of the diluted sample was used. A non-template control containing 15 µL of sterile water was also used. The analysis was carried out on a 3730 DNA Analyzer (Applied Biosystems), using the CC5 Internal Lane Standard 500 (CC5 ILS-500) and allelic ladder mix provided with the PowerPlex® 21 System. The results were then analysed with the GeneMapper® Software (Applied Biosystems) and GeneMarker® Software v2.6.7 (SoftGenetics), with a minimum calling threshold of 100 rfu.

Results

DNA Quantification

The quantification results of each sample are contained in Table 1. The total and mean amount of DNA recovered for each area (driver-side inner door handle, driver-side door buttons, steering wheel, gear, handbrake and seat belt buckle) are also shown.

Table 1.

Amount of DNA (ng/µL) recovered from six different areas of three different vehicles.

	RAV4	**Corolla**	**Falcon**	**Mean**
Door handle	0.022	0.006	—	0.0093
Door buttons	0.019	0.005	0.002	0.0087
Steering wheel	0.058	0.069	0.264	0.1303
Gear	0.049	0.111	0.013	0.0577
Handbrake	0.017	0.015	0.031	0.0210
Seat Belt Buckle	0.033	0.048	0.002	0.0277

The greatest amount of DNA was recovered from the steering wheel, followed by the gear, seat belt buckle, handbrake, door handle and door buttons. DNA recovered from the steering wheel was about 2.26 times more than the amount recovered from the gear.

DNA Profiling

Artifacts were observed in the no template control at AMEL (84.9 bp) and D3S1358 (100.2 bp) of the fluorescein channel, and D16S539 (86.3 bp) of the JOE channel, as depicted in Figure 1.

The number of called alleles varied greatly between the other samples. The number of alleles observed for each locus was tabulated and the mean number of alleles per locus per area was calculated for each sample and recorded in Table 2. Samples that contain a greater amount of DNA generally also yielded a greater number of alleles (eg. RAV4 gear)

than samples with lower levels of DNA (eg. Falcon door buttons). Figure 2 compares the profiles of the Falcon door buttons (A) and RAV4 gear (B). More peaks of higher RFUs are clearly observed in B than in A.

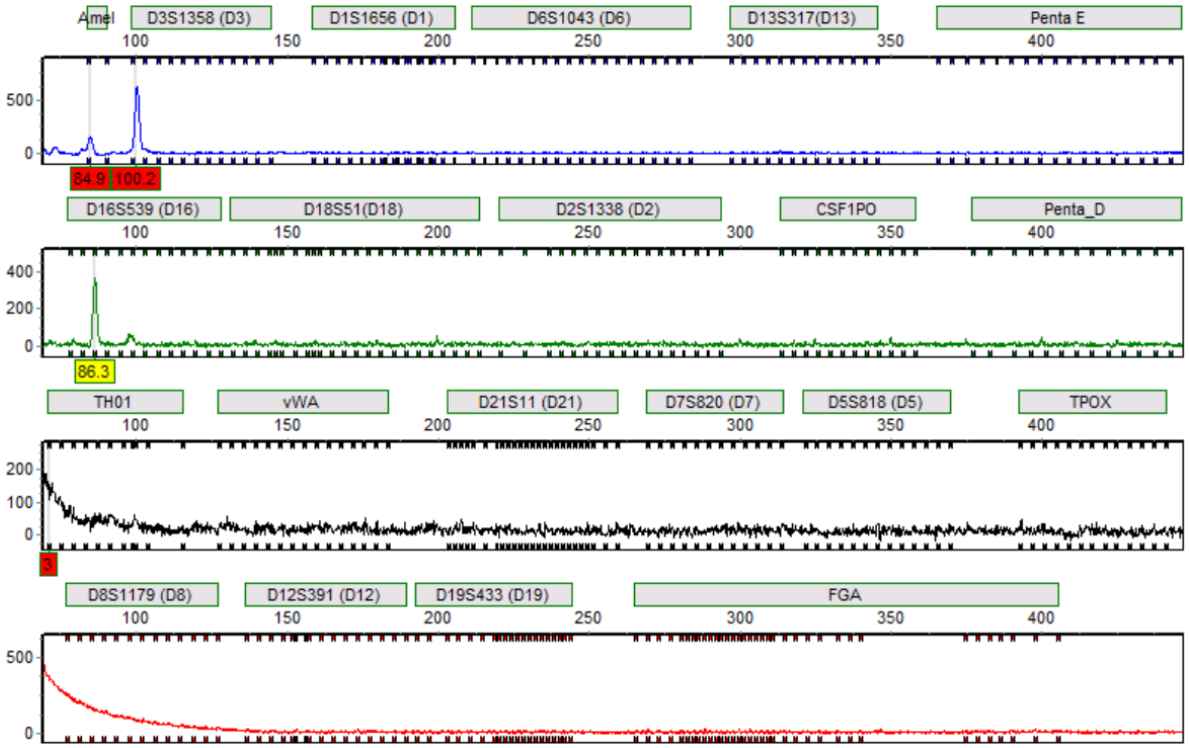

Figure 1. Electropherogram of the no-template control showing artifacts in the fluorescein and JOE channels.

Table 2.

Mean number of observed alleles per locus (excluding amelogenin) for each vehicle and area sampled

	RAV 4	Corolla	Falcon
Door Handle	3.40	1.98	-
Door Buttons	2.30	1.80	0.40
Steering Wheel	5.70	4.70	4.75
Gear	6.85	5.00	3.55
Handbrake	5.30	4.30	4.60

Seat Belt Buckle	6.05	4.55	0.85

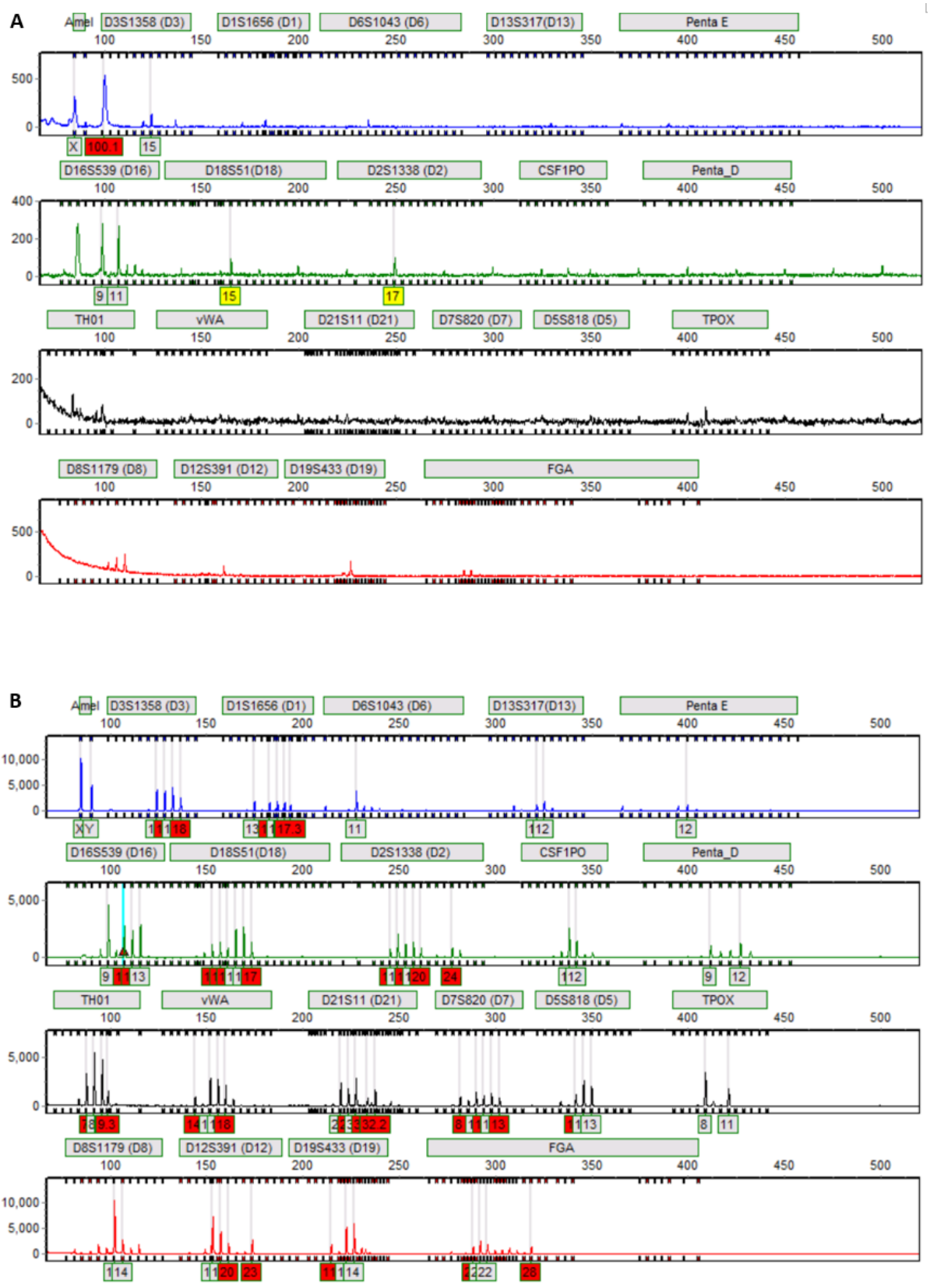

Figure 2. Comparison of the electropherograms between: A. Falcon door buttons – sample with the least number of called alleles, and B. RAV4 gear – sample with the most number of called alleles.

Discussion

Since forensic vehicles were not available for investigation in this study, university vehicles were used instead. Although these vehicles were not used for forensic purposes, they were available for use by university staff and students. Therefore, the vehicles would have been handled by a large number of people, which is similar to that of forensic vehicles, and can thus be used to estimate the background levels of DNA.

Due to limitations in the types of instrumentation and reagents available, the 3730 DNA analyser was used in this study, together with the Performance Optimised Polymer 7 (POP-7™) polymer which was developed specifically for use with the instrument. However, the use of POP-7™ was advised against in the PowerPlex® 21 System technical manual, due to possible artifacts within the fluorescein and JOE channels (Promega Corporation 2016). This would likely account for the spikes observed in loci AMEL, D3S1358 and D16S539 (Figure 1), which were noted to be known artifacts when analysing PowerPlex® 21-amplified products with POP-7™ polymers (Promega Corporation 2012). This indicates that any such spikes observed in the samples were due to anomalies in the run and not due to contamination of the samples themselves.

From the quantification results, the greatest amount of DNA was recovered from the steering wheel and gear, which are most possibly areas that are heavily and frequently handled by people. A relatively greater amount of pressure and friction would also likely be required when operating them. The buttons on the door yielded the least amount of

DNA, which was expected as the buttons would most possibly be handled the least frequently and also require the least friction to operate.

The samples which yielded less DNA amounts also tended to generate profiles with much lower numbers of called alleles. In addition, these samples also had lower peak heights. Therefore, the areas with low quantities of DNA (such as the door buttons or handles) would likely pose a lower risk of contamination to evidence samples, as the amount of background DNA present at those areas may not even be quantifiable. However, areas with higher background levels of DNA (such as the steering wheel) may pose a greater risk of contamination to evidence samples. As most forensic samples contain degraded and/or low amounts of DNA, the profiles may get swamped by high levels of background DNA if contaminated, which would result in ambiguous mixed profiles that are difficult to interpret.

Furthermore, a mean of 6.85, 5 and 3.55 alleles per locus were observed for the RAV4, Corolla and Falcon gear samples respectively (Table 2). Therefore, the lowest possible number of DNA contributors is 4 for the RAV4, 3 for the Corolla and 2 for the Falcon gear samples. However, it is difficult to correctly determine the actual number of contributors or to ascertain which alleles were contributed by each contributor (Paoletti et al. 2012). Thus, it would be difficult to identify a suspect from such samples. In addition, assuming a lower number of contributors than there actually was might skew an investigation in favour of the prosecution (Cowell et al. 2015), which could then lead to a miscarriage of justice.

Conclusion

The findings of this study highlighted the areas within a vehicle that may pose to be of high contamination risk, such as the steering wheel and gear. Therefore, measures could be undertaken to ensure that environmental DNA levels within these areas are kept to the minimum, such as implementing cleaning regimes and ensuring that gloves are worn before contact with evidence samples. It has also demonstrated that the level of background DNA may affect interpretation of profiles. Low background DNA levels may be insignificant, especially if the questioned exhibit sample has much higher amounts of good quality DNA. In contrast, high background levels of DNA may swamp DNA from the forensic sample, making interpretation of data difficult, which would thus affect the outcome of an investigation.

However, as the sample size in this study is small, further investigation with a larger sample size should be done to conclusively determine the risk of contamination of environmental DNA within vehicles on evidence samples. In addition, other areas could also be sampled, such as the trunk of forensic vehicles where consumables and field kits are stored for crime scene investigation use. Monitoring the level of DNA in such areas would allow investigators to ensure that there would be minimal cross contamination between different crime scenes.

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