An Evaluation of the Transfer and Persistence of Deoxyribonucleic Acid (DNA) Evidence

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"For every complex problem, there is a simple solution.......and it is wrong."

Henry Louis Mencken, Mencken's Law

Abstract

DNA analysis is now a sufficiently sensitive technique to enable identification of an individual from an extremely small amount of biological material. Exhibits are routinely submitted to forensic laboratories for recovery and analysis of 'touch DNA', in order to link an offender to the crime scene. One such exhibit type is spent cartridge cases, where DNA transferred from the handler to the exterior surface of the casing may be the only evidence available for identification of the handler. Alternatively the firearm itself may be recovered, which could also have potential for uncovering the identity of the shooter by means of 'touch DNA' profiling. However, the analysis of minute amounts of DNA introduces additional interpretational challenges. The ability to identify the source of a low level DNA sample and the relevance of a recovered DNA profile to the crime scene are not comprehensively understood. The variations in DNA deposition, recovery, transfer and persistence were examined, through a series of controlled laboratory experiments. Volunteers were asked to take part in DNA deposition studies that involved handling items for set periods of time, to determine the variability in the quality of DNA deposited. They were also asked to take part in handshaking studies, where the persistence of DNA, as well as the primary and secondary transfer of DNA, was studied. Additional variables were considered in relation to DNA recovered from spent cartridge cases, including the effect of firing and gunshot residue on DNA quality.

DNA was extracted using QIAamp® DNA Mini Kit (Qiagen) and Chelex® (Bio-Rad) protocols and amplified with the AmpF/STR® SGM Plus® Kit and the AmpF/STR® Identifiler® Kit (both Applied Biosystems). DNA profiles were analysed on the ABI PRISM™ 310 Genetic Analyser and the ABI PRISM™ 3500 Genetic Analyser (both Applied Biosystems).

It was possible to recover a usable DNA profile from a handled item and the quality of DNA deposited after repeated contacts was comparable. The quality of DNA recovered from 'touch DNA' samples from different individuals varied, and specific methods for recovery based on surface type were found to increase the likelihood of generating a successful DNA profile. Where an item was handled by more than one individual, the major contributor to the profile was not always that of the final handler. Furthermore, secondary transfer of DNA was observed to some degree in every test sample. This

research also highlighted the challenges of interpreting mixed profiles, especially with low levels of DNA present.

Identification of the handler of a spent cartridge case was not possible using DNA profiling techniques, due to the increased DNA degradation as a result of conditions experienced during the firing process. However, where a higher yield of DNA was present prior to firing, there was the possibility of recovering an interpretable DNA profile from this type of evidence.

The findings of this research should be considered when submitting items for DNA analysis, when considering best practice for recovery of 'touch DNA' samples and when attempting to interpret 'touch DNA' evidence profiles.

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Abbreviations of Technical Terms

ACPO	Association of Chief Police Officers
AFSP	Association of Forensic Science Providers
bp	base pairs
CAST	Centre for Applied Science and Technology
CJ	Criminal Justice
CNA	Cell free Nucleic Acids
CPS	Crown Prosecution Service
CRS	Cambridge Reference Sequence
DNA	Deoxyribonucleic Acid
EDNAP	European DNA Profiling
ENFSI	European Network of Forensic Science Institute
ESS	European Standard Set
FoSciSIG	Forensic Science Special Interest Group
FSS	Forensic Science Service
GF	Genotype Frequency
GSR	Gun Shot Residue
HVI	Hypervariable region I
HVII	Hypervariable region II
LCN	Low Copy Number
K	Kelvin
ml	millilitre
mRNA	messenger RNA
ms	milliseconds
MSY	Male Specific Y DNA
mtDNA	mitochondrial DNA

NCA	National Crime Agency
NSA	National Strategic Assessment
NDNAD	National DNA Database
ng	nanogram
nt	nucleotide
OL	Off Ladder
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
pg	picogram
POP	Performance Optimised Polymer
RFU	Relative Fluorescent Units
RNA	Ribonucleic Acid
SD	Standard Deviation
SOC	Scene of Crime
SOCA	Serious and Organised Crime Agency
SGM+	Second Generation Multiplex Plus
SNP	Single Nucleotide Polymorphism
STR	Short Tandem Repeat
v/v	volume to volume
WGA	Whole Genome Amplification
w/v	weight to volume
µg	microgram
µl	microlitre
x *g*	times gravity
^{o}C	degrees centigrade

One. Introduction

Forensic science is the application of science to the resolution of legal disputes, whether in criminal or civil cases. Whilst forensic science is by no means a new discipline, some of the fundamental principles and technologies that are routinely used today are still relatively 'young'. One such technology is that of DNA (deoxyribonucleic acid) analysis, with the first use of DNA for the purposes of identity verification through familial DNA comparison occurring in 1985 (Jeffreys, Wilson & Thein, 1985). This was closely followed by the first use of DNA in a criminal investigation in Leicestershire, England in 1987. Since its emergence, DNA analysis has evolved in the past thirty years to become one of the most frequently used forensic identification tools. So much so that in 2005, GeneWatch, an independent public interest organisation who review developments in genetic developments, reported that in a typical month the DNA database linked suspects to approximately 30 murders, 45 rapes and 3,200 volume crimes (Staley, 2005). Current DNA profiling techniques are sufficiently sensitive that they enable identification of human remains, meaningful comparisons of minute crime scene samples with control samples from a suspect, as well as familial relationship testing (Li, 2008). The development of more sensitive methods for analysing DNA has led to increased reliance on this type of evidence in cold case reviews and appeal cases, as well as standard criminal investigations.

The primary goal of most criminal investigations is to link a piece of evidence or an individual to a location at the time of a specific event. The central principle governing the ability to identify these links is Locard's principle of exchange, which states that when two items come into contact with one another, exchange of material between the two items will occur (Jackson and Jackson, 2011). The potential for transfer of DNA evidence is relatively high as a result of the wide variety of possible biological sources ranging from DNA rich sources, such as blood and semen, through to samples with a lower anticipated DNA yield, such as hairs and skin cells.

In 1995, the UK launched the first National DNA Database (NDNAD), enabling comparison of Scene of Crime (SOC) samples to control DNA samples collected from suspects (Thompson and Black, 2007). Between 1st April 2011 and 31st March 2012, the NDNAD

produced 124 matches in murder investigations, 522 matches in rape investigations and 28,996 matches in the investigation of other crimes (National Policing Improvement Agency, 2011), indicating the huge part DNA evidence plays in criminal investigations.

However, whilst DNA continues to play a hugely significant part in aiding justice, there are still challenges to be overcome, issues to be investigated and caution that needs to be heeded with this type of evidence. As with any type of forensic evidence, there must be confidence in the admissibility of that evidence in court (Budowle et al., 2003). All methods used in forensic DNA analysis must undergo a rigorous validation process to ensure that they are fit for purpose (International Accreditation Laboratory Cooperatino, 2002; National Research Council, 1992; National Research Council, 1996; Baechtel, Presely and Smerick, 1995; Cotton et al., 2000). As developments in DNA analysis arise, the admissibility of the evidence must be re-evaluated. One of the most highly debated arguments in forensic science was the admissibility of Low Copy Number (LCN) DNA analysis. From 1999, LCN analysis was used successfully in many forensic cases, up until 2007 when its use in UK criminal cases was suspended (Williams and Johnson, 2008). The reasoning behind the suspension of the use of LCN analysis in criminal investigations was as a result of evidence challenged in the Omagh bombing case suggesting that LCN DNA analysis was not a reliable technique for use in criminal investigations. An internal review of the admissibility of LCN DNA evidence was carried out by the Crown Prosecution Service (CPS) and the overall conclusion was that this type of evidence would be admissible in court, with the proviso that the strength of this evidence was to be considered and reported appropriately (Buckleton, 2009; Crown Prosecution Service, 2008).

As DNA has become one of the most prevalent evidence types presented in court, and as techniques used in the analysis of DNA evidence have become more sensitive, defence lawyers have devised new arguments to challenge the admissibility of DNA evidence in court. A recent incident involving a procedural error whilst processing DNA evidence resulted in an innocent individual, Adam Scott, being charged with the offence of rape, based on the occurrence of a partial DNA profile match. The presence of Scott's DNA was found to be the result of contamination arising from the accidental re-use of disposable equipment in the laboratory, and the case was later withdrawn by the CPS (Rennison, 2012). This case led to the review of the quality control procedures employed by one of

the major forensic science providers, LGC Forensics. A further incident arising as a result of an error with the processing of DNA samples occurred in 2012; this time with another one of the major forensic science providers, Cellmark. A mishandling of two samples during the dilution stage of processing led to one individual being linked to two separate offences, when actually there were two different assailants. An internal review was carried out on 550,000 samples to ensure this error had not occurred in any other case (Home Office, 2013). Another incident relating to challenges to the quality of DNA evidence in court resulted in the acquittal of a suspect in a murder case, where DNA from David Butler had apparently been recovered from the victim (Barnes, 2012). Transfer and persistence of DNA were key issues in this case as Mr Butler had a rare skin condition which caused him to shed flakes of skin and, in his role as a taxi driver, the potential for secondary transfer of DNA, by way of him coming into contact with intermediary people, was increased. In this case Mr Butler was acquitted of murder, in part due to the poor quality and questioned provenance of the DNA evidence.

These three incidents highlight the caution that is needed when interpreting and reporting DNA 'matches' and the requirement for a comprehensive understanding of the circumstances surrounding the recovery of the DNA evidence.

1.1 DNA as an Identification Tool

Since the discovery of the structure of DNA in 1953, scientists have been continually exploring how we can unlock the mysteries of DNA for a variety of purposes such as tackling diseases, exploring genealogy, understanding epigenetics and evolution, maximising the nutritional value of food and, more recently, for data storage (Goldman et al., 2013). With the increasing interest in the structure and function of DNA, it was perhaps inevitable that the attention would eventually focus on the use of DNA to identify an individual. In 1944, Oswald Avery characterised the role of DNA as 'the vehicle of generational transference of heritable traits' (Rudin and Inman, 2002), and as a result, DNA is often referred to as the 'genetic blueprint of life' as an individual's genetic make-up is encoded in their DNA. What is meant by this is that DNA is transmitted from parents to their offspring (Snustad and Simmons, 2010), and the phenotypic

characteristics exhibited by an individual, such as eye and hair colour, are a result of their genetic makeup (Kirby, 1993).

There are a wide variety of technologies we can apply to human DNA samples to enable identification of, or differentiation between, individuals. The choice of method is dependent on circumstances and requirements (i.e. whether you are wanting to exploit the variable or conservative nature of DNA).

In the majority of cases where the aim is to identify the donor of DNA evidence, it is not imperative that we study the entire genome sequence. This would be a very costly and time-consuming process. In addition, approximately 99.5% of the human genome is identical for all individuals so, for the purposes of human identification, has no individualising function (Rudin and Inman, 2002). Instead, we routinely analyse polymorphic markers, at specific sites (loci) within the nuclear genome, which differ between individuals. The markers that are used currently are known as Short Tandem Repeats (STRs), which are length polymorphisms. STRs are short repeat sequences found predominantly in the non-coding regions of DNA, either within or between genes (Butler, 2005). STRs are present in every nucleated cell in the body and are identical whether from a blood stain, a saliva sample or a hair root from an individual.

The features of STRs that lend themselves to being a useful tool for forensic identification are plentiful, and include the small size of the alleles which means analysis is still applicable in cases where the DNA yield is expected to be small or degraded. The small size also enables rapid and accurate amplification which results in a quick turnaround of samples. A further advantage of STRs is that the frequencies for the individual alleles are known for different population groups which enable statistics to be calculated for an individual's genotype.

Predominantly DNA profiling of nuclear STRs is the method routinely carried out on evidential samples, with the AmpFLSTR® SGM Plus® PCR (Polymerase Chain Reaction) Amplification Kit (SGM+) generally the system of choice in the United Kingdom, although alternative kits with an increased number of loci are gaining operational popularity for certain submissions. The SGM+ amplification kit analyses 10 STR loci, plus the sex-determining marker Amelogenin. The SGM+ multiplex system has been designed and optimised such that all 11 loci are amplified in one reaction, therefore saving time and

money. Amplification kits with an increased number of loci, such as AmpF*l*STR® Identifiler™ (Applied Biosystems) and PowerPlex®16 HS (Promega) which both amplify 16 loci, can reportedly provide "enhanced sensitivity, a cleaner baseline, improved performance on mixtures and the ability to overcome high levels of PCR inhibition" (Applied Biosystems, 2014). From July 2014, these kits with an increased number of loci have been used as standard by forensic science providers in the UK.

The presence of PCR inhibitors in a sample, unless removed completely during DNA extraction protocols, can result in a complete failure to amplify any loci or can produce profile issues such as a reduced detection of larger STR loci (Butler, 2010). Inhibitors, such as haemoglobin or melanin, can act in a number of ways (Li, 2008). They can interfere with cell lysis resulting in incomplete or poor DNA extraction. They can degrade the DNA present, resulting in the production of poor quality DNA profiles, or they can bind to the active site of *Taq* polymerase impacting on its ability to function (Butler, 2010). Ions present in high concentrations inhibit PCR by inhibiting *Taq* polymerase whereas chemicals such as EDTA (ethylenediaminetetraacetic acid, used in some extraction methods) inhibit PCR by binding magnesium ions that are essential for the success of the PCR reaction (Goodwin, Linacre and Hadi, 2007). The presence of PCR inhibitors often result in the production of partial DNA profiles, similar in appearance to those produced with degraded DNA samples (Butler, 2005), and therefore any systems capable of reducing this issue are very appealing to forensic science providers .

The move towards including a greater number of loci in standard DNA profiling systems for casework samples in the United Kingdom has been initiated by Forensic Science Northern Ireland, who launched 'DNA 17' in December 2013, a new DNA profiling service that examines 17 loci (Department of Justice, 2013). It is anticipated that this system will be implemented throughout the rest of the UK in 2015 and will have a significant impact on success rates from degraded or low yield evidential samples.

DNA degradation refers to the breaking of DNA molecule into smaller fragments (Li, 2008). DNA degrades as a result of enzymes called nucleases that break down the strands – exonucleases degrade from the end of the DNA strand and endonucleases break through the double strand at random intervals (Robertson, Ross and Burgoyne, 1990). Any process which encourages hydrolytic cleavage (breaking of the glycosidic base sugar

bond) or oxidative base damage, such as heat or humidity, will also result in an increase in the degradation rate of DNA (Butler, 2010).

Degraded DNA samples often result in the production of failed profiles if the 'breaks' in the DNA occur in a primer binding site. The quality of the resulting profile is directly related to the degree of degradation; the more degraded a sample, the less intact the DNA will be, therefore the less chance of the target DNA being intact (Butler, 2005). Breaks in the DNA at the flanking regions (where the primer would bind during PCR amplification) or within the target region of DNA itself, will result in failure of PCR amplification.

Partial profiles are often produced when degraded DNA samples are amplified (Naughton and Tan, 2011). The larger alleles are generally less likely to be successfully amplified resulting in a profile with over amplification of the smaller loci and a gradual decline in the peak height as the loci increase in size (Li, 2008; Goodwin, Linacre and Hadi, 2007). This can also be true of alleles at the same loci, where extreme peak imbalance can result in heterozygous loci being incorrectly perceived to be homozygous. This may be a result of preferential amplification of alleles resulting in allele drop out (Alaeddini, Walsh and Abbas, 2010). Systems that can reduce the potential production of partial profiles, by the inclusion of smaller target loci, will be a valuable tool for forensic science providers.

The adoption of comparable systems for DNA profiling between different countries became more widespread as a result of the Prüm Treaty in 2005 (European Commission, 2013), with the aim being to promote efficient sharing of unidentified DNA profile information across borders. In order to facilitate the sharing of DNA data between EU member states, the European Network of Forensic Science Institutes (ENFSI) DNA working group and the European DNA Profiling (EDNAP) group published recommendations to increase the number of loci available (Gill et al., 2006a; Gill et al., 2006b). This has led to the development of new chemistries that incorporate the expanded European Standard Set (ESS) loci, consisting of 12 standard loci (Welch et al., 2012). In addition to enabling cross-border sharing of DNA profiles, the specific combination of loci were also chosen for inclusion into the ESS as a result of their potential to increase success rates with degraded samples and to reduce the likelihood of obtaining advantageous matches (Welch et al., 2012).

Goodwin and Peel (2012) studied the effectiveness of the expanded ESS loci, in conjunction with the AmpF*l*STR® Identifiler™ kit, and found that the potential for producing a correct identification was significantly greater. A further concordance study was organised by the ENSFI DNA working group where the latest commercial STR multiplex kits (that include the 12 expanded ESS loci) were compared against the current, validated systems for consistency of genotype results (Welch et al., 2012). The new chemistries included the AmpFℓSTR® NGM™ and NGM SElect™Kits (Applied Biosystems), the PowerPlex® ESX-16, ESI-16, ESX-17 and ESI-17 Systems (Promega) and the ESSplex® and ESSplex SE® kits (QIAGEN). The results of this study indicated that the discordance rates for all of the kits were very low and therefore the new kits were fit for purpose.

In addition to increasing the number of loci amplified, there have been further developments aimed at enhancing the sensitivity of DNA profiling systems, namely introducing additional cycles of amplification.

Theoretically the PCR reaction doubles the amount of target DNA at each cycle, but in reality this process is not 100% efficient (Hughes and Moody, 2007). After 28 cycles of amplification, the standard cycle number for most forensic analytical techniques, there would be approximately 67,000,000 copies of the PCR product or amplicon (Goodwin, Linacre and Hadi, 2007). When very low starting amounts of DNA exist, typically less than 100 pg of template DNA, an increased number of cycles is often used to improve the yield of DNA produced during DNA amplification. This is often referred to as 'Low copy number', 'Low level' or 'Low template' DNA analysis; for the remainder of this thesis it will be referred to as Low Copy Number (LCN) DNA. This form of analysis involves additional cycles of PCR amplification. Generally the cycle number is increased from the standard '28' cycles of amplification, to between 34 and 40 cycles (Buckleton, 2009). After 34 cycles of amplification there would theoretically be approximately 4294 million copies of the PCR product (Goodwin, Linacre and Hadi, 2007).

Gill et al. (2001) have defined LCN analysis as profiling of DNA samples that contain less than 100 pg of DNA. In a single cell there is approximately 6 pg of genomic DNA, therefore 100 pg of genomic DNA would equate to approximately 30 copies of the locus (with diploid cells) (Butler, 2010). The normal range of template for standard DNA analysis is between 500 and 2500 pg (Goodwin, Linacre and Hadi, 2007).

Although the practical aspects of the technique do not incur a great deal of additional time or cost, the analysis and elucidation of the resulting DNA profile requires specialist interpretation. LCN analysis is specifically sensitive to the stochastic effects of amplification. The stochastic effect, or stochastic fluctuation, is where a random selection of alleles are amplified, generally as a result of a limited amount of template DNA being available (Butler, 2010). LCN analysis can be defined as any DNA analysis where the results are over the limit of detection, but under the stochastic limit for reliable interpretation (Budowle et al., 2002). Common features indicating stochastic fluctuation, which are observed in electropherograms of LCN samples, are (Buckleton, 2009; Budowle et al., 2002):

- Locus dropout, when no alleles are observed at a specific locus.
- Allele drop out, defined as a situation where one allele in a heterozygote is not visible, giving the appearance of a homozygote. It is not completely understood what the cause of allelic dropout is, but it has been suggested it could be an extreme case of preferential allele amplification or heterozygote imbalance (Li, 2008; Butler, 2005). Whitaker, Cotton and Gill (2001) suggest the incidence of allele drop out is not related to the size of the loci. They state that “When allelic drop out was observed there was a slight tendency for the low molecular weight allele to drop out in preference to the high molecular weight” (Whitaker, Cotton and Gill, 2001). This is in contrast with the findings for heterozygote imbalance reported by one of the same authors previously. Gill et al. (2000) reported that it was possible to apply LCN amplification to samples that contained as little as 25 – 50 pg (equivalent to between 4 and 10 cell nuclei) without the incidence of allele drop out.
- Preferential allele amplification, where one allele in a heterozygous pair is preferentially amplified (Gill et al., 2000; Walsh, Erlich and Higuchi, 1992). Walsh, Erlich and Higuchi (1992) found that preferential amplification of shorter allelic products occurred, especially when samples were degraded or when low template DNA was analysed. Preferential allele amplification often results in heterozygote imbalance (Li, 2008).
- Heterozygote imbalance, when the two peaks of a heterozygote are not equal in height, as expected in standard DNA profiles (Li, 2008). Rather, one of the peaks is

significantly larger than the other. It is often defined as $(\phi a - \phi b)/\ \phi a \geq 0.2$ where ϕa and ϕb are the areas of the larger and smaller peak (irrespective of molecular weight) (Gill et al., 2000). Ideally the two peaks of a heterozygote would have a 1:1 peak height/area ratio, but generally it is accepted that the smaller peak is 60-90% of the size of the larger peak in good quality samples (Goodwin, Linacre and Hadi, 2007). Incidence of heterozygote imbalance is higher with low amounts of template DNA material and with increased cycles of amplification (Gill et al., 2000; Anjos, et al., 2006). Research by Whitaker, Cotton and Gill (2001) indicated that in samples with a low template level there was a tendency for the low molecular weight allele to be preferentially amplified. This was also observed by Kloosterman and Kersbergen (2003). Detection of heterozygote imbalance is complicated by the fact that a similar profile is produced when a DNA sample has more than one contributor (Budowle et al., 2002). This is especially pertinent when one considers that a mixed DNA profile would be more likely to be encountered during LCN analysis due to an increased likelihood of contamination occurring (Gill, 2001).

- Allele drop in, the non-specific generation of extra alleles which are often smaller in peak height. Buckelton (2009) suggests they are often a result of contaminating fragmented DNA from the laboratory environment or consumables. However, Budowle, Eisenberg and van Daal (2009a) suggest that allele drop in may also be the result of strand slippage during the PCR. Goodwin, Linacre and Hadi (2007) states that these products are spurious amplification products which may not be evident in replicate reactions.
- Stutter products occuring as a result of strand slippage occurring during the extension stage of PCR amplification and are evident in the form of a small peak, usually one repeat unit smaller than the true (parent allele) peak (Goodwin, Linacre and Hadi, 2007). Stutter products can also be one repeat unit larger than the parent allele peak, but this is much less common (Li, 2008). STR loci should have a stutter peak incidence of less than 15% of the peak height or area of the parent allele peak in order to be validated for use in forensic DNA profiling kits. DNA profiling software will discount peaks it interprets as stutter products during analysis. However, the relative proportion of stutter is often increased with LCN analysis (Gill, 2001; Budowle et al., 2002; Gill et al., 2000) and if a stutter peak is

observed in more than one replicate analysis it may be misinterpreted to be a true allele (Budowle, Eisenberg and van Daal, 2009a).

Because very low levels of DNA produce these stochastic effects, it can often be difficult to determine if the DNA is present is evidentially valuable or if it is a result of DNA contamination (Ballantyne, Poy and van Oorschot, 2013).

Reproducibility of peaks in DNA profiles from samples with a low template level is poor, and even when duplicate amplification is carried out, often the generation of a consensus profile is not possible [Buckleton, 2009; Budowle, 2002; Budowle, Eisenberg and van Daal, 2009a). This may be due to increased potential for contamination (Gill, 2001). Gill (2000) also raised the issue of a true negative control not existing for LCN analysis. This is due to the fact that the negative control is generally included to detect contamination within a reagent. If LCN analysis can detect a single molecule of DNA, then the presence of that single DNA molecule in one sample does not indicate that it also exists in all other samples (as a single DNA molecule can only be present in one sample).

Additional care must be taken during the preceding stages of DNA processing, as due to increased sensitivity the potential for detection of extraneous DNA is increased. Incidental DNA from the scene or evidential item as well as contamination from the crime scene personnel or laboratory personnel or other sources is an increased issue with LCN DNA analysis (Goodwin, Linacre and Hadi, 2007). When cold cases are reviewed or cases predate the implementation of LCN analysis, such as the Omagh bombing, the scientist must take into account when interpreting the evidence that crime scene and laboratory personnel would not have been aware of the more stringent anti-contamination procedures required with this type of analysis (Budowle, Eisenberg and van Daal, 2009a).

Buckleton (2009) suggests that more research needs to be carried out to determine methods of reducing the stochastic effect on low template DNA samples. Sorensen et al. (2004) suggested, on the basis of their research findings, that Whole Genome Amplification (WGA) could be used to enable unbiased amplification of the template DNA. However, only four alleles in total were amplified in this research, therefore its effectiveness with a larger number and range of alleles would need to be determined.

The challenges associated with LCN analysis have led to the introduction of an additional level in the hierarchy of propositions when reporting DNA evidence. The three pre-existing levels of hierarchy were source level, activity level and offence level. The source level would indicate the origin of a stain; for example, the bloodstain originated from the suspect. The activity level would indicate the type of transfer of evidence; for example, the suspects' blood was present as a result of an arterial breach. The offence level would generally be the decision of the jury; for example, the suspect committed the offence. The additional level, initiated as a result of LCN analysis, is a sub-source level; for example, the DNA profile originated from the suspect (Gill, 2001).

When reporting DNA evidence that has been amplified using LCN conditions, it is suggested that multiple repeats of each amplification reaction is carried out and alleles are only reported if the allele occurs in each replicate. A further recommendation is discounting of any alleles that are also seen in any corresponding negative controls (Butler, 2005). Retesting of the sample is also suggested if a single allele not matching the suspect is identified, although this approach could suggest bias in interpretation.

There have been recommendations to move away from the term 'LCN' as there is confusion as to whether the term refers to the amount of DNA present or the actual technique used when analysing samples with a low amount of template DNA. In some literature, LCN refers to the protocols whereas low template DNA (LtDNA) refers to a sample containing low amounts of DNA (Whitaker, Tully and Sullivan, 2009). However, the terms are often used interchangeably within published literature.

There is ongoing debate as to the general acceptance of LCN analysis, with discussions continuing as to whether this type of analysis should only be applied to single-source samples rather than evidential samples, and the issues of a lack of standard protocols in interpreting LCN profiles (Budowle, Eisenberg and van Daal, 2009b; Caragine and Prinz, 2011; Budowle, Eisenberg and van Daal, 2011; Buckleton and Gill, 2011). Some attempts have been made to introduce interpretation guidelines, through software (Gill, Kirkham and Curran, 2007) or frameworks (Balding and Buckleton, 2009) but there are still challenges to overcome. The lack of a clear directive on the situation has led more widespread discussion as to the inclusion of LCN DNA evidence in trials (Naughton and Tan, 2011).

Any developments in DNA technology that increase the sensitivity of the profiling system will have an inevitable impact on the quality of the profile, as previously outlined. The interpretation of these profiles can be more challenging, especially in instances where mixed profiles occur. When two or more individuals have contributed to a DNA sample, the resulting profile will reflect the contributions of the donor individuals. This is evident by the presence of more than two alleles at any given loci (Li, 2008). Initial detection of a mixture may be difficult depending on the number of contributors, the quality of the DNA profile and the respective ratio of DNA from each source (Butler, 2010).

Where there is a clear major and minor component to a mixture, the two profiles may be separated by the peak height ratio (Goodwin, Linacre and Hadi, 2007) as the peak height is representative of the relative amounts of DNA present (Butler, 2010). Often in cases where low levels of DNA are sampled, the distinction between individual components is not clearly defined. This is exacerbated when the low levels of DNA result in stochastic amplification (Butler, 2010). Even in cases where there is a significant difference between the contributions of two individual components, the presence of artefacts such as stutter can complicate designation of alleles (Goodwin, Linacre and Hadi, 2007).

Individuals may share many alleles; a mixture of DNA from two individuals may contain two, three or four peaks at each loci. This makes the process of interpreting profiles and determining the number of contributors to a mixed profile extremely complex (Naughton and Tan, 2011). For example, if two individuals at the FGA locus have genotypes of 23, 24 and 24, 24, respectively, then the ratio of peaks seen in a mixture (if the two individuals contribute an equal amount of DNA to the mixture) would be 1:3 for the 23:24 loci. This could resemble a homozygous peak with a large stutter product. In instances like this, a broader examination of the profile as a whole should give some indication that a mixture exists (Butler, 2010).

Schneider et al. (2009) have defined mixtures as belonging to one of three types; Type A, Type B or Type C. The process of classifying which type of mixture has been produced, and therefore determination as to whether statistical analysis is appropriate, has been summarised by Butler (2010) (Figure 1.1).

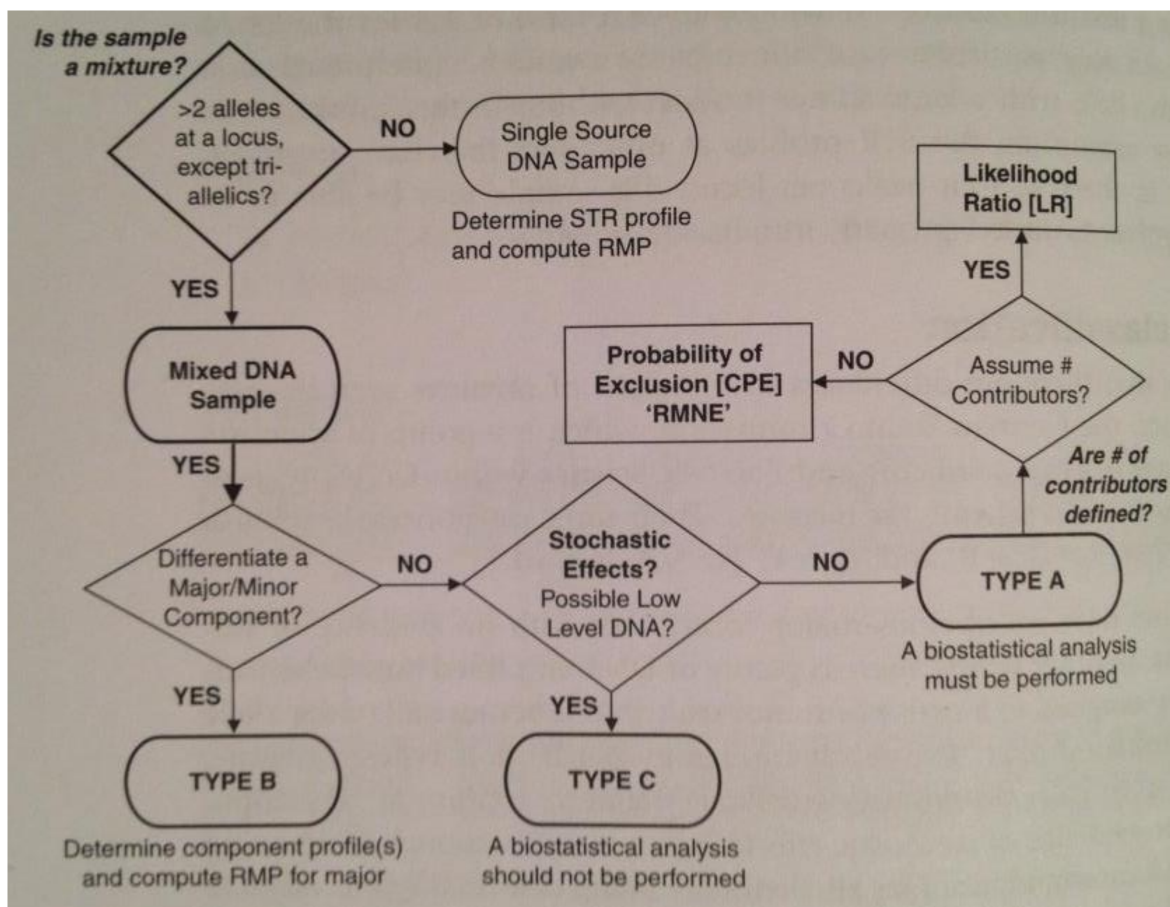


Figure 1.1 Mixed DNA profile classification scheme from the German Stain Commission (Butler, 2010).

Some forensic laboratories simply state that if the DNA profile of an individual matches a component of the mixed profile then that individual cannot be excluded as a contributor and a combined probability of exclusion can be calculated (using the frequentist approach) (Li, 2008; Butler, 2010; Buckleton, Triggs and Walsh, 2005).

There is yet to be a universal consensus on how mixed DNA profiles should be interpreted (Naughton and Tan, 2011). It is generally perceived that if the minor component of a mixed profile represents over 5 -10% of the total DNA, it is still detectable (Butler, 2010; van Oorschot, 1996). Several different approaches have been proposed for interpreting mixtures. The likelihood ratio approach has been suggested (Weir, 1997; Bär, 2003; Buckleton, Curran and Gill, 2007; Haned et al., 2011; Ladd et al., 2001), the use of peak area ratios (Ladd et al., 2001; Gill et al., 1998; Cowell , Lauritzen and Mortera, 2007; Cowell , Lauritzen and Mortera, 2011) and the probability of exclusion (Ladd et al., 2001). Despite each technique having its own advantages and limitations, debate is still ongoing as to the most appropriate technique to use (Torres et al., 2003; Bill et al., 2005; Gill et al.,

2006; Wickenheiser, 2006; Budowle et al., 2009; Gill and Buckleton, 2010; Budowle, Chakraborty and van Daal, 2010). Alternatively, a statistical approach using quantitative probability modelling, such as that exhibited by the TrueAllele® computer interpretation system, could ensure the process of mixture interpretation is much more reliable and defensible (Perlin et al. 2011).

Alongside the advances in comprehending how to go about interpreting mixed profiles, there have been developments in the ability to 'search' against this type of profile on the NDNAD. In December 2012, the NDNAD Unit was granted ministerial approval to allow for specialised searches of mixed DNA profiles, using a new technology called 'DNA Boost' (Home Office, 2013). It was perceived that this service, currently available to police forces in England and Wales as of June 2013, would positively impact on detection rates and enhance the effectiveness of the NDNAD, however there is little documented evidence on how successful this technology has been.

A novel suggestion as to how the issue of two person DNA admixtures could be resolved is through the use of laser capture micro-dissection (Ballantyne, Hanson and Perlin, 2013). This process involves isolating groups of cells from a mixture and profiling the individual cell groups independently, much like differential extraction, where sperm and non-sperm cells are separated from one another. This method has been applied successfully when separating distinguishable cell types from one another, such as spermatozoa from epithelial cells (Sanders et al., 2006) but additional challenges arise when the two donor samples originate from the same source material (e.g. epithelial cells). The issue with not being able to differentiate between non-distinguishable cell types, such as epithelial cells, originating from two different donors was overcome by using binomial sampling of the samples. This process involves sub-sampling of the cells, rather than all cells being sampled at once, which results in different proportions of the donors DNA being recovered enabling easier distinction of the two donors' individual genotypes. This method was determined to assist in deconvoluting the individual genotypes in a two person DNA admixture, and it was suggested that combining this with RNA analysis to identify the tissue source would result in an extremely powerful identification technique (Ballantyne, Hanson and Perlin, 2013).

In addition to the standard STR profiling systems available, there are a plethora of alternative or supplementary methods of DNA analysis which have been developed with forensic applications in mind and which may be better suited to generating viable identification information in specific situations; for example in cases where the DNA is extremely degraded.

Once such method utilises the Y-chromosome, unique to males, which consists of approximately 60 million bps including over 400 STRs (Li, 2007; Gunn, 2006). However, only a maximum of seventeen Y-STRs are routinely used in forensic identification (Decorte, 2010). Profiling of Y-STRs is a particularly valuable technique in cases where there is an admixture of male and female components, such as in cases of alleged sexual assault (Thompson and Black, 2007; Rudin and Inman, 2002; Savino and Turvey, 2011), as it allows separation of the male-specific information from the mixture. An additional situation where Y-STRs are a powerful tool is in cases of sexual assault where the assailant does not produce spermatozoa, as a profile can be isolated from the male epithelial cells present which would, in traditional STR analysis, be vastly overpowered by the female epithelial cells (Gunn, 2006). They are also particularly useful in identification of human remains, as well as for paternity investigations, especially in cases where the alleged father is not available for testing (Rapley and Whitehouse, 2007). Y-STRs can be analysed to establish paternity as the region that is studied on the Y chromosome, the MSY (Male Specific Y) region, does not undergo recombination and therefore is identical between all male relatives (Li, 2008; Rapley and Whitehouse, 2007). This, however, means that the discriminatory power of the Y-STR profile is much lower, which must be taken into account when presenting this type of analysis as evidence. Often Y-STR information is used as supporting intelligence in criminal cases or as an exclusion tool. However, in cases such as the identification of human remains in the 2004 Asian Tsunami and in identifying President Jefferson as the likely father to one of his slaves' children, Easton Hemmings Jefferson, Y-STR analysis has proved its validity as a technique for forensic identification (Rapley and Whitehouse, 2007). In the future it may be possible to exploit the Y choromosome for intelligence information by dating Y-chromosomal lineage changes or predicting surnames based on sequence data (using Y-STR haplotypes) but these technologies are still in their infancies (Wei et al., 2013; Burgess, 2013).

Another more recently explored alternative to STR analysis is the examination of Single nucleotide polymorphisms (SNPs). SNPs are sequence variations at specific locations within the human genome, and arise as a result of a single base pair mutation. This mutation can be in the form of a deletion, insertion or substitution of a nucleotide (Li, 2008; Decorte, 2010). SNPs differ from STRs in that they are sequence variations as opposed to length variations therefore only have four potential variants for each SNP (Thompson and Black, 2007), as opposed to STRs which have between 8 and 15 possible alleles for each locus (Rapley and Whitehouse, 2007). This means that the rate of discrimination from an individual SNP is very low, so between 50 and 100 SNPs are required to give an equivalent discriminatory power to that of SGM+ (Hughes and Moody, 2007). Approximately 15 million SNPs have been characterised to date (Decorte, 2010) so there is no issue with locating sufficient SNPs to analyse. The primary benefit SNPs have over STRs is the size of the DNA fragment required to be intact for analysis. The total length of DNA required to be intact for successful STR analysis can be up to 500bp long, whereas SNP fragments are on average less than 100 bp in length (Rapley and Whitehouse, 2007). In cases of severely degraded DNA samples, where the average fragment length is less than 150 bp, there will be an increased likelihood of gaining SNP information rather than an STR profile. SNPs are present in both the autosomal and gender specific genomes, so mitochondrial and Y-chromosome SNP analysis can be utilised in solving cases of disputed parentage, as well as in providing ancestry information on the ethnic origin of specific evidence types (Li, 2008). However, there are limitations to the use of SNPs in certain situations, such as that of the identification of individual donors in a mixture, which is almost impossible in the case of multiple contributors (Gunn, 2006). A further issue with using SNPs routinely is the fact that current databases are made up of STR profiles, so cross referencing to current entries could not be carried out. In addition, there are ethical implications to using SNPs as phenotypic information of the individual can be revealed, which may contravene the regulations relating to the use of genetic markers in certain countries (Rapley and Whitehouse, 2007).

Previous advances in SNP technology include the development of SNaPshot, a primer extension-based method that enables multiplexing of SNPs (Applied Biosystems, 2012). Examples of potential forensic applications for SNP technology include analysis of

mitochondrial coding regions for haplotyping (Quintáns et al., 2004), differentiating between drug and non-drug forms of Cannabis (Rotherham and Harbison, 2011) and predicting eye colour (Kastelic, 2013). The future mapping of SNPs is being overseen by the Wellcome Trust, through their global partnership initiatives, the SNP Consortium and the International HapMap Project (Wellcome Trust, 2014). These two projects are involved in identifying common SNP variations, exhibited both at an individual level as well as in combination (known as haplotypes). The Wellcome Trust has also continued to fund further development of the 1000 Genomes Project, an initiative that aims to identify genetic variants in different populations through the use of DNA sequencing. Although this project was completed in 2015, recognition of the requirement to maintain and extend this resource led to the development of The International Genome Sample Resource (IGSR) (European Bioinformatics Institute, 2015).

SNPs have been widely used in forensic cases such as the identification of human remains after the terrorist attacks on the World Trade Center on September 11th 2001 (Decorte, 2010; Thompson and Black, 2007) as well as in more specialised cases, such as in the prosecution of Derek Todd Lee for seven counts of rape and murder in Louisiana in 2003. The intelligence on the genetic origin of the offender, provided by DNAWitness™ SNP analysis, indicated correctly that the offender had an ethnic origin that was 85% sub-Saharan and 15% Native American, which was in contrast to the eye-witness testimonies stating that the offender was a white male (Gunn, 2006).

Mitochondrial DNA (mtDNA) is located in the mitochondria organelles contained within the cytoplasm of the cell, unlike autosomal and Y-chromosome DNA which is located within the nucleus of the cell. A further distinction between nuclear and mtDNA is the copy number in which they are present within each cell; there is only one copy of the nuclear genome per cell whereas there are between 100 and 10,000 copies of the mtDNA genome present (Iborra, Kimura and Cook, 2004). The mtDNA genome is 16,569 bp in length and each copy is identical for that individual, barring any mutations. The section that is studied in relation to identification is the control region, the D loop, which is approximately 1100 bp in length and is situated between 15971 bp and 484 bp of the genome (Kirby, 1993; Lincoln and Thomson, 1998). Within the D-loop there are two regions that are polymorphic and therefore lend themselves well to forensic exploitation; these are known as Hypervariable regions I and II (HVI and HVII). These regions are of

particular interest as they have a mutation rate 5-10 times that of nuclear genes (Budowle, 2003). Both HVI and HVII are approximately 350 bp in length and both can be sequenced for identification purposes (Thompson and Black, 2007). HVI can be found within the locations 16,024 bp to 16,365 bp and HVII within positions 73 bp to 340 bp (Budowle et al., 2003) (Figure 1.2).

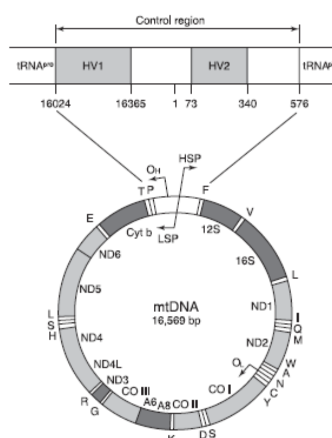


Figure 1.2 Hypervariable regions of the D loop in mtDNA (with nucleotide positions) (Melton and Sensabaugh, 2000).

The main benefit of mtDNA is its prevalence in very old or degraded samples – mtDNA has been successfully sequenced from samples from the skeletal remains of World War II victims (Zupanic, Gornjak and Balazic, 2010) and played a part in several high profile forensic identification cases (Holland and Parsons, 1999; Holland et al., 2003; US National Library of Medicine, 2011). However, the major limitation to mtDNA analysis, which prevents it from being an investigators' tool of choice in all identification cases, is that the mtDNA sequence is not unique to an individual. Due to the mechanism of inheritance, mtDNA is maternally inherited; therefore, barring mutations, all maternally related individuals will exhibit the same mtDNA sequence (Decorte, 2010).

As opposed to nuclear DNA profiles, where direct comparison can be carried out between the evidential and reference samples, mtDNA sequences are compared to the Cambridge Reference Sequence (CRS), which was the first complete mtDNA genome to be sequenced (Thompson and Black, 2007). This allows any differences from the CRS to be identified, and if two samples exhibit identical sequence variations they can be said to be a match and the statistical significance of that match can then be calculated. This is currently

done by stating the rarity of that sequence by determining the number of times that a particular sequence, or haplotype, is seen in a particular database. The choice of database used may be determined by the ethnicity of the individual and/or be one created by or made available to a specific forensic provider (Budowle, 2003; Holland and Parsons, 1999; Holland et al., 2003; Mozayani and Noziglia, 2006).

The number of nucleotide differences that must be demonstrated in order to exclude a maternal relationship between two samples is debated – the most accepted view is that two or more nucleotide differences are sufficient to be classed as an exclusion as this allows for natural heteroplasmy occurring. Heteroplasmy is where there is a mixture of normal and mutated DNA present due to the high mutation rate at a specific locus and is thought to occur in between 1-15% of individuals, depending on the sensitivity of the sequencing method utilised (Thompson and Black, 2007; Decorte, 2010; Melton and Sensabaugh, 2000; Mozayani and Noziglia, 2006). The number of nucleotide differences anticipated varies between racial groups – Caucasians have on average 8 differences between individuals and Africans have on average 15 (Budowle et al., 2003; Melton and Sensabaugh, 2000).

Well established technologies are continually being pushed to new frontiers to attempt to address pre-existing issues with human identification. For example, until recently it has not been possible to differentiate between identical (monozygotic) twins through standard forensic DNA testing methods. However, a recent publication by Weber-Lehmann et al. (2014) suggests that by applying the standard Sanger sequencing method to DNA samples from identical twins it would be possible to differentiate between the individuals through detection of rare mutations. When ultra-deep sequencing methods were applied to DNA samples taken from identical twins and the child of one of the twins, five SNPs were detected enabling differentiation of the twins, and accurate determination of the father of the child (Weber-Lehman et al., 2014). Although in its early stages, with only one set of twins having been tested, this could be a very significant step in cases involving identical twin suspects.

Another exciting development that has recently been reported is the potential to predict an individual’s facial features based on their genetic make-up. Research in 2012 led by the International Visible Trait Genetic(VisiGen) Consortium identified five loci that influenced

facial morphologies in Europeans (Liu et al., 2012) and this has been further developed by Claes et al. (2014) who have developed a statistical model for predicting 3-Dimensional facial shape. The research undertaken by Claes et al. (2014) involved studying the genes that cause facial abnormalities when mutated, reasoning that these genes were likely to play a part in the formation of facial features when expressed regularly. They report that by identifying the relationship between facial variation and gender, genomic ancestry and craniofacial genotype using bootstrapped response-based imputation modelling (BRIM), it is possible to predict the variation in facial features caused by the effects of a specific allele. Again, like the identical twin discrimination this technology is still some distance from being routinely used by forensic laboratories. However, both of these developments demonstrate the future potential for DNA analysis in human identification.

1.2 Research Questions

A decade ago, DNA evidence was routinely accepted in courts in England and Wales with very few challenges to the interpretation of the evidence; if a profile from the crime scene matched that of the suspect, it appeared to be an 'open-and-shut' case. However, as techniques surrounding DNA analysis have become steadily more sensitive, defence lawyers have become aware of the fallacies associated with this type of evidence. Now, it is not enough that a DNA profile from a crime scene is the same as that taken from a suspect; forensic scientists are expected to be able to answer questions such as when and how the DNA was deposited at the scene.

In order to be able to answer these questions, scientists need to have an understanding of the processes involved in, and the inherently variable nature of, transfer and persistence of DNA. The research question this thesis aims to address is whether the DNA recovered from a piece of evidence is representative of primary transfer of DNA (i.e. that person handled that specific item), and if so, is it indicative of the final handler?

In addition to understanding the mechanisms for how the DNA has been deposited, there also needs to be standardisation in the techniques used to collect any DNA that has been deposited, to enable accurate and informative interpretation of that evidence. Variation in the quantity and quality of DNA evidence has compelled governing bodies to undertake

research into optimal ‘best practice’ standards in the recovery and interpretation of DNA evidence. A further research question addressed by this thesis is which method of DNA recovery is optimal for firearms related evidence?

1.3 Research Aims and Objectives

1.3.1 Aims of the research

This research aims to:

- Evaluate the potential for transfer and persistence of trace levels of DNA;
- Establish a protocol for optimising the retrieval of DNA evidence from firearms and related paraphernalia.

1.3.2 Objectives of the research

- To determine the variability in an individuals’ tendency to deposit DNA onto inanimate objects;
- To evaluate any potential differences in DNA recovery methods in relation to DNA quantity and profile quality;
- To study the persistence of DNA on handled objects, especially after repeated contacts from different handlers;
- To study the potential for secondary transfer of DNA through an intermediary person or object;
- To develop a protocol for the retrieval and amplification of DNA from firearms and firearms related articles, such as spent cartridge cases;
- To determine the influence that firearms type, calibre or Gun Shot Residue (GSR) has on the ability to retrieve DNA information;

1.4 Significance of the Study and Original Contribution to Knowledge

As a direct result of the recommendations of the Silverman Report, published in 2011, to the Technology Strategy Board (TSB), the Forensic Science Special Interest Group (FoSci SIG) was formed. The purpose of the FoSci SIG was to enable communication and collaboration across the forensic community. To facilitate this, a challenge catalogue was developed, giving forensic scientists the opportunity to share issues that need to be overcome with the wider research community. Some of the challenges identified by the forensic community include improving biological sample collection, improving the quality of evidence resulting from 'touch' DNA and giving greater consideration to the problem of DNA persistence (Technology Strategy Board Network, 2011).

In 2012, the Association of Chief Police Officers (ACPO) published the 'Live-time Forensics Document', which provided an insight in to some of the key issues with the application of forensic science to criminal investigations (ACPO, 2012). One key aspect that was highlighted was the need for "development of the current procedures for the existing technology for locating and recovering biological evidence at scenes" (ACPO, 2012, p. 24).

A report published by the FoSci SIG in 2013 stated that it would be "extremely useful to determine whether the analysed nucleic acids were deposited as a result of direct physical contact…..or whether there has been an intermediate vector that has permitted transfer of the donor DNA to the item without any direct contact" (Forensic Science Special Interest Group, 2013, p. 48). This report also identified the importance of considering the potential for DNA persistence when interpreting DNA evidence.

These three publications highlight the potential impact this research could have on the collection and evaluation of evidence within the forensic science discipline. The findings of this research could contribute towards recommendations on how to recover and interpret DNA evidence produced by the Association of Forensic Science Providers (AFSP), the Centre for Applied Science and Technology (CAST) and the Association of Chief Police Officers (ACPO) Criminal Use of Firearms (ACPO CUF) Working Group. It is also hoped that this research could lead to the development of a statistical approach to predict which samples are likely to produce a DNA profile that can be loaded on to the NDNAD, which could inform best practice for DNA intelligence in the future.

1.5 Structure of the Thesis

This chapter has introduced the discipline of forensic DNA analysis, identified the importance of this evidence type in the forensic science arena and highlighted the main techniques, both current and emerging, that have resulted in this being one of the most prevalent types of evidence being utilised in criminal investigations across the globe. It has also identified some key research questions that need addressing to assist interpretation of DNA evidence and to attempt to resolve disputes in criminal courts.

Chapter 2 will introduce the key literature that has been published in the areas surrounding the research questions. The emphasis of this chapter will be on DNA recovered from handled items, DNA deposition, UK crime priorities and evidence recovery from firearms.

Chapter 3 outlines the standard techniques that have been utilised in the individual research studies undertaken.

Chapters 4 to 8 detail the scientific approaches, experimental results and discussions associated with the experiments that have been designed to answer the research questions outlined in Chapter 1. These chapters focus on research experiments undertaken to address questions related to DNA deposition, DNA recovery techniques, DNA transfer and persistence, and recovery of DNA evidence from firearms and spent cartridge cases.

Chapters 9 and 10 provide an overview of the main conclusions gathered from this research, including a philosophical discussion of the implications of this research to the wider arena of forensic science and beyond. Any recommendations that have arisen from the findings of this research will be outlined, as well as any other avenues for further development.

Two. Literature Review

2.1 DNA from Handled Items

2.1.1 Source of DNA on Handled Items

Epithelial cells cover the entire surface of the body and epithelial tissue found in the skin is known as stratified epithelium (The University of Western Cape, 1996). Stratified epithelial tissue is compiled of several layers of cells, as it needs to be able to withstand a large degree of sustained contact. Mammalian skin is made up of dry, keratinised, stratified epithelial – that is the top cells have usually lost their nuclei and instead contain a resistant protein called keratin (Figure 2.1).

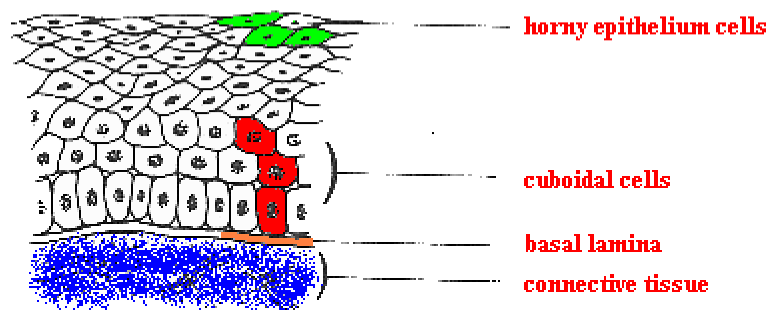


Figure 2.1 The stratified epithelium, as observed in mammalian skin (The University of Western Cape, 1996).

The epidermal layer (the outer layer of cells in the skin) renews itself continually as the basal cells undergo mitotic division and slow maturation (Kita et al., 2008). The process of epidermal differentiation starts with mitosis, resulting in the production of epidermal cells at the basal layer. These cells migrate towards the outer layers, changing shape and composition, resulting in the cytoplasm being released and keratin being incorporated. As they continue to move outwards, the cell nucleus degenerates and the keratinocytes become flatter. At the top of the cornified layer, where the cells have become anucleate (or nuclei-free) corneocytes, the cells are then shed or desquamated (Bhoelai, de Jong

and Sijen, 2013). The stratum corneum, the upper epidermal layer, is made up of terminally differentiated nuclei-free cells called corneocytes or horny epithelial cells (Balogh et al., 2003a). The loss of nuclei is related to apoptosis (programmed cell death) and the DNA within the cell is degraded during keratinisation (Kita et al., 2008).

DNA left behind when a surface has been handled is generally recognised to be epithelial cells that have been sloughed off and deposited onto a surface during contact (Williams and Johnson, 2008). Microscopic examination of stained latent fingerprints deposited on glass slides indicated the presence of nuclei-free corneocytes as well as nucleated cells (Alessandrini et al., 2003). Although the incidence of nucleated cells was minimal, Balogh et al. (2003a) indicated that there would be sufficient numbers present to enable generation of a DNA profile. Bohnert et al. (2001) studied the cells deposited on ligatures used in cases of hanging and strangulation and found that predominantly horny calls from the stratum corneum were recovered, as well as, on occasion, nuclei-containing basal cells. The authors also identified that extensive deposition of epithelial cells improved the success rate of DNA profiling.

Kita et al. (2008) identified both high molecular weight and degraded DNA present in samples tape lifted from human skin. A higher amount of degraded DNA, in relation to high molecular weight DNA, was recovered from swabs collected from the skin surface. Through immunoelectron microscopy, the authors demonstrated that the fragmented DNA was localised in the cornified layers of the epidermis.

Gršković et al. (2014) suggests that trace deposits contain DNA from corneocytes, anucleate keratinocytes, other nucleated cells and sweat containing cell-free DNA. However, more recently Zoppis et al. (2014) have suggested that the inference that the yield of DNA deposited onto a surface is related to the number of keratinocytes shed is merely an assumption, and has no scientific basis. In fact they suggest that sebaceous fluid is a more likely vector for DNA deposition. This is based on their observations that sebaceous glands, located over almost all of the body, produce sebum which would contain a mixture of cellular debris. Their research involved the detection of single stranded DNA using immunohistochemical techniques and their findings suggest the presence of this DNA in the majority of cells that are present in the sebaceous gland but not in the epidermis layers.

Research has been undertaken to try and identify the composition of contact traces using mRNA markers (Hanson et al., 2012; Bhoelai, de Jong and Sijen, 2013). The research published by Hanson et al (2012) aimed to identify novel messenger RNA (mRNA) biomarkers for the identification of human skin. The authors reported that they were able to identify five mRNA markers that exhibited a high degree of specificity for human skin and they were able to detect these markers in human skin samples with as little as 5 picograms (pg) of RNA present. However, although it was possible to detect all of these markers in skin samples and samples recovered from handled objects, none of the samples exhibited co-expression of all five markers at once. Therefore, more research is needed in order to understand the behaviour of nucleic acids, both RNA and DNA, in skin samples. Bhoelai, de Jong and Sijen (2013) hypothesised that based on the degree and duration of contact, different mRNA markers would be detected relating to the different epidermal layers present. They identified six markers that could be present in the various layers of the human epidermis, these being the basal, spinous, granular, translucent and cornified layers. However, their research findings suggested that it wasn't possible to differentiate between different degrees of contact (in this case incidental contact or grip contact) based on the presence of specific mRNA markers. An interesting point to note is that the two research groups examined different mRNA markers, with Hanson et al. (2012) focussing on late cornified envelope, interleukin 1 family member and chemokine ligand 27 genes, and Bhoelai, de Jong and Sijen (2013) analysing corneodesmosin, loricrin, filaggrin, keratin 9, involucrin and keratinocyte differentiation-associated protein genes. Therefore, although both research groups produced possible multiplex kits for identifying skin samples, there is currently no concordance between the different research groups as to the optimal markers to use for identification of human skin samples.

Other techniques that could be employed to assist in the determination of cell types from trace deposits have been suggested by Sijen (2014). In this article, a range of approaches including analysis of messenger RNAs, micro RNAs, DNA methylation and microbial markers have been reviewed in relation to their degree of success in identifying different biological traces. Although some of this research reviewed is still in its infancy, there are clear benefits of a combined approach and the use of these techniques could provide useful evidence for addressing source level questions. Currently, there is no standard mechanism utilised to determine the source of touch DNA. The informally agreed practice

currently is that when reporting Forensic Scientists present their evidence in court, if the DNA quantification reading is below a certain threshold (for one provider this value is 0.06 $ng.ml^{-1}$) they would state that the source could not be attributable, as such a low reading is not consistent with DNA from body fluids.

2.1.2 Variables impacting DNA deposition

The ability to recover DNA from epithelial cells when an item is handled by an individual was initially reported by van Oorschot and Jones in 1997. This resulted in an increased potential for DNA evidence in a vast number of different cases, including sexual assaults, rapes and murders, where previously DNA recovery had not been attempted or considered (Williamson, 2012). There are now numerous research studies available in the literature where DNA has been demonstrated to be recovered from handled items such as handbags, clothing, jewellery, weapons and car steering wheels (Findlay et al., 1997; Schulz and Reichert, 2000; Pizzamiglio et al., 2004b; Barbaro, Cormaci and Barbaro, 2006; Andrèasson et al., 2006; Petricevic, Bright and Cockerton, 2006; Franke, Augustin and Püschel, 2008; Sewell et al., 2008; Aditya et al., 2011).

In relation to case investigations, there are references in many publications to the DNA being recovered from handled items (Pizzamiglio et al., 2004a; Zamir, Cohen and Azoury, 2007; Taupin and Cwilklik, 2011). However the actual success rate may be lower than anticipated depending on sample type, as Williams and Johnson (2008) report that only 18% of samples collected from watch straps were successfully profiled by the Forensic Science Service (FSS). The general opinion is that DNA analysis can be used to determine if an individual has handled an item although caution must be heeded.

Another issue to be considered when interpreting profiles generated from handled items is the distinction between two terms that are often used interchangeably; touch DNA and trace DNA. Touch DNA refers to samples recovered from a handled item that may contain DNA from nucleated cells that have been transmitted from other areas of the body such as eyes, nose and mouth (Taupin and Cwiklik, 2011). Trace DNA refers to a DNA profile recovered from an evidential item where the source of the DNA (in terms of a specific body fluid) cannot be determined (Taupin and Cwiklik, 2011). Although to some

degree these definitions are overlapping, for the remainder of the report DNA recovered from samples that have been handled will be referred to as touch DNA.

Williamson (2012) highlighted the potential evidentiary value of DNA from handled items, but also identified several evidence types where collection of touch DNA would not be recommended, such as severely degraded samples, items that have been washed or that have been exposed to extreme environmental conditions or samples where one individuals' DNA is likely to be present in very high amounts thereby masking any other potential DNA sources. Williamson (2012) also suggested that items that have been contacted by multiple individuals, such as cash machines and lift buttons, are not likely to generate probative or interpretable DNA profiles.

When considering the ability to recover DNA evidence from handled items it is important to consider that the quantity and quality of this deposited DNA is not constant and there are many variables that could account for this disparity. These variables will be discussed in detail in Chapter 4.

2.2 United Kingdom Crime Statistics and Priorities

In 1995, the UK launched the first National DNA Database (NDNAD). Control samples, collected from individuals in the form of buccal swabs, are entered on to the NDNAD and are referred to as Criminal Justice (CJ) swabs (Thompson and Black, 2007). The legislation behind whose DNA is collected and the retention of DNA profiles on the NDNAD has recently undergone some major changes (National Policing Improvement Agency, 2011); discussion of this legislation is outside the remit of this research but can be found on the Home Office NDNAD Delivery Unit report entitled 'The NDNAD Strategy Board Policy for Access and Use of DNA Samples, Profiles and Associated Data' (Home Office, 2013a). Evidential samples recovered from crime scenes are referred to as Scene of Crime (SOC) samples. Profiles from SOC samples can be compared to the CJ samples stored in the database and any positive matches will be reported. Between the 1st April 2011 and 31st March 2012, the NDNAD produced 124 matches in murder investigations, 522 matches in rape investigations and 28,996 matches in the investigation of other crimes (National

Policing Improvement Agency, 2011). This indicates the huge part DNA evidence plays in criminal investigations.

The most recent Home Office crime statistics indicate that the number of recorded crimes where a firearm was reported to have been used was 11,227 in 2010/11 (Smith et al., 2012). This value is 13% less than the previous year, and is continuing the gradual decline in firearms offences since the peak in 2002/2003 (Figure 2.2). However, gun crime is still perceived to be a serious issue in the UK, especially with communities that have a prevalent gang culture, and is one of the identified threats of serious and organised crime identified in the National Strategic Assessment (NSA) (National Crime Agency, 2014).

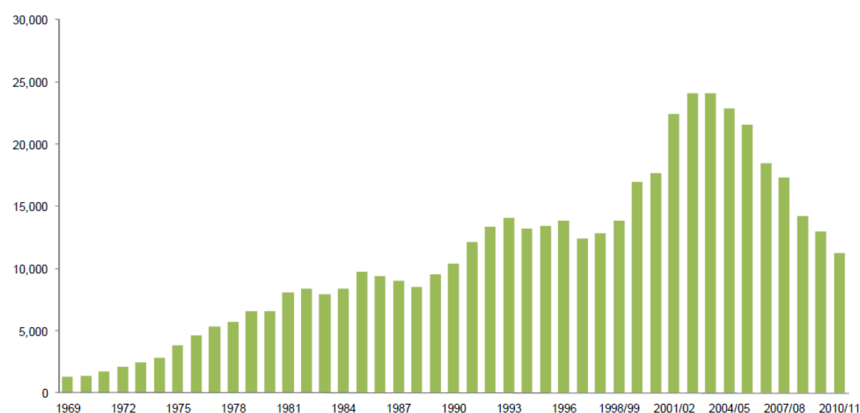
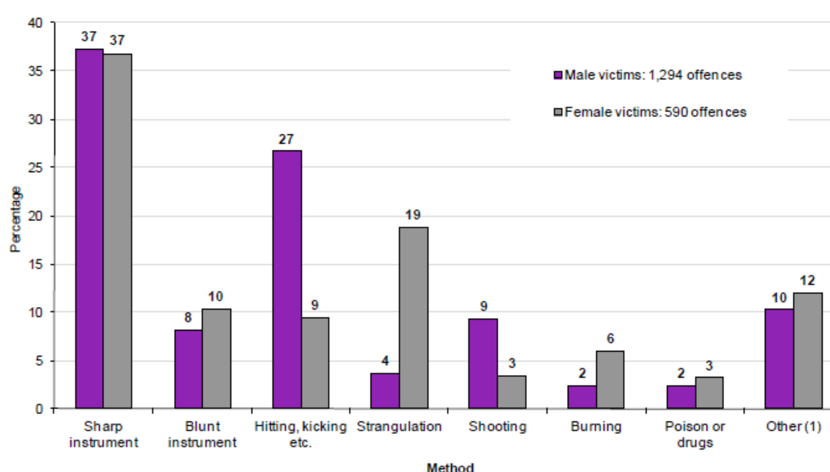


Figure 2.2 Offences recorded in which firearm use was reported, 1969 to 2010/11, England and Wales (Berman, 2012).

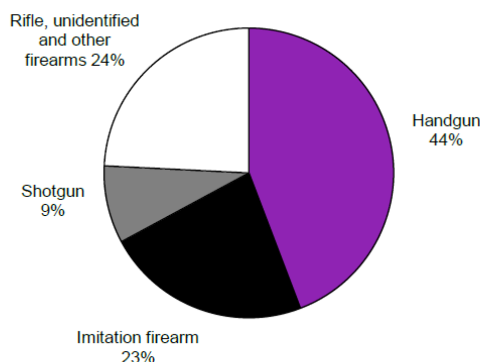
The majority of homicides recorded in the UK for 2010-2011 were predominantly caused by injuries from sharp instruments (Figure 2.3(a)) (Smith et al., 2012). However, shooting of the victim was still observed in a number of these cases. The National Crime Agency (NCA), preceded by the Serious Organised Crime Agency (SOCA), reports that firearms are continually the preferred weapon of in organised crime group disputes, with a strong link to drug related incidents (National Crime Agency, 2014).

One of the main reasons shooting is believed to be a popular choice of homicide is that the degree of contact between the assailant and victim(s) is minimal and the firearm user

can control multiple people from a safe distance (Matthews, 2002). Handguns (revolver and pistols) are generally more prevalent in firearms related offences (Mozayani and Noziglia, 2006) accounting for 44% of non-air weapon firearms offences recorded (Figure 2.3(b)) (Berman, 2012; Smith et al., 2012). The popularity of handguns could be a combination of availability, cost and ability to conceal the weapon (Matthews. 2002). The numbers of converted firearms (blank firing weapons modified to enable firing of bulleted ammunition) has increased. Between September 2003 and September 2008, a total of 8887 guns of all types were submitted, with 21% of those being converted weapons (Hannam, 2010).



(a)



(b)

Figure 2.3 (a) and (b). (a) Percentage of offences currently recorded as homicide by apparent method of killing and sex of victim, combined years 2008/9 to 2010/11. (b) Offences recorded by the police in which firearms were reported to have been used, by type of principle weapon (excluding air weapons), 2010/11 (Smith et al., 2012).

In 1920, legislation known as the Firearms Control Act was introduced which stated that the British 'right to bear arms' was no longer legal. A firearms certificate was required if you wished to purchase, possess, use or carry any type of firearm or ammunition (Squires, 2000). The term 'firearm' is defined by the Firearms Act 1968, s.57 as a "lethal barrelled weapon of any description from which any shot, bullet or missile can be discharged" (Hill, 1995).

The National Ballistics Intelligence Service (NaBIS) currently provides ACPO with information on the use of firearms in the UK, by examining ballistic material recovered from crime scenes and suspects to identify linked incidents, trends and emerging threats across the country. Even with this national intelligence, estimates of the number of legal weapons present in the UK are thought to be an imprecise picture of the actual gun problem, due to the scale of illegal distribution of firearms and the increased practice of converting blank firing guns to firearms capable of discharging live ammunition.

2.3 Firearms and Related Paraphernalia

Firearms generally fall into two broad categories – handguns and long guns. The main types of handgun available are revolver and pistols (which are either fully- or semi-automatic). Long guns include shotguns, rifles, machine guns and submachine guns (Mozayani and Noziglia, 2006; Hill, 1995).

Revolvers, first seen in their current form in 1870, rely on a revolving cylinder to hold cartridges and place them into the firing position. The capacity of the cylinder is generally 6 cartridges and once these have all been discharged, the spent cartridge cases will be manually ejected (Mozayani and Noziglia, 2006; Hill, 1995).

Self-loading pistols do not have a revolving cylinder; instead the cartridges are stored in a magazine in the handle of the weapon (Hill, 1995). The first round of ammunition is placed into the barrel by pulling back and releasing the slide. The weapon uses the energy of the discharged ammunition to automatically eject the fired cartridge case and the next round of ammunition is then fed into the barrel ready for firing (Heard, 1997; Warlow, 1996).

Rifles have a long barrel with spiral grooves running along the length of the inside of the barrel. This enables a higher degree of accuracy when firing. Rifles are single shot, or magazine fed in the case of bolt action rifles. The spent cartridges are ejected manually or semi-automatically depending on the rifle action (Hill, 1995; Heard, 1997).

Shotguns are smooth bore, meaning the barrel contains no lands or grooves (Mozayani and Noziglia, 2006; Hill, 1995; Heard, 1997). They can be either single- or double-barrelled weapons and spent cartridges are manually ejected after firing.

Machine and submachine guns are generally designed for military use, and can rapidly fire continuous rounds of ammunition in a short space of time (Heard, 1997; Warlow, 1996).

The ammunition for firearms consists of the bullet and the cartridge case. Cartridge cases are a metallic casing containing a primer, a propellant and a projectile (Figure 2.4) (Mozayani and Noziglia, 2006). The bullet is the projectile driven out of the weapon by the propellant (Hill, 1995; Heard, 1997).

Cartridge cases are predominantly made of brass with a 75:25 Copper/Zinc alloy (Heard, 1997). They are usually one of three shapes – straight cased, bottle-necked or tapered case. Shotgun cartridge cases are generally composed of a brass base with a plastic casing (Figure 2.4).

The primer is struck by the hammer during the firing action producing a flame which acts as the means for igniting the propellant (Heard, 1997; Warlow, 1996). Predominantly the primer is made up of barium nitrate, lead styphnate and antimony trisulphide (Warlow, 1996).

The propellant (referred to as gunpowder in Figure 2.4) is a mixture of chemicals that, when ignited, produces a huge amount of gas which forces the projectile along and out of the barrel (Heard, 1997).

The projectile, or bullet, is most commonly lead or lead alloy, combining antimony and tin with the lead. Jacketed bullets will consist of a lead core, and a coating of Copper/Zinc alloy (Heard, 1997).

Shotgun cartridges contain both the propellant and the shot, separated by a layer of wadding. The shot is usually a number of small lead or steel balls, or a solid slug, dependant on the type used (Hill, 1995).

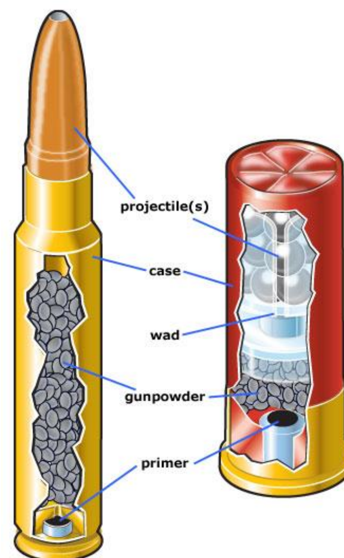


Figure 2.4 Components of ammunition. The cartridge on the left is typical rifle ammunition. The cartridge on the left is a shotgun cartridge (Michigan Department of Natural Resources, 2011).

2.4 Identification Information from Firearms and Cartridge Cases

One of the benefits of shooting as a mode of killing, from the perspective of the assailant, is that the weapon can be discharged from a distance. This means the potential for evidence of the assailant to be left behind at the crime scene is reduced, when compared to other methods such as stabbing or beating. This may also result in limited forensic evidence such as biological or other trace evidence being present on the firearm.

Spent cartridges are often found at crime scenes were firearms have been discharged. In 1996, it was reported that spent cartridge cases were recovered from scenes in 80 – 90% of terrorist shooting incidents in Northern Ireland (Bentsen, 1996). Spent cartridge casings can be used to connect a weapon to a crime, utilising marks made by the

mechanism of the weapon, or to connect a weapon to a person, using latent fingerprints. However, the enhancement of fingerprints on spent cartridge cases is often unsuccessful, especially with smaller calibre cartridges.

Given (1976) first investigated the effect that firing a cartridge had on the quality of a latent fingerprint in 1976. He found that the effects of detonation, more specifically the blowback of hot gases on the external surface of the cartridge case, seriously degraded the fingerprints deposited.

Bentsen et al. (1996) examined the quality of fingerprints post firing and found that several factors could result in poor fingerprint quality. The physical contact that occurred during the loading and ejection of the case, as well as issues relating to the gaseous blowback appeared to contribute to fingerprint damage. The authors also suggested the reduced area present for fingerprint deposition on smaller cartridge cases could impact on successful identification.

Several different research groups have looked into optimising the enhancement methods used, including metal vapour deposition (Migron et al., 1998), dusting with magnetic fingerprint powder (Freeman, 1999), 3-Dimensional laser topography (Kinder and Nys, 2000) and a range of other methods (Williams, McMurray and Worsley, 2001; Bersellini et al., 2001; Bond and Phil, 2008; Bond, Phil and Heidel, 2009; Bond and Phil, 2009; Edminston and Johnson, 2009; Lévesque and Bond, 2011). However, the success rate for identification of fingerprints recovered from spent cartridge cases remains relatively low.

The findings of research carried out by Barnum and Klasey (1997) reported that the success rate for identification of fingerprints on firearms was also very low, with identifiable prints being obtained in only 10% of cases. Some suggestions for potential enhancement techniques were proposed by the authors in a later publication (Klasey and Barnum, 2000).

In cases where it is not possible to identify the individual who handled the cartridges prior to firing through finger marks (either due to poor ridge detail or smudging), an alternative method of identification would be required. DNA profiling has the potential to individualise and offers some level of persistence, through DNA deposition whilst loading the weapon. However, the processes that the cartridge undergoes during the firing

process are not conducive to the survival of DNA. High temperatures and high pressures experienced as a result of firing the weapon, as well as the presence of a mixture of chemicals in the form of gunshot residue (GSR), may inhibit DNA profiling success. This will be discussed in detail in Chapters 7 and 8.

Three. Methods

The methods outlined in this chapter were used to conduct the research outlined in Chapters 4, 5, 6, 7 and 8. Any modifications to these methods will be identified in the individual chapters. The methods were selected based on their widespread use both in published research in the field of forensic DNA analysis, as well as their semblance to standard operating procedures within Forensic Provider laboratories in England and Wales.

During all laboratory work the researcher wore appropriate personal protective clothing (laboratory coat, double gloves, hair net, and face mask), which was changed regularly throughout all experiments. All pieces of equipment were autoclaved where possible (121 ^{o}C at 1 bar excess pressure), wiped with 2% Virkon (bactericide, fungicide and virucide), 96-100% ethanol and double distilled water to remove any contaminating DNA from the surface. Aerosol barrier pipette tips were used throughout all experiments to prevent cross-contamination between samples.

3.1 DNA Deposition

DNA was deposited onto the target surfaces by either seeding with a standard saliva solution, or handling for a specified duration of time. Specific details of the method utilised in each experiment will be detailed in later chapters.

3.2 DNA Collection

3.2.1 Buccal Swabs

Control DNA profiles were retrieved from the subject by collecting a buccal swab from the inside of the mouth. The serrated edge of the buccal swab (Whatman Sterile Omni Swab) was scraped on the inside of the cheek 15 times to collect oral epithelial cells. The

serrated end of the buccal swab was ejected into a 2 ml Eppendorf tube for DNA extraction.

Where stated in specific experiments, buccal swabs were used to collect DNA from subjects' hands. The buccal swab was pre-wetted by immersing in molecular grade water (Eppendorf) for 10 seconds and then scraped across the palm of the subjects hand twenty times (unless stated otherwise). The serrated end of the buccal swab was then ejected into a 2 ml Eppendorf tube for DNA extraction.

3.2.2 Swabbing with Cotton Swabs

Where stated, cotton swabs were used to retrieve DNA from substrates. The double swabbing technique, as recommended by Sweet et al. (1997), was used to maximise DNA retrieval. This technique involved immersing one cotton swab in molecular grade water for 10 seconds then wiping this pre-wetted swab over the surface of the object ten times (unless stated otherwise). This was followed by wiping the same surface ten times with a second, dry cotton swab. The cotton ends of these two swabs were removed using sterile scissors and were placed together into a single 1.5 ml Eppendorf tube for DNA extraction.

3.2.3 Swabbing with Cotton Square

Where stated, 1 cm^2 squares of cotton wool were used to collect DNA from specific objects. The double swabbing technique was also used in these instances in order to maximise DNA retrieval. This technique involved immersing one cotton square in molecular grade water for 10 seconds then wiping this pre-wetted square over the surface of the object ten times (unless stated otherwise), using sterile forceps. This was followed by wiping the same surface ten times with a second, dry cotton square. The two cotton squares were then placed together into a single 1.5 ml Eppendorf tube for DNA extraction.

3.2.4 Mini-Taping

Where stated, DNA was retrieved from specific objects using tape strips, as recommended by personnel at the DNA department of Strathclyde Police Force (Murray, 2005), and as outlined by Li and Harris (2003) and Wickenheiser (1996). The strips were prepared as detailed in Appendix One. After applying the tape to the object 20 times, in order to collect any DNA present, the strip of tape was then cut up into 4 mm^2 pieces using sterile scissors and all of the pieces were placed together, using sterile forceps, into a single 1.5 ml Eppendorf tube for DNA extraction.

3.3 DNA Extraction

3.3.1 Chelex Extraction

Chelex extraction was carried out using Chelex® 100 Chelating Ion Exchange Resin (Bio-Rad). The general method for Chelex extraction is detailed below, with any modifications from this method detailed when employed.

a. 1 ml of sterile double distilled water was added to the sample (buccal swab/cotton swab/cotton square/tape). The sample was vortexed briefly and was then incubated at room temperature for 30 minutes, vortexing occasionally.
b. The swab/tape was removed and the remaining supernatant was centrifuged at 20,000 x *g* for 3 minutes. The supernatant was discarded (into 10% v/v bleach solution), leaving approximately 20 µl without disturbing the pellet.
c. Using a 200 µl tip with the end 5 mm cut off, 180 µl of a 5% v/v Chelex solution was added to the sample.
d. 2 µl of Proteinase K (10 $mg.ml^{-1}$) was added, the sample was vortexed briefly and then incubated at 56 ^{o}C for 30 minutes.
e. The sample was then incubated at 100 ^{o}C for 8 minutes and centrifuged at 20,000 x *g* for 3 minutes.
f. 150 µl of the supernatant was removed and aliquoted into a labelled 1.5 ml Eppendorf. This DNA extract was stored at -20 ^{o}C until required.

The protocol for preparing the 5% v/v Chelex Solution can be found in Appendix Two.

3.3.2 Qiagen Spin Column Extraction

Where stated samples were extracted using the QIAamp® DNA Mini Kit spin column (Qiagen), according to the manufacturer's Buccal Swab Spin Protocol.

a. The sample (buccal swab/cotton swab/cotton square/tape) was placed into a 2 ml eppendorf tube. 400 µl PBS (Phosphate Buffered Saline) was added to the cotton swab, cotton square or tape, and 600 µl PBS was added to the buccal swab.
b. 20 µl Proteinase K was added to each sample. 400 µl Buffer AL was added to the cotton swab, cotton square or tape, and 600 µl Buffer AL was added to the buccal swab. The sample was then mixed immediately by vortexing for 15 seconds.
c. The sample was incubated at 56 ^{o}C for 10 minutes, then briefly centrifuged to remove any drops from the inside of the lid.
d. 400 µl of 96-100% ethanol was added to the cotton swab, cotton square or tape, and 600 µl of 96-100% ethanol was added to the buccal swab. The sample was then mixed by vortexing, then centrifuged to remove any drops from the inside of the lid.
e. 700 µl of the mixture from step d was added to the QIAamp Spin Column (in a 2 ml collection tube) without wetting the rim, the cap was closed and centrifuged at 6,000 x *g* for 1 minute. The QIAamp Spin Column was placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded.
f. Step e was repeated by applying up to 700 µl of the remaining mixture from step d to the Spin Column.
g. The QIAamp Spin Column was carefully opened and 500 µl Buffer AW1 was added without wetting the rim. The cap was closed and the sample was centrifuged at 6,000 x *g* for 1 minute. The QIAamp Spin Column was placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded.
h. The QIAamp Spin Column was carefully opened and 500 µl Buffer AW2 was added without wetting the rim. The cap was closed and the sample was centrifuged at full speed (20,000 x *g*) for 3 minutes.

i. The QIAamp Spin Column was placed in a clean 1.5 ml microcentrifuge tube and the collection tube containing the filtrate was discarded. The QIAamp Spin Column was carefully opened and 150 µl of double distilled water was added. The tube was incubated at room temperature for 3 minutes and then centrifuged at 6,000 x *g* for 1 minute.
j. The filtrate (DNA extract) was then stored at -20 ^{o}C until required.

3.4 DNA Quantification

DNA quantification was performed using the Quant-iT™ Assays on the Qubit™ Fluorometer (both Invitrogen). For all reference/control DNA samples, the Quant-iT™ Broad Range buffer was used and for all other samples, the Quant-iT™ High Sensitivity buffer was used.

3.4.1 Preparation of Calibration Standards for DNA Quantification

400 µl of the Quant-iT™ Working Solution was prepared by diluting the Quant-iT™ reagent 1:200 in Quant-iT™ buffer. 190 µl of the Quant-iT™ Working Solution was then aliquoted into two thin-wall, clear 0.5 mL Qubit™ assay tubes and 10 µl of the appropriate Quant-iT™ Standard was added (Standard #1 to Tube One and Standard #2 to Tube Two, respectively). The tube was then briefly vortexed and incubated for 2 minutes at room temperature. The two standards were then placed into the Qubit™ Fluorometer, as directed, to complete the two point calibration prior to the reading of the reference and other samples.

3.4.2 Preparation of Samples for DNA Quantification

Once calibration of the Qubit™ Fluorometer was complete, 200 µl of the Quant-iT™ Working Solution per sample was prepared by diluting the Quant-iT™ reagent 1:200 in Quant-iT™ buffer. 190 µl of the Quant-iT™ Working Solution was then aliquoted into a thin-wall, clear 0.5 mL Qubit™ assay tube and 10 µl of the appropriate sample was then

added. The tube was briefly vortexed and incubated for 2 minutes at room temperature. The sample tubes were then placed into the Qubit™ Fluorometer, as directed, and a sample reading was taken. All readings were multiplied by a dilution factor of 20 to calculate the concentration of the original sample.

3.5 DNA Profiling

3.5.1 DNA Amplification

DNA amplification was performed using the AmpF/STR® SGM Plus® kit and the AmpF/STR® Identifiler® Kit (both Applied Biosystems).

3.5.1.1 Preparation of Samples for DNA Amplification

3.8 µl of the extracted DNA sample was added to 6.2 µl of the PCR mastermix in prelabelled 0.2 µl PCR tubes. The components of the PCR mastermix are detailed in Table 3.1.

Table 3.1 Components of PCR Mastermix.

Reagents	Volume per Sample (µl)	Number of samples	Total Volume Required (µl)
AmpF/STR® PCR Reaction Mix	4.0	n + 2	4.0 x (n + 2)
AmpF/STR® SGM Plus/Identifiler Primer Set	2.0	n + 2	2.0 x (n + 2)
AmpliTaq Gold® DNA Polymerase	0.2	n + 2	0.2 x (n + 2)

It was necessary to prepare n + 2 reactions as for each set of PCR reactions, as it was important to include a positive control (1.9 µl AmpF*l*STR® Control DNA 007 plus 1.9 µl Molecular Grade Water, in place of a DNA extract) and a negative control (3.8 µl Molecular Grade Water, in place of a DNA extract).

3.5.1.2 PCR Thermal Cyclers

Several different PCR Thermal Cyclers were used for PCR amplification throughout this research. These were:

- Tetrad® Peltier Thermal Cycler (Bio-Rad Laboratories, Inc.)
- MJ Research PTC-200 Peltier Thermal Cycle (Bio-Rad Laboratories, Inc.)
- AB GeneAmp PCR System 2400 (Applied Biosystems)

3.5.1.3 Standard PCR Amplification Cycle Parameters

The samples were placed in the PCR Thermal Cycler and the DNA was amplified using the following cycling conditions:

1 cycle 95 ^{o}C for 11 minutes

28 cycles 94 ^{o}C for 1 minute

59 ^{o}C for 1 minute

72 ^{o}C for 1 minute

1 cycle 60 ^{o}C for 45 minutes

Hold 4 ^{o}C indefinitely

Unless otherwise stated PCR amplification was carried using the standard 28 cycle parameters.

3.5.1.4 Low Copy Number (LCN) Amplification Cycle Parameters

In cases where additional cycles were required, for example when trace levels of DNA were present, Low Copy Number (LCN) amplification was utilised. LCN amplification involved additional cycles of amplification, with the following cycling conditions being utilised:

1 cycle	95 ^{o}C for 11 minutes
34 cycles	94 ^{o}C for 1 minute
	59 ^{o}C for 1 minute
	72 ^{o}C for 1 minute
1 cycle	60 ^{o}C for 45 minutes
Hold	4 ^{o}C indefinitely

3.5.2 Capillary Electrophoresis

The ABI PRISM™ 310 Genetic Analyser and the ABI PRISM™ 3500 Genetic Analyser (both Applied Biosystems) were used to separate the fragments of amplified DNA. The system was prepared for sample analysis prior to use according to the manufacturer's guidelines.

3.5.2.1 AB 310 Sample Preparation

A 2 µl aliquot of amplified PCR product was added to 25 µl of the 310 mastermix in 0.5 ml prelabelled genetic analyser tubes. The 310 mastermix was prepared as stated in Table 3.2.

Table 3.2 Components of the 310 mastermix.

Reagents	Volume per Sample (µl)	Number of samples	Total Volume Required (µl)
Deionised Formamide (Applied Biosystems)	24.5	n + 2	24.5 x (n + 1)
GS500-ROX Internal Size Standard (Applied Biosystems)	0.5	n + 2	0.5 x (n + 1)

It was necessary to prepare n + 1 reactions as for each set of profiling reactions, as it was important to include an allelic ladder (AmpF/STR® SGM Plus/Identifiler Allelic Ladder) for each run.

The samples were heat denatured at 95 ^{o}C for 3 minutes, immediately followed by cooling on ice for 5 minutes. Samples were then placed into the 48 well autosampler tray and positioned in the machine.

The 310 Genetic Analyser used Performance Optimised Polymer 4 (POP-4) (Applied Biosystems). The conditions utilised were 30 minute run duration, specific analysis parameters and a 5 second injection.

3.5.2.2 AB 3500 Sample Preparation

A 1 µl aliquot of amplified PCR product was added to 9 µl of the 3500 mastermix in 0.2 ml prelabelled eppendorf tubes. The 3500 mastermix was prepared as stated in Table 3.3.

Table 3.3 Components of the 3500 mastermix.

Reagents	Volume per Sample (µl)	Number of samples	Total Volume Required (µl)
Deionised Formamide (Applied Biosystems)	8.8	n + 2	8.8 x (n + 1)
GS500-ROX Internal Size Standard (Applied Biosystems)	0.2	n + 2	0.2 x (n + 1)

It was necessary to prepare n + 1 reactions as for each set of profiling reactions, as it was important to include an allelic ladder (AmpF/STR® SGM Plus/Identifiler Allelic Ladder) for each run.

The samples were heat denatured at 95 ^{o}C for 3 minutes, immediately followed by cooling on ice for 5 minutes. Samples were then placed into a 96 well plate and positioned in the machine.

The 3500 Genetic Analyser used Performance Optimised Polymer 4 (POP-4) (Applied Biosystems). The conditions utilised were a capillary length of 36 cm, specific analysis parameters and a HID (Human Identification) plate type.

3.5.3 Profile Electropherogram Analysis

The results from the capillary electrophoresis were presented in electropherogram format. The electropherogram was analysed using GeneMapper® ID-X Software. The sample type was assigned (either sample, allelic ladder, positive control or negative control) and the ROX in each sample was assigned the correct standard sizes (75, 100, 139, 150, 160, 200, 300, 340, 400).

All peaks with a peak height of over 50 Relative Fluorescence Units (RFU) were recognised as profilable by the 310 Genetic Analyser, and all peaks with a peak height of over 100 RFU were recognised by the 3500 Genetic Analyser. The Genetic Analyser automatically

assigns the repeat number to the peak, by analysing the repeat numbers included in the allelic ladder and comparing the values. Where possible the electropherogram results were presented in tabular form in this report, with exemplar electropherograms included to demonstrate data quality or the presence of specific profile phenomena.

3.5.4 Data Analysis

Where applicable statistical comparisons were performed with SPSS v22.0 (SPSS Inc.) using the Kolmogorov Smirnov (KS) and Shapiro-Wilk test for normal distribution followed by significance testing with a 95% confidence interval.

Four. DNA Deposition

4.1 Introduction to DNA Deposition

As outlined in Chapter 2, a handled item has the potential to retain sufficient DNA to generate a DNA profile, thereby enabling identification of the handler. However, the mechanisms by which DNA is deposited onto a handled item are complex and the scientific principles governing DNA deposition are not completely understood. Variables that have been identified as potentially impacting on the amount of DNA deposited onto a surface during handling include surface type, gender, age, contact time, degree of contact (i.e. friction), an individuals' tendency to 'shed' DNA and the time since hands have been washed. Other variables that could influence the success of DNA profiling from touch DNA could include any enhancement methods applied, the duration since deposition, the environmental conditions and the subsequent recovery and analysis methods utilised.

Gršković et al. (2014) evaluated the effect that donor age, gender, handling time and surface type had on the quantity of DNA recovered from a handled object. Their experiments compared the concentration of DNA recovered from paper, plastic and plastic-covered metal after one, two and five minutes of handling. Donors were classified according to gender and age (grouped from 25-34, 35-44 and 45-54). Their results suggested that higher concentrations of DNA were recovered from plastic and plastic-coated metal surfaces, that the handling time had no significant impact on DNA concentration, that men tended to deposit more DNA than women and that there was no statistically significant difference between the concentration of DNA deposited by different age groups. However, their findings are not completely in agreement with other research groups results previously published.

Research undertaken by Daly Murphy and McDermott (2012) suggested that there was no statistically significant difference observed between the amount of DNA deposited by men and women. These findings are supported by those reported by Lowe et al. (2002) and Raymond (2010). Research undertaken by van Daly, Murphy and McDermott (2012) suggested that there was a significant difference in the amount of DNA deposited depending on the surface type, although they used difference substrates than those

utilised by Gršković et al. (2014). Pesaresi et al. (2003) also identified that the substrate surface may influence the potential for successful DNA profiling, as differing quantities of DNA and qualities of profile were recovered from wood, metal and glass surfaces. Possible explanations for this variation in DNA deposition due to substrate type have included the variation in the incidence in perspiration, the variation in ability to recover the DNA (based on absorbency of the surface) and the propensity for DNA to be inadvertently removed during the handling process.

Wickenheiser (2002) and Alessandrini et al. (2003) both reported that the amount of DNA deposited was independent of handling time, which has been supported by the findings of Gršković et al. (2014). In contrast, Raymond (2010) observed a positive correlation between the time an item was held and the average DNA quantity recovered, although the research time periods in this study were considerably greater than any of the others reported (with a maximum duration of 1 week contact). Linacre et al. (2010) also suggested that there is a correlation between the amount of cellular material transferred and the contact time and pressure applied.

It is also important to note that the research published by Gršković et al. (2014) was solely looking at quantification values, not profile quality, whereas some of the other research used profile percentage as a measure of DNA deposition.

Sandoval et al. (2013) have suggested that the potential for the successful recovery of a DNA profile from a handled object depends on “the shedder status of the individual, the hand used, the activities of the individual prior to contact and the nature of the surface”. The authors suggest that the degree of contact would also play a part, as they predicted that if the donor used their dominant hand, they would be able to impart a stronger grip and therefore deposit a great number of skin cells. Research undertaken by Sandoval et al. (2013) into the potential for recovering male DNA after simulation of an assault on a female revealed that no useable profiles were produced using either the AmpFlSTR® Identifiler® PCR Amplification Kit or the Yfiler™ Kit. The Yfiler™ kit has been designed specifically to enable detection of male DNA components in a sample that predominantly consists of female DNA. They did however report that, when comparing DNA yields from grab and struggle scenarios, the amount of DNA recovered was greater when there was an increased degree of contact (i.e. the struggle scenario).

In relation to the quantity of DNA deposited, Sandoval et al. (2013) state that 'cellular material and associated DNA from contacts often involved no more than five to six epitheliam cells". van Oorschot and Jones (1997) reported that swabs from hands yielded between 2 and 150 nanoggrams (ng) DNA, whereas handled items yielded between 1.1 and 75 ng DNA. Furthermore, they stated that there was no significant increase in the amount of DNA deposited as a result of increased contact time. Prinz et al. (2006) quantified DNA from 109 touched samples and reported that in 57.8% of the samples, less than 40 pg of DNA was recovered. Quinones and Daniel (2012) reported that an average yield of 11.5 ng DNA could be recovered from 1 mL cell-free sweat samples (cells removed through repeated centrifugation). The authors propose that as a result of the keratinisation process the epidermal cells deposited are enucleated (contain no nuclear material). Therefore, although keratinocytes may have residual amounts of DNA present, the origin of the majority of the DNA recovered from handled items could be from cell free nucleic acids (CNAs) present in sweat. The authors' results indicate that DNA could be transferred from both nucleated cells and CNAs present in sweat.

A possible method that could be used to indicate the presence, and possible amount, of DNA within a fingermark has been proposed by Haines et al (2013). They report that by applying a fluorescent dye that intercalates between the DNA backbone, such as SYBR® Green I or GelGreen™, to fingermarks it was possible to detect latent DNA. Although this may be a useful tool to predict the likelihood of a handled item yielding a DNA profile, more research would need to be carried out to determine if there is any correlation between the degree of fluorescence and the quantity of DNA present. It would also be necessary to ensure that the DNA being detected is human DNA and not bacterial DNA.

Variations in the amount of DNA recovered from handled items led to the definition of 'Shedder Status'. The concept of an individuals' shedder status was introduced in 2002 by Lowe et al. Lowe et al. (2002) defined a good shedder as an individual who deposited sufficient DNA 15 minutes after hand washing to produce a full DNA profile, and denoted individuals who produced only a partial profile, under the same conditions, to be a poor shedder. This definition was adopted by other researchers undertaking experiments involving DNA from handled items (Linacre et al., 2010; Murray et al., 2002). However, a criticism with this system is there is no differentiation between a partial profile where

10% of the alleles are present, and one where 90% of the alleles are present, although clearly the evidential value of the two samples is very different.

Other researchers have reported their own definitions for what constitutes a good or poor shedder. Allen et al. (2008) divided shedder status in to light, intermediate and heavy. An individual who deposited less than 50 pg DNA (which resulted in a negative to poor profile being produced) would be classified as a light shedder and an individual who deposited between 50 – 300 pg DNA would be an intermediate shedder, as this would generate somewhere between 10 and 80% of the complete DNA profile. A heavy shedder would be an individual who deposited more than 300 pg DNA, which would be sufficient to generate over 80% of the complete DNA profile. Criticisms with this particular system are that it relies on quantification systems generating accurate values and it does not account for the stochastic fluctuation known to affect DNA samples containing less than 100 pg DNA.

Alessandrini et al. (2003) proposed that an individuals' shedders status depends on the number of nuclei-free corneocytes present in their stratum corneum. The same authors proposed that differences in the amount of DNA deposited could be due to a combination of external factors, such as the surface area of contact, and a variety of internal factors. These internal factors could include an increased turnover of epidermal maturation as a result of the overexpression of epidermal growth factors, as well as other factors that may influence the keratinocyte cycle times. Lowe et al. (2003) suggested that an individuals' propensity for depositing DNA was reproducible; good shedders would consistently deposit a full DNA profile after only a 10 second contact whereas poor shedders deposited very little DNA. Decorte (2010) states that only 60% of the population shed sufficient skin cells to enable generation of a DNA profile. Rutty, Hopwood and Tucker (2003) stated that an estimated 45% of the population can be categorised as good shedders, whilst 70% of the population are likely to deposit sufficient amounts of DNA to generate over 70% of their complete DNA profile. Results reported by Allen et al. (2008) suggest that a partial or complete profile may be generated from touch evidence from approximately 80% of the population.

Pesaresi et al. (2003) reported that both clean and unwashed hands produced profiles that were either negative (no alleles present), partial (not all alleles present) or full

profiles (all alleles present). There was a decreased incidence in full profiles when hands were washed prior to the experiments. This was supported by Alessandrini et al. (2003) who stated that hand washing prior to contact drastically reduced the potential for a useable DNA profile to be recovered.

van Oorschot et al. (2003) identified that the amount of DNA deposited was dependent on a range of variables, including the individual and the area of contact. The authors also suggested caution when interpreting quantification results of trace amounts of DNA as the accuracy of available techniques is limited and positive DNA profiling results were observed when a negative quantification result had been recorded.

Zamir, Springer and Glattstein (2000) found that fingerprint enhancement techniques didn't have an adverse effect of the quality of DNA profile produced. They utilised several different enhancement techniques, including alternative light source (ALS), crystal violet staining and cyanoacrylate fuming followed by basic yellow staining. Their findings are in general agreement with Pesaresi et al. (2003), Schulz and Reichert (2002), Raymond et al. (2004), Balogh et al. (2003b), van Hoofstat et al. (1999) and Lowe et al. (2003).

Schulz and Reichert (2002) determined that it was possible to recover profiles from fingerprints enhanced with soot or magnetic powder, as well as from the adhesive tape used to lift the enhanced print. However, although it could be determined that the enhancement technique did not interfere with the DNA analysis, the success rates for producing a DNA profile were relatively poor (29% profiled successfully when directly swabbed, 19% profiled successfully when extracted from adhesive tape). It is also important to note that in this research the authors only amplified one locus, FGA. Raymond et al. (2004) examined several different fingerprint enhancement techniques and determined the recovery of DNA is more dependent on surface type as opposed to recovery technique, which is in agreement with the findings of Pesaresi et al. (2003), but contradicts the findings of Lenz et al. (2006). The authors reported that plastic, glass and adhesive tape had a limited impact on the success of DNA analysis, whereas paper and aluminium foil produced negligible DNA results. They also highlighted the issue, which has already been discussed in this section, of the variation in the amount of DNA deposited by individuals.

Balogh et al. (2003) found that chemical enhancement of fingerprints on paper (using Ninhydrin and iodine) had a detrimental impact on DNA profiling success rates (only 47% of samples were successfully profiled). They also identified that the proportion of the DNA profile that was successfully typed was not dependant on the amount of contact time between an individual and the surface. Interestingly, the authors also reported that increasing the amplification cycle number in their analysis (to 38 cycles) did not increase the incidence of amplification artefacts or stutter products, which is not supported elsewhere in the literature.

van Hoofstat et al. (1999) reported that although some fingerprint enhancement techniques had no adverse effect on the DNA profile produced, contamination could be introduced to samples through the use of dactyloscopic (fingerprint) powders. They reported that there was a variation in the quality of DNA profile dependent on the type of powder used; metallic powders completely inhibited DNA profiling, whereas magnetic, white and black powders produced full profiles (albeit with varying peak intensity). Lowe et al. (2003) investigated the effect of a wide range of enhancement techniques and demonstrated that although DNA profiles could be obtained after treatment with all of the techniques applied, in some cases as little as 33% of the donor profile was recovered.

Popa, Potorac and Preda(2010) demonstrated, in their fingerprint research, that the constituents of an epithelial cell degrade over time, as a result of both the substrate they are deposited onto as well as the environmental conditions they are exposed to. They also demonstrated the quantity of DNA recovered from a finger-mark significantly decreased over time.

Stouder et al. (2001) reported that the method of DNA recovery impacted on the quantity of DNA recovered, as did the item the DNA was recovered from. During their research, DNA swabbed from the inside of a pillbox (a small, round metal or cardboard box routinely used for storing collected trace evidence such as hairs and fibres) containing the debris scraped from the surface was compared to DNA recovered by direct swabbing of the surface, and the amount of DNA recovered was greater using the pillbox collection technique. The authors also reported variation in the amount of DNA recovered from different items of evidential clothing, with more DNA being recovered, on average, from t-shirts over hosiery items.

Lenz et al. (2006) determined that the DNA recovery technique used could account for differences in success rates. However, they didn't consider the variability in the amount of DNA deposited in the initial instance therefore their conclusions that the difference in success rates "could be contributed only to the sampling technique...." should be considered with caution. The impact that recovery method has on the success of DNA profiling has been discussed in greater detail in Chapter 5 of this thesis.

In conclusion, although much research has been carried out into DNA deposition, the factors that determine the quantity and quality of DNA deposited on a surface are extremely complex and incompletely defined. It also appears that the assignation of shedder status is somewhat arbitrary, and the degree to which this designated status would impact on casework interpretations is unknown. The key aspect to consider when studying DNA from epithelial cells is that the samples are such that the amount of DNA is sufficiently low that any of the phenomena outlined in Chapter 1.1 could be observed.

In comparing the research carried out by different authors, a clear distinction can be seen in what one considers to be successful DNA profiling. Some authors deemed incomplete profiles to be unsuccessful, whereas others considered a partial profile to be successful as some form of identification information could be gained from such results.

Finally, a significant challenge when comparing research reported by different groups is that many of the publications report the use of different numbers of donors, different variables and different methods for analysis (deposition, recovery, extraction, quantification, and amplification) which makes direct comparison between research studies almost impossible. Furthermore, and arguably the most significant issue that is often not considered when attempting to evaluate the effect that specific variables have on the amount of DNA deposited, is that the methods used to collect, extract, quantify and amplify the DNA may have more of an impact on DNA success rates than any other variable discussed here. Most publications compare 'DNA deposition' quantification values or percentage profiles, when in fact they are most likely comparing 'DNA recovery' or 'DNA extraction' yields. It is not possible to determine whether during handling, the amount of DNA deposited is equivalent across several replicates of the same variable, so this should be taken into account when concluding the apparent success of any one method.

4.2 DNA Deposition Research Questions

This chapter aims to address the overall objective to determine the variability in an individuals' tendency to deposit DNA onto inanimate objects.
Based on the previously published experimental work undertaken by different research groups, as outlined in the introduction to this chapter, several research questions were identified:

a. Is it possible to detect DNA from the handler of an inanimate object immediately after a short period of contact?
b. If so, are there significant differences in the quality of the DNA recovered from the first object handled and subsequent items handled?
c. Is there a difference in the potential for recovery of a successful DNA profile based on the item being handled (i.e. size, shape, etc.)?
d. Is there any difference in the quality of the resultant DNA profile recovered from an item handled by a person's right or left hand?
e. Is it possible to detect DNA from the handler on another person immediately after a short period of contact?
f. If so, is it still possible to detect DNA from the handler on another person 30 minutes after the short period of contact?
g. Are there any modifications that can be introduced to the protocol to reduce any stochastic fluctuation based on the fact that very low yields of DNA are anticipated from touch DNA?

4.3 Experimental Design

To address these research questions the following experiments were designed, as outlined below.

4.3.1 Three Point Deposition Study

For this study, 1.5 ml Eppendorf tubes were used. All Eppendorf tubes were autoclaved, then cleaned with 2% Virkon, 100% ethanol and double distilled water prior to use.

Subjects were instructed not to wash their hands or come in contact with another individual for at least 1 hour prior to the experiment.

Subjects were asked, using their right hand, to hold Eppendorf tube 1 for 30 seconds, followed immediately by holding Eppendorf tube 2 for 30 seconds, then finally holding Eppendorf tube 3 for 30 seconds.

All DNA samples from the objects were collected by swabbing the outer surface of the Eppendorf tube with cotton square swabs.

Chelex extraction was used to extract the DNA from the sample cotton squares. Both standard and LCN PCR amplification were carried out on the samples, followed by Capillary Electrophoresis, with the only modification being that the kit used for DNA amplification was the AmpF*l*STR® Identifiler™ kit (Applied Biosystems).

4.3.2 Five Point Deposition Study

For this study, 50 ml glass beakers were used. All beakers were autoclaved, then cleaned with 2% Virkon, 100% ethanol and double distilled water prior to use.

For each repeat experiment, a pair of beakers to be handled (R 1-5 for beakers for the right hand, and L 1-5 for beakers for the left hand) were swabbed prior to the experiment as a negative control.

Subjects were instructed not to wash their hands or come in contact with another individual for at least 2 hour prior to the experiment.

Subjects were asked which was their dominant hand prior to deposition, to examine whether the amount of DNA deposited was dependant on which predominantly used.

Subjects were asked, using their right hand, to hold glass beaker R1 for 15 seconds, followed immediately by holding glass beaker R2 for 15 seconds, then glass beaker R3 for 15 seconds, then glass beaker R4 for 15 seconds, finally holding glass beaker R5 for 15 seconds. This process was replicated with the subjects' left hand.

DNA from the glass beakers was recovered by swabbing the outer surface with cotton square swabs. The only modification to the stated method was that each glass beaker was swabbed with the wet cotton square 20 times, followed by the dry cotton square for a further 20 times.

Chelex extraction was used to extract the DNA from the sample cotton squares. LCN SGM+ PCR amplification was carried out on the samples, followed by Capillary Electrophoresis .

4.3.3 DNA Deposition onto Another Individual (Primary Transfer of DNA)

Subjects were instructed not to wash their hands or come into contact with another individual for at least 1 hour prior to the experiment.

Subject A and Subject B manufactured a scenario involving primary transfer of DNA, by way of shaking hands for 30 seconds.

DNA samples from the hands of the subjects were collected after contact by swabbing the hands using pre-wetted buccal swabs.

Hand swabs were collected from Subject A and B's hands after contact.

Chelex extraction was used to extract the DNA from the hand swabs. Both standard and LCN PCR amplification were carried out on the samples, followed by Capillary Electrophoresis, with the only modification being that the kit used for DNA amplification was the AmpF*l*STR® Identifiler™ kit (Applied Biosystems).

4.3.4 DNA Deposition onto Another Individual with Time Delay (Primary Transfer of DNA with Time Delay)

Subjects were instructed not to wash their hands or come into contact with another individual for at least 1 hour prior to the experiment.

Subject A and Subject B manufactured a scenario involving primary transfer of DNA, by way of shaking hands for 30 seconds. They were then asked to return to work (limited to desk-based activities) for 30 minutes, before returning to have their hands swabbed. During this 30 minute interval they were instructed not to wash their hands or come into contact with another individual.

All DNA samples from the hands of the subjects were collected after contact by swabbing the hands using pre-wetted buccal swabs .

Hand swabs were collected from Subject A and B's hands 30 minutes after initial contact.

Qiagen extraction was used to extract the DNA from the hand swabs. LCN SGM+ PCR amplification was carried out on the samples, followed by Capillary Electrophoresis.

4.3.5 Stochastic Effect Study

For this study, 50 ml glass beakers were used. All beakers were autoclaved, then cleaned with 2% Virkon, 100% ethanol and double distilled water prior to use.

Subjects were instructed not to wash their hands or come into contact with another individual for at least 1 hour prior to the experiment. Subject A and Subject B manufactured a scenario involving secondary transfer of DNA, by way of Subject A and B shaking hands for 30 seconds. This was followed immediately by Subject A holding glass beaker 1 for 30 seconds and Subject B holding glass beaker 2 for 30 seconds.

DNA from the glass beakers were collected by swabbing the outer surface of the beaker with a cotton square.

Chelex extraction was used to extract the DNA from the cotton squares. LCN SGM+ PCR amplification was carried out on the samples. The only variation from the method stated was that 5 replicates of each PCR amplification were set up (so 5 x amplification reactions per sample were prepared). These 5 replicates were amplified for the first 3 cycles of PCR cycling, and then the samples were pooled (so 5 x 10 µl reactions pooled to make 1 x 50 µl reaction). The samples were then returned to the PCR machine for the remaining 31 cycles of PCR. Capillary Electrophoresis was then carried out on the samples.

4.4 Analysis and Interpretation of DNA Deposition Results

In order to determine if the amount/quality of DNA deposited by an individual was constant with each contact, experiments were designed where the individual had repeated contacts with specific objects. The rationale behind being able to determine if the amount/quality of DNA deposited by an individual is constant is that in DNA transfer studies it is important to understand the variables that control DNA deposition. Many other research groups have tried to define individuals as 'good' or 'poor' shedders (Linacre et al., 2012; Alessandrini et al., 2003; Lowe et al., 2003; Lowe et al., 2002; Murray et al., 2002; Allen et al., 2008; Rutty, Hopwood and Tucker, 2003), thereby suggesting that the amount of DNA an individual deposits remains constant irrespective of time or number of previous contacts. This would indicate that the source of the DNA being deposited was from epithelial cells being 'sloughed' off the hands of the donor individual during contact.

However, statistical analysis performed by Alessandrini et al. (2003) on the correlation between the number of nucleated cells present in a fingerprint and the amount of DNA recovered indicated a low correlation of 0.43 using Spearman's test. This suggests that there are potentially other sources of DNA that contribute to the amount of DNA recovered from a fingerprint. Wickenheiser (2002) supports this idea of the DNA that is deposited through contact having originated from multiple sources, including the mouth, nose and eyes. The eye in particular would be a good source of DNA, due to the constant regeneration of cells (total regeneration every 6 – 24 hours) from the corneal epithelium and bulbar epithelium (cells from the eyeball and the interior of eyelids, respectively). If

the DNA recovered from a handled item originates from sources other than the donors hand, this suggests that the amount of DNA an individual deposits will not be constant and therefore the ability to define an individual's shedder status would be inconsequential in understanding the significance of their DNA on an item.

4.4.1 Three Point Deposition Studies

The profiles for the three point deposition samples are present in Appendix Three (A3.1).

In this experiment, different donors were asked to hold three Eppendorf tubes in succession, to determine if the amount of DNA reduced with consecutive contacts. Five different donors took part, each using their right hand only. Figure 4.1 summarises the results gained in this experiment.

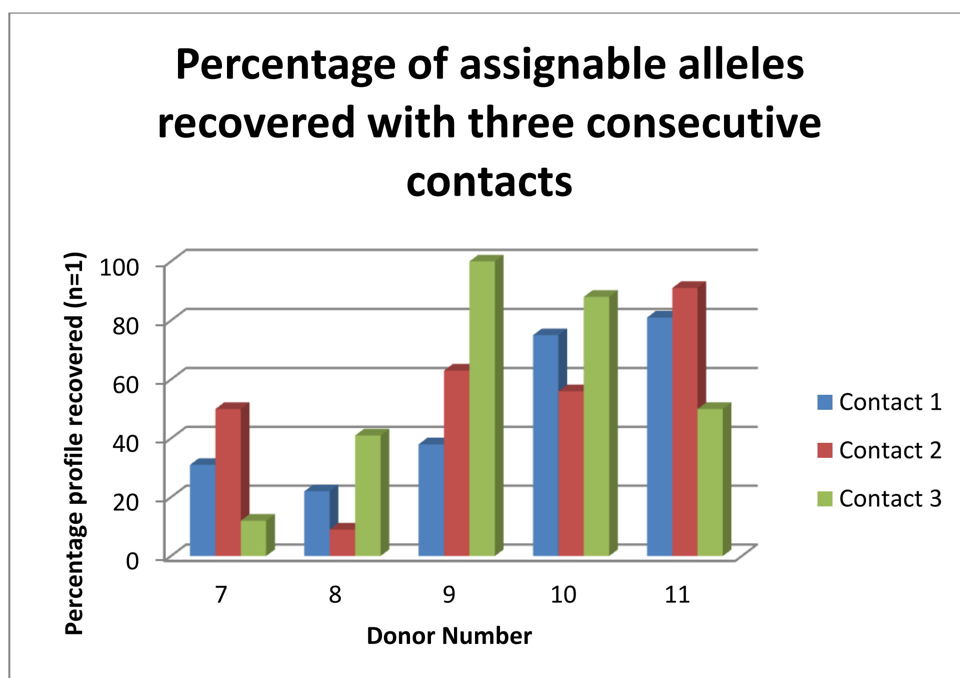


Figure 4.1 Percentage of profile recovered from Eppendorf tubes after contact with donor. Contact number refers to the order in which the tube was handled (i.e. Contact 1 was tube one, Contact 2 was tube two which was handled after tube one, and so on).

Based on the percentage of assignable alleles recovered, these results indicated that the amount of DNA deposited was not uniform and there was no correlation between the percentage of profile observed and the number of previous contacts.

It was anticipated that as the contact number increased, the potential to deposit sufficient DNA to produce a profile would decrease. This was expected as DNA present on an individual's hand was believed to have originated from other sources (Wickenheiser, 2002) in addition to the epithelial cells present on the surface of the skin (Balogh et al., 2003). If this was the case, a proportion of this DNA bearing material would be deposited on the initial contact and therefore would not be present for subsequent contacts. The number of epithelial cells sloughed off with each contact would not generate the same yield of DNA, due to the limited number of nucleated cells present, and therefore the amount of DNA deposited would decrease with each subsequent contact. The results gained in this experiment do not support this hypothesis, as the percentage of profile recovered varies considerably. This suggests that the amount of DNA deposited by an individual is not constant, and the DNA deposited is not predictable based on the number of previous contacts.

There also appears to be considerable variation between individuals with respect to the number of alleles recovered. Samples recovered from some of the donors only generated a maximum of 50% of the total alleles available, whereas others consistently produced over 50% of their profile on each contact. These findings support the theory that some individuals are better DNA 'shedders' than others, although larger sample numbers would be needed to determine if these findings were significant. It also supports the findings of Gršković et al. (2014), that there is great variation in DNA deposition between donors, although it must be noted that Gršković et al. used DNA quantification as an indicator of DNA deposited, not profile quality as used by the author of this thesis. Quantification of the DNA samples recovered from each tube would have been useful, as it may have enabled determination as to whether the amount of DNA deposited was constant with each contact, or whether the quantities reflected the profile quality. However, in this instance DNA quantification was not undertaken but would be a recommendation for further work. Gršković et al. (2014) attempted to clarify the mechanism by which the variation in DNA deposited could be explained. They proposed the mechanical movement of the hand during contact could disturb any loosely adhering corneocytes. This would be difficult to demonstrate, especially if the DNA that has been deposited has

originated from other cellular sources (Wickenheiser, 2002). They also proposed that any DNA originating from sweat could contribute towards the variation in DNA deposited, as there has been research published indicating inter- and intra-individual variation in DNA concentrations in sweat (Quinones and Daniel, 2012). This is also supported by findings reported by Zoppis et al. (2014) who have suggested that sebaceous fluid, containing cellular debris, could be responsible for DNA being transferred onto a surface.

It is also important to highlight that the data included in Figure 4.1 does not include any alleles that were not assignable to the donor. As can be seen in Appendix Three, there are alleles present in each sample that are not consistent with those of the donor. Although some of these peaks could potentially be identified as stutter products or allele drop in, phenomena often observed in DNA samples with a very low yield (Budowle et al., 2002), they could also potentially be contamination from other sources such as laboratory consumables (Buckleton, 2009) or inanimate objects. By observing the electropherogram for Donor 7, Contact 1 (Appendix Three, A3.2) it can be observed that the true alleles from the donor are not significantly discernible in peak height from the non-assignable alleles. In fact some of the peaks that have been identified are only just over the baseline of 50 RFU, so if this was a casework situation it is extremely unlikely this individual would be identifiable from the DNA deposited on the handled item. In this experiment the donors had a time interval of one hour prior to handling where they were asked not to wash their hands or contact another person, but this doesn't exclude the potential to collect DNA from other sources (i.e. door handles, computer keyboards, etc.) (Goray and van Oorschot, 2015). Later chapters in this thesis will be examining the potential for secondary transfer of DNA in more detail.

If this is contrasted with the electropherogram for Donor 9, Contact 3 (Appendix Three, A3.3) it is evident that the peak heights for the true alleles are significantly higher than any spurious alleles identified by the computer software. Please Note – for this sample the baseline had been set at 20 RFU (as these samples were analysed in the GENA Institute of DNA Analysis, Norway) which explains the large number of Off Ladder (OL) peaks that have been flagged up. Based on the strength of the profile and the number of alleles that have been identified, it is extremely likely this individual would be identified in a casework application.

These results suggest that although it is possible to recover DNA deposited onto a handled object, the quality of that DNA is very variable and there is no correlation between the number of preceding contacts and the potential for a successful DNA profile being recovered. These results also support the research findings of Wickenheiser (2002), Gršković et al. (2014) and Zoppis et al. (2014), who have all suggested DNA deposited via 'touch DNA' is unlikely to originate solely from sloughed epithelial cells.

4.4.2 Five Point Deposition Studies

The profiles for the five point deposition samples are present in Appendix Three (A3.4). Charts depicting the number of assignable and non-assignable alleles for each donor can be found in Appendix Three (A3.5)

The deposition study was expanded to include five different contact points, and a comparison of the left and right hand of the donor to determine if the 'Three Point Deposition Study' results were reproducible and to examine the requirement for a process to standardise the starting amount of DNA.

In this experiment, donors were asked to hold five glass beakers in succession, carrying out this process using both their right and left hands. Six different donors took part and the results are summarised in Table 4.1 and Figure 4.2.

Table 4.1 The percentage of assignable alleles for different DNA donors (right and left hand) over five consecutive deposition contacts.

Contact Number	Percentage of assignable alleles observed (%)												
	Donor 5 L	Donor 5 R	Donor 8 L	Donor 8 R	Donor 12 L	Donor 12 R	Donor 13 L	Donor 13 R	Donor 14 L	Donor 14 R	Donor 15 L	Donor 15 R	Mean (SD)
1	25	31	9	44	22	13	3	6	28	22	16	22	20.6 (11.9)
2	16	44	9	19	44	16	13	13	22	28	25	16	22.1 (11.5)
3	25	6	16	47	25	3	9	16	28	31	25	25	21.3 (12.2)
4	25	16	13	25	47	6	22	6	25	28	28	9	20.8 (11.7)
5	25	28	19	13	47	16	3	22	25	47	0	22	22.3 (14.4)

The mean values for the percentage of assignable alleles are consistent for all five contact points, indicating almost no variation in the percentage of assignable alleles recovered after each contact. This is supported by the very small standard deviation of the means for Contacts 1 to 5, (SD = 0.7), demonstrating very little spread of data across the mean values for the different contacts.

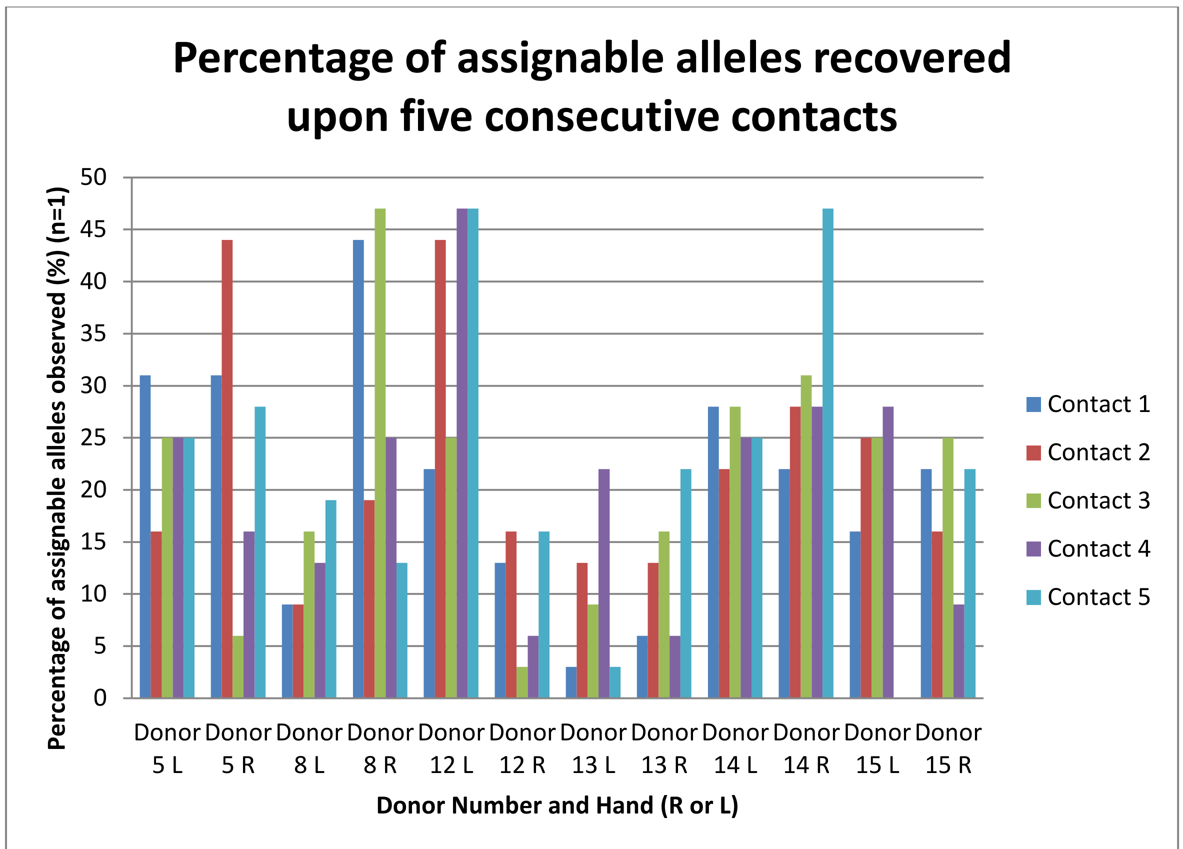


Figure 4.2 Percentage of assignable alleles observed when recovered from glass beakers after contact with donors right or left hand. Contact number refers to the order in which the tube was handled (i.e. Contact 1 was tube one, Contact 2 was tube two which was handled after tube one, and so on).

The results appear to support the findings of the 'Three Point Deposition Study', which indicated that the amount of DNA deposited was not predictable and that there appeared to be no correlation between the number of contacts and the number of alleles observed. The mean value for the percentage observable alleles for Contact 1 was 20.6 (SD = 11.9) and for Contact 5 was 22.3 (SD = 14.4) (n=12). A one-way, independent-measures Analysis of Variance (ANOVA) was undertaken on the percentage of assignable alleles over five consecutive contacts, followed by the Bonferroni Post-Hoc test, and according to the results there was no statistically significant difference between contact numbers ($p>0.05$). The large standard deviations observed across each data set demonstrate the poor reproducibility in the data, which is consistent with other groups findings of DNA yield from touch DNA (Daly, Murphy and McDermott, 2012; Raymond et al., 2009a). There also appeared to be little parity between the number of alleles recovered from samples from the left and right hands of the donor. The donors involved in the research

all stated that their right hand was their dominant hand. The results do not support the hypothesis that an individual's dominant hand would deposit a larger amount of DNA, although quantification of the DNA may have provided more information on this matter. These results contradict the findings of Phipps and Petricevic (2007) who reported that individuals 'shed' more DNA from their dominant hand when compared to their non-dominant hand. However, Phipps and Petricevic (2007) do conclude that variable results can be observed in relation to the amount of DNA deposited by an individual. These findings also contradict the suggestion by Sandoval et al. (2013) that a person is more likely to deposit more skin cells when using his or her dominant hand, due to being able to have a 'tighter grip' of the object.

Although there are consistencies in the trends observed within this data set and that generated from the 'Three Point Deposition Study', there are also some differences that should not be overlooked. One significant difference is the overall success rate of the profile recovery. Although it is not possible to carry out a direct comparison with the results from the 'Three Point Deposition Study', due to other variables differing (donor, surface type, surface area, etc.) there does appear to be a lower success rate for this study, in terms of generating more complete profiles. The objects in this study were held for a shorter duration, but it would be irresponsible to infer that the difference is solely due to the differences in handling time. The profiling system used in this study (SGM+) is not as sensitive as the system used in the 'Three Point Deposition Study' which may explain the difference in success rates observed. However, if you observe the peak heights achieved for these samples they are predominantly comparable to those observed in the previous study (Appendix Three, A3.6). Only one of the Donors (13) consistently produced DNA profiles with peak heights of less than 1000 RFU. This can be seen in the electropherogram for their sample from Contact 4 with their right hand, (Appendix Three, A3.7) which exhibit peak heights of less than 1000 RPU. However, this is reflected in the consistently low percentage of assignable alleles that were recovered from both samples from this donor.

Another potential explanation for the lower percentage recovery of alleles could be that in this study the contact area was larger, so although a comparable amount of DNA may have been deposited, it may have been distributed over a greater surface area (Gršković et al., 2014). However, if that were the case, it would be anticipated that all samples

recovered in this study would have consistent success rates, which was not observed. Alternatively the recovery method may have impacted negatively on the data generated. Although double swabbing was used in this study, it may have not had the positive impact on the recovery yield that would be anticipated, due to limited space for buffer absorbency/DNA release with having two cotton squares within one sample tube.

In this study, contaminating peaks were identified in the negative control swabs taken from some of the glass beakers prior to testing. This indicated that the anti-contamination measures in place were not sufficiently robust to remove all previous traces of DNA. As a result the anti-contamination measures were modified to try to reduce the incidence of this in the future.

In addition to the peaks that appeared to be a result of contamination, either from the equipment or from contaminating DNA already present on the donor's hands, there also appeared to be stutter products evident in the profiles. An example of this can be seen in Figure 4.3.

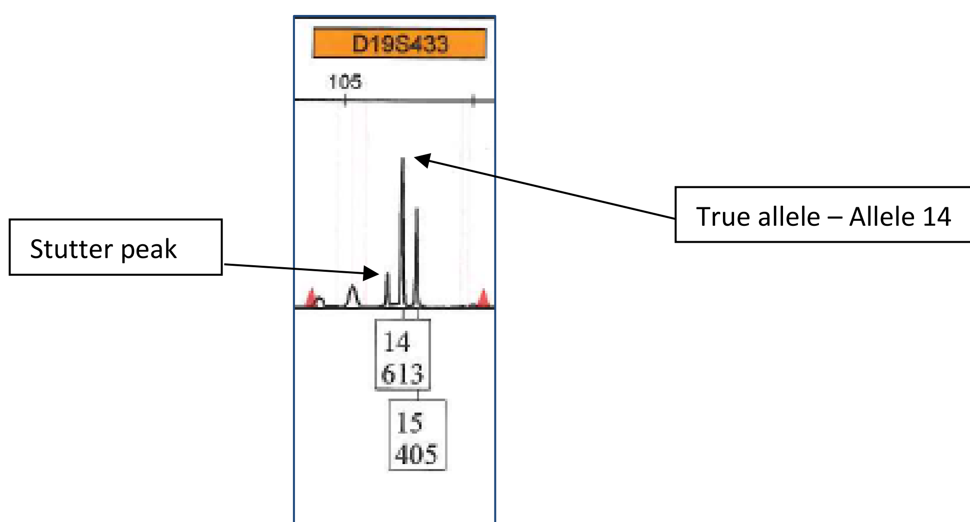


Figure 4.3 Example of stutter product evidenced as a minor peak one repeat unit smaller than the true allele.

The incidence of stutter products is often higher in samples that are amplified using LCN conditions, and this may have resulted in some peaks being identified as an allele when in fact they were stutter products that had exceeded the 15% of parent allele peak height

allowance. Alternatively, as suggested by Wickenheiser (2002), stutter products could mask true alleles from a minor contributor when dealing with mixed samples.

Although natural variation in DNA deposition is one possible explanation for the apparent randomness exhibited in regards to the number of alleles recovered for each contact, another possibility could be stochastic fluctuation. The incidence of allele and locus dropout and stutter products exhibited in the samples suggests that stochastic fluctuation may be occurring in these samples. In support of the natural variation theory, Balogh et al. (2003) suggest that the reduction in the incidence of larger alleles supports a mechanism of enzymatic DNA degradation by a $Ca^{2+/}Ma^{2+}$ dependent endonuclease that breaks DNA into fragments of approximately 200 bp. The authors suggest that the differences in the amount of DNA successfully profiled could be a result of the amount of catabolic enzymes produced by an individual, as a result of the biochemical difference between different individuals' rates of terminal cell differentiation. However, as the samples being amplified in this study are low yield DNA samples, due to the source being 'touch DNA', stochastic variation is an equally likely theory.

Finally, there appears to be a correlation between the number of assignable alleles and the number of non-assignable alleles present in the samples (Appendix Three, A3.5), with a slightly higher number of non-assignable alleles detected than assignable (difference of 0.77 for the mean percentage of alleles per sample between observed assignable and non-assignable). This could indicate that any 'contaminating' DNA present on the donors hand is as likely to be deposited as the donors own DNA. This would suggest that defining an individual based on their apparent 'shedder status' could be misleading, as the DNA they are depositing is as likely to originate from another source as from the donor themselves. The limitations of a classification system for shedder status have also been identified by Graham and Rutty (2008) who have stated that the determination of an individual's shedder status may not be as straightforward as initially believed. This is supported by Meakin and Jamieson (2013) who conclude that the complexities involved in the processes governing DNA deposition mean that to attempt to define an individual as consistently a good or poor shedder is oversimplifying the situation.

4.4.3 DNA Deposition onto Another Individual (Primary Transfer of DNA)

The profiles for the primary transfer samples are present in Appendix Three (A3.8).

This experiment was designed to determine the potential for DNA to be detected if that DNA was deposited on to another person, i.e. when two donor individuals have been in contact with one another. Two donors were asked to shake hands with one another, in order to enable deposition of DNA to occur. The hands of both donors were swabbed individually to determine the degree to which DNA was deposited onto the hand of another person. The results for these samples, highlighting the source of the alleles, are shown in Figures 4.4. Where an allele was shared between the two donors, it was only counted once in the shared allele total. This was because it was not possible to determine which individual was the donor of that allele, and therefore it was interpreted to be equally as likely to originate from either of the donors.

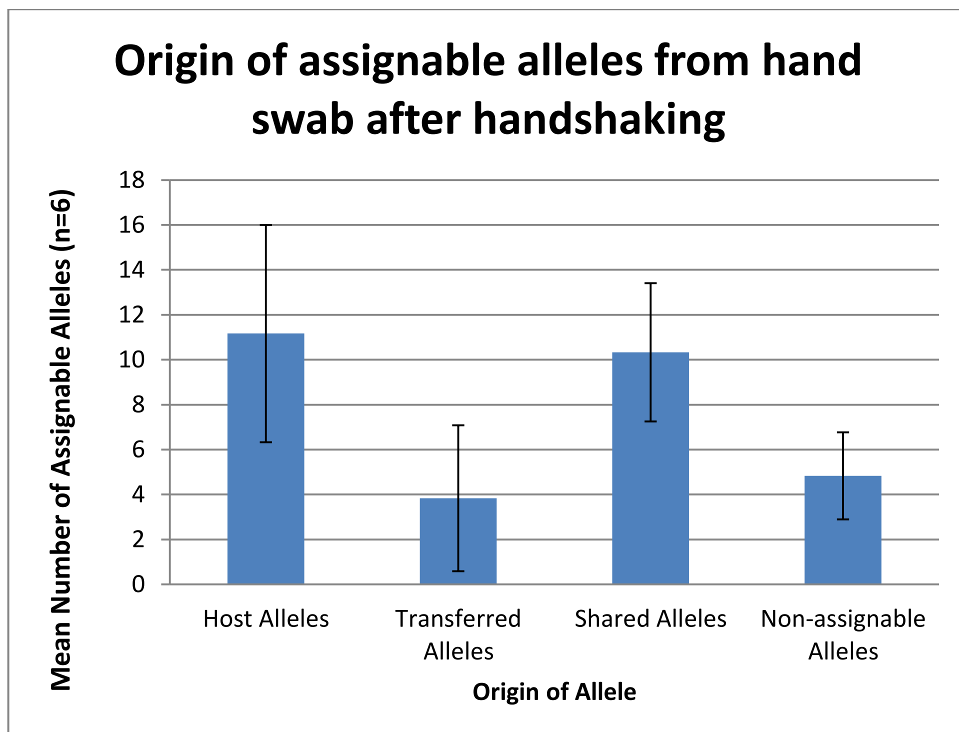


Figure 4.4 Number and origin of assignable alleles present on hand swab after handshaking. Host alleles are those originating from the person whose hand was swabbed, whereas transferred alleles are those originating from the partner whose hand was shaken.

One issue with interpreting the data generated during this experiment, as well as in other experiments where more than one DNA source was present, was the occurrence of shared alleles between the two donors involved. Attempts were made to pair up donors with as few shared alleles as possible, but this was very challenging due to the frequency of certain alleles. One potential method for overcoming this issue in the future could be to use donors from different ethnic backgrounds, as all the donors in this study were Caucasian and therefore are more likely to share common alleles. The use of only Caucasian donors resulted in a higher incidence of shared alleles than would have been desirable. This has led to more conservative conclusions being drawn as it is not possible to definitively state the source of all alleles.

Another point to note when examining this data is the variability demonstrated between repeats. The standard deviation for each group of alleles was relatively high, which is a good indicator of the degree of variation observed when studying DNA deposition on to another individual. Nevertheless, some interesting observations were identified upon examination of the data.

The results from this study demonstrate that in all cases DNA was deposited onto the hand of the host (also known as primary transfer). The degree to which an individual transfers his or her DNA on to another individual varies, but there was a significant difference between the proportion of DNA that could be identified as belonging to the host and that from the handshaking partner. Although the majority of published research in primary transfer of DNA involves deposition of DNA onto an object (Alessandrini et al., 2003; Abaz et al., 2002; Lowe et al., 2002; Murray et al., 2002; Phipps and Petricevic, 2007) the processes involved are comparable. The varying degree to which an individual transfers his or her DNA during contact, as observed in this study, is in agreement with other published research in this area. The results presented here indicated that it was possible to identify DNA from a second individual even with the higher background levels of DNA from the donor individual's hand. This supports the other findings reported in this chapter, in that primary transfer of DNA is also likely to be detectable on objects that have been handled, as the background levels of DNA are not anticipated to be as high as those recovered from a donor's hand.

It is worth noting that in this study the hand of the donor was swabbed. It would be anticipated that in that instance a larger proportion of the DNA profile recovered would belong to the donor whose hand was swabbed (higher background levels present on their hand), with a smaller proportion coming from the second individual involved in the contact. This contribution trend was observed in the majority of the samples collected. However, with one of the donors, it was apparent that they had contributed the majority of the DNA to the swab recovered from his partners' hand. This would suggest that in this instance this donor transferred a large amount of his DNA, or that his partner transferred a particularly low amount of her DNA, or perhaps both statements were true to some degree. Research published by Graham at al. (2014) examined the background levels of DNA found on the skin of young children. There results indicated that there was a great deal of variation between the amount of child and non-child DNA on the swabs recovered from the skin of the child. Interestingly they attempted to determine if there was any correlation between the amount of DNA recovered and specific variables (number of contacts, incidents of washing, number of people in close contact with the child, etc.) and found no relationship between the amount of DNA recovered and these variables. Although this research examined young children, the mechanisms for DNA transfer would be expected to be similar. Wiegand and Kleiber (1997) demonstrated that it was possible to detect DNA of the assailant on the neck of the victim after manual strangulation and although in most cases DNA from both individuals was detected, it was still possible to identify another person through their deposited DNA. Graham and Rutty (2008) also carried out research into the background levels of DNA on adult necks and found the presence of DNA not originating from the donor.

Herein lies a key issue with the transfer of DNA. Although in this study the donors were asked to control their behaviour in an attempt to standardise the starting amount of DNA present (i.e. through hand washing, rubbing of hands and restricted contact with other individuals), the variables that can influence the amount of starting DNA cannot be completely controlled. Some of the variables are internal (e.g. number of nucleated epithelial cells produced, temperature, skin conditions, etc.) and others are external (e.g. contact with DNA containing surfaces, contact with eyes and mouth, etc.). Although this research has attempted to address and prioritise some of the variables that influence the

deposition and transfer of DNA, it has also identified the limitations with this approach to interpreting casework, which will be discussed later on in this thesis.

4.4.4 DNA Deposition onto Another Individual with Time Delay (Primary Transfer of DNA)

The profiles for the primary transfer samples, where a 30 minute delay was introduced between contact and sampling, are present in Appendix Three (A3.10).

This experiment was designed to determine the potential for DNA to be detected if that DNA was deposited on to another person, when there is a delay of 30 minutes between the time of contact between two individuals and the sample recovery. This would mimic a situation where DNA has been recovered from a victims hand 30 minutes after contact with an assailant.

In this experiment Subject A shook hands with Subject B. After 30 minutes of 'regular' activity, during which the participants were asked not to contact any other individuals or wash their hands, both Subject A and B's hand were swabbed. The results of the DNA recovered from Subject A and B's hand swabs are summarised in Figure 4.5.

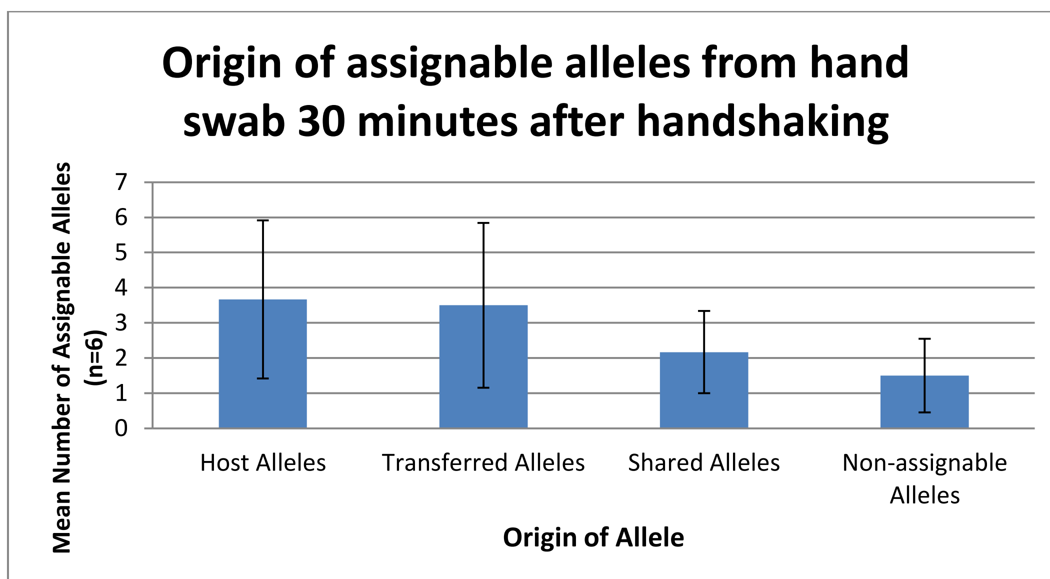


Figure 4.5 Number and origin of assignable alleles present on hand swab 30 minutes after handshaking. Host alleles are those originating from the person whose hand was swabbed, whereas transferred alleles are those originating from the partner whose hand was shaken.

The results from this study demonstrate that in all cases DNA was deposited onto the hand of the host (also known as primary transfer) and was still possible to detect this 30 minutes after initial contact. Although the degree to which an individual transfers his or her DNA on to another individual varies, as was found when samples were collected immediately after contact, in this case there was no significant difference between the proportion of DNA that could be identified as belonging to the host and that from the handshaking partner. This is unexpected, as it would be presumed that the 'loosely adhering' DNA deposited from the partner on the hand of the host would be more easily lost. However, it is important to note the small sample size and the very low numbers of alleles that were recovered in general from these samples, so it would be difficult to make a conclusive inference from these results.

The anticipated result would be that the host was the dominant contributor to the swabs collected from their hand. However, the findings of this study indicate that this may not always be the case as in one sample the dominant contributor to the profile was not the individual whose hand was swabbed but was instead that of the contact partner. In all cases, excepting one sample which failed to produce a profile at all, there was evidence of primary transfer of DNA detectable 30 minutes after the original contact.

With this study, the subjects were asked to refrain from washing their hands for at least an hour prior to taking part in the experiment. After the initial contact (where they shook hands for 30 seconds), they were then asked to return 30 minutes later to have samples collected. During this 30 minute interval, the subjects returned to their offices to continue desk based activities. Although there was evidence of the ability to detect alleles from the initial contact, there was also evidence of contaminating alleles. This may have an effect on the ability to determine the contributors to a profile; in this instance, where a controlled experiment was undertaken, the profiles of the two contributors were known. In practice, this is unlikely to be the case and therefore interpretation of the mixed profile will be more challenging. Although the amount of DNA transfer is dependent on many variables, it is hypothesised that the longer the duration between the initial contact between individuals and the subsequent deposition of a sample, the less likelihood there is of being able to confidently identify the DNA of the partner on the

hosts' hand. Rutty (2002) reported the potential for detection of an individuals' DNA on the neck of a 'victim' up to 10 days after initial contact, in this case relating to a simulated manual strangulation experiment. However, he also noted the issue of this DNA originating from another transfer event. Transfer of DNA through indirect mechanisms will be discussed further in the following chapters.

4.4.5 Addressing Stochastic Effect Study

The profiles for the stochastic effect samples are presented in Appendix Three (A3.11).

This study was designed to determine if pooling PCR products after the initial three cycles of amplification would result in a reduction in the incidence of stochastic effect observed in many of the samples generated from 'touch' DNA samples. The rationale behind this approach was that during the initial stages of PCR, the random selection of which DNA molecules will be amplified occurs. In instances where the starting amount of DNA is low, this random process often leads to only a selection of DNA molecules being amplified (Butler, 2012). It was perceived that if it was possible to initiate the amplification process, and then increase the number of DNA molecules available for amplification, then the random selection would be more representative of all the fragments of DNA present.

In this study, 5 amplification reactions were prepared from one DNA extract and these underwent the initial three cycles of PCR amplification. The samples were then pooled together and the remainder of the PCR amplification was carried out. The results of these reactions are summarised in Figure 4.6.

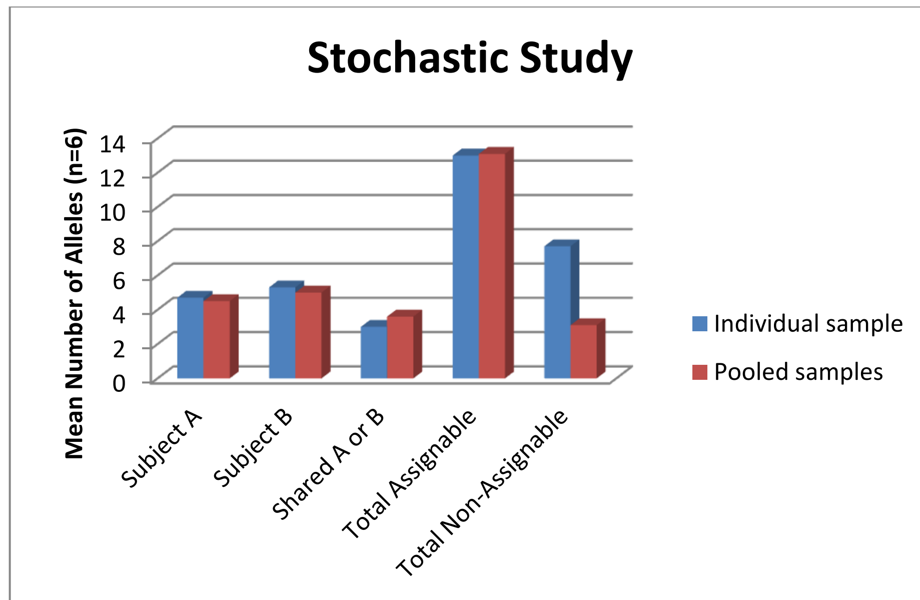


Figure 4.6 Mean number of alleles originating from Subject A or B in the original sample and the pooled samples.

In order to evaluate this method, the mean number of alleles donated by each donor was calculated, as well as the mean number of assignable and non-assignable alleles present. The mean values for the original samples were compared with the mean values for the pooled samples in order to determine if the method proposed would result in an overall increase in the total number of assignable alleles identified. This overall increase ideally should represent all possible alleles available, which is why the individual components of the mixed profile were separated for evaluation. It was also necessary to monitor the potential for increased amounts of contamination, especially as the samples were opened mid-way through the process of amplification.

From the results observed there appears to be no significant difference between the original sample amplified individually (total assignable alleles mean =13) and the samples that have been pooled during amplification (total assignable alleles mean =13.1). The ratio of alleles from the different donors is consistent between the two approaches, and the total number of assignable alleles is comparable. One apparent benefit of the pooling process is that it has resulted in a reduced number of non-assignable alleles present in the sample. This could be a result of reduced stochastic effect so the non-assignable alleles are less likely to be preferentially amplified. However, in evaluating the potential

for this method to be used as a feasible alternative to the current system, the benefit of reduced numbers of non-assignable alleles does not counteract the increased cost element (5 amplification reactions as opposed to the current verification system of 3 reactions). Also, this method would use more of the DNA extract and therefore could cause problems where there is only a limited sample available for testing.

4.5 Conclusions

This chapter aimed to address several research questions in relation to the deposition of DNA. The research aimed to determine if it was possible to detect the DNA of a handler on an inanimate object after only a short duration of contact. The results indicated that it is possible to detect touch DNA on an object that had been handled. The quality of DNA recovered was very variable, but there was no statistically significant correlation between the quality of DNA recovered from the first object handled and any subsequent items handled. Insufficient repeat data was collected to allow determination of the impact that surface type may have on the potential for DNA recovery, but the large degree of variation in the quality of DNA that has been observed on a single item would suggest that substrate shape and size may not be the primary variable in determining on the success of DNA profiling from touch DNA. In addition to examining the quality of the data, quantification of the DNA may have proved useful to enable a greater understanding of DNA deposition and would be recommended for future studies into this subject. Further discussion on DNA recovery from handled items, with specific reference to firearms and spent cartridge cases, will be included in Chapters 7 and 8. There was no statistically significant difference between the quality of the resultant DNA profiles recovered from an item handled by a person's right or left hand, so this is another variable that would not allow indication of the potential for recovery of a successful DNA profile. It was found to be possible to detect DNA of another person, immediately after and 30 minutes after handshaking, but the presence of additional non-assignable alleles and the incidence of shared alleles could cause issues with being able to confidently identify the other person. However, it was noted in one incidence the hand swab taken from the donor had a larger proportion of alleles belonging to the other person in the handshaking pairing, rather than that of the donor himself. This suggests that DNA deposited on to a person can be

detected and will not be completely masked by the excessive contribution of DNA from the person being swabbed. Further discussion on DNA transfer and persistence on hands, including research into the daily variation of DNA transfer, will be included in Chapter 6. Modifications to the amplification process were attempted to determine it if was possible to counteract any stochastic fluctuations brought about by the low levels of DNA that were anticipated. It was found that these modifications have no significant different to the number of assignable alleles that were produced, so it would not be recommended to implement these changes into casework practice.

Five. Recovery of DNA Evidence

5.1 Introduction to DNA Recovery

The optimal method for recovering the maximum yield of DNA from an exhibit depends on the substrate the DNA is to be collected from as well as the source of the DNA sample e.g. blood, saliva, handled item (Li, 2008). If the evidential item can be collected and taken to the laboratory, the recovery of DNA can be performed during forensic examination of the item. The benefit to recovering the DNA in a laboratory environment is a reduction in the potential for contamination, as well as optimal storage conditions for the DNA sample. However, if the DNA sample is present on an immovable item then it may be necessary to collect the sample at the scene.

Wet bodily fluids are generally collected with a dry sterile cotton swab, unless there is sufficient volume for the liquid to be transferred into a securely sealed plastic container (with or without a preservative, as outlined by the forensic provider). Dry bodily fluids are routinely collected using a sterile cotton swab pre-wetted with a suitable liquid. Dry samples may also be collected by scraping the sample off the substrate, which is beneficial in that the sample remains dry, therefore less prone to degradation. However, scraping can lead to a loss of sample and therefore swabbing is the preferred method of choice for most scene examiners (Rudin and Inman, 2002; Goodwin, Linacre and Hadi, 2007). The choice of liquid for wetting the swab is predominantly sterile DNA-free water, although ethanol can also be used (Williams and Clarke, 2010).

Published research has demonstrated the widespread use of cotton swabs for DNA recovery. Rivera et al. (2009) reported that cotton swabs had higher DNA recovery rates than foam swabs when swabbing samples from stainless steel and brass.

Recently, research has been carried out into alternative materials for swabbing – nylon flocked swabs, in place of the traditional cotton swab are one of the most recent developments. Copan Innovation produces a flocked swab that they report increases sample collection and elutes the sample more efficiently resulting in a more effective recovery and extraction process (Funagalli and Vaněk, 2008; Benschop et al., 2010; Saskova et al., 2008). The nylon swabs have an open fibre structure that enables rapid

sample absorption during collection and sample release during extraction (Funagalli and Vaněk, 2008; Benschop et al., 2010; Saskova, Giambra and Vaněk, 2008). Research carried out by Benschop et al. (2010) suggests that recovery of a male donor profile from a post-coital vaginal sample is improved by using nylon flocked swabs. The effect of nylon flocked swabs on DNA recovery rates in samples with atypically low yields of DNA is yet to be published.

In circumstances where there is an anticipation that a low yield of DNA will be recovered, such as in the instance of collecting epithelial cells from lip prints, fingerprints and handled items, a technique known as double swabbing is employed (Butler, 2009). This entails first swabbing the item with a wet swab (moistened with sterile DNA-free distilled water) followed by swabbing the same area with a dry swab, then extracting the samples together. This is believed to maximise recovery by first rehydrating the cells during the initial swabbing, which enables the second swab to readily collect the loosened cells. This technique, suggested by Sweet et al. (1997), showed an increase in the recovery of DNA from saliva on human skin when compared to swabbing with a wet swab and swabbing with a piece of wet filter paper. More recently, it has been shown to increase the quantity of DNA, as well improving the quality of the DNA profile, recovered from touched evidence (Pang and Cheung, 2007). Research published by Castella and Mangin (2008) also supports the assertion that double swabbing is more effective at DNA recovery than either a wet or dry single swab. Williamson (2012) recommends that the double swabbing method should be used when swabbing hard, non-porous surfaces.

Reference samples, collected from an individual for comparison purposes, are customarily collected using a buccal swab, consisting of either a compressed cotton serrated head, a foam-tipped swab or a filter paper device (Butler, 2009; Burger, Song and Schumm, 2005). The swab is placed in an individual's mouth and rubbed against the inside of the cheek several times to ensure a sufficient amount of oral epithelial cells are collected. In some countries, blood samples are still taken as a reference source of DNA, but due to the health and safety issues with collecting blood samples, this has been replaced for the most part by the use of buccal swabbing (Jackson and Jackson, 2011).

Hydrophilic Adhesive Tape (HAT) lifting is an alternative technique to swabbing and it involves pressing a piece of HAT repeatedly on a surface in order to recover DNA (Li,

2008; Zech, Malik and Thali, 2012). The identified benefits of this technique are that it keeps the sample in a dry state, thereby reducing the potential for degradation, it enables recovery of more than one evidence type at once, such as Gunshot Residue (GSR) and DNA together, and remains stable for a longer period at room temperature (Li and Harris, 2003). Previous studies have demonstrated the ability to recover DNA from clothing and handled items using HAT lifting (Hall and Fairley, 2004; Barash, Reshef and Brauner, 2010; Jones, 2007; Wickenheiser, 1996; Bright and Petricevic, 2004).

Gelatine lifts are similar to adhesive tapes in that they rely on an adhesive action for retrieval. However, they are more frequently used for the recovery of fingermarks as opposed to DNA evidence. This may be due to the fact that in order to recover the fingermark deposits only a low level of adhesion is required, but DNA recovery techniques have historically involved some degree of pressure during contact. Their relative lack of popularity with respect to DNA recovery may also be due to a more logistical concern – the gelatine lifts are approximately 0.5 mm thick, which introduces challenges in terms of placing these into 1.5 ml Eppendorf tubes for extraction purposes. This lack of application is reflected in the published literature, with no evidence of any research specifically reported on DNA recovery using gelatine lifters. Norlin et al. (2013) compared a range of different fingerprint recovery techniques to determine the impact of these on subsequent DNA profiling success. They reported that it was possible to recover usable DNA, both nuclear and mitochondrial, from gelatine lifters, but as this method was one of several they were testing, little emphasis was put on determining the relative success of this method when compared to other DNA recovery systems. As previously outlined for adhesive tape, the potential for this method to facilitate dual recovery of evidence makes this recovery technique an attractive consideration.

Biological samples are, where possible, packaged in paper evidence bags, envelopes or cardboard boxes. This packaging method is used in order to prevent bacterial degradation of the samples, which can occur when samples are packaged incorrectly in plastic evidence bags (Butler, 2009). However, when recovering wet samples these are placed into plastic evidence bags and frozen, to prevent loss of the sample or contamination of other evidential items. Swabs used to collect wet or dry body fluids are placed back into their plastic casing and then into a plastic evidence bag. Ideally samples should be dried prior to packaging to prevent transfer of the evidence onto other areas of

the exhibit and bacterial degradation. Wet, warm conditions speed up the growth of bacteria and encourage the activity of enzymes, known as DNases, that break down DNA (Butler, 2005). Biological evidence upon receipt should be stored either at 4°C, in a freezer at -20°C, or at -70 ^{o}C for longer term storage. This is true for all stages prior to, and post, extraction.

An alternative storage medium that is increasing in popularity is FTA™ paper, due to no requirement for refrigerating or freezing the samples. Evidential samples, such as blood, or reference buccal samples can be stored by pressing the swab (or in the case of liquid a small aliquot is pipetted) onto the paper. FTA™ paper was developed in the late 1980s and is a cellulose-based collection card treated with chemicals to prevent nuclease degradation and bacterial growth. The paper lyses the cells and binds the DNA onto the medium where it can be stored at room temperature for many years (Goodwin, Linacre and Hadi, 2007; Butler, 2005).

5.2 Recovery of DNA Research Questions

This chapter aims to address the overall objective to evaluate any potential differences in DNA recovery methods in relation to DNA profile quality and DNA quantity.
Based on the review of the previously published experimental work undertaken by different research groups, as outlined in the introduction to this chapter, several research questions were identified:

a. Is swabbing or lifting the most effective method of DNA recovery?
b. Does the type of swab or lifting material (i.e. the material used for collected the sample) impact on the success rate?
c. Does the surface the DNA is being recovered from have an impact on the success of DNA recovery?
d. If the surface is such that inhibitors are also present (i.e. rusted metal), is the most effective recovery method the same as when inhibitors are not present? In other words, does the swab/lifter type also influence the recovery of potential PCR inhibitors?

5.3 Experimental Design

To address these research questions the following experiments were designed, as outlined below.

For this study, all slides were exposed to UV light (Mineralight® Lamp, multiband UV, 254/366 nm, 215-250 volts) for 1 hour prior to use. The glass and polished metal slides were also cleaned with 2% Virkon, 100% ethanol and double distilled water prior to UV exposure.

0.5 µl aliquots of blood were pipetted onto glass slides, polished metal slides and rusted metal slides and allowed to dry at room temperature for two hours. Blood was collected from the researcher using a finger pricking device (Roche Safe-T-Pro, Roche) and all samples were deposited at the time of collection.

Samples were recovered using the following swabs/lifters:

- Cotton Swab (Invasive sterile EUROTUBO® Collection Swab, Deltalab)
- Nylon Flocked Swab (4NGFLOQSwabs™ Crime Scene, Copan Flock Technologies)
- Viscose Swab with polystyrene stem (Forensic Swab, Sarstedt Ltd.)
- Mini-tape lifters (Scenesafe FAST™, SceneSafe Ltd.)
- White gel-lifters (WA Products).

DNA samples were recovered either by swabbing 5 times with a wet swab or by pressing the lifter onto the surface 20 times. The end of the swab was then cut off and placed into a 2 ml Eppendorf tube. Mini-tapes were placed directly into a 2 ml Eppendorf tube with the adhesive side facing the inside of the tube. Gel-lifters were cut into 0.5 x 0.5 cm pieces and these were placed into a 2 ml Eppendorf tube.

Qiagen extraction was used to extract the DNA from the samples. DNA quantification was carried out using the Qubit Fluorometer (Invitrogen™). Standard SGM+ PCR amplification was carried out on the samples, followed by Capillary Electrophoresis (with a baseline threshold of 50 RFU).

5.4 Analysis and Interpretation of DNA Recovery Results

The quantification values and profiles for the DNA Recovery study are presented in Appendix Four (A4.1 and A4.2).

Blood was used in this study as opposed to touch DNA to enable a consistent amount of DNA to be deposited. This would allow conclusions to be made as to the optimal DNA recovery method, without having to consider that the amount of DNA material being deposited may not have been uniform for each variable. 0.5 µl aliquots of blood were used as it has been reported this volume of blood contains approximately 10 ng of DNA, and therefore can be considered to fall within the low-template region for DNA samples (Lee and Ladd, 2001).

This research was undertaken to determine which method of DNA recovery was optimal for the recovery of low amounts of DNA. Figure 5.1 shows the mean concentrations of the DNA samples recovered from glass, metal and rusted metal slides using cotton wet swabs, cotton double swabbing, viscose wet swabs, nylon flocked wet swabs, mini-tapes and gel-lifters. Table 5.1 summarises the mean concentrations and the standard deviations for each variable.

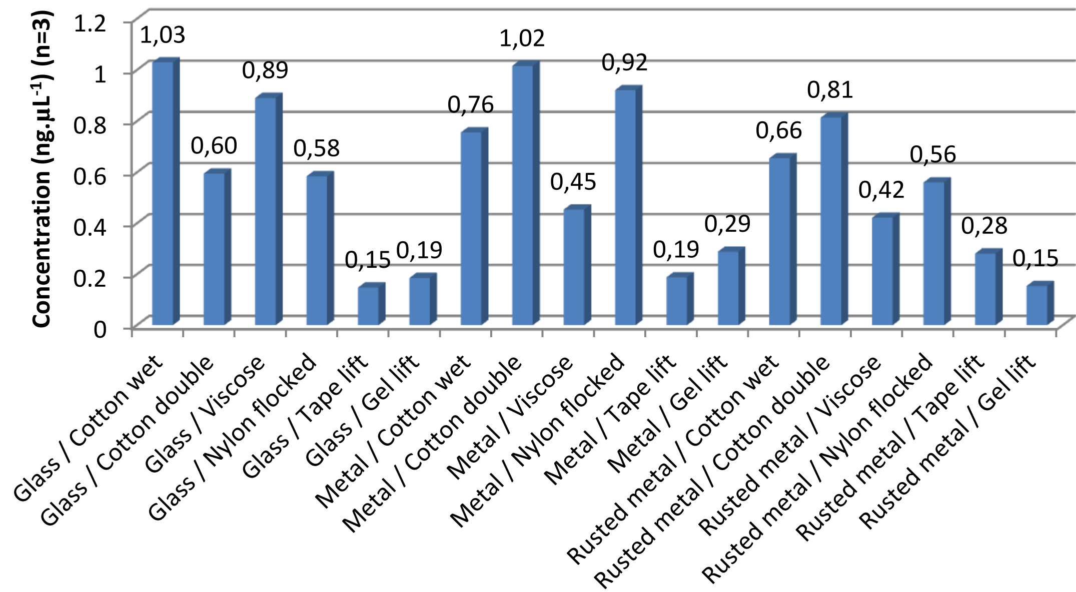


Figure 5.1 Mean concentration (ng/µl) of DNA recovered from glass, metal and rusted metal slides.

Table 5.1 The mean and standard deviation values for the DNA concentration of samples recovered from glass, metal and rusted metal slides.

Surface	Recovery method	Min conc. ng.µL^{-1}	Max conc. ng.µL^{-1}	Mean conc.	Standard deviation
Glass	Cotton wet	1.00	1.06	1.03	0.03
	Cotton double	0.31	1.01	0.60	0.37
	Viscose	0.77	0.97	0.89	0.11
	Nylon flocked	0.52	0.70	0.58	0.10
	Tape lift	0.10	0.23	0.15	0.07
	Gel lift	0.11	0.23	0.19	0.07
Metal	Cotton wet	0.10	1.09	0.76	0.57
	Cotton double	0.79	1.13	1.02	0.20
	Viscose	0.17	0.78	0.45	0.31
	Nylon flocked	0.88	0.95	0.92	0.04
	Tape lift	0.19	0.19	0.19	0.00
	Gel lift	0.23	0.37	0.29	0.07
Rusted metal	Cotton wet	0.46	0.84	0.66	0.19
	Cotton double	0.68	0.98	0.81	0.15
	Viscose	0.41	0.43	0.42	0.01
	Nylon flocked	0.44	0.70	0.56	0.13
	Tape lift	0.24	0.35	0.28	0.06
	Gel lift	0.05	0.29	0.15	0.12

If the mean concentrations of DNA are examined, the top performing recovery method overall appears to be cotton, with double swabbing generating the highest yields when recovering DNA from polished metal and rusted metal and wet cotton swabbing recovering the greatest yield from glass. The reproducibility of the results is questionable, with cotton double swabbing from glass giving a standard deviation (SD)

value of 0.37 and cotton wet swabbing from glass having a SD of 0.57. This could suggest that these methods are less reliable on these specific surface types, or that more repeats would be needed to determine how reproducible these methods truly are.

Viscose swabs produced very variable results, with consistently good yields from the glass samples, consistently average yields from the rusted metal samples but very inconsistent results on the polished metal surface. In contrast, the nylon flocked samples performed very well in relation to DNA recovery from the polished metal surface and average yields from the other two surfaces.

Gel lift and tape lift appear to be consistently low performing, across all surface types. The standard deviation values for each of these methods are also low, which demonstrate that the mean value is representative of all of the repeats collected. However, DNA quantification results can only provide limited information and therefore the resulting DNA profiles should be scrutinised alongside these results before attempting to explain any trends observed in the quantification data.

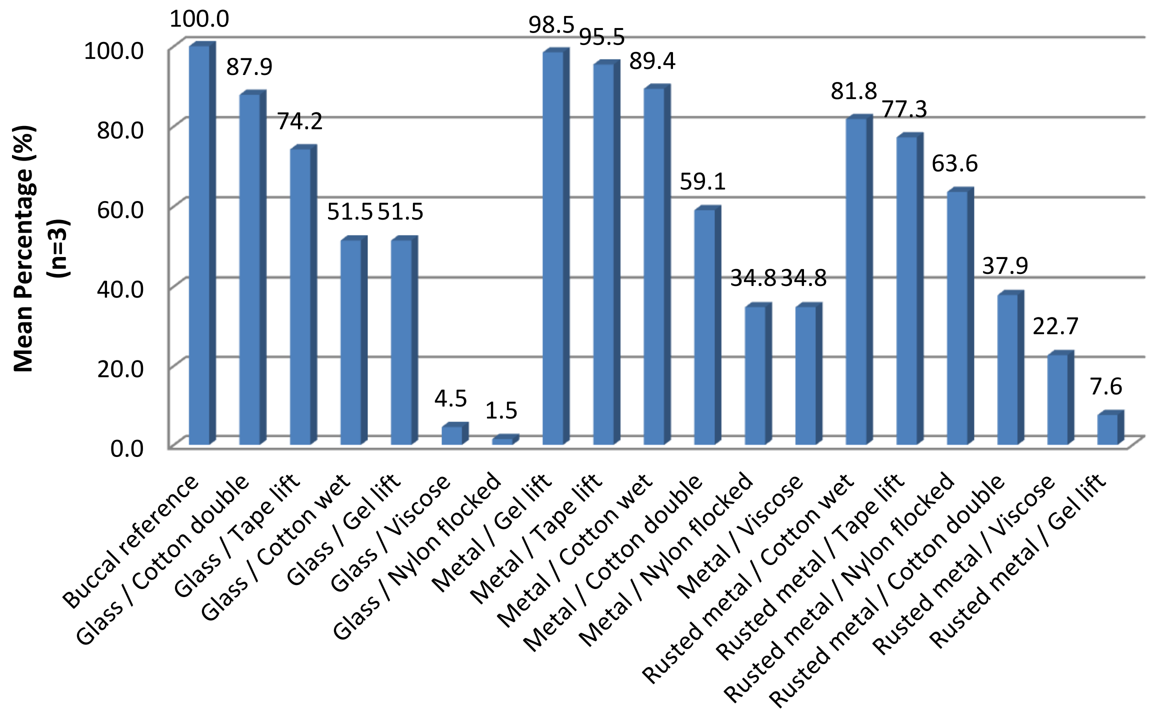


Figure 5.2 Mean percentage of assignable alleles from DNA samples recovered from glass, metal and rusted metal slides.

Figure 5.2 shows the mean percentage of assignable alleles recovered from 0.5 µl aliquots of blood that had been deposited on each of glass, metal and rusted metal slides and then recovered using cotton wet swabs, cotton double swabbing, viscose wet swabs, nylon flocked wet swabs, mini-tapes and gel-lifters. The rankings for the individual samples for the DNA Recovery study, ranked according to percentage of profile present, are presented in Appendix Four (A4.3). Table 5.2 summarises the mean and standard deviations for the peak heights for each variable.

Table 5.2 The mean and standard deviation values for the peak height values for assignable alleles recovered from glass, metal and rusted metal slides.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Reference	25	50,00	18691,00	5251,7200	4116,53116
Positive control	22	811,00	2637,00	1732,0909	495,18115
Cotton double Glass	54	50,00	413,00	184,5741	98,69041
Cotton double Metal	36	51,00	389,00	165,2222	89,23264
Cotton double Rusted metal	23	63,00	559,00	179,7826	101,44365
Cotton wet Glass	29	55,00	452,00	142,9310	96,51015
Cotton wet Metal	53	51,00	2075,00	478,2642	509,52309
Cotton wet Rusted metal	48	61,00	545,00	186,0625	91,62212
Gel lift Glass	30	50,00	355,00	147,1667	78,57660
Gel lift Metal	59	101,00	5236,00	1529,5763	1128,41950
Gel lift Rusted metal	4	98,00	112,00	107,0000	6,16441
Nylon flocked Glass	1	100,00	100,00	100,0000	.
Nylon flocked Metal	20	53,00	719,00	164,3000	154,56870
Nylon flocked Rusted metal	12	50,00	138,00	99,3333	27,97185
Tape lift Glass	44	59,00	731,00	243,9318	146,20301
Tape lift Metal	58	62,00	1335,00	460,1379	280,24053
Tape lift Rusted metal	47	51,00	1951,00	485,7660	434,19727
Viscose Glass	3	60,00	100,00	73,3333	23,09401
Viscose Metal	23	52,00	698,00	204,6957	159,21410
Viscose Rusted metal	4	56,00	181,00	116,5000	53,04401
Valid N (listwise)	1				

If the mean percentages of assignable alleles are examined, the gel lift and mini-taping on metal appear to produce the most complete DNA profiles, which contradicts the DNA quantification results for these recovery methods. This could suggest the DNA quantification method is not very reliable, or it could indicate that although low quantities of DNA were recovered there was sufficient good quality DNA present in the sample to generate a strong DNA profile. The mean peak heights for these samples are relatively high, which demonstrates that the profiles are good quality, and this is supported by the clear electropherogram examples included in the Appendix Four (A4.5 and A4.6).

According to the mean percentages of assignable alleles, the lowest performing recovery methods were the viscose swab on glass, the nylon flocked swab also on glass and the gel lift on rusted metal. All of these samples also gave the lowest peak height values, which supports the poor profile quality findings (Appendix 4, A4.7 – A4.9). Although the results for the gel lifter on rusted metal are what would be expected based on the quantification results, the other two results are unexpected as both gave good quantification readings. The poor quality profiles suggest that either very little DNA was present (which is not supported by the quantification values in all of the cases) or that the DNA that was present was unsuitable for DNA profiling. As the DNA quality and quantity deposited was controlled, through use of a blood sample, this would be an unlikely explanation. The SD values for viscose swab and nylon flocked swab on glass were both relatively small (SD of 0.11 and 0.10, respectively) which does not support any claims that the DNA quantity wasn't relatively reproducible across samples. A more likely explanation is that the results for the quantification were unreliable.

Generally the mini-taping recovery process appears to have generated good quality DNA profiles which alleviates any concerns raised by other authors of the adhesive inhibiting DNA (Williamson, 2012; Verdon, Mitchell and van Oorschot, 2014). As the mini-tapes used in this research (Scenesafe's FAST™ minitape) are designed for the recovery of DNA material, there should be no issue of inhibition. Furthermore, the use of tapes removes any requirement for a solution to be applied, and therefore reduces the potential for PCR inhibitors to be collected alongside the sample (Verdon, Mitchell and van Oorschot, 2014). One potential explanation for the high success rate observed with the tape lifting method could be that as large surface areas can be sampled, there is an increased chance of obtaining a successful DNA profile (Williamson, 2012). In this instance, although the surface area was quite small, because the blood was dried when it adhered to the tape, it may have been easier for it to be released into the extraction buffer when compared with the ease of substance release from the swab materials.

In comparison, the results from the gel lifters were more variable and therefore less reliable. Although gel lifters produced the highest quality profile from polished metal, they generated the poorest quality profile from the rusted metal, as previously highlighted. The gelatine in the lifters is soluble in the DNA extraction buffers so this should not cause any issues in relation to releasing the DNA (Yamamoto et al. 2010).

There may be the potential for any dissolved gelatine to cause issues with the spin-column extraction method by blocking the silica filter preventing DNA from binding or from eluting from the column. There is also the possibility that the gel lifter collected small pieces of rusted metal alongside the DNA sample, leading to PCR inhibition, which could explain the poor result for this sample.

The results from the cotton wet swabs are encouraging, with good quality profiles being recovered from the metal and rusted metal surfaces. Double swabbing with cotton swabs in these instances produced a lower quality profile than when using wet swabs, but on glass this trend was reversed. These results are a complete contradiction to the quantification data generated, which suggests that DNA quantification data should be viewed with caution if it is to be used to estimate the potential for DNA profiling success.

The quality of profiles from the samples recovered using viscose swabs were generally the poorest on each surface type. The contrast between the profile quality and DNA quantity was most stark when observing the samples recovered from glass. Similarly, the quality of profiles from the samples recovered using nylon swabs was not as expected from the quantification results. The nylon swabs generated poor quality profiles on metal and glass surfaces, with a significantly improved quality of profile being generated from the rusted metal.

Published research that compared the ability to recover a DNA profile when cotton and nylon swabs were used reported that despite the manufacturers' claims, flocked nylon swabs are not as effective as cotton swabs at DNA recovery (Brownlow, Dagnall and Ames, 2012). However, the researchers did state that variations in this trend were observed when specific swab types were combined with different extraction methods, so research into optimal combinations of methods to identify a complimentary approach may be required.

Whilst evaluating the quality of the profiles from these samples, it was noted that there was practically no evidence of spurious or contaminating alleles in almost all of the samples. Very few additional peaks were observed, but when detected could be easily distinguished as contamination as opposed to stutter products or background noise. One example of this is given in Appendix Four, A4.10 where an additional peak can be

observed on a mini-tape sample recovered from rusted metal. It is not unexpected that out of the three surfaces there may be some contamination on the rusted metal surface, as this could not be cleaned to the same extent as the other surfaces due to the nature of the material. The lack of contamination present could be due to the fact that standard PCR amplification cycling conditions were applied thereby reducing the potential for contamination. Alternatively, perhaps the collection of background, contaminating DNA was reduced as the sample for collection was easily visualised in this research. In traditional cases involving touch DNA, the source material is not easily distinguished from other material, so the potential for collection of contaminating DNA samples may be increased.

This research also utilised the QIAamp spin-column extraction method which could account for the high success rate in these samples. Research carried out by Castella et al. (2006) reported that the QIAamp DNA extraction procedure, when used in conjunction with the sample homogeniser QIAshredder, was more efficient at successfully recovering DNA profiles from several different types of evidential samples than either Chelex or Phenol-Chloroform procedures. This combined system of homogenisation and extraction increased the yield of DNA recovered as well as improving the quality of the resultant DNA profile for sample types such as cigarette butts, food remains, blood swabs and saliva swabs. Although the sample homogeniser was not used in this research, the success rate of the sample set as a whole appears to be quite high.

Another indicator or DNA quality is the heterozygote balance of samples, especially in low yield DNA samples. Table 5.3 shows the mean heterozygote balance values for assignable alleles recovered from glass, metal and rusted metal slides using cotton wet swabs, cotton double swabbing, viscose wet swabs, nylon flocked wet swabs, mini-tapes and gel-lifters. The peak heterozygote balance values for each allele in the DNA Recovery study are presented in Appendix Four (A4.4).

Table 5.3 The mean heterozygote balance values for assignable alleles recovered from glass, metal and rusted metal slides.

Surface	Recovery method	Mean heterozygote balance (%)
-	Buccal reference	87.17
-	Positive control	88.09
Glass	Cotton double	60.24
Glass	Cotton wet	66.93
Glass	Gel lift	86.34
Glass	Tape lift	63.95
Metal	Cotton double	67.67
Metal	Cotton wet	66.61
Metal	Gel lift	71.80
Metal	Nylon flocked	71.21
Metal	Tape lift	66.98
Metal	Viscose	58.78
Rusted metal	Cotton double	58.93
Rusted metal	Cotton wet	65.53
Rusted metal	Nylon flocked	91.23
Rusted metal	Tape lift	61.43

Examination of the results for heterozygote balance indicates that almost all of the mean values are greater than 60%, which demonstrates that the majority of profiles are of good quality (Goodwin, Linacre and Hadi, 2007). The only recovery methods where the samples have exhibited a mean value of less than 60% are viscose swabs on metal and cotton double swabbing on rusted metal. These results are supported by the mean profile percentage values, as viscose swabs produced the lowest quality profile to be recovered from the metal surface and rusted metal was the least successful surface for the cotton double swabbing technique in relation to profile percentage. However, if the

standard deviation values for the mean heterozygote balance are examined in more detail (Table 5.4), the results may not be as reliable as they first appear.

Table 5.4 The mean and standard deviation values for the heterozygote balance for assignable alleles recovered from glass, metal and rusted metal slides.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Reference	9	72,37	94,72	87,1744	7,64389
Positive control	11	67,56	98,94	88,0900	11,09727
Cotton double Glass	21	25,25	83,75	60,2371	20,43780
Cotton double Metal	9	21,79	99,14	67,6656	23,96759
Cotton double Rusted metal	6	37,94	78,76	58,9300	17,26281
Cotton wet Glass	6	28,00	85,98	66,9333	22,85963
Cotton wet Metal	21	18,41	96,55	66,6114	22,73716
Cotton wet Rusted metal	17	34,78	96,73	65,5282	16,40442
Gel lift Glass	5	73,64	98,25	86,3400	10,20151
Gel lift Metal	26	22,90	99,93	71,7969	21,65371
Nylon flocked Metal	5	41,38	98,15	71,2140	22,67185
Nylon flocked Rusted metal	1	91,93	91,93	91,9300	.
Tapelift Glass	16	23,93	97,46	63,9494	23,92607
Tapelift Metal	26	24,95	97,28	66,9773	22,48171
Tapelift Rusted metal	16	31,60	94,47	61,4275	18,74750
Viscose Metal	6	34,18	94,52	58,7800	22,46513
Valid N (listwise)	1				

The standard deviation values for some of the samples are quite large, for example double swabbing with cotton from glass. The mean value for this sample type is 60.2% so appears to fit the criteria of a good profile, but the minimum value observed in this data set was 25.3% which would suggest a poor quality profile was produced. However, the profile percentage value for this sample type was high, suggesting a successful DNA recovery result. Upon closer inspection of the profiles (Appendix Four, A4.11 – A4.12), it was evident that although the majority of alleles were detected, there was some clear indications of heterozygote imbalance, that haven't caused an issue in this case of identification, but with lower yields of DNA or with the presence of contamination, could be very problematic. This was also the case with many of the other sample types, where the minimum heterozygote balance observed was as low as 18.4%, however this

imbalance appears not to have negatively impacted on the process of profile identification. This suggests that although heterozygote balance should be noted when observed, the impact of imbalance may not be as significant as it first appears if the peak height values are sufficiently robust to manage this imbalance.

Although several different types of swabs were considered in this study, other methods are available and have been considered with regards to use in DNA recovery.

Mulligan, Kaufamn and Quarino (2011) compared the use of different fabrics (not in swab format) for the recovery of cellular DNA. They reported that swabbing substrates that were dry or wet with water recovered higher yields of DNA than those wet with isopropanol. They also identified that woven fabrics with a low thread count generally performed well in a number of different circumstances (when wet, in a highly utilised environment, etc.).

Most recently, Verdon, Mitchell and van Oorschot (2014) have published research evaluating the success of DNA recovery from fabrics using tape lifting when compared to recovery using cotton swabs. Their findings were that in certain cases the tape lifting collected more DNA than swabbing, but advised against the use of tape in cases where the fabric is made up of loose fibres (e.g. flannelette). These results are in agreement with those reported by Hansson et al. (2009) who also found tape to be more efficient than cotton, flocked or foam swabs when recovering touch DNA from cotton material. However, Williamson (2012) doesn't advocate the use of the tape-lifting recovery method at the crime scene due to an increased chance of contamination, therefore evaluation of this method in a crime scene environment should be carried out before recommending its widespread use.

Thomasma and Foran (2013) compared a range of swabbing solutions to determine the effect these may have on the recovery of touch DNA and found that the detergent based solutions demonstrate a significant increase in DNA yield when compared to swabbing with water. Vacuum systems for DNA recovery have also been suggested (Van Oorschot, Ballantyne and Mitchell, 2010), with most recently the M-Vac liquid based system being proposed as an effective technique for recovery of trace levels of DNA evidence (M-Vac Systems® Inc).

There have been suggestions that the application of laser microdissection techniques or flow cytometry could be applied to samples where there is a believed to be a significant amount of DNA-containing material from other sources, and where the aim is to recover a DNA profile from a single source. Van Oorschot, Ballantyne and Mitchell (2010) discuss the use of this technique but identify the limitations of its applications to the forensic field. It would appear that although these are both extremely effective techniques the potential for widespread application is limited due to the time required and costs associated with both.

5.5 Conclusions

This chapter aimed to address several research questions in relation to the recovery of DNA. The research aimed to determine what method was the most effective at recovery DNA from inanimate objects. The conclusions drawn will be based predominantly on the DNA profiling results, as the DNA quantification results do not appear to support the trends realised by the DNA profiling. Swabbing (using wet cotton, double cotton, wet viscose and wet nylon swabs) was compared with lifting (using mini-tapes and gel lifters) of a bloodstain on three different surfaces (glass, polished metal and rusted metal). It was found that swabbing in general did not generate consistently better profiles than lifting. The swabbing material appeared to have a significant effect on the success rate, with cotton swabs generally performing well, viscose swabs performing poorly consistently and nylon swabs exhibiting very variable results (very poor profiles on some surfaces and very good profiles on other surfaces). The two methods of lifting also gave different results with mini-taping generally performing well but gel lifters generating more variable results, including some very poor profiles on some surfaces and very good profiles on other surfaces.

In relation to the surface type having an impact on the success of DNA recovery, there appeared to be a strong correlation between surface types and certain recovery methods. On glass surfaces, the optimal methods appear to be cotton double swabbing (with a mean percentage profile of 87.9%) followed by mini-taping (74.2%). These findings are well supported in the published literature. On the polished metal surface, gel lifters

performed the best, closely followed by mini-tapes and wet cotton swabs (mean percentage profiles of 98.5, 95.5 and 89.4%, respectively). This is a surprising finding as there is currently little research published on the use of gel lifters specifically for DNA recovery, so would be an interesting area to conduct more research into. Mini-tapes have been recommended for recovery of DNA from firearms (Murray, 2005) and therefore these research findings support those recommendations. On rusted metal surfaces, the optimal methods appear to be cotton wet swabbing and tape lifting, with mean percentage profiles of 81.8 and 77.5%, respectively. The tape lifting findings may be supported by the recommendation that tape lifting be used to recover touch DNA from textured surfaces (such as clothing) (Hansson et al., 2009; Verdon, Mitchell and van Oorschot, 2014). However, the wet cotton swab findings are unexpected as the use of a liquid is believed to increase the potential for inhibitors to be co-recovered.

In relation to the potential recovery of inhibitors alongside trace levels of DNA, the results indicated that gel lifters and double swabbing using cotton swabs would results in poor quality profiles, perhaps as a result of collecting PCR inhibitors during recovery. However, more repeats for each swab and lifting material would be needed to determine definitive recommendations for optimal recovery methods for different surfaces. More discussion on DNA recovery methods will be included in later sections, with specific reference to firearms and cartridge case evidence.

Six. Persistence and Transfer of DNA

6.1 Introduction to DNA Persistence and Transfer

6.1.1 Introduction to DNA Persistence

When discussing the degree to which DNA persists on an item, there must be consideration of three potential issues; contamination, the number of potential donors and profile dominance. In this instance, DNA persistence relates to the ability to detect the DNA on a particular item after a significant event.

Contamination is a particularly important issue when analysing DNA evidence, especially when low levels of DNA are anticipated. Contaminating peaks on a DNA profile could arise as a result of background levels of DNA being recovered alongside the sample of interest, as this extraneous DNA would be co-extracted and amplified. Alternatively, contamination can be introduced during the DNA analysis process, at any stage from the DNA recovery to capillary electrophoresis.

Whilst every effort is taken to reduce potential contamination throughout DNA analysis, little can be done to address the issue of extraneous DNA contamination. For research experiments, procedures such as UV light irradiation or wiping surfaces with ethanol or bleach (Linacre, 2010; Raymond et al., 2004; van Hoofstat et al., 1999) are carried out prior to experimentation in order to remove as much DNA from the surface. A negative control swab (also referred to as an 'environmental sampling control') is then collected prior to experimentation, to determine the success of the decontamination procedure. For evidential samples a negative control swab is often collected from a visually clean area on the exhibit, to give an idea of the potential background levels of contamination. When interpreting profiles recovered from handled items, any peaks identified in the sample swab which corresponding with peaks in the negative control swab are discounted, as these are determined to originate from extraneous background DNA.

DNA contamination introduced by crime scene or laboratory personnel, or by consumables used during DNA analysis techniques can be monitored. Negative controls are often included at different stages of the DNA profiling process to monitor the incidence of contamination. Whilst research has been carried out into the different

decontamination procedures available (Preuβe-Prange, 2009) there is still no standard decontamination method used which can eliminate DNA contamination completely, without interfering with downstream analysis. This could be a result of the multitude of potential stages where DNA could be accidentally introduced and the lack of general awareness of the potential issue of contamination by individuals involved (Rutty, Hopwood and Tucker, 2003; Rutty, 2000; Toledano et al., 1997; Port et al., 2006; Rutty, 2002; Finnebraaten, Granér and Hoff-Olsen, 2008; Toothman et al., 2008; Tolliver and Sobieralski, 2008; Schwark et al., 2012; Poy and van Oorschot, 2006; Proff et al., 2006; Raymond et al., 2008).

A recent study examining potential DNA contamination through environmental monitoring (sampling equipment and surfaces to determine the background level of DNA in forensic laboratory settings) identified that methods currently perceived to be fit-for-purpose in terms of reducing DNA contamination, may not be sufficient for the new, more sensitive DNA profiling chemistries (Ballantyne, Poy and van Oorschot, 2013). The authors recommended that in addition to post-manufacture treatments such as ethylene-oxide, gamma irradiation and UV irradiation, forensic scientists must consider applying more stringent cleaning methods such a Virkon-S, DNA *ZAP* or DNAway.

Research indicates that there are many factors involved in the persistence of deposited DNA on a particular item. As outlined previously, DNA can be removed or degraded as a result of internal and external influences, and as different environmental conditions are experienced at different scenes, the ability to predict the potential for DNA persistence is extremely unlikely (Rudin and Inman, 2007; Raymond et al., 2008; Raymond et al., 2009b). Raymond et al. (Raymond et al., 2008; Raymond et al., 2009b) suggested that a profile successfully recovered from an outside environment would indicate that DNA was likely to be from a recent contact, due to the rate at which successful recovery of DNA decreases in this environment. However, when looking at the persistence of DNA on an item when there is more than one handler, the ability to determine who handled the item last would be evidentially very useful. Murray et al. (2002) reported that it was possible to recover a full profile from a good DNA shedder four months after the original contact. However, they observed a “marked decrease in the recovery of the poor shedder’s DNA” after the same time period.

Predominantly in casework, the most commonly cited reasons for examining touch DNA is to determine the habitual wearer of an item of clothing, or the last person to handle a specific item (Rudin and Inman, 2007). This assumes that the individual who handled the item last (or most often) would have the dominant profile present in the DNA profile recovered from the item. This model suggests that successive handling of an object either removes or replaces DNA deposited by a preceding handler. Therefore the persistence of DNA will depend on the degree of contact of the same object/area by other individuals (Gill, 2002).

Research carried out by Peel and Gill (2004) studied the ability to recover a DNA profile from a body fluid stain when the object had been handled either before or after stain deposition. The research indicated that handlers' DNA was dominant when a stain was small (no specific size was published) and the more dilute or degraded a stain was, the greater the contribution of the handler's DNA to the overall profile. It must be noted that in this research, the handler was deemed to be a good DNA shedder, with a high tendency to deposit DNA onto items.

Murray et al. (2002) carried out research into the persistence of DNA on wrist watches, by exchanging watches worn by individuals to determine the duration of wear required for the original wearer's DNA to be completely replaced by that of the new wearer. Different combinations of poor and good shedder status were used, with results indicating the major component of the mixed profile originating from the good DNA shedder after just three days of wear, whereas the poor DNA shedder typically required approximately two weeks of wear to become the major contributor. After two weeks of wear by the good DNA shedder, the DNA profile recovered contained no observable DNA from the original wearer. Lowe et al. (2003) also indicated that the shedder status of an individual could determine the success to which their DNA profile could be detected. Research completed by the authors during a simulated assault, where an area of t-shirt was grabbed, indicated that the profile of the good shedder was the major component in the profiles recovered. Furthermore, in samples recovered from items handled by more than one individual, van Oorschot and Jones (1997) reported that the major contributor to the profile was not always that of final handler. This research was also supported by findings published by Balogh et al. (2003).

Research undertaken by Ballantyne, Poy and van Oorschot (2013) identified that DNA was present in higher quantities on pored or pitted surfaces when compared to smooth, non-porous surfaces. Although the research they were undertaking was in relation to removing background DNA contamination, their findings are still pertinent when considering the variables that could influence the degree of DNA persistence.

6.1.2 Introduction to DNA Transfer

Transfer of DNA can exist in multiple scenarios. Primary transfer of DNA occurs when an individual handles an object and DNA from that individual is transferred directly on to the object. Secondary transfer occurs in one of four ways:

- An individual (Person A) comes in to contact with another individual (Person B), who then comes into contact with an object. The presence of Person A's DNA on the object would indicate secondary transfer of DNA.
- An individual (Person A) comes in to contact with another individual (Person B), who then comes into contact with another individual (Person C). The presence of Person A's DNA on Person C's hand would indicate secondary transfer of DNA.
- An individual (Person A) comes in to contact with an object. A second individual (Person B) then comes in to contact with the same object. The presence of Person A's DNA on Person B's hand would indicate secondary transfer of DNA.
- An individual (Person A) comes in to contact with an object (Object 1). A second object (Object 2) comes in to contact with Object 1. The presence of Person A's DNA on Object 2 would indicate secondary transfer of DNA.

In the first two modes of transfer, a person acts as the vector or intermediary for DNA transfer. In the final two modes of transfer, an object acts as the vector for DNA transfer. Either an object or an individual can be the final substrate, or surface, that the transferred DNA is recovered from.

Tertiary DNA transfer involves an additional stage to those proposed for secondary transfer. For example, the process occurs if an individual (Person A) comes into contact with a second individual (Person B) who then comes into contact with a third individual

(Person C). Person C then comes into contact with an object. If Person A's DNA is present on the object then this would indicate tertiary DNA transfer. Secondary transfer is considered more of a casework issue than tertiary transfer is, as a profile resulting from the number of individuals involved in tertiary transfer would likely consist of multiple donors indistinguishable from each other. With secondary transfer, the majority of transfer scenarios involve two donors, making profile resolution more straightforward.

The potential issue of DNA transfer, and it relevance to casework interpretation, was not widely reported until DNA analysis techniques developed to such a degree of sensitivity that it was possible to recover a DNA profile from handled items. In 1997, van Oorschot and Jones (1997) suggested that transfer of DNA could exist both when an object or a person was acting as the vector for transfer. Lee et al. (1998) first reported secondary transfer of DNA in 1998, but this was more as an investigative aide (to link an individual to a scene) as opposed to an interpretational complexity. In 1999 Ladd et al. (1999) first presented a detailed analysis of the potential for secondary transfer of DNA and reported that their findings did not support the potential for secondary transfer to occur to such a degree that it would interfere with interpretation of forensic casework. However, in a future publication by Lee and Ladd (2001), it was suggested that secondary transfer could be an issue with more sensitive techniques such as LCN PCR or mtDNA analysis. The potential for secondary transfer to impact on forensic casework was first reported by Rutty (2002) in simulated manual strangulation scenarios. A partial DNA profile from a victim in a mock strangulation scenario was found on the control hand swabs of the offender, despite the fact the offender had never had direct contact with the victim. Secondary transfer from inanimate objects present in the work space of the two individuals (and therefore likely to be handled by both parties) was suggested as a potential mechanism for how the victims DNA had been observed on the hand of the offender. By 2002, there had been reports of unpublished data to support secondary transfer of DNA from an individual to an object, but no evidence of secondary transfer between objects (Gill, 2002).

In 2002, Lowe et al. (2002) introduced the idea that the amount of DNA deposited by individuals varied, and that people could be classified as good or poor shedders dependant on the amount of DNA they deposited through contact with a surface. They also reported secondary transfer of DNA, through contact of one individual with another,

followed by contact with an object, could occur. They stated that the potential for secondary transfer could not be determined when a time delay of 30 minutes or longer occurred between the initial contact and sample collection.

In a later publication in 2003, Lowe et al. (2003) reported that secondary transfer of DNA was observed when experiments involving poor and good shedders were carried out. Furthermore, with one pairing of subjects where a good shedder shook hands with a poor shedder and the poor shedder held an object, only the DNA of the good shedder was observed on the object. When a time delay of 30 minutes was introduced between contact and handling of the object, 70% of the total DNA from the mixed profile that was recovered belonged to the good shedder.

Wickenheiser (2002) published a comprehensive review of the published literature relating to DNA transfer in 2002. Wickenheiser reports that the average human being sheds approximately 400,000 skin cells per day and that secretions produced by the hand may be a source of DNA due to the large amount of DNA containing cells the secretions are exposed to en route to the skins surface. This review concluded two key statements in relation to DNA transfer. Firstly, it suggested that "it is extremely unlikely for the vector individual to inadvertently transfer only the first person's DNA without also leaving his or her own DNA in a larger amount". Secondly, in addition, it states that "The previous contributor will often be replaced by subsequent contact by a second individual. A trace DNA profile is indicative of the last individual to contact the substrate". In other words, DNA recovered from an item indicates the final person to handle that item, although mixed profiles that include components from previous handlers may be observed.

There have also been publications outlining the potential for secondary transfer of DNA to occur through contact with an object, more specifically through the use of fingerprint brushes when enhancing latent fingerprints (Proff et al., 2006; van Oorschot et al., 2005). Both publications suggested that DNA contamination could occur via secondary transfer, especially if a surface containing body fluids had been in contact with the brush previously, and advocated the use of decontamination techniques on used brushes.

More recently, research groups have investigated other variables that could influence transfer of DNA such as the effect of surface type (Farmen et al., 2008; Goray, Mitchell

and van Oorschot, 2010; Goray, Mitchell and van Oorschot, 2012; Goray, van Oorschot and Mitchell, 2012; Graham and Rutty, 2008; Daly, Murphy and McDermott, 2012), and the type of sample transferred (e.g. blood, saliva, semen, etc) (van Oorschot et al., 2009; Goray et al., 2010; Kenna et al., 2011; Wiegand et al., 2011). In one of the most recent publications, Daly et al. (2012) states that "Although secondary transfer is possible the profiles obtained from touched objects are more likely to be as a result of primary transfer rather than a secondary source".

Ballantyne, Poy and van Oorschot (2013) identified that although issues relating to DNA transfer such as the type of substrate, the type of biological material and the degree of contact had been considered, there was not yet "sufficient data to draw firm conclusions regarding the magnitude of risk this increased detection of environmental DNA poses to evidentiary material". The authors acknowledged however, that based on published research, recommendations could be made to reduce the potential of DNA transfer occurring during examination of evidential items.

The degree to which DNA transfer is a potential issue could depend on many variables including the number of contacts, the number of individuals involved in the transfer process and the type of vector for transfer. One could propose, if the mode of transfer involves object to object contact theoretically the amount of DNA present will be reduced, and will not get replaced by other DNA, therefore mixed profiles would not always be a result of DNA transfer. This concept is eluded to in Wickenheiser (2002) where it is stated that loosely adhering cells would be removed, resulting in any background DNA that was present prior to contact to become the dominant contributor once again. In this instance, the statement refers to the amount of DNA present on a car steering wheel if the final contact with the wheel was by an individual who was wearing gloves. There are many questions relating to DNA transfer that currently remain unanswered.

However, the implications that DNA transfer could have on the interpretation of casework are now widely disputed, especially with defence lawyers (Savino and Turvey, 2011; Jamieson and Meakin, 2010; Jamieson, 2009; Jamieson, 2012; Day, N.D; Tasker, 2006; Haesler, N.D). Tertiary transfer of DNA was used as a potential defence in the murder trial of MA v. Greineder (Thompson et al., 2003; Ryan, 2009; The Boston Channel,

N.D.; Taylor, N.D.) although the results provided to the court as evidence of possible tertiary transfer have not be published and Dr Greineder was later convicted. Court reports, produced by expert witnesses, which discuss secondary and tertiary transfer of DNA are now being presented in criminal trials. Specific results, from experiments designed to investigate the likelihood of DNA transfer occurring, are now being considered when interpreting the presence of the suspects DNA on an evidential item (Taylor and Johnson, 2001; Benzinger, 2005).

A further review of research into DNA transfer was published by Meakin and Jamieson in 2013 which drew together the previous published research in this field. This report evaluated the factors affecting deposition, persistence and transfer of DNA as well as the implications these factors have on the use of trace DNA in casework. They stated that the current situation in relation to DNA transfer is that published research has raised more questions than it has answered, and there remains a level of ambiguity relating to transfer of DNA when interpreting case work. They also identify that the current practice in giving evidence varies between forensic reporting officers; some feel an opinion as to the likelihood of transfer can be given during expert witness testimony, whereas others do not.

6.2 Research Questions

This chapter aims to address the overall objectives to study the persistence of DNA on handled objects, especially after repeated contacts and to study the potential for secondary transfer of DNA through an intermediary person or object.

Based on a review of the previously published literature of experimental work undertaken by different research groups, as outlined in the introduction to this chapter, several research questions were identified:

a. Will DNA deposited onto an object persist if that object is subsequently handled by another person?
b. Is it possible for subsequent handling of an object to completely remove previous traces of touch DNA deposited by the prior handler?

c. Is the DNA profile recovered from an object indicative of the final handler?
d. Does secondary transfer of DNA occur when the vector for transfer is an object?
e. Does secondary transfer of DNA occur when the vector for transfer is another person?
f. Is the process of secondary transfer reproducible or do donors exhibit daily variation in DNA deposition?
g. Is secondary transfer of DNA still evident after a time delay of 30 minutes?

6.3 Experimental Design

To address these research questions the following experiments were designed, as outlined below.

6.3.1 Persistence and Secondary Transfer of DNA with Person as Vector for Transfer and Final Substrate

Subjects were instructed not to wash their hands or come into contact with another individual for at least 1 hour prior to the experiment.

Subject A, Subject B and Subject C manufactured a scenario involving secondary transfer of DNA, by way of Subject A and B shaking hands for 30 seconds, followed immediately by Subject B and C shaking hands for 30 seconds.

DNA samples from the hands of the subjects were collected after contact by swabbing the hands using pre-wetted buccal swabs.

Hand swabs were collected from Subject A, B and C's hands after contact.

Chelex extraction was used to extract the DNA from the hand swabs. Both standard and LCN SGM+ PCR amplification were carried out on the samples, followed by Capillary Electrophoresis, with the only modification being that the kit used for DNA amplification was the AmpF*l*STR® Identifiler™ kit (Applied Biosystems).

6.3.2 Persistence and Secondary Transfer of DNA with Person as Vector for Transfer and Object as Final Substrate

For this study, 50 ml glass beakers were used. All beakers were autoclaved, then cleaned with 2% Virkon, 100% ethanol and double distilled water prior to use.

Subjects were instructed not to wash their hands or come into contact with another individual for at least 1 hour prior to the experiment.

Experiment 1: Subject A and Subject B manufactured a scenario involving secondary transfer of DNA, by way of Subject A and B shaking hands for 30 seconds, followed immediately by Subject B holding a glass beaker for 30 seconds.

Experiment 2: Subject A and Subject B manufactured a scenario involving secondary transfer of DNA, by way of Subject A and B shaking hands for 30 seconds. This was followed immediately by Subject A holding glass beaker 1 for 30 seconds and Subject B holding glass beaker 2 for 30 seconds.

DNA samples from the hands of the subjects were collected, as outlined below, by swabbing the hands using pre-wetted buccal swabs. DNA from the glass beakers were collected by swabbing the outer surface of the beaker with a cotton square.

Experiment 1: Hand swabs were collected from Subject A's hands after contact with Subject B. Hand swabs were collected from Subject B's hands after contact with the glass beaker. Object swabs (from the glass beakers) were collected after contact with Subject B.

Experiment 2: Hand swabs were collected from Subject A's hands after contact with glass beaker 1. Hand swabs were collected from Subject B's hands after contact with glass beaker 2. Object swabs were collected from glass beaker 1 after contact with Subject A and from glass beaker 2 after contact with Subject B.

Chelex extraction was used to extract the DNA from the cotton squares and hand swabs. Both standard and LCN SGM+ PCR amplification were carried out on the samples,

followed by Capillary Electrophoresis. Both AmpF*l*STR® Identifiler™ kit and AmpFlSTR® SGM Plus® kit (both Applied Biosystems) were used for DNA amplification.

6.3.3 Persistence and Secondary Transfer of DNA with Object as Vector for Transfer

For this study, 50 ml glass beakers were used. All beakers were autoclaved, then cleaned with 2% Virkon, 100% ethanol and double distilled water prior to use.

Subjects were instructed not to wash their hands or come into contact with another individual for at least 1 hour prior to the experiment.

Subject A and Subject B manufactured a scenario involving secondary transfer of DNA, by way of Subject A holding a glass beaker for 30 seconds, followed immediately by Subject B holding the same glass beaker for a further 30 seconds.

DNA samples from the hands of the subjects were collected by swabbing the hands using pre-wetted buccal swabs. Subject A and Subject B's hands were swabbed after contact with the glass beaker. DNA from the glass beakers was collected by swabbing the outer surface of the beaker with a cotton square. Object swabs (from the glass beakers) were collected after contact with Subject B.

Chelex extraction was used to extract the DNA from the cotton squares and hand swabs. Both standard and LCN SGM+ PCR amplification were carried out on the samples, followed by Capillary Electrophoresis. Both AmpF*l*STR® Identifiler™ kit and AmpFlSTR® SGM Plus® kit (both Applied Biosystems) were used for DNA amplification.

6.3.4 Secondary Transfer of DNA with Time Delay

For this study, 50 ml glass beakers were used. All beakers were autoclaved, then cleaned with 2% Virkon, 100% ethanol and double distilled water prior to use.

Subjects were instructed not to wash their hands or come into contact with another individual for at least 1 hour prior to the experiment.

Subject A and Subject B manufactured a scenario involving secondary transfer of DNA, by way of Subject A and B shaking hands for 30 seconds. Subject A and Subject B manufactured a scenario involving secondary transfer of DNA, by way of Subject A and B shaking hands for 30 seconds. They were then asked to return to work (limited to desk-based activities) for 30 minutes, before returning to complete the experiment. During this 30 minute interval they were instructed not to wash their hands or come into contact with another individual. After the 30 minute interval, Subject A held glass beaker 1 for 30 seconds and Subject B held glass beaker 2 for 30 seconds.

DNA samples from the hands of the subjects were collected, as outlined below, by swabbing the hands using pre-wetted buccal swabs. DNA from the glass beakers was collected by swabbing the outer surface of the beaker with a cotton square.

Hand swabs were collected from Subject A's hands after contact with glass beaker 1. Hand swabs were collected from Subject B's hands after contact with glass beaker 2. Object swabs were collected from glass beaker 1 after contact with Subject A and from glass beaker 2 after contact with Subject B.

Qiagen extraction was used to extract the DNA from the cotton squares and hand swabs. LCN SGM+ PCR amplification was carried out on the samples, followed by Capillary Electrophoresis.

6.4 Analysis and Interpretation of DNA Persistence and Transfer Results

The following sections discuss the results gained during the transfer and persistence experiments. Although there appears to be contaminating alleles present in many of the profiles, especially the secondary transfer studies, this is not a result of poor anti-contamination measures. The subjects were requested to not wash their hands or come into direct contact with any other individual in the two hours prior to taking part in the experiment. This was to ensure 'normal' levels of DNA (that is, as true to real life as possible) were present on the individuals hands for the experiments. Any 'foreign' alleles (that is, alleles that could not be assigned to the individuals involved in the study) were perceived to be a result of transfer of DNA picked up through the individuals' normal,

everyday activities. Lowe et al. (2002) reported that in samples collected 6 hours after hand washing, 100% of the individuals DNA profile was recovered. Their results indicated that in the eight donor individuals tested, samples collected 2 hours after hand washing generated a minimum of 60% of the donor profile. For the purposes of this research, 2 hours was decided upon as it was felt that the donors would be able to refrain from contact and hand washing for this duration. It was felt that requesting a longer duration would mean that donors would be unlikely to refrain from using the toilet (where they would then wash their hands) and that they would be more likely to come in contact with a third person.

In all experiments, other than the DNA deposition studies, donor individuals were asked to rub their hands together for 30 seconds prior to the commencement of any experiments. The aim of this was to attempt to standardise the amount of DNA present on the individual's hands to ensure reproducibility throughout the repeats. Allen et al. (2008) reported that there was variability in the amount of DNA deposited by an individual's right and left hand. In contrast, van Oorschot et al. (2003) reported that the amount of DNA deposited was similar for both the right and left hand of a donor. He stated that the variation in the amount of DNA deposited was greater between individuals, than between one individual's right and left hand. In a more recent publication, Quinones and Daniel (2012) suggested a glass bead collection method for standardisation of the starting amount of DNA. They have proposed that rubbing the hands together with glass beads ensured creation of identical samples. As the experiments carried out in this study were designed to address issues of transfer and persistence through regular (non-intimate) contact, the use of rubbing hand together prior to any handshaking was deemed an appropriate measure to standardise the staring amount of DNA.

6.4.1 Persistence and Secondary Transfer of DNA with Person as Vector for Transfer and Final Substrate

The profiles for the persistence and secondary transfer samples where a person was the vector for transfer and the final substrate are present in Appendix Five (A5.1)

This experiment was designed to determine the potential for an individual to act as a vector for DNA transfer. This would mimic a situation where a suspect has claimed their DNA profile has been recovered from a victim's body through mutual contact with an intermediary person. In this experiment Subject A shook hands with Subject B. Subject B then went on to shake hands with Subject C. All three subjects' hands were swabbed after their final contact. The results are summarised in Appendix Five (A5.1) and in Figure 6.1.

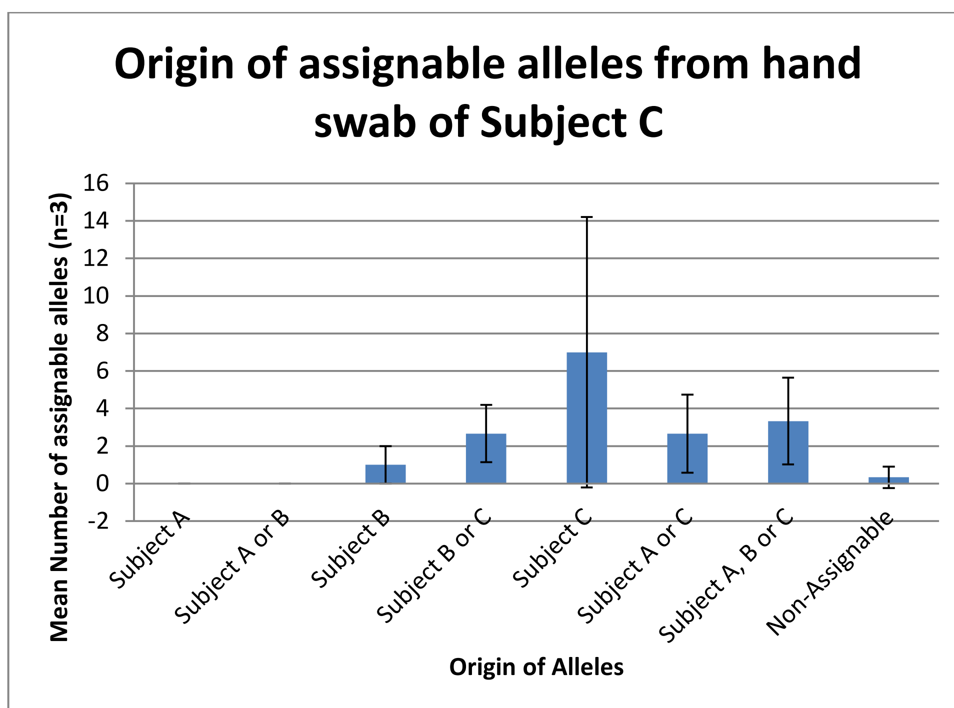


Figure 6.1 Mean number of assignable alleles from swabs recovered from Subject C's hand after transfer process (Subject A ↔ Subject B ↔ Subject C)

The results indicate that the potential to identify secondary transfer of DNA where a person acts as the vector for transfer, and an individual is the final substrate, was minimal. There was no instance where an allele that could only have originated from Subject A was detected on Subject C's hand. However, it is not possible to state that this mechanism of secondary transfer is not feasible. This is due to the incidence of shared alleles, more specifically alleles that could have originated from any of the three donors involved in the transfer experiments. This issue of shared alleles makes it impossible to determine the exact source of these alleles, and therefore impossible to determine the degree of transfer of DNA. There is no reference to this mechanism of transfer in any

publications currently available, possibly as a result of the complexities in interpreting mixed profiles where three individuals are potential contributors. However, in a related publication, Rutty (2002) identified the potential for secondary or tertiary transfer of DNA through manual strangulation (which mimics the skin to skin to skin transfer investigated here). The potential for secondary transfer through contact between individuals was also reported by Graham and Rutty (2008) where they stated that secondary transfer of DNA could be observed.

Another observation from the data collected was that, although the sample number tested was small, there was significant variation (see error bars on Figure 5.2) in the detection of the subjects' alleles between samples. For example, one sample recovered 15 alleles originating from Subject C on the hand swab taken from Subject C. This is not surprising given the location the sample has been collected from. However, in one of the other samples, only 1 allele originating from Subject C was identified in the hand swab taken from Subject C. This was not expected as it would be anticipated that the DNA from the donor of the hand being swabbed would far outweigh any other DNA donors in this instance. This phenomenon of low levels of self DNA being recovered from an individual has been reported by Graham and Rutty (2008) when swabbing the neck area, but was identified as potential sampling error. In this instance it may also be suggested that the profile was not strong for this sample, with an average peak height of 62 RFU across the electropherogram, indicating a poor quality/quantity DNA sample.

It was also possible to determine the potential for persistence of DNA from other sources on an individual's hand by examining the percentage of alleles present on Subject B's hand, after contact with both Subject A and then Subject C. The results are summarised in Appendix Five (A5.1) and in Figure 6.2.

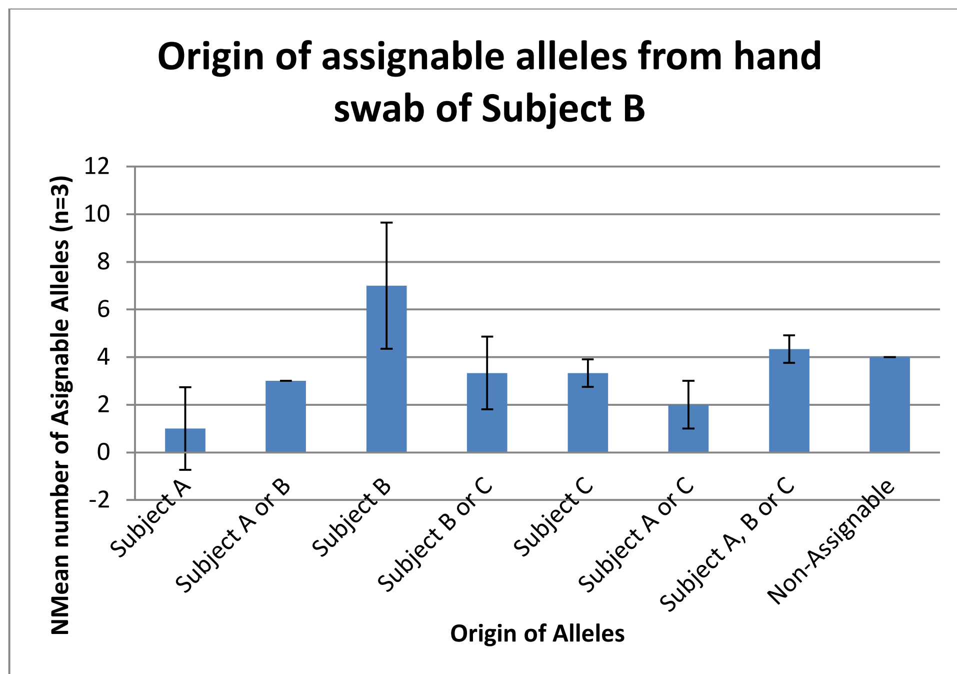


Figure 6.2 Mean number of assignable alleles from swabs recovered from Subject B's hand after transfer process (Subject A ↔ Subject B ↔ Subject C)

Interpretation of the results generated from the swab of Subject B's hand suggests that the persistence of a specific individuals DNA is variable depending on the donors involved.

When persistence is referred to in these studies it is not in relation to how long an individual's DNA will remain on an object for. Instead it relates to the potential for one individuals DNA to persist on an object, even after contact of another individual with that same object. In this experiment, DNA persistence was examined by analysing the total number of alleles that could have originated from each of the donor individuals.

To ensure that there was no allele bias (i.e. to ensure that one donor is not under-represented due to the incidence of shared alleles between donors), Figure 6.3 summarises the total percentage of alleles that each donor may have contributed by also including the presence of any shared alleles within the mixture. This means that the total percentage may add up to more than 100% as shared alleles, for example, between Donor A and B will be included in Donor A's total, as well as Donor B's total. However, it was felt that this figure may be more representative of the situation given the issue of shared alleles.

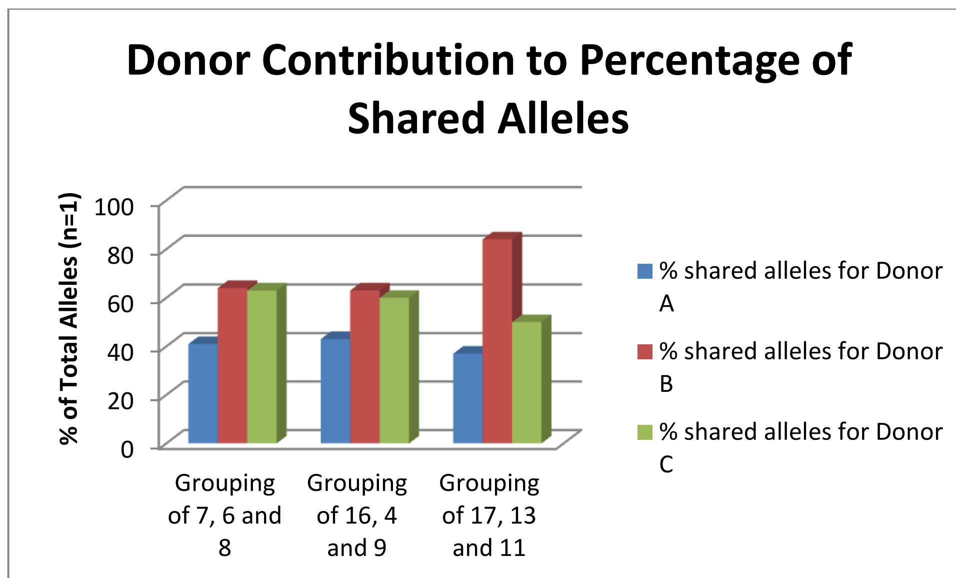


Figure 6.3 Percentage of shared alleles that could have originated from Donor A, B or C recovered from Subject B's hand swab.

These results indicate that even after the donor had further contact with another individual (with Subject C), DNA from the initial contact (with Subject A) was present on the donor's hand (Subject B). In all instances, DNA from the donor (Subject B) was present in excess of Subject A, even with consideration of any shared alleles. However, Subject C's contribution to the mixture was comparable to Subject B's, and in one instance Subject C exceeded that of Subject B (when considering only alleles that could be designated as being definitively contributed by a specific donor). This suggests that DNA was transferred from one individual to another through contact, with DNA from some individuals persisting better than others. In order to determine the degree to which DNA persists on an individual's hand, a further experiment was designed studying the effect of subsequent contacts on DNA recovery.

One other observation from this data set is the presence of non-assignable alleles that did not originate from any of the subjects in this study. The presence of these alleles is likely to be a result of one or more of the subjects collecting this contaminating DNA through contact with another item or person. The presence of these alleles indicates the challenges associated with interpreting profiles that are likely to have arisen as a result of

transfer and support the more cautious approach of presenting evidence of this nature in a court environment.

6.4.2 Persistence and Secondary Transfer of DNA with Person as Vector for Transfer and Object as Final Substrate

The profiles for the persistence and secondary transfer samples where a person was the vector for transfer and an object was the final substrate are present in Appendix Five (A5.2).

This experiment was designed to determine the potential for an individual to act as the vector for DNA transfer, but in place of a third individual the final substrate was an object. This would mimic a situation where a suspect has claimed their DNA profile has been recovered from an item at a scene as a result of secondary transfer from an intermediary person. One such case where this has been presented as a defence was in the murder of Mabel Greineder (The Boston Channel, N.D;Taylor and Johnson, 2001).

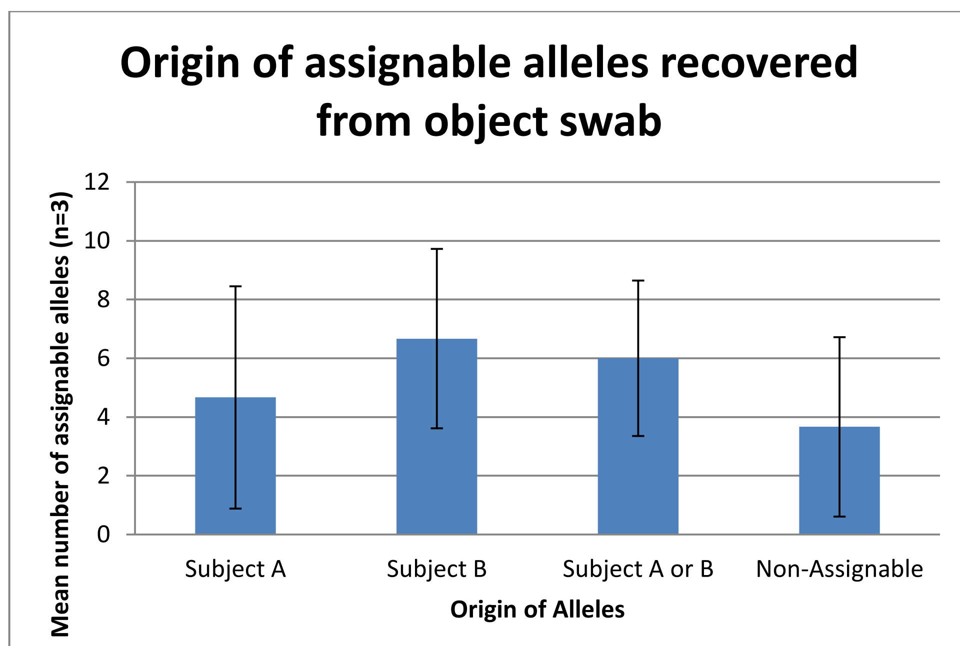


Figure 6.4 Mean number of assignable alleles from swabs recovered from the object swab after transfer process (Subject A ↔ Subject B ↔ Object)

These results indicate that DNA can be transferred from an individual to an object, by contact with an intermediary individual. These findings are in contrast with those of Ladd et al. (1999) who reported that their research did not support the conclusion that secondary transfer could compromise DNA profiling results. Results published by Murray et al. (2002) were somewhat consistent with the results presented in this thesis, in that they reported that secondary transfer could occur from an individual on to an object when a second person acted as the vector for transfer. However, they based their donor pairing on shedder status for their experiments and found when Subject A in the pairing was a 'good' shedder and Subject B was a 'poor' shedder then in some instances only the profile of Subject A was recovered from the object swab. This phenomenon has not been observed in the research carried out by the author. Lowe et al. (2002) reported findings that were consistent with the results reported in this thesis, in that secondary transfer of DNA onto an object was observed. They also reported the isolated occurrence of an individual's full DNA profile being recovered from an object solely as a result of secondary transfer by a second individual. However, they did identify that this occurred in designed 'ideal' conditions and therefore it was unlikely to routinely occur in casework. Lowe et al. (2002) also based their experiments on the individual's shedder status to create a 'worst case scenario'. Zoppis et al. (2014) report that secondary transfer of DNA was possible from person to person and then to object and also try and suggest that variation in the amount of DNA transferred could relate to whether sebaceous or non-sebaceous skin had previously been touched.

Wickenheiser (2002) discusses a number of case examples where secondary transfer of DNA has been reported. He concluded that in instances where secondary transfer occurs, the individual acting as the intermediary would contribute the major component to the profile, stating "it is extremely unlikely for the vector individual to inadvertently transfer only the first person's DNA without also leaving his or her own in a larger amount' (Wickenheiser, 2002). Although in all instances observed in this research the vector individual's DNA profile contributed to the object swab, it was not always the major contributor to the profile, which contradicts Wickenheiser's statement.

To determine whether the vector individual would routinely be the major contributor to the profile recovered from the object swab, one pairing of subjects carried out the transfer experiment over 6 different days. These profiles are presented in Appendix Five (A5.3).

The results of the DNA recovered from the object swabs are presented in Table 9.10 and summarised in Figure 6.5.

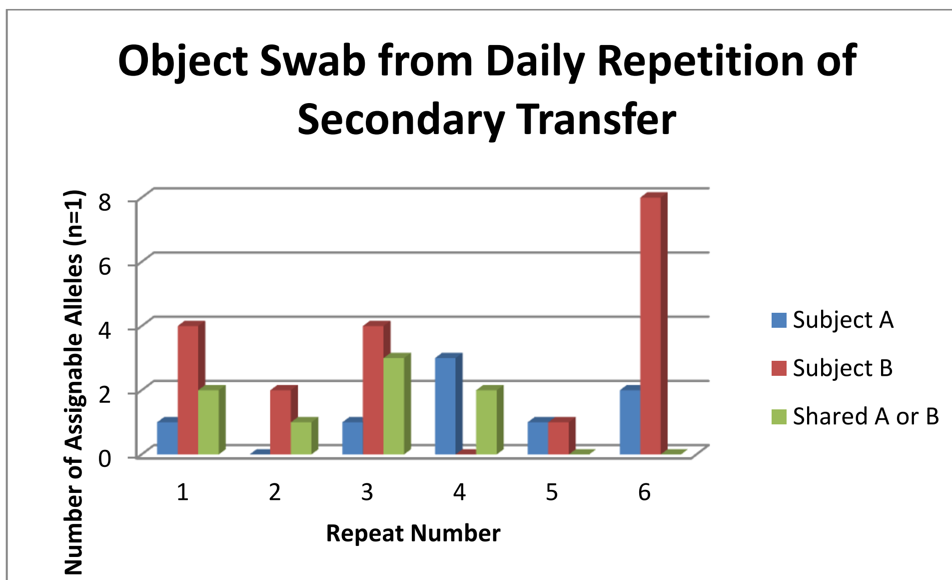


Figure 6.5 Number of alleles originating from Subject A or B recovered from Object swab in Daily Repetition Study.

Before attempting to reach any conclusions in relation to the data, it is important to note than in all samples for the daily repeats of this transfer process the success rate of the DNA recovery was very poor. The number of assignable alleles was very low and therefore would likely fall below what would be an interpretable profile in casework. However in this instance, as both contributors were known, the degree to which each donor contributed towards the profile was considered. It was found that the degree to which one individual contributed to the profile varied considerably between different contact events. This suggests that the amount of DNA an individual deposits and transfers is unpredictable. This supports the previous proposition by the author that the 'shedder status' of an individual is not a constant and therefore the attempt to define an individual's propensity to deposit DNA would not be indicative of the likelihood of secondary transfer occurring.

It was also possible to determine the potential for the persistence of DNA from other sources on an individual's hand after subsequent contact with an object by examining the percentage of alleles present on Subject B's hand, after contact with both Subject A and then the object. These results are presented in Figure 6.6.

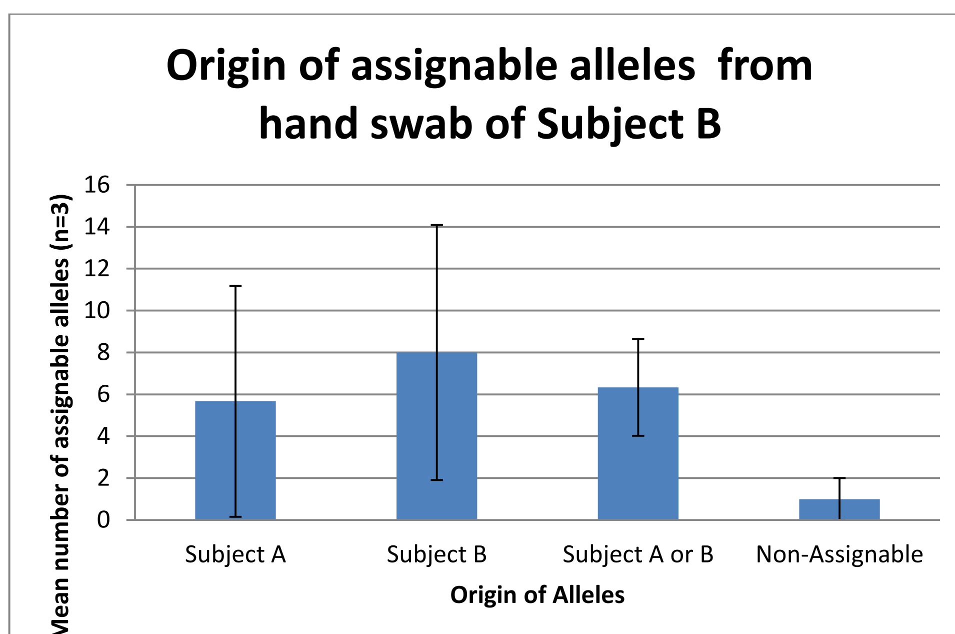


Figure 6.6 Mean number of assignable alleles from swab recovered from Subject B's hand after transfer process (Subject A ↔ Subject B ↔ Object)

These results indicate that even after the donor had further contact with an object, DNA from the initial contact (with Subject A) was present on the donor's hand (Subject B). In all instances, both DNA from the donor (Subject B) and DNA from the previous contact with Subject A were present. This suggests that DNA transferred through previous contact persists on an individual's hand even after contact with an object. This may give an insight into the mechanism by which DNA is transferred. Wickenheiser (2002) proposed that samples of DNA that consisted of a mixture of more than one individual would be deposited in the same ratio that they were present in. If the ratios of Subject A and B's DNA present on the object swab are compared with the ratios present on the hand swab of Subject B, it can be observed that there is some correlation between the

ratios of DNA present from each donor. The results reported here support the proposition of transfer suggested by Wickenheiser.

A further consideration in relation to these results is the potential for Subject B to transfer Subject A's DNA in consecutive contacts, as some DNA from Subject A had persisted on Subject B's hand even after contact with an object. Kenna et al. (2011) studied the persistence of salivary DNA on human skin and found that DNA could be recovered from fabrics that had been in contact with saliva on skin. This transfer of DNA from saliva to skin to fabric resulted in the recovery of sufficient DNA from the fabric to be able to generate a DNA profile. Although the researchers in this publication did not explore the ability to recover DNA from the skin post-contact with the fabric, the reported findings suggest the potential for transfer and persistence of DNA.

In order to further examine the issue of DNA persistence on items recovered from a scene a further experiment was designed studying the transfer of DNA when an object was the intermediary.

6.4.3 Persistence and Secondary Transfer of DNA with Object as Vector for Transfer

The profiles for the persistence and secondary transfer samples where an object was the vector for transfer and the final substrate are present in Appendix Five (A5.5).

This experiment was designed to determine the potential for an object to act as the vector for DNA transfer between two individuals. This would mimic a situation where a suspect has claimed their DNA profile has been recovered from a victim as a result of secondary transfer from an intermediary object.

In this experiment Subject A held an object (glass beaker) followed by Subject B then handling the same object. Subject A's hand was swabbed after contact with the object, Subject B's hand was swabbed after contact with the object and the object was swabbed after contact with Subject B. The results of the DNA recovered from Subject B's hand swabs are presented in Figure 6.7.

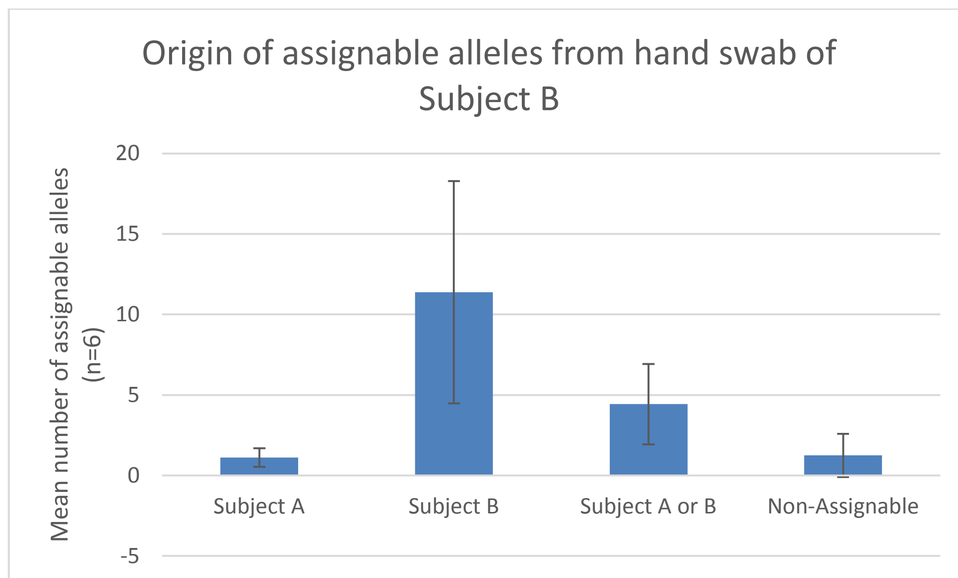


Figure 6.7 Mean number of assignable alleles from swab recovered from Subject B's hand after transfer process (Subject A ↔ Object↔ Subject B)

These results indicate that secondary transfer of DNA can occur when the vector for transfer is an object, but the dominant contributor to the DNA profile recovered from the second individual's hand is typically the donor himself. The contribution of transferred DNA in this instance was found to be minor in all cases, although there was no tangible trend in the mean peak heights for the contributions from both subjects (See Table 6.1).

Table 6.1 The mean and standard deviation values for the peak heights for assignable alleles recovered from Subject B's Hand.

Origin of Alleles	Donor Pairings					
	One		Two		Three	
	Mean RFU	SD RFU	Mean RFU	SD RFU	Mean RFU	SD RFU
Subject A	635.5	120.9	160	N/A	45.7	14.8
Subject B	366.3	338.1	369	240.8	533.9	457.8
Subject A or B	555.2	304.4	61.3	433.4	702.7	774.9
Non-assignable	N/A	N/A	146	12.7	105.5	65.8

By examining these values and the distribution of peak heights on the electropherogram, it can be concluded that it would not be possible to differentiate between DNA from different sources. The issue in this instance is not the presence or spurious alleles originating from someone other than the two subjects, as this is not a significant issue. The challenge with these samples is the variation within peak heights, so it is not possible to determine the number of contributors based on peak height ratios. For example, for donor pairing one at D8S1179, the peaks observed are depicted in Figure 6.8.

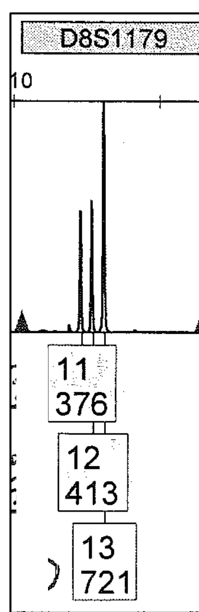


Figure 6.8 Electropherogram of locus D8 from swab recovered from Subject B's hand after transfer process in donor pairing one (Subject A ↔ Object↔ Subject B)

In the above example, allele 11 originates from Subject B, allele 12 could originate from Subject A or B and allele 13 originates from Subject A. If Subject B has heterozygote balance (so alleles 11 and 12 originate from her), then it could be assumed Subject A has allele drop out (so only allele 13 originates from her and allele 12 has 'dropped' out). However, the converse could also be true and it is not possible to determine this from the DNA profiles observed.

These findings were consistent with results published by other research groups, such as Poy and van Oorschot (2006) who identified that gloves and other objects could act as vectors for DNA transfer. Goray, van Oorschot and Mitchell (2012) also identified the potential for DNA transfer from objects, in this case transfer of DNA from evidential items to the forensic packaging used. Wickenheiser (2002) reports findings from Bellefeuille et

al. that confirmed transfer from objects to individuals, as well as from individuals to objects. The results outlined here have indicated that DNA can be transferred onto an object, and then from that object onto another individual. In a related field, a number of research groups have been examining the potential for transfer of body fluids from different surfaces which could have an impact on the interpretation of a DNA profile when presumptive or confirmatory testing has indicated the presence of a specific body fluid (van Oorschot et al., 2009; Goray et al., 2010; Kenna et al., 2011). This introduces a potential additional challenge with DNA interpretation, as previously DNA profiles originating from body fluids have not been disputed to the same degree that DNA profiles generated from 'touch DNA' samples have.

It was also possible to determine the potential for persistence of DNA on an object by examining the percentage of alleles present on the object swab, after contact with both Subject A and then Subject B. These results are summarised in Figure 6.9.

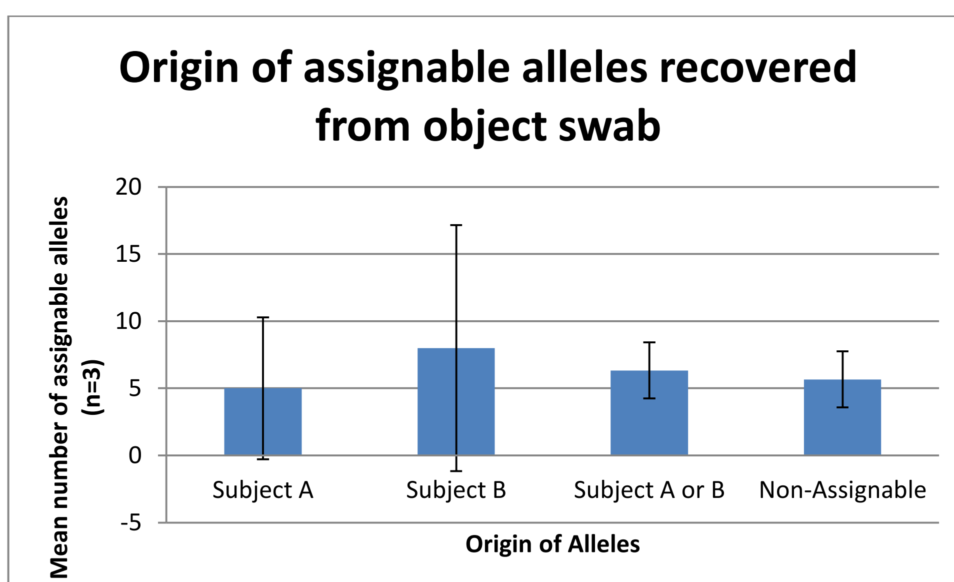


Figure 6.9 Mean number of assignable alleles from swab recovered from the object swab after the transfer process (Subject A ↔ Object↔ Subject B)

Three of the object swabs have not been considered due to poor profiling results.

These results indicate the persistence of DNA on an object is very variable when more than one individual has handled the item (as indicated by the large error bars depicting

the standard variation in assignable alleles between repeats). The proportions to which each subject contributed to the profile recovered can be seen in Appendix Five. In some instances the final person to handle the object contributes the major component to the DNA profile recovered, but at other times the dominant contribution is from the initial handler. These findings contradict those published by other research groups. Murray et al. (2002) reported that in instances where a second individual came into contact with a piece of clothing that was being worn, the second individual consistently contributed the dominant component to the sample. Wickenheiser (2002) also proposed that the DNA profile recovered from an item was indicative of the last person to handle that object, although mixed profiles were often observed. He suggested that during contact with an item, the mechanism by which DNA was deposited would result in any epithelial cells present from previous contacts being removed by subsequent contacts. The findings presented in this thesis would suggest that this mechanism is not accurate, as in some instances the initial donor's DNA profile contributes the major component to the object swab, indicating that their DNA has not been removed by successive contacts. Van Oorschot, Glavich and Mitchell (2014) examined items that had been used by one individual for an extended period and were then used by a second person for a specified duration. They found that "the profile percentage contribution of the first user relative to the second user of an object declines in a linear manner over time". They also identified that the persistence of the first users DNA was influenced by the type of object used, which could help inform forensic scientists evaluations relating to casework in the future.

If the ability to define an individual's shedder status was to be accepted, this could account for the variations in the persistence of an individual's DNA on an object. However, when two of the different pairings were compared, as both had the same individual acting as Subject B in the pairings it was clear that the degree to which this donors DNA profile contributed to the overall mixture varied considerably. This suggests that there is some other mechanism influencing the degree to which a DNA profile persists and is transferred.

To determine whether the final handler would consistently be the major contributor to the profile recovered from the object swab, one pairing of subjects carried out the transfer experiment over 6 different days. The profiles of this study are presented in Appendix Five (A5.6).

The results of the results for the DNA recovered from the object swabs are summarised in Figure 6.10.

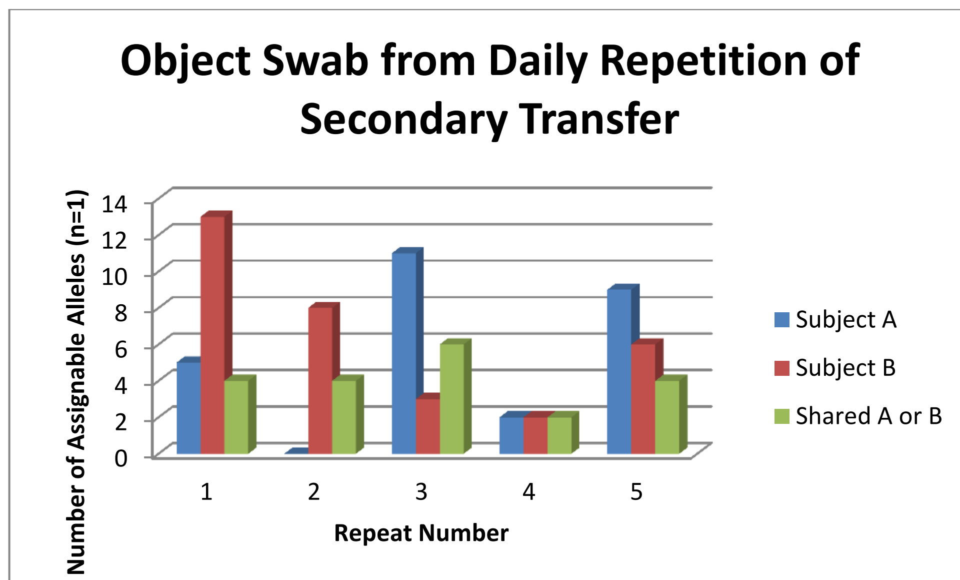


Figure 6.10 Number of alleles originating from Subject A or B recovered from Object Swab in Daily Repetition Study where object was vector for transfer.

One of the samples was not included as the sample failed to amplify. However, consideration of the remainder of the results from the daily repetition study suggest that the major contributor to DNA recovered from an object handled by two individuals was not constant. Therefore, it is not safe to conclude that the DNA profile recovered from a handled item is indicative of the last person to handle that object. It is also not possible to predict, based on the individuals involved, which person would be likely to contribute the major component of a mixed DNA profile, as the amount of DNA deposited is not reproducible.

Other research groups have examined the background levels of DNA that persist at scenes (Raymond et al., 2008; Raymond et al., 2009b), on indoor surfaces (Toothman et al., 2008), car steering wheels (Pizzamiglio et al., 2004) and on bedding (Petricevic, Bright and Cockerton, 2006), suggesting that DNA persists at levels that may influence case work interpretations, especially in cases where a mixed profile is recovered. This issue has proved especially pertinent in a murder case where DNA from David Butler was apparently recovered from the victim (Barnes, 2012). Transfer and persistence of DNA

were key issues in this case as Mr Butler has a rare skin condition which causes him to shed flakes of skin and, in his role as a taxi driver, the potential for secondary transfer of DNA, by way of him coming into contact with intermediary people, is increased. In this case Mr Butler was acquitted of murder, in part due to the poor quality and questioned provenance of the DNA evidence.

6.4.4 Secondary Transfer of DNA with Time Delay

The profiles for the secondary transfer samples, where a 30 minute delay was introduced between contact and sampling, are present in Appendix Five (A5.7).

This experiment was designed to determine the potential for secondary transfer of DNA to be detected when there is a delay of 30 minutes between the time of contact between two individuals and the subsequent contact with an object. This would mimic a situation where DNA has been recovered from a crime scene and the suspect claims the DNA has been transferred through an intermediary individual who had been in contact with the suspect 30 minutes previous to the DNA being deposited at the scene.

In this experiment Subject A shook hands with Subject B. After 30 minutes of 'regular' activity, during which the participants were asked not to contact any other individuals or wash their hands, both Subject A and B handled an object. After this contact, both the objects and the Subjects' hands were swabbed. The results of the DNA recovered from Subject A and B's hand swabs and object swabs are summarised in Figures 6.11 and 6.12.

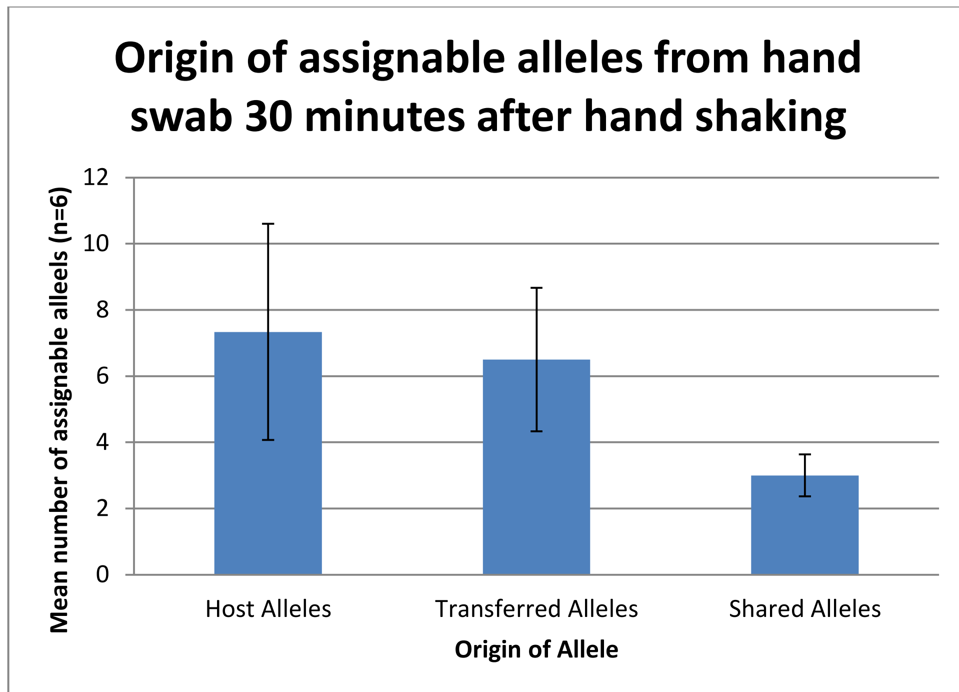


Figure 6.11 Number and origin of assignable alleles present on hand swab 30 minutes after handshaking. Host alleles are those originating from the person whose hand was swabbed, whereas transferred alleles are those originating from the partner whose hand was shaken.

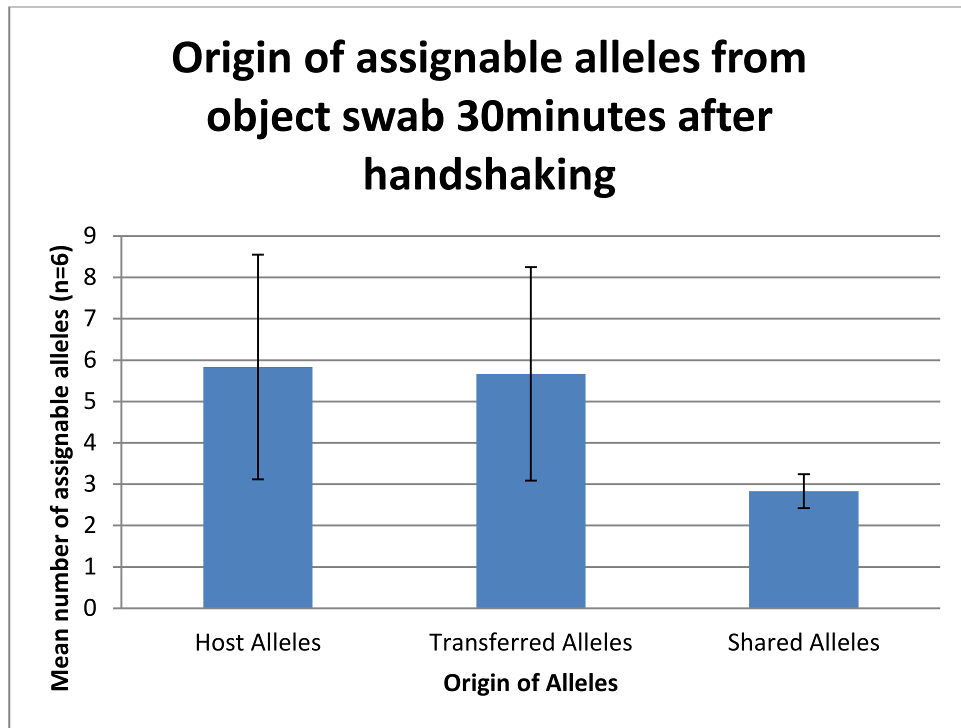


Figure 6.12 Number and origin of assignable alleles present on object swab when object was handled 30 minutes after handshaking. Host alleles are those originating from the person whose hand was swabbed, whereas transferred alleles are those originating from the partner whose hand was shaken.

The anticipated result would be that Subject A was the dominant contributor to swabs collected from Hand A (i.e. hand swabs from Subject A) and Subject B was the dominant contributor to swabs collected from Hand B. However, this trend was not observed in the results collected. In the majority of samples Subject A contributed the major component of the profile, both when the swab was collected from his hand or from the hand of Subject B.

In relation to the object swabs, secondary transfer of DNA was observed in all cases, for both Subject A and B. In agreement with the hand swabs, Subject A contributed the major component for all samples. If the ratios of Subject A and B's DNA present on the object swab are compared with the ratios present on the hand swabs, it can be observed that there is some correlation between the ratios of DNA present from each donor. The results reported here support the earlier outlined proposition of transfer suggested by Wickenheiser (2002).

These findings are consistent with results published by other research groups. Murray et al. (2002) reported that when a 30 minute time delay was introduced with a good and poor shedder pairing of individuals, secondary transfer was still observed (when the good shedder was Subject A in the pairing). Lowe et al. (2002) also reported mixed profiles recovered from objects where a time delay of 30 minutes or one hour between transfer and subsequent contact with the object had been introduced.

However, although the results are in line with other research groups findings the quality of the electropherograms was quite poor. There was a clear reduction in overall peak height, and evidence of allele drop out, especially at the longer STR loci. There was also an increased incidence of stutter peaks which would influence the accuracy in which contributing alleles were accurately identified. This was likely to be a result of stochastic fluctuation, caused by a low amount of template DNA.

As with the primary transfer time delay samples, there was an increase in alleles that could not be assigned to either donor. These contaminating alleles are likely to be a result of DNA transfer from other sources, and make profile interpretation more challenging.

6.5 Conclusions

This chapter aimed to address several research questions in relation to the transfer and persistence of DNA. The research aimed to determine if DNA that had previously been deposited onto an object would persist even after subsequent handling by another individual. The results gained in this research suggested that DNA from previous handlers could persist, but the degree to which the first handlers' DNA would dominate the profile recovered could vary. The research also aimed to determine if subsequent handling of an object could completely remove previous traces of touch DNA deposited by the prior handler. In one sample no DNA that could be solely attributed to the first handler was detected, although caution must be given when concluding the significance of this. Likewise, in one sample no DNA that could be solely attributed to the second handler was detected on an object but DNA that could be solely attributed to the first handler was observed. The issue with concluding too much from these samples is that in both cases,

shared alleles that could have belonged to either were present so it is not possible to definitive conclude that subsequent handling could completely remove previous traces of touch DNA. The results have also indicated that the dominant donor present in a mixed profile is not always indicative of the final handler.

The research also aimed to determine if secondary transfer of DNA could occur when the vector for transfer was an object or another person. The results gained suggest that DNA transfer could occur when the vector for transfer was another person and the final substrate was an object, but where the final substrate was another person (i.e. person to person to person) secondary transfer of DNA was not detectable. The results also suggest that DNA transfer could occur when the vector for transfer was an object, but in this case the first person's DNA would form the minor contribution to any profiles recovered.

The results identified significant variation in the degree to which secondary transfer was observed, and this variation was observed at both an inter- and intra-sample level. For example, when the same pair of donors conducted the same transfer experiments over several days, the results were not reproducible, suggesting the amount of DNA an individual deposits and transfers is not uniform. This would suggest that previous suggested mechanisms for the deposition and transfer of DNA evidence may not be accurate.

Finally the research aimed to determine whether secondary transfer of DNA could still be detected after a time delay of 30 minutes and the results indicated that it was still possible to detect the transferred DNA after this time period.

Seven. Recovery of DNA from Cartridge Cases

7.1 Introduction to DNA Recovery from Cartridge Cases

In firearms related cases where it is not possible to identify the individual who handled firearms cartridges prior to firing through fingerprints (either due to poor ridge detail or smudging), an alternative method of identification would be beneficial. DNA profiling would fulfil these requirements as it has the potential to individualise and offers some level of persistence. However, the processes that the cartridge undergoes during the firing process are not conducive to the persistence of DNA. High temperatures and high pressures experienced as a result of firing the weapon, as well as the presence of a mixture of chemicals in the form of gunshot residue (GSR), may inhibit DNA profiling success.

54% of the total propellant energy is converted to thermal energy, and 10 – 25% of that energy is detected as heat in the barrel wall of the weapon (Given, 1976). The barrel and cartridge case retain the heat, and are referred to as heat sinks (Warlow, 1996). However, the majority of the thermal energy produced from the ignition of the propellant is not believed to be retained in the cartridge case. Therefore the temperature increase on the external surface of the cartridge case should be minimal and very short in duration. This theory is supported by findings reported by Gashi et al. (2010) who measured the temperatures experienced by cartridge cases during firing. Gashi et al. (2010) recorded temperatures of between 313 and 372 K for a duration of 1.2 ms, which the authors stated was not sufficient to degrade DNA. These findings were supported by Allen et al. (2008) who stated that DNA deposited on a casing is not affected by the heat and pressure experienced during firing a handgun. Research carried out by Esslinger et al. (2004), Bille, Cromartie and Farr (2009) and Foran, Gehring and Stallworth (2009) into DNA recovered from exploded pipe bomb devices also reported some success with DNA analysis under flash burning conditions.

However, literature reporting the success rates of DNA analysis from cartridge cases suggests recovery of usable DNA is extremely variable (Williams and Clarke, 2010; Szibor et al., 2000; Karger, Meyer and DuChesne, 1997; Soares-Vieira et al. 2000; Polley et al.,

2006; Horsman-Hall et al., 2008; Horsman-Hall et al., 2009; Ferreira et al., 2009; Dieltjes et al., 2011).

One hypothesis for the degradation of this DNA is the incidence of gaseous blowback (where gas, released through propelling the projectile from the casing, is forced between the cartridge casing and the wall of the firing chamber) could dramatically increase the temperatures experienced on the external surface of the cartridge case (Given, 1976).

During the process of firing, the cartridge case swells with the release of gas pressure (Warlow, 1996). This swelling could result in mechanical abrasion of the exterior surface of the cartridge case with the wall of the firing chamber (Given, 1976). The degree of abrasion would depend on the calibre of ammunition, the fit of the cartridge in the chamber and the specific area of the casing (expansion is more pronounced at the base of the cartridge case due to the increased thickness of the metal) (Given, 1976).

Another possible explanation for poor success rates for DNA analysis recovered from spent cartridges cases could be the presence of GSR. The specific chemical composition of GSR is variable but is generally composed of unburned and partially burnt propellant, primer particles and metals from the projectile or barrel (Dalby, Butler and Birkett, 2010; Meng and Lee, 2007; Morales and Vázquez, 2004; Romolo and Margot, 2001; Brożek-Mucha, Zadora and Dane, 2003; Schwoeble and Exline, 2000; Wallace, 2008). Heavy metals, including lead, antimony and barium, derived predominantly from the primer mixture are often used for positive identification of the presence of GSR (Morales and Vázquez, 2004; Romolo and Margot, 2001) as the combination of these three elements is only observed in GSR (Schwoeble and Exline, 2000). Heavy metals are known to cause breakage of phosphodiester bonds in the DNA molecules (Li, 2008) and therefore prolonged exposure to GSR could have a negative effect of success rates for DNA analysis.

Relatively few papers have been published studying the effects of GSR on DNA analysis. Torre and Gino (1996) reported that DNA from stubs used to collect GSR from suspects' hands could be typed at a single DNA locus. Research completed by Hall and Fairley (2004) suggests that a tape lifting method could be used for the collection of DNA and GSR evidence, which results in an 80% DNA recovery success rate. Zamir et al. (2004) demonstrated that adhesive lifters used to recover GSR from the entrance wound of a shooting victim could be a source of DNA.

In a related field, the DNA from plants exposed to heavy metal solutions (including Lead, Cadmium and Manganese) displayed polymorphic bands which were not detectable in DNA of control plants (Conte et al., 1998). This could suggest exposure to heavy metals in GSR could degrade the DNA present.

7.2 Research Questions for DNA Recovery from Firearms Related Paraphernalia

This chapter aims to address the overall objective to determine the influence that firearms type, calibre or Gun Shot Residue (GSR) has on the ability to retrieve DNA information from spent cartridge cases.

Based on a review of the previously published literature of experimental work undertaken by different research groups, as outlined in the introduction to this chapter, several research questions were identified:

a. Is it possible to recover DNA from a spent cartridge case?
b. Does the firing process impact on the ability to recover DNA from a saliva seeded cartridge case?
c. Does the firing process impact on the ability to recover DNA from a handled cartridge case?
d. Does the weapon type or calibre of the weapon influence the potential for successful recovery of DNA from fired cartridge cases?
e. Does the presence of gunshot residue inhibit DNA profiling?

7.3 Experimental Design

To address these research questions the following experiments were designed, as outlined below.

All information regarding the ammunition and firearms used in these experiments can be found in Appendix Six (A6.1)

7.3.1 DNA Recovery from Fired and Unfired Cartridge Cases

For this study, live ammunition and weapons were used. All weapons and ammunition were cleaned with 2% Virkon, 100% ethanol and double distilled water prior to use.

DNA application and firing of the live weapons was carried out at the firing ranges of the respective Police Forces, for health and safety reasons.

7.3.1.1 Fired Samples

7.3.1.1.1 Saliva Seeded Samples

For the samples that were to be seeded with saliva, the subject was asked to rinse his/her mouth with 20 mls PBS for 1 minute to collect an oral fluid sample containing DNA. The PBS/oral fluid sample (which will be referred to as saliva for the remainder of this report) was spat back into the container and mixed by inverting at regular intervals.

The saliva was applied to the cartridges by immersing the cartridge into the solution for 10 seconds, and then allowing the cartridge to air dry on sterile bench coat for 30 minutes. Any further handling of the cartridge was carried out by the subject whilst wearing gloves to ensure no additional DNA was applied to the cartridges.

Samples that were to be fired from a semi-automatic weapon were loaded into the magazine by the Firearms Officer who wore gloves throughout the loading process. The maximum capacity of ammunition was loaded into the magazine at one time. A cartridge case was individually ejected after each discharge of the weapon.

Samples that were to be fired from a revolver weapon were loaded into the barrel by the Firearms Officer who wore gloves throughout the loading process. The maximum capacity of ammunition was loaded into the barrel at one time. Once all the ammunition had been discharged, the cartridge casings were removed from the barrel of the weapon.

Samples that were to be fired from a rifle were loaded into the chamber of the weapon by the Firearms Officer who wore gloves throughout the loading process. Two rounds of

ammunition were loaded at a time, discharged, and then the casings were removed from the chamber of the weapon.

After discharging the weapons, all of the saliva seeded, fired cartridge casings were placed in the labelled storage devices. Upon returning to the laboratory, all samples were stored at -80 ^{o}C until required for DNA collection.

7.3.1.1.2 Handled Samples

For the samples that were to be seeded with fingerprints, the subject was asked to load the cartridges into the weapon or magazine as they would routinely, not wearing gloves.

Samples that were to be fired from a semi-automatic weapon were loaded into the magazine by the Firearms Officer who handled the ammunition as would be routine whilst loading this type of firearm. He/she did not wear gloves during the handling of the ammunition or during the loading of the magazine. The maximum capacity of ammunition was loaded into the magazine at one time. A cartridge case was individually ejected after each discharge of the weapon.

Samples that were to be fired from a revolver weapon were loaded into the barrel by the Firearms Officer who handled the ammunition as would be routine whilst loading this type of firearm. He/she did not wear gloves during the handling of the ammunition or during the loading of the magazine. The maximum capacity of ammunition was loaded into the barrel at one time. Once all the ammunition had been discharged, the cartridge casings were removed from the barrel of the weapon.

Samples that were to be fired from a rifle were loaded into the chamber of the weapon by the Firearms Officer who handled the ammunition as would be routine whilst loading this type of firearm. He/she did not wear gloves during the handling of the ammunition or during the loading of the magazine. Two rounds of ammunition were loaded at a time, discharged, and then the casings were removed from the chamber of the weapon.

After discharging the weapons, all of the handled fired cartridge casings were placed in the labelled storage devices. Upon returning to the laboratory, all samples were stored at -80 ^{o}C until required for DNA collection.

7.3.1.2 Unfired Samples

As it is not permitted to bring live ammunition away from the firing ranges, the cartridge cases that were to remain unfired were modified cartridges. The cartridge case had been modified to remove any propellant and bullet so only the casing remained.

7.3.1.2.1 Saliva Seeded Samples

The saliva was applied to the cartridges by immersing the modified cartridge casing into the solution for 10 seconds, and then allowing the cartridge casing to air dry on sterile bench coat for 30 minutes. Any further handling of the cartridge casing was carried out by the subject whilst wearing gloves to ensure no additional DNA was applied to the cartridge casings.

After seeding the modified cartridge casings with saliva and allowing to dry, they were placed in the labelled storage devices. Upon returning to the laboratory, all samples were stored at -80 ^{o}C until required for DNA collection.

7.3.1.2.2 Handled Samples

For the samples that were to be seeded with fingerprints, the subject was asked to load the cartridges into the weapon or magazine as they would routinely, not wearing gloves.

The cartridge casings for the Semi-Automatic weapon were loaded into the magazine by the Firearms Officer who handled the ammunition as would be routine whilst loading this type of firearm. He/she did not wear gloves during the handling of the ammunition or during the loading of the magazine. The maximum capacity of ammunition was loaded

into the magazine at one time. The unfired cartridge casings were then removed from the magazine without discharging the weapon.

The cartridge casings for the revolver weapon were loaded into the barrel by the Firearms Officer who handled the ammunition as would be routine whilst loading this type of firearm. He/she did not wear gloves during the handling of the ammunition or during the loading of the magazine. The maximum capacity of ammunition was loaded into the barrel at one time. The unfired cartridge casings were then removed from the barrel without discharging the weapon.

The cartridge casings for the rifle were loaded into the chamber of the weapon by the Firearms Officer who handled the ammunition as would be routine whilst loading this type of firearm. He/she did not wear gloves during the handling of the ammunition or during the loading of the magazine. Two rounds of ammunition were loaded at a time, and then the casings were removed from the chamber without discharging the weapon.

All cartridge cases were placed in the labelled storage devices. Upon returning to the laboratory, all samples were stored at -80 ^{o}C until required for DNA collection.

7.3.1.3 DNA Collection

DNA was recovered from the cartridge cases by swabbing with a cotton swab. The only modification from the stated method was that each cartridge case was swabbed with the wet swab 50 times, followed by swabbing with the dry swab 50 times.

Both methods of extraction (Chelex extraction and Qiagen extraction) were used for the sample swabs. The results from both extraction techniques were compared to determine which was most suitable for extracting from this type of sample. Both standard and LCN SGM+ PCR amplification were carried out on the samples, followed by Capillary Electrophoresis.

7.3.2 Gun Shot Residue (GSR) Experiments

These experiments were carried out at Staffordshire University Forensic and Crime Science Department. Therefore, due to health and safety restrictions, for this study, blank ammunition and blank firing weapons were used. All weapons and ammunition were cleaned with 2% Virkon, 100% ethanol and double distilled water prior to use.

7.3.2.1 Inhibition of DNA Profiling by GSR

A GSR suspension was prepared by washing out the inside of a spent cartridge case (308 calibre) with 1 ml of sterile molecular grade water. This suspension was stored at 4 ^{o}C and left to equilibrate for 96 hours before use.

The GSR suspension was used to spike samples at different stages of the DNA profiling process. The stages at which the suspension was added were as follows:

1. Control: Non-seeded saliva was applied to the clean cartridge case, and the DNA was collected, extracted, amplified and profiled.

2. Extract: GSR-seeded saliva was applied to the clean cartridge case, and the DNA was collected, extracted, amplified and profiled.

3. Amplify: Non-seeded saliva was applied to the clean cartridge case, the DNA was collected, extracted, and the DNA extract was seeded with GSR suspension. The sample was then amplified and profiled.

4. Separate: Non-seeded saliva was applied to the clean cartridge case, and the DNA was collected, extracted, and amplified. The PCR product was then seeded with GSR suspension and placed on the 310 Genetic Analyser for capillary electrophoresis.

The samples were seeded with GSR in the following quantities:

1. No GSR seeding required (this sample was the No GSR Control).

2. 500 µl of GSR suspension was added to 2000 µl of saliva and the mixture was left to equilibrate at 4 ^{o}C for 24 hours before use.

3. 40 µl of GSR suspension was added to 150 µl of DNA extract and the mixture was left to equilibrate at 4 ^{o}C for 24 hours before use.

4. 2 µl of GSR suspension was added to 10 µl of PCR product and the mixture was left to equilibrate at 4 ^{o}C for 24 hours before use.

7.3.2.1.1 Sample Deposition

For the 'Control', 'Amplify' and 'Separate' samples, a saliva sample was applied to the cartridges by immersing the cartridge into the solution for 15 seconds, and then allowing to air dry on sterile benchcoat for 30 minutes. Any further handling of the cartridge was carried out by the researcher whilst wearing gloves to ensure no extraneous DNA was applied to the cartridges.

For the 'Extract' samples, the GSR suspension - saliva mixture was applied to the cartridges by immersing the cartridge into the solution for 15 seconds, and then allowing to air dry on sterile benchcoat for 30 minutes. Any further handling of the cartridge was carried out by the researcher whilst wearing gloves to ensure no extraneous DNA was applied to the cartridges.

DNA was recovered from the cartridge cases by swabbing with a cotton square. The only modification from the stated method was that each cartridge case was swabbed with the wet cotton square 20 times, followed by swabbing with the dry cotton square 20 times.

Both methods of extraction (Chelex extraction and Qiagen extraction) were used for the sample cotton squares.

At the end of the DNA extraction, the GSR suspension was added to the DNA extract from the 'Amplify' samples.

The results from both extraction techniques were compared to determine which was most suitable for extracting from this type of sample.

Both standard and LCN SGM+ PCR amplification were carried out on the samples, followed by Capillary Electrophoresis.

At the end of the SGM+ amplification, the GSR suspension was added to the PCR product from the 'Separate' samples, prior to placing on the 310 Genetic Analyser for capillary electrophoresis.

7.3.2.2 Short-term Exposure to GSR Study

For this study glass slides were used in place of cartridge cases. This was due to a limited supply of cartridge cases being available. All glass slides were cleaned with 2% Virkon, 100% ethanol and double distilled water prior to use.

For the 'No GSR Control' samples, a saliva solution was prepared by mixing 200 μl of neat saliva with 200 μl of molecular grade water. 25 μl of this saliva solution was pipetted onto a glass slide and was allowed to dry for 20 minutes.

A GSR suspension was prepared by washing out the inside of a spent cartridge case (308 calibre) with 1 ml of sterile molecular grade water. This suspension was stored at 4 ^{o}C and left to equilibrate for 96 hours before use.

200 μl of the GSR suspension was mixed with 200 μl of neat saliva. 25 μl of this GSR suspension – saliva mixture was pipetted onto a glass slide and was allowed to dry for 20 minutes.

DNA was recovered from the glass slide by swabbing with a cotton square. The only modification from the stated method was that each glass slide was swabbed with the wet cotton square 5 times, followed by swabbing with the dry cotton square 10 times.

DNA collection took place on both the 'GSR seeded' samples and the 'No GSR Control' samples at 24 hours after application and 1 week after application.

Qiagen extraction was used to extract DNA from the sample cotton squares. LCN SGM+ PCR amplification was carried out on the samples, followed by Capillary Electrophoresis.

7.3.2.3 Exposure of DNA to Discharged Levels of GSR

For this study, blank ammunition and blank firing weapons were used. All weapons, ammunition and glass slides were cleaned with 2% Virkon, 100% ethanol and double distilled water prior to use.

A negative control sample was collected from each weapon prior to use.

Subjects were instructed not to wash their hands or come in contact with another individual for at least 1 hour prior to the experiment.

Subjects were asked to rub their hands together for 1 minute prior to experimentation in order to attempt to equilibrate the amount of DNA present on both hands.

Two fingerprints (one from the left index and one from the left middle finger) were deposited and a hand swab was taken from the subjects' left hand prior to the experiment for a 'No GSR Control'.

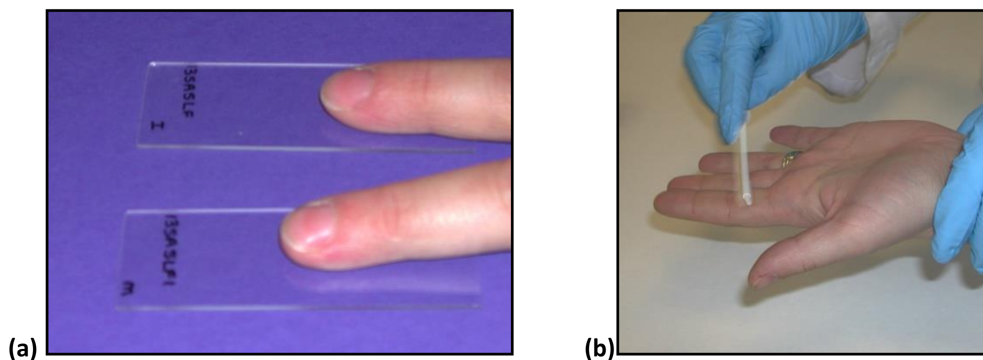
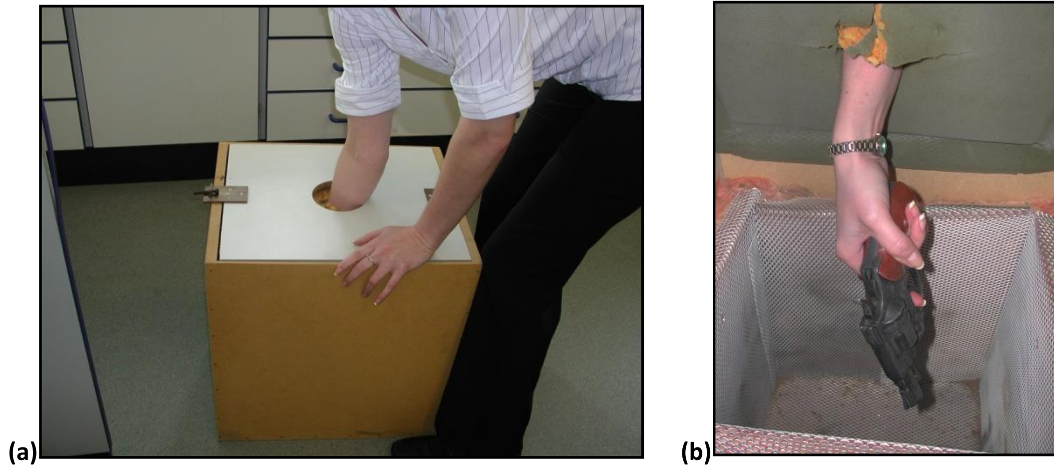


Figure 7.1 (a) and (b). (a) Fingerprint deposition. The index and middle finger were placed down onto a sterile glass slide for 5 seconds to deposit a fingerprint. (b) Hand Swab taken using a pre-wetted buccal swab.

The subject then fired three blanks from either a revolver or semi-automatic weapon, using their right hand.



(a) (b)

Figure 7.2 (a) and (b). (a) Firing Box. The firearm is placed on the floor of the box, the lid is sealed in place and the subject places his/her hand through the designated arm hole at the top in order to fire the weapon. (b) Inside view of Firing Box.

The subject then deposited a further two fingerprints (from the index and middle finger of the subjects right hand) and a hand swab was collected from their right hand after firing. DNA was also collected from the weapon to examine the amount of DNA deposited during the firing process.

DNA was recovered from the weapon by swabbing with a cotton square or mini-taping.

DNA was recovered from the glass slides by swabbing with a cotton square or mini-taping.

DNA was recovered from the subjects hand by swabbing with a buccal swab.

The results from both DNA collection techniques were compared to study which was most suitable for obtaining DNA from this type of sample.

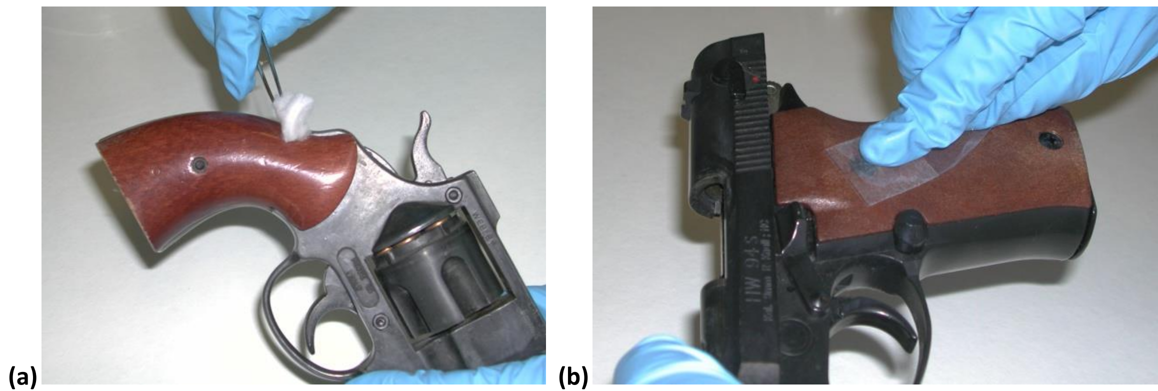


Figure 7.3 (a) and (b). (a) DNA collection using cotton square swabbing technique. (b) DNA collection using taping technique. Both techniques were used on both weapons at both collection intervals.

DNA collection from the hand, weapon and index finger glass slides took place immediately after application. DNA collection from the middle finger glass slides took place 1 week after application.

Qiagen extraction was used to extract DNA from the sample cotton squares and tapes.

LCN SGM+ PCR amplification was carried out on the samples, followed by Capillary Electrophoresis.

7.4 Analysis and Interpretation of DNA Persistence and Transfer Results

7.4.1 DNA Recovery from Fired and Unfired Cartridge Cases

As some of the sample collection aspect of the firearms work was carried out at specified Firing Ranges, every attempt was made to reduce the potential for contamination at these sites. Bench Coat was placed on surfaces and this was wiped with 2% Virkon, 100% ethanol and double distilled water prior to any experimental work commencing.

One issue that could not be overcome was the potential contamination introduced as a result of the cartridge ejection by the semi-automatic weapon. The cartridges are introduced to the firing chamber of the semi-automatic weapon by way of a magazine. Once one cartridge has been fired, the casing is ejected so that the next cartridge can

then enter the chamber. This ejection process is automatic, so there is no control of where the cartridge case will be ejected to. Health and safety restrictions, both in terms of the temperature of the cartridge case upon ejection and in relation to the safe proximity of the researcher to the Firearms Office whilst discharging the weapon, resulted in the cartridge cases being ejected onto the ground. Therefore any contaminating alleles present in the samples fired from a semi-automatic weapon could be a result of this ejection technique.

7.4.1.1 Saliva Seeded Samples

The profiles for the DNA recovered from saliva seeded fired and unfired cartridge cases are present in Appendix Six (A6.2 and A6.3).

The results indicate that the firing process has a detrimental effect on the ability to retrieve a DNA profile from saliva seeded cartridge cases. Figure 7.4 compares the mean number of alleles recovered from saliva seeded cartridge cases (alleles that were assignable to the donor) for fired and unfired cartridge cases. This data includes samples from three different individuals, two different types of weapon (semi-automatic handgun and rifle) and three different calibre cartridge case types.

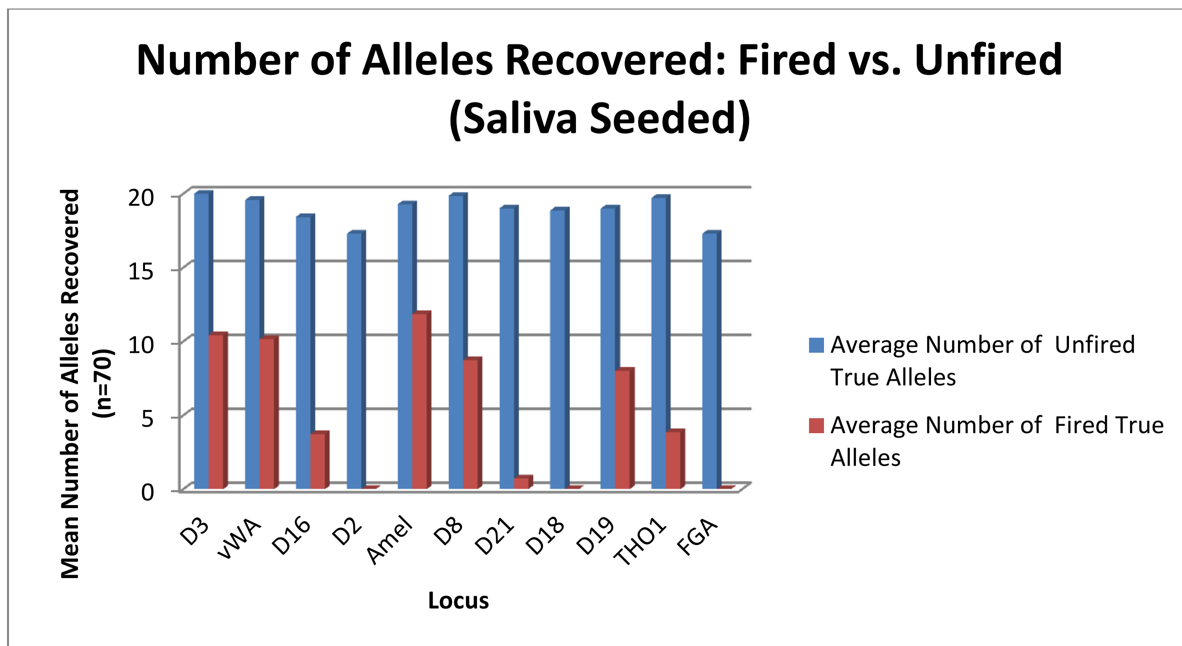


Figure 7.4 Comparison of average number of alleles assignable to the donor for fired and unfired saliva-seeded cartridge cases.

The rationale behind seeding the cartridge cases with saliva was to try to deposit a constant amount of DNA onto each case, making comparison between replicates possible. The number of alleles was chosen as an indicator of the quality of DNA, as this was deemed to be a more useful guide to casework success than the quantity of DNA present. If the number of alleles recovered from the fired cartridge case samples is compared to the amount recovered from the unfired cartridge case samples, it is apparent that the firing process causes a dramatic reduction in the number of profilable alleles.

There is little published work on DNA recovered from saliva samples on spent cartridge cases. Polley et al. (2006) seeded cartridge cases with saliva samples and their findings, published in 2006, were in agreement with those reported here. Karger, Meyer and DuChesne (1997) published a case report where they reported amplification of 5 STR loci from bullets believed to be stained with blood from the victims. Their results indicated some success in producing a useable DNA profile, and suggested an increased yield of DNA was recovered from hollow point bullets, as opposed to the smooth surface of full metal jacket bullets. Soares-Vieira et al. (2000) have also published findings from a case report where DNA information from a blood stained projectile was useful in parentage testing. Similar findings have been published by Ferreira et al. (2009) where samples were extracted using a modified organic phenol-chloroform extraction protocol and then amplified using the AmpF/STR® Identifiler kit,

Although the published data examines bullets as well as cartridge cases (which will undergo different conditions as a result of the firing process), and blood as well as saliva, the literature indicates that the processes involved in gaining a successful DNA profile from firearms related paraphernalia are extremely complex, and no standard protocol is currently in existence.

In terms of the number of alleles recovered from the different donors, there was no significant difference between the average values with either the fired or unfired samples (Table 7.1).

Table 7.1 The number and percentage of assignable alleles for different DNA donors.

	N° of Assignable Alleles		% Total Allele N°	
	Saliva Fired	Saliva Unfired	Saliva Fired	Saliva Unfired
Donor 1	54	204	25	93
Donor 2	57.5	215.5	26	98
Donor 3	62.5	207	35	94

A Z-score test for two population proportions was carried out to determine if there was a significant difference between the two populations of data (fired and unfired). The results indicated that there was a statistically significant difference between the number of alleles recovered pre- and post-firing ($n=30$, $p<0.01$, two tailed), which is reflected in the percentage of alleles presented in Table 7.1.

With the fired saliva seeded samples, there was a notable reduction in the number of alleles present at the larger loci. To demonstrate this, the loci have been ranked roughly according to size (starting with the smallest amplicon, D19) and have been plotted against the average number of assignable alleles present (Figure 7.5). The average number of alleles for the unfired samples appears to remain relatively constant across the range of different size amplicons. This reduction in the number of larger alleles present post-firing may indicate a reduction in the amount of DNA present in the fired samples, resulting in an increased incidence of stochastic fluctuation.

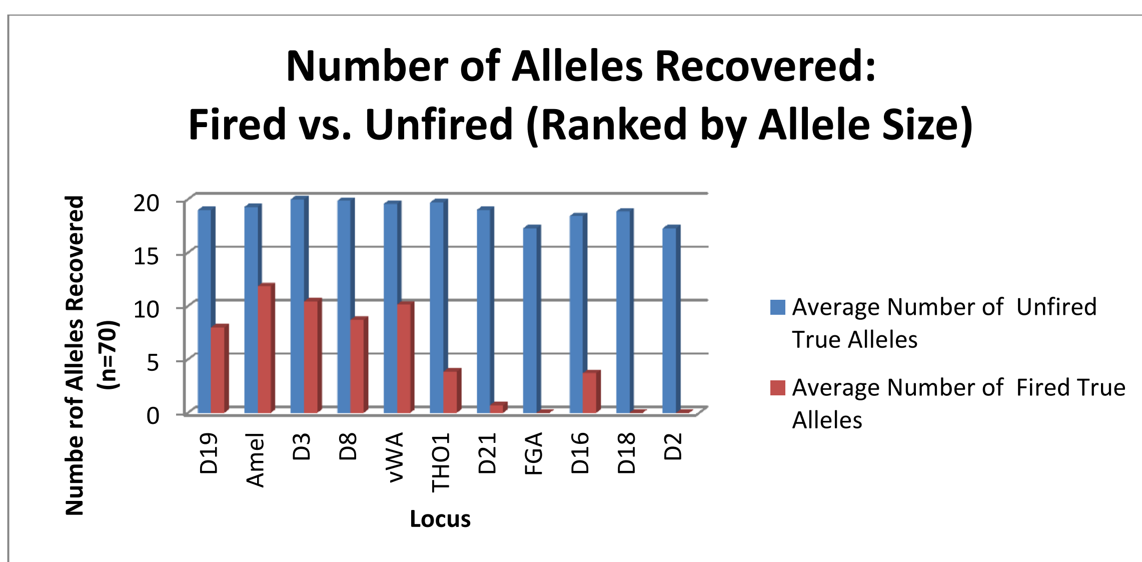


Figure 7.5 Comparison of the average number of assignable alleles recovered from fired and unfired saliva seeded cartridge cases, with loci ranked in size order (smallest to largest).

However, although a general trend can be observed in the sample results, there is a large amount of overlap in allele size across the different STR loci. FGA, especially, has a wide range of potential number of repeats present in the population. Therefore this system of ranking may not be completely accurate. If, at a locus where an individual was homozygous, there was generally a higher observed incidence of those alleles (Donor 1: vWA; Donor 2: D3 and vWA; Donor 3: D8, D18, D19, THO1 and FGA) when compared to heterozygous alleles, this could be a contributing factor to the differences in the number of alleles observed between fired and unfired samples. This could be due to firing reducing the amount of DNA present, resulting in increased heterozygote imbalance causing allele drop out. However, there appears to be a stronger correlation between the number of alleles and the size of the amplicon, rather than between the number of alleles and whether the locus was homozygous or heterozygous. The use of DNA amplification systems that use smaller STR loci, such as mini-STRs may offer an alternative approach to DNA recovery from fired cartridge cases if the starting quantity of DNA was relatively high.

In addition to assignable alleles belonging to the donor, there were varying numbers of non-assignable, or false, alleles present in the samples recovered (Figure 7.6). The presence of contaminating peaks may have been a result of insufficient anti-contamination procedures, as the cartridge cases were provided by Police Forces.

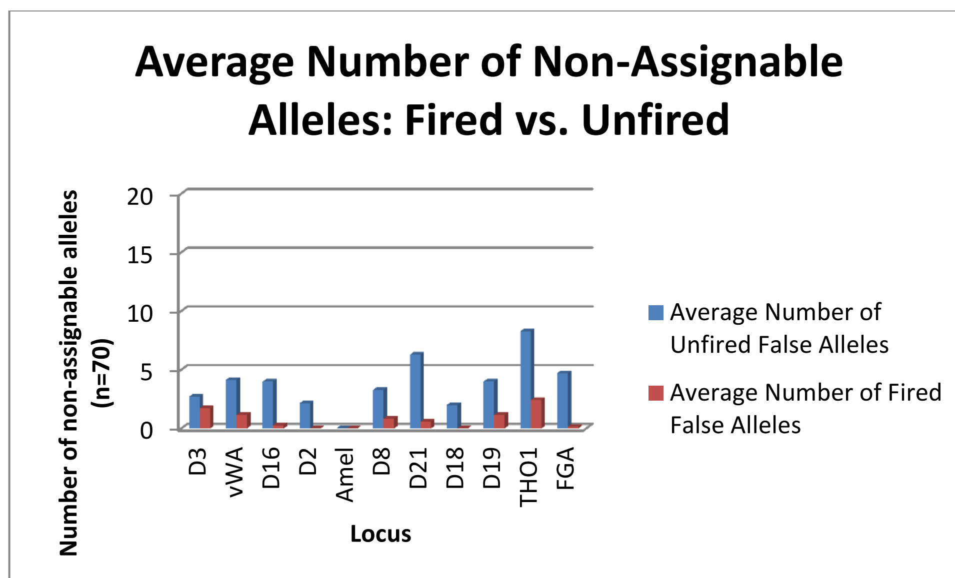


Figure 7.6 Comparison of average number of non-assignable alleles for fired and unfired saliva-seeded cartridge cases.

The Amelogenin locus does not have any non-assignable alleles as all three donors used in this study were male, and therefore all possible alleles at this locus are assignable to the donor.

Although the incidence of non-assignable alleles on average was higher in the unfired samples, as a percentage of the total number of alleles 13% of all alleles in the fired samples were non-assignable compared with 17% of all alleles in the unfired samples. There may be a slightly higher incidence of non-assignable peaks in the unfired samples due to an increased amount of DNA which could result in an increase in stutter products being misidentified as peaks and increased adenylation.

Adenylation, or non-template addition, is seen when the DNA polymerase adds an additional nucleotide to the 3’ end of the PCR product during replication of the template strand (Butler, 2005). In approximately 85% of cases, the additional nucleotide is adenosine (Goodwin, Linacre and Hadi, 2007), and so this is often referred to as the ‘+A’ version of the amplicon (Butler, 2005). This process is usually assisted by the inclusion of a final extension stage at the end of the PCR amplification protocol, which enables complete adenylation of all double stranded PCR products. However, if too much DNA is added to the amplification reaction, or if the *Taq* polymerase is not working at an optimal efficiency, the potential for incomplete 3’ adenylation is increased (Butler, 2005;

Goodwin, Linacre and Hadi, 2007). In this instance, where partial adenylation occurs, there will be a mixture of +A and –A products, resulting in split peaks and broad peaks in the electropherogram (Figure 7.7 (a) and (b)).

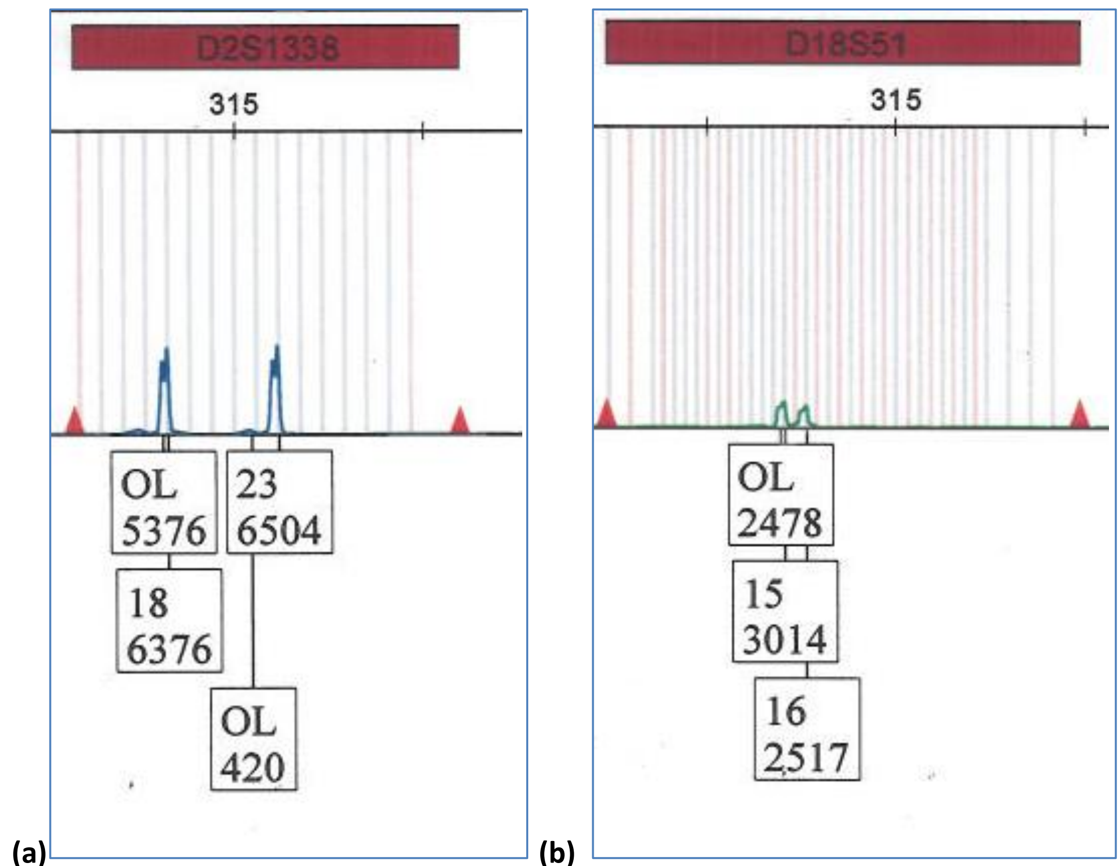


Figure 7.7 (a) and (b). (a) Split peaks as a result of incomplete adenylation. (b) Broad peaks due to incomplete resolution.

The 'OL' labels in Figure 7.7 (a) and (b) refer to alleles that are 'Off Ladder'. This means that upon comparison of the data point for the peak with the data points provided by the allelic ladder, the software has been unable to assign an allele number to the peak. This is often seen where split peaks and broad peaks are present, as the peak falls outside of the grey bins, which are the areas in which certain alleles are recognised.

Split peaks can cause issues where both peaks are recognised as a true allele. For example, at THO1 where 5.3 and 6 are observed alleles, a split peak at either of those alleles could result in both alleles being called, which would not be a true representation of the correct genotype of the donor/sample.

If the main cause of reduction in DNA quality post-firing was the temperature and pressure experienced during detonation, there may be a difference in the degree of

degradation based on the type and calibre of weapon used. For example, a high calibre weapon will generate more heat and pressure (caused by an increased amount of burning propellant resulting in expansion of the casing). This may cause friction between the outside of the cartridge case and the inside of the chamber, resulting in a transfer of DNA from one surface to the other. These issues will be exacerbated in the case of handled samples, where the amount of DNA present is anticipated to be at a very low level.

In this research, two types of weapons were used (semi-automatic pistol and semi-automatic rifle) with three different ammunition types (9mm, .223 and .308). As each of the weapons had the same firing mechanism (i.e. cartridge automatically loaded into a single chamber prior to discharging the weapon) any differences observed may be a result of the ammunition type. However, the results indicate there is no significant difference between the total number of alleles recovered from each of the ammunition types (average total number of alleles = 59 for 9mm; 57 for .223; 56 for .308).

7.4.1.2 Handled Samples

The profiles for the DNA recovered from handled fired and unfired cartridge cases are present in Appendix Six (A6.4 and A6.5).

The results indicate that the firing process has a detrimental effect on the ability to retrieve a DNA profile from handled seeded cartridge cases. Figure 7.8 compares the average number of alleles recovered from handled cartridge cases (alleles that were assignable to the donor) for fired and unfired cartridge cases. This data includes samples from three different individuals, two different types of weapon (semi-automatic handgun and rifle) and three different calibre cartridge case types.

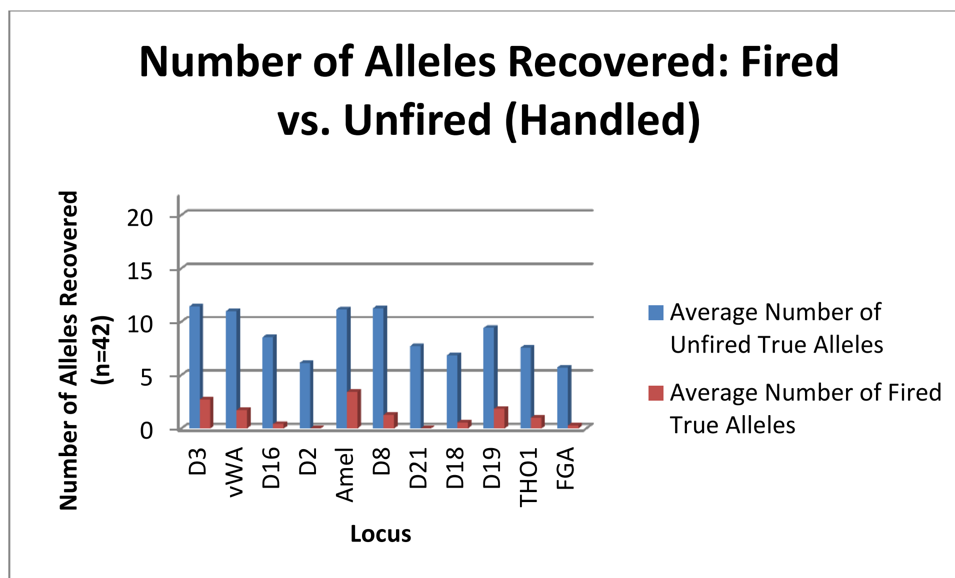


Figure 7.8 Comparison of average number of alleles assignable to the donor for fired and unfired handled cartridge cases.

If the number of alleles recovered from the fired cartridge case samples is compared to the amount recovered from the unfired cartridge case samples, it is apparent that the firing process causes a dramatic reduction in the number of profilable alleles.

These findings contradict Allen et al. (2008) who state that DNA deposited on a cartridge case was stable to the temperatures and pressures experienced during the firing process. However, the data presented by Allen *et al.* indicates that only 15% of pre-fired and 17% of post-fired samples generated a full profile. Although overall the success rate for post-fired samples was higher, the fact that 46% of pre-fired samples failed to generate a profile indicates that there are issues with recovering useable DNA profiles from handled items, even before any additional variables (such as high temperatures or pressures) are introduced.

These findings also contradict those of Gashi et al. (2010) who recovered on average 74.09% of the donors DNA profile post-firing. However, the researchers in this paper used cartridge cases that had been knurled (series of grooves present on the surface) which has been demonstrated to increase the amount of DNA deposited (Xu et al., 2010). They used a sonication method to remove the DNA from the cartridge case, as well as a micro-concentrator device to concentrate the DNA present. As these cartridges with a

knurled brass coating were only developed in 2010, it is unlikely that they would be used widely in firearms related crimes currently.

Dieltjes et al. (2011) (using an immersion-swab recovery technique, QIAamp® DNA Mini extraction kit and the Powerplex® 16 system, the AmpF/STR® Identifiler kit and MiniFiler™ kit) and Horsman-Hall et al. (2009) (using four different extraction techniques and three different amplification kits). These publications all reported differing levels of success with the different amplification kits.

In terms of the number of alleles recovered from the different donors, the variation between the donors for the unfired samples was higher than that observed in the unfired saliva seeded samples (Table 7.2). This increase in the degree of variation would be expected, as it has been reported that different individuals deposit different amounts of touch DNA (Lowe et al. 2002; Murray et al., 2002).

Table 7.2 The number and percentage of assignable alleles for different DNA donors.

	N° of Assignable Alleles		% Total Allele N°	
	Handled Fired	Handled Unfired	Handled Fired	Handled Unfired
Donor 1	12	96	9	73
Donor 2	13	87.5	10	66
Donor 3	15.5	107.5	12	81

A Z-score test for two population proportions was carried out to determine if there was a significant difference between the two populations of data (fired and unfired). The results indicated that there was a statistically significant difference between the number of alleles recovered pre- and post-firing ($n=30$, $p<0.05$, two tailed). The degree of variation for the handled fired samples was actually slightly less than that observed in the saliva-seeded fired samples (handled samples calculated a Z-Score of 15.86 compared to the saliva-seeded samples which generated a Z-Score of 25.46). This could be due to the low levels of DNA present in handled samples; therefore if conditions were such that degradation could occur, the likelihood is that the degradation would be more uniform across the samples. With the saliva seeded samples, the amount of DNA present appeared to be much larger, so the degree to which degradation would occur may be much more variable (depending on the total amount of DNA present in each sample).

With the fired handled samples, there was a notable reduction in the number of alleles present at the larger loci. This can be observed in Figure 7.9. The average number of alleles for the unfired samples also appears to reduce as the amplicons increase in size. This reduction in the number of larger alleles present post-firing may indicate a reduction in the amount of DNA present in the fired samples, resulting in an increased incidence of stochastic fluctuation. This hypothesis was supported by the differences observed when the saliva-seeded samples and the handled samples were compared. The stochastic fluctuation was observed in both pre- and post-firing samples with the handled samples, as low levels of DNA were present in both variables, whereas with the saliva samples this stochastic effect was only visible in the post-firing samples where the DNA was degraded.

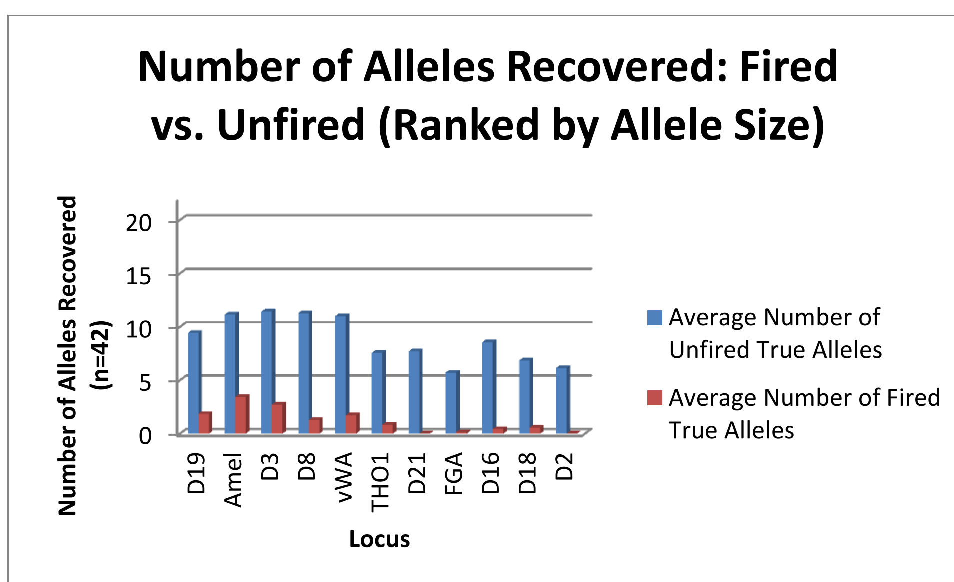


Figure 7.9 Comparison of the average number of assignable alleles recovered from fired and unfired handled cartridge cases, with loci ranked in size order (smallest to largest).

In addition to assignable alleles belonging to the donor, there were varying numbers of non-assignable, or false, alleles present in the samples recovered (Figure 7.10). The number of false alleles amplified in the fired samples was lower than in the unfired samples. The presence of non-assignable alleles in handled samples was not unexpected as the donors were asked not to wash their hands for at least 1 hour prior to the experiment. They were also asked not to come into direct contact with anyone else

during that time period but, as will be outlined later in the 'Results and Discussion' section, there is the potential for the donors to pick up extraneous DNA from other sources.

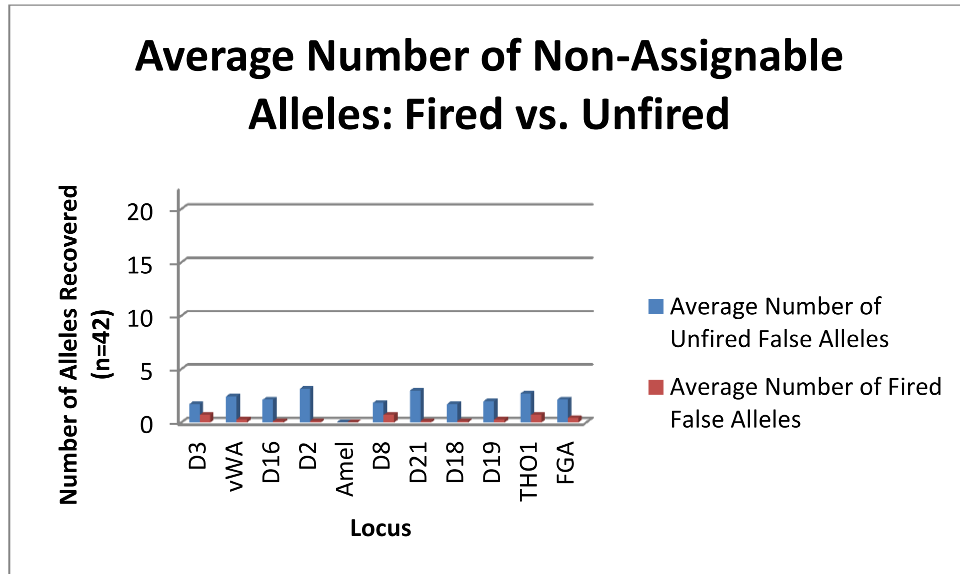


Figure 7.10 Comparison of average number of non-assignable alleles for fired and unfired handled cartridge cases.

With the handled samples the incidence of non-assignable alleles on average was lower in the unfired samples when compared to the fired samples. As a percentage of the total number of alleles 22% of all alleles in the fired samples were non-assignable compared with 19% of all alleles in the unfired samples. These percentages are higher than those observed with the saliva-seeded samples, possibly as a result of LCN amplification being used. There may be a slightly higher incidence of non-assignable peaks in the fired samples due to a decreased amount of DNA being present, resulting in an increase in the prevalence of issues arising as a result of LCN amplification (as outlined in Chapter 1), such as stutter product formation and allele drop in.

With the handled samples a lower number of replicates were used to ensure DNA was deposited on to all of the samples. As these experiments were carried out using Firearms Officers, with limited availability and donation of ammunition, this was the maximum number of samples available.

7.4.1.3 Gunshot Residue (GSR Experiments)

As multiple authors had previously suggested that the firing process was not detrimental to the amount of DNA recovered from spent cartridge cases, another possible explanation for the observed reduction in DNA quality was the presence of GSR. As a weapon discharges it releases vast quantities of GSR, which is made up of partially combusted and unburned propellant, primer and metals. The presence of GSR would expose any DNA present to heavy metals, which are known to have an inhibitory effect on the activity of DNA polymerases. This could hinder the successful amplification of DNA.

7.4.1.3.1 Inhibition of DNA Profiling by GSR

The profiles for the DNA recovered from GSR seeded samples are present in Appendix Six (A6.6).

Initially two methods of DNA extraction were compared in order to determine if one method would be more successful at extracting DNA when GSR was present in the sample. Chelex and Qiagen extraction techniques were both used and Qiagen was found to be the more successful method. Only 22% of the profile was observed when Chelex extraction was used on the GSR seeded DNA samples, compared to 67% of the profile with the Qiagen extracted samples. However, the success rate for the No GSR control samples was only 35% for Chelex and 67% for Qiagen, so the GSR appears not to have affected the Qiagen samples adversely. With the samples extracted using Qiagen, there were consistent issues in this study with the longest allele in each dye not being successfully amplified. This may be evidence that the GSR is degrading the amplicons with a larger molecular weight.

One possible explanation for the poor results produced by the Chelex-extracted samples could be that Chelex works by binding magnesium ions. This aids DNA extraction because DNA nucleases, which degrade DNA, require magnesium in order to work. In this situation, where the sample contains several heavy metals, the Chelex may be overloaded

with binding heavy metals so may be unable to bind any magnesium ions present, leaving the DNA exposed to potential degradation.

Additional peaks, not assignable to the donor, were only observed in the Qiagen samples, not in the Chelex samples. As the success rate for Chelex was relatively low, it could be that there was a smaller yield of DNA using this method, which resulted in less additional peaks being amplified. These non-assignable alleles could be determined in some instances to be stutter product formation, but in other cases this was not the case (e.g. vWA: allele 22). In cases where stutter product formation was not a plausible explanation for the allele being present, there was potential that contamination may have been introduced, either from the researcher or from the cartridge case itself.

To determine the effect that would be observed if the GSR was not successfully separated from the DNA during the extraction process, samples at different stages throughout the DNA profiling process were seeded with GSR. These stages were:

1. No GSR Control;
2. Saliva seeded with GSR, then extracted;
3. DNA extract seeded with GSR, then amplified;
4. PCR product seeded with GSR, then placed on capillary electrophoresis.

The process of introducing GSR at different stages of the DNA profiling process does not seem to have had any real effect on the quality of DNA profile in terms of number of alleles, when looking at the Qiagen extracted samples. However, one thing that hasn't been taken into account when looking at the quality of the DNA profile is the peak height of each of the alleles. Table 7.3 shows the approximate peak height of each of the samples in the Qiagen extracted samples.

Table 7.3 Approximate maximum peak heights for GSR seeded samples.

Variable	Maximum Peak Height
1	Up to 7000 RFU
2	Up to 4000 RFU
3	Up to 4000 RFU
4	Up to 5000 RFU

The approximate maximum peak height for each sample shows a reduction from Variable 1 (No GSR control) to Variable 2 (GSR seeded Saliva). If the extract was seeded with GSR after this DNA extraction stage the impact on the resulting profile peak height was not as significant, although a reduction in the overall peak height was observed when compared to the No GSR Control. The reduction in peak height could have been due, to some degree, to a factor of dilution (as at each stage the sample was diluted by adding the GSR solution). However, it appears that GSR did have some detrimental effect on the quality of the DNA profile. It is important to consider that peak height may not be consistent throughout a DNA profile and so for more accurate determination each individual peak height should be looked at. In this case, as a potential dilution factor may have affected the peak heights, so this data was not included in the report.

Based on the data observed in this initial study, the presence of GSR throughout the DNA profiling process does not appear to have an adverse effect, especially when the DNA is extracted using the Qiagen extraction kit. Furthermore, the heavy metals present in the GSR do not appear to have an inhibitory effect on the activity of *Taq* Polymerase.

7.4.1.3.2 Short Term Exposure to GSR Study

The profiles for the DNA recovered from short term exposure to GSR samples are present in Appendix Six (A6.7).

This experiment was designed in order to determine the effect that exposure to GSR for time periods up to one week would have on the DNA profile recovered from a sample. Saliva samples were used to enable a constant amount of DNA to be deposited, so any results would be able to be interpreted. Glass slides were used for two reasons. Firstly, the number of cartridge cases that had been made safe by the Firearms Officers was limited, so there were not sufficient numbers to use in this study. Secondly, the interaction of saliva with the metallic surface of the cartridge case would have introduced an additional variable to the experiment, so an unreactive surface was used in its place.

The average number of assignable alleles for the samples exposed to GSR for 24 hour and 1 week durations were compared to control samples of saliva that were stored for the same time periods, but with no GSR present. These values are shown in Table 7.4.

Table 7.4 Average Number of Assignable Alleles for Saliva samples exposed to GSR for 24 hours and 1 week.

	Average Number of Assignable Alleles	Standard Deviation
24 Hours - No GSR	4	2.94
1 Week - No GSR	5.25	3.59
24 Hours + GSR	1.75	1.25
1 Week + GSR	2.75	3.09

One issue when interpreting this data was the quality of the DNA control samples (the No GSR controls). The samples were stored at room temperature, so although not ideal for DNA storage, this level of degradation after such short time periods would not be expected. One explanation for this poor success rate in profiling a saliva sample could be that the DNA sample collected contained low amounts of nucleated buccal epithelial cells. In retrospect, collecting a buccal scrape and re-suspending the collected cells in a Phosphate Buffered Saline (PBS) solution may have generated more optimal samples to work with (Mulot et al., 2005). Research has shown that if an individual brushes their teeth prior to providing a buccal cell sample, the yield of DNA is reduced (Feigelson et al., 2001). This could be a result of the epithelial cells in the buccal cavity being 'stripped' from the mouth. This could account for poor results.

Interpretation of the results indicates a possible reduction in the quality of DNA recovered when exposed to GSR, but the poor quality of DNA present in the saliva samples could have also accounted for the poor results observed. The standard deviation of the allele number present is greater than the difference between the sample types (no GSR and GSR Seeded) for both time periods, therefore the confidence in the data sets would be low.

7.4.1.3.3 Exposure of DNA to Discharged Levels of GSR

The profiles for the DNA recovered from discharged levels of GSR samples are present in Appendix Six (A6.8).

This study was designed to corroborate the research of Torre and Gino (1996) in regards to collecting GSR and DNA samples at the same time. It would also further substantiate or refute the previous work reported in this thesis in relation to the inhibitory effect GSR may have on DNA.

This study was carried out using non-live ammunition (referred to as blanks in this thesis) in the laboratories at Staffordshire University. The blanks were fired using blank firing weapons in a firing box. This was designed to reflect the results that would be observed in a scenario where a weapon has been discharged. GSR produced by blank ammunition was determined by researchers to be undistinguishable from GSR, but has been found to produce very few lead-only particles (Romolo and Margot, 2001). However, there were additional limitations with the design of this experiment, as blanks are likely to produce a different distribution of GSR to when compared to that of normal ammunition. This is due to blanks having a port where the gas can escape from (where the projectile would be placed in live ammunition) (Wikimedia Commons, 2005; Bolton-King, 2013). Another limitation would have been the firing box used, as it was a contained environment so the levels of GSR present after firing may have been artificially high.

There was also the potential variable of the amount of DNA deposited. Whilst attempts were made to try and control the amount of DNA deposited (by the rubbing of hands

together for defined period of time) it was not possible to ensure that the amount of DNA deposited before and after firing hands was equivalent, both in quality and quantity.

Figures 7.11 – 7.14 demonstrate the quality of DNA recovered prior to and after discharging the weapon.

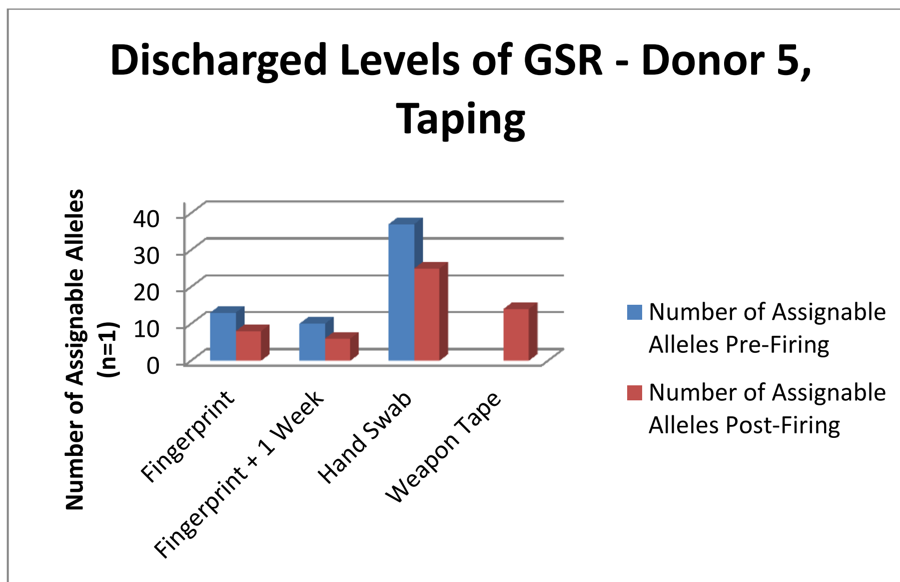


Figure 7.11 Number of alleles recovered from samples deposited pre- and post- firing for Donor 5, with taping used as the DNA recovery method.

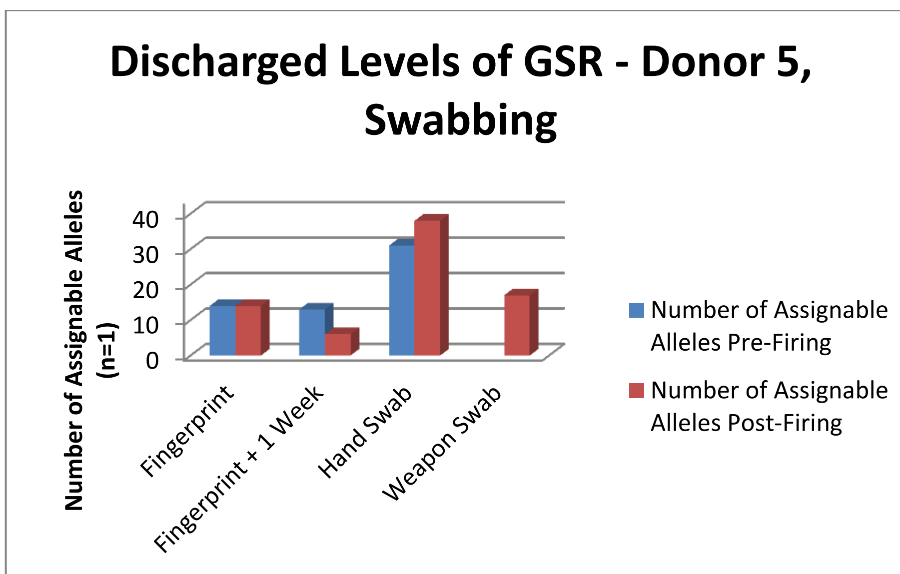


Figure 7.12 Number of alleles recovered from samples deposited pre- and post- firing for Donor 5, with swabbing used as the DNA recovery method.

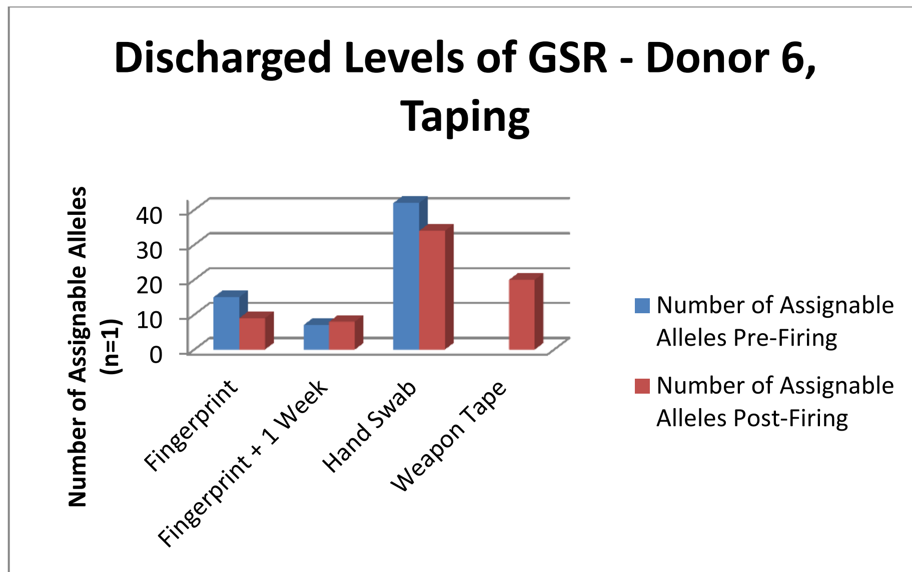


Figure 7.13 Number of alleles recovered from samples deposited pre- and post- firing for Donor 6, with taping used as the DNA recovery method.

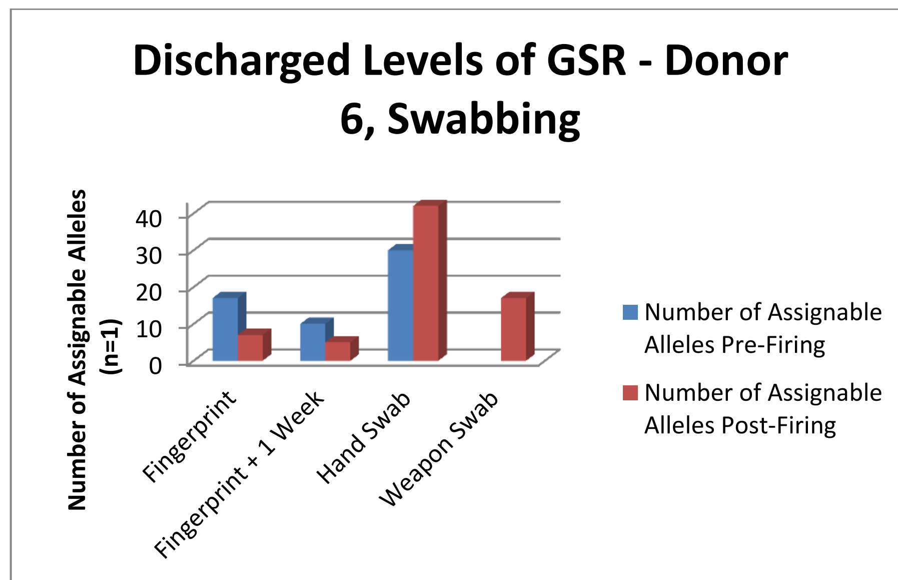


Figure 7.14 Number of alleles recovered from samples deposited pre- and post- firing for Donor 6, with swabbing used as the DNA recovery method.

The results have indicated that neither method of recovery, taping or swabbing, performs significantly better when recovering DNA from fingerprint samples or weapons (Figure 7.15). When the results from both donors and weapon types are pooled together, it is clear to see that the swabbing method has a marginally higher recovery rate for the fingerprint samples but an equal rate to taping for the weapons. However, the

differences between these results were not statistically significant ($p>0.05$ for One-Way Independent Measures ANOVA). The benefits and limitations of these recovery methods are discussed further in the 'Recommendations' Section of this thesis.

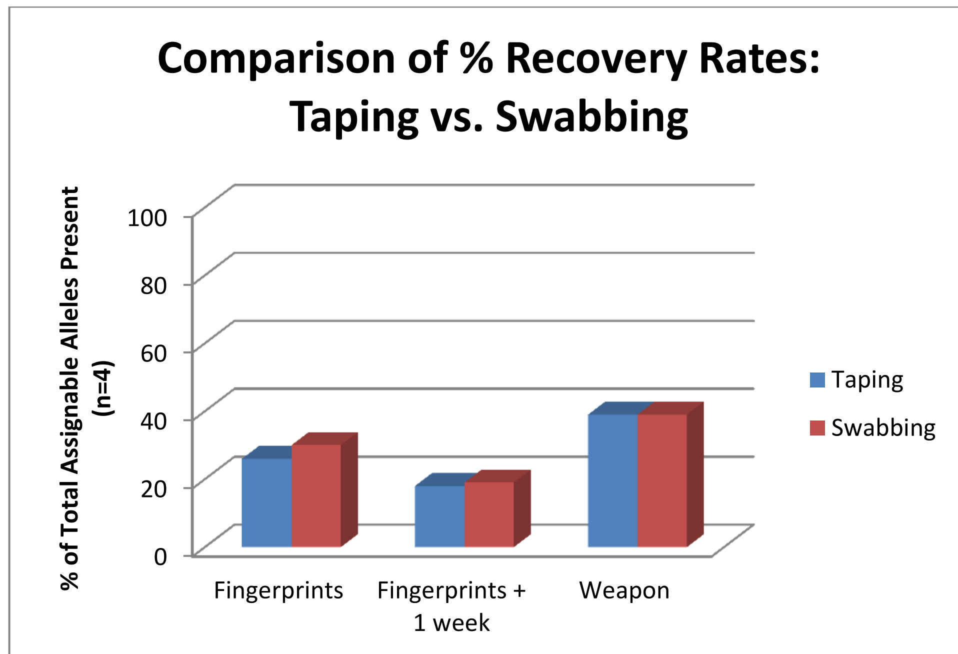


Figure 7.15 Percentage recovery rates for DNA, comparing taping and swabbing as the recovery method.

When attempting to determine the likelihood that GSR presence has on the quality of DNA, the optimal samples to compare are the hand swabs. This is due to the fact that the amount of DNA present is expected to be higher and more constant between donors in the hand swabs than in the fingerprint samples. This is partly due to surface area, but also due to the DNA being collected from the source, not from a primary transfer event. The results indicate that the difference in the quality of DNA between pre- and post-fired hand swab samples was minimal (Figure 7.16). Although the standard deviation for the post-fired samples was marginally higher, the average number of alleles was comparable between the two variables. A paired T-test was undertaken and the results indicated that there was no statistically significant difference between the DNA samples collected from hand swabs prior to, or directly after, discharging a weapon ($p>0.05$). This suggests that

either the DNA present is in sufficient quantity that GSR does not have an effect, or simply that the presence of GSR does not inhibit successful DNA profiling.

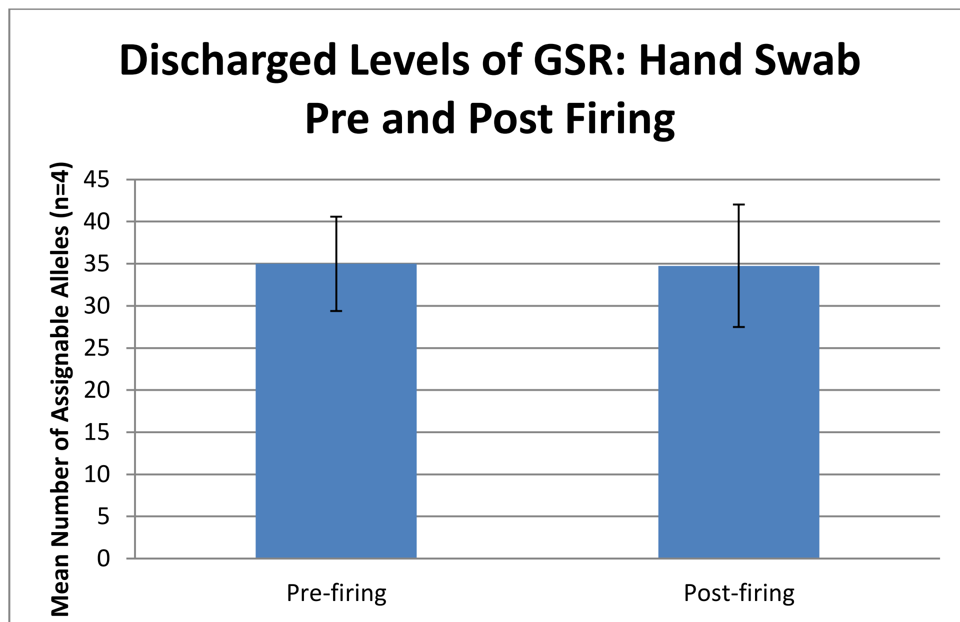


Figure 7.16 Average number of alleles recovered from hand swabs, pre- and post-firing.

The results also indicated that the type of weapon used was not a significant factor in the quality of DNA recovered from fingerprints or hand swabs collected post-firing. This was to be anticipated as the ammunition used for both weapon types was the same, and therefore there would be no reason for the quality of DNA to be affected.

However, the quality of DNA recovered from the weapon itself was variable. Figure 7.17 indicates that the number of assignable alleles recovered from the revolver is lower that the number recovered from the semi-automatic weapon. This may indicate that the surface type has an impact on the DNA recovery rate (the semi-automatic handle was plastic, compared to the polished wooden surface of the revolver handle) or that the degree of contact when loading the ammunition into the weapon varied, resulting in differing amounts of DNA being deposited. Overall, the recovery of DNA from the handled weapon was relatively poor, which supports the work of Polley et al. (2006) and Horsman-Hall et al. (2009) who report poor levels of DNA recovered from weapons. The amount of DNA deposited and transferred through handling is discussed in detail later in this thesis.

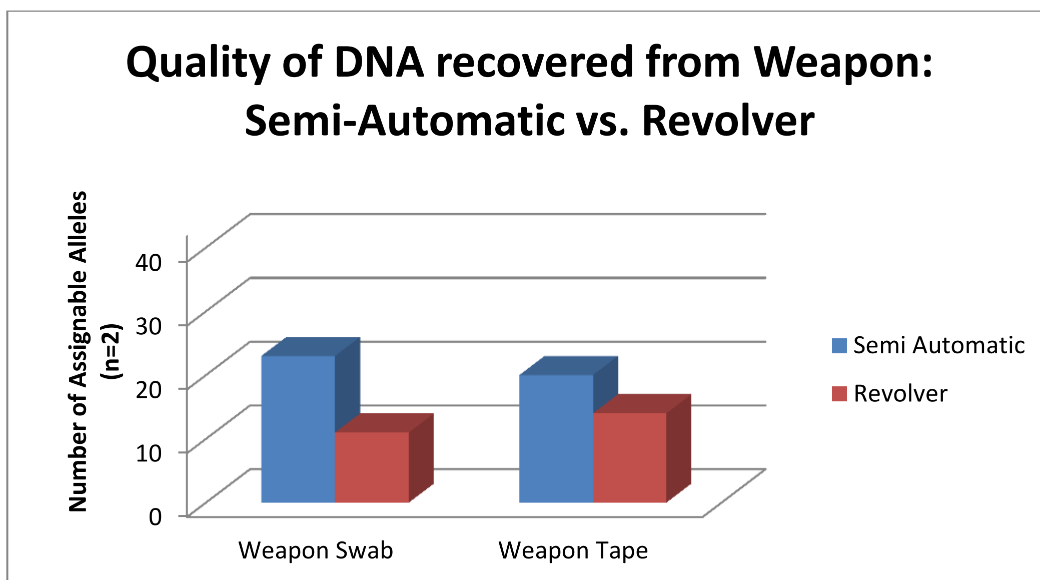


Figure 7.17 Number of alleles recovered from semi-automatic and revolver weapons.

Based on these results, it appears that the majority of DNA degradation was not due to exposure to GSR. This suggests that either the amount of DNA deposited on handled cartridge cases was insufficient to produce a DNA profile, the DNA present is being transferred off the cartridge case during the firing process, that the firing process has a major effect on the quality of DNA profile recovered or that another process was having a detrimental impact on DNA quality. Results gained during this research indicated that the amount of DNA deposited on handled items was low level, but was sufficient to produce partial, if not full, profiles. The inside of the barrel was not swabbed during this research, so it is not possible to determine if transfer of DNA from the cartridge case to the chamber or barrel was a factor in the amount of DNA recovered. However results gained during this research would suggest that the firing process is the key factor in the DNA degradation observed. These results contradict those of Allen et al. (2008) and Gashi et al. (2010), as in this research poor quality profiles were recovered from spent cartridge cases. One of the issues is comparing research from different groups is the variation in methodology used. Differences in the sample recovery method, DNA extraction method, concentration method (if used), amplification protocols, electrophoresis method and analysis parameters used could account for variations in the profiling success rate. The potential that another process could be having a detrimental effect on the DNA has been

suggested by Sermon et al. (2012). They have proposed that copper and zinc ions from the brass cartridge casings have been found in sweat residue deposited by handling, and that these may bind to and fragment any DNA present. This hypothesis has not been rigorously tested and therefore cannot currently be verified.

The results from this research appear to support those reported by police officers, who have had consistently low success rates with this type of evidence.

In June 2012, the New Scientist (Marks, 2012) announced a research publication that reported that modified cartridge cases were being developed that would 'protect' deposited DNA. This research by Sermon et al. (2012) has involved altering the surface of the cartridge by immersing it in a solution of aluminium oxide and urea, which produces a more abrasive surface. This was carried out to increase the amount of DNA that would be deposited onto the surface. Their results indicated the mean number of assignable alleles recovered from these modified cartridge cases was 11.2 (with a standard deviation of 5.5) which was higher than the number recovered from a standard cartridge case (7.1, with a standard deviation of 6.1). They have also reported that coating the surface of the cartridge case with a nanotag that could be transferred during handling would enable the detection of the handler.

Very recently Montpetit and O'Donnell (2015) have reported that soaking cartridge cases in lysis solution as opposed to swabbing the surface of a casing with a moistened swab has resulted in an increased yield of DNA being recovered. They also recommend the use of more sensitive DNA profiling chemistries when processing very low yield samples.

7.5 Conclusions

This chapter aimed to address several research questions in relation to the recovery of DNA from cartridge cases. The research aimed to determine if it is possible to recover DNA from a spent cartridge case once it has been ejected from a firearm. Based on the research findings, the firing process has a statistically significant impact on the quality of DNA that can be recovered from a cartridge cases (profiling success rates reduced from 96% to 26% for saliva-seeded samples and from 75% to 10% for handled samples). The

samples seeded with saliva showed a significant decrease ($p<0.01$) in the profile quality when pre- and post-firing samples were compared. However, sufficient alleles were observed in the saliva seeded fired samples to enable identification. It would be recommended that the use of amplification systems with a greater sensitivity or the application of systems that amplify smaller amplicons could be utilised to enhance success rates, for example DNA 17 or mini-STR profiling systems. The potential to recover sufficient DNA to enable identification of the handler is very low, as evidenced by the poor quality profiles produced from the handled fired cartridge case. The firing process appears to have a negative impact on the ability to recover DNA from handled cartridge cases, although the difference was not as statistically significant between pre- and post-firing as with the saliva seeded samples. There is no evidence to suggest that the weapon type or calibre appears to have any effect on the DNA success rate, although only a limited selection of firearms and ammunition were used for this research. Finally, the presence of gunshot residue did not appear to significantly impact on the potential for DNA profiling success. It is believed that the DNA is more likely to be affected by the high temperatures during firing as opposed to any inhibitory action brought about by the gunshot residue present in the sample.

Eight. Recovery of DNA from Firearms

8.1 Introduction to DNA Recovery from Firearms

The potential for evidence recovery from firearms varies depending on how the firearm has been handled and when it was discharged. Finger marks, gunshot residue, trace evidence, biological material and DNA have all been recovered from firearms with the purpose of identifying the shooter. However, the success rate for fingerprint recovery on firearms is relatively low given the requirement to handle the weapon prior to and during discharge. One reason for this could be due to the textured, grooved and knurled gripping surfaces most commonly used when operating the firearm. Barnum and Klasey (1997) have reported that the traditional methods for enhancing finger-marks are unlikely to be appropriate for use on firearms, as a result of the surface textures encountered on this type of exhibit. However, although the textured surface is not optimal for fingerprint recovery, it may be a good source of DNA. Nunn (2013) calculated that touch DNA provided more probative evidence than fingerprints from firearms evidence, and when identification outcomes were compared the two methods were equal.

Barash, Reshef and Brauner (2010) examined the use of tape lifting as a method for DNA recovery from a rusty revolver in forensic casework. They sampled four areas on the revolver (right- and left-hand side of the handle, the cylinder and the trigger/hammer area) and were able to generate a profile of suitable quality to be loaded onto a national DNA database, subsequently implicated the suspect in a number of armed robberies based on the DNA evidence. Their aim in this research was not to consider the optimal method for DNA recovery from firearms evidence; rather, their focus was aimed at the comparison of tape lifts for recovery of DNA evidence in general.

Research published by Polley et al. (2006) investigated the recovery of DNA from firearms and used cotton swabs and 50% methanol as their chosen recovery method. A targeted approach to swabbing was used, where four locations on each weapon were identified as most likely to have come into contact with the handler. They found that the area most likely to consistently produce the best quality DNA profiles was the grip with an 80%

success rate. They also identify potential issues associated with transfer and persistence of DNA impacting on the interpretation of DNA evidence recovered from firearms.

Horsman-Hall et al. (2009) undertook research into the recovery of DNA from firearms and fired cartridge cases and examined the potential for DNA evidence to be transferred from a handled cartridge case to the chamber of the barrel, ejection port and breech face. Although they were able to obtain a DNA profile from the barrel, they acknowledged the quality of the profile was not sufficient to aid an investigation as a result of allele drop in and out, as well as contaminating peaks. When attempting to recover DNA from cartridge cases they adopted a targeted approach and utilised double swabbing to maximise evidence recovery.

Ryan and Kelepecz (2008) stated that the optimal locations for recovery of DNA from the handler of a weapon will be on rough, textured surfaces such as the grip, hammer and trigger, supporting the research reported by Polley et al. (2006). Richert (2011) suggested that as opposed to using a targeted approach when recovering DNA from firearms, the use of single swab for the entirety of the firearm enhanced the collection of cellular material. He suggested that lower yields were generated from the individual swabs, and recommended that a firearm be swabbed in its entirety, using no more than two swabs. This is supported by Wickenheiser (2002) who suggests that using a single swab to collect trace DNA from multiple areas will increase the likelihood of recovering a useable DNA profile. The counterargument for swabbing a larger area with a single swab is that there will be an increased chance of obtaining mixed profiles (Montpetit and O'Donnell, 2015), but Wickenheiser (2002) suggests that areas should only be swabbed jointly if there is reasonable belief that a single person will have come into contact with these areas.

Reducing the number of personnel required to handle a firearm will also enhance the potential for successful DNA recovery. ACPO (2005) guidelines state that firearms officers must assist in the safe recovery of firearms under the guidance of a Crime Scene Investigator (CSI). With recent changes to the provision of forensic services in England and Wales, most police forces will store their firearms exhibits once they have been made safe and packaged appropriately. They will then use their own in-house forensic investigators to swab for DNA, and these samples will then be sent to their tendered Forensic Provider for analysis. Currently there is no one approved method for

recovering DNA evidence from a firearm within the UK. Scottish Police personnel use mini-taping (Murray, 2005), West Midlands Police personnel use a maximum of two swabs for the whole of the firearm whereas Durham Police personnel typically target eight different areas for swabbing (Outhwaite, 2012).

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8.2 Research Questions for DNA Recovery from Firearms

This chapter aims to address the overall objective to develop a protocol for the optimal retrieval and amplification of DNA from firearms.

Based on a review of the previously published literature of experimental work undertaken by different research groups, as outlined in the introduction to this chapter, several research questions were identified:

a. Is there an optimal method (double cotton swabbing, nylon swabbing or mini-taping) for the recovery of DNA from firearms?
b. Is a zoned approach more likely to generate a better quality DNA profile or is a targeted approach more appropriate?

8.3 Experimental Design

To address these research questions the following experiments were designed, as outlined below.

For this study, Generation 3 'Glock 17' self-loading pistols were used. All weapons were cleaned prior to use with 'Super Sani-ClothPlus' disposable disinfection wipes for medical devices and control swabs were taken prior to handling.

Subjects were instructed not to wash their hands or come in contact with another individual for at least 1 hour prior to the experiment.

Subjects were asked to handle the firearm for 1 minute, during which they were instructed to work the slide to the rear and release, to cock the action and pull the

trigger. They were asked to repeat this process three times and then place the firearm onto a sterile sheet of paper.

Samples were recovered using the following swabs/lifters:

- K650 Standard Kit containing two Cotton Swabs and sterile water (Scenesafe FAST™, SceneSafe Ltd.)
- K542 Enhanced DNA Recovery Kit containing a Nylon Flocked Swab and Reagent (Scenesafe FAST™, SceneSafe Ltd.)
- Mini-tape lifters (Scenesafe FAST™, SceneSafe Ltd.)

DNA samples were recovered using either a zoned approach (Figure 8.1) or a targeted approach (Figure 8.2).

Figure 8.1 Identification of two zoned areas for swabbing of the firearm.

Table 8.1 The zoned areas for swabbing of the firearm.

1] Green Zone (Handling)	Pistol grip including front strap, back strap, thumb rest
2] Red Zone (Using)	Magazine catch, slide stop lever, rear slide gripping area, trigger guard, trigger and trigger safety
3] Mag	Remove magazine and swab base-plate, sides of magazine walls and magazine lips

Figure 8.2 Identification of six targeted areas for swabbing of the firearm.

Table 8.2 The targeted areas for swabbing of the firearm.

1	Pistol grip including front strap, back strap, thumb rest
2	Magazine catch and slide stop lever
3	Rear slide gripping area
4	Trigger guard inside and outside
5	Trigger and trigger safety
6	Remove magazine and swab base-plate, sides of mag walls and mag lips

For the zoned approach, the swab or tape was applied to the surface of the areas indicated 20 times. For the targeted approach, the swab was wiped across the surface areas indicated 5 times.

The end of the swab was then cut off and placed into a 2 ml Eppendorf tube (for the double swabbing the two swab ends were combined into a single tube). Mini-tapes were placed directly into a 2 ml Eppendorf tube with the adhesive side facing the inside of the tube.

Qiagen extraction was used to extract the DNA from the samples. DNA quantification was carried out using the Qubit Fluorometer (Invitrogen™). Standard SGM+ PCR amplification was carried out on the samples, followed by Capillary Electrophoresis (with a baseline threshold of 150 RFU).

8.4 Analysis and Interpretation of DNA Recovery from Firearms

The profiles for the DNA recovered from firearms samples are present in Appendix Seven.

This study was designed to determine if any methods of DNA recovery would generate high quality and quantity DNA samples, thereby increasing the chances of a successful profile being generated. Figure 8.3 illustrates the mean concentration values recorded from cotton swabs, nylon swabs and mini-tapes in both the targeted and zoned DNA recovery approach.

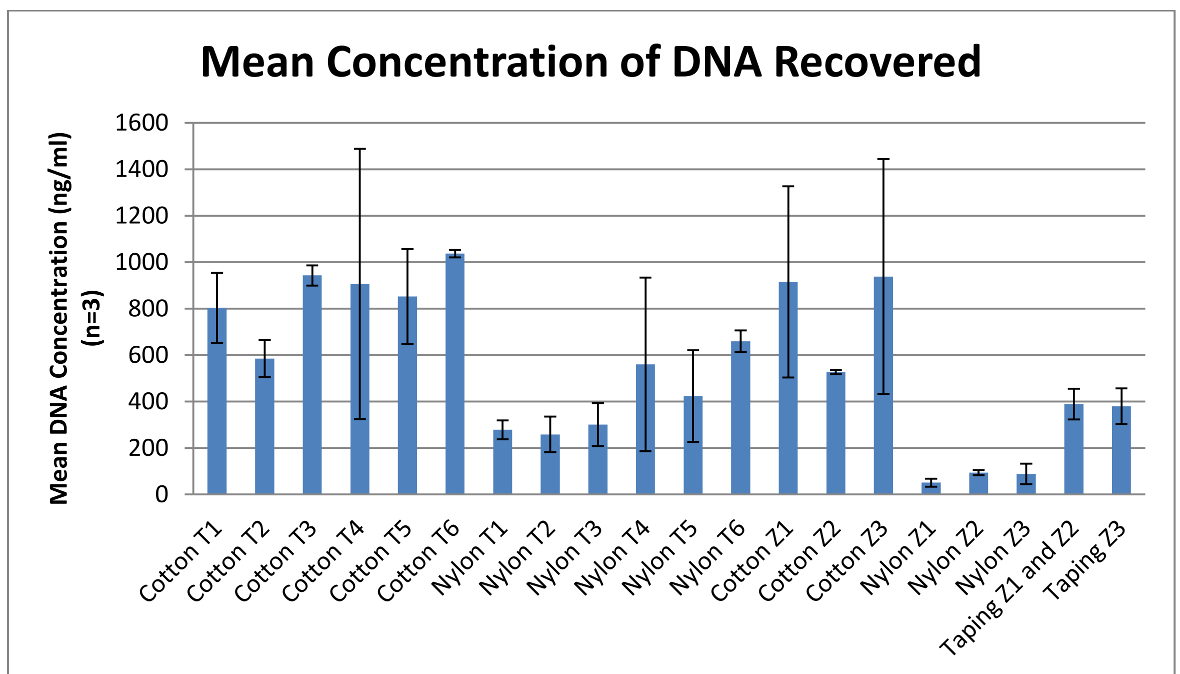


Figure 8.3 Mean concentrations (ng/mL) of DNA recovered using nylon swabs, cotton swabs or tape lifts, comparing the zoned and targeted approaches of recovery.

Based on the quantification results, it appears that cotton double swabbing recovers the largest quantity of DNA from firearms, for both the targeted and zonal approach. The quantification values obtained from the nylon swabs are very low, which is consistent with other results previously reported in this thesis (Chapter 5). The tape lift values are not as high as the cotton swab values, but are higher than for the respective nylon samples. A one-way, independent-measures Analysis of Variance (ANOVA) was undertaken on the mean concentrations of DNA recovered, followed by the Bonferroni Post-Hoc test. For cotton swabs, the results suggested that there was a statistically significant difference between T2 and all other areas, apart from T4 ($p<0.05$) but no statistically significant difference between the other areas ($p>0.05$). This would be anticipated as T2, the magazine catch and slide stop lever, would not be in prolonged contact with the handler as much as some of the other areas may be. Similarly, little contact would be anticipated with the trigger guard. There was no statistically significant difference between the targeted cotton swabs and the zoned cotton swabs. There was a statistically significant difference when the different methods of recovery from zoned areas was compared (zoned cotton swabs, zoned tapings and zoned nylon swabs) but here was not a statistically significant difference between the samples within those zoned areas.

It would be expected that higher quantities of DNA would be recovered using the zonal approach but this is not a trend consistently observed in this data set. When comparing the quantity of DNA recovered from the zoned samples with the targeted samples, the nylon swabs appear to be less successful at recovering DNA from the zoned regions. This could be due to the design of the swab head and its affinity for DNA retention. One of the attractions of the nylon flocked material is that it is able to release the DNA at time of extraction, ensuring maximum DNA recovery (Fumagalli and Vaněk, 2008). It may be that excessive repeat swabbing causes any DNA that has been collected to be prematurely released from the swab back onto the surface.

The quantification values recorded for the cotton swabs are not significantly different between the two approaches, suggesting that once DNA has been collected onto the swab, it will not be redeposited. These results further support the claim that double swabbing increases the yield of DNA recovered (Pang and Cheung, 2007). It must be highlighted that the taping method was only applied using the zonal approach, due to the

small surface areas of the targeted approach not being appropriate for tape-lifting. Another observation from the results recovered was that the variability in some of the datasets was quite significant. Three of the cotton samples and one of the nylon samples demonstrated extremely high standard deviation values, suggesting significant variability in the samples. There doesn't appear to be any meaningful trends relating specifically to these samples; two are from the targeted approach and two are from the zonal approach samples, so this is not an explanation for the results observed.

In the DNA Recovery chapter, there was poor correlation between the quantification results and their respective DNA profiles causing concern as to the reliability of this quantification method. This poor correlation has also been observed in these samples, as evidenced in Figure 8.4.

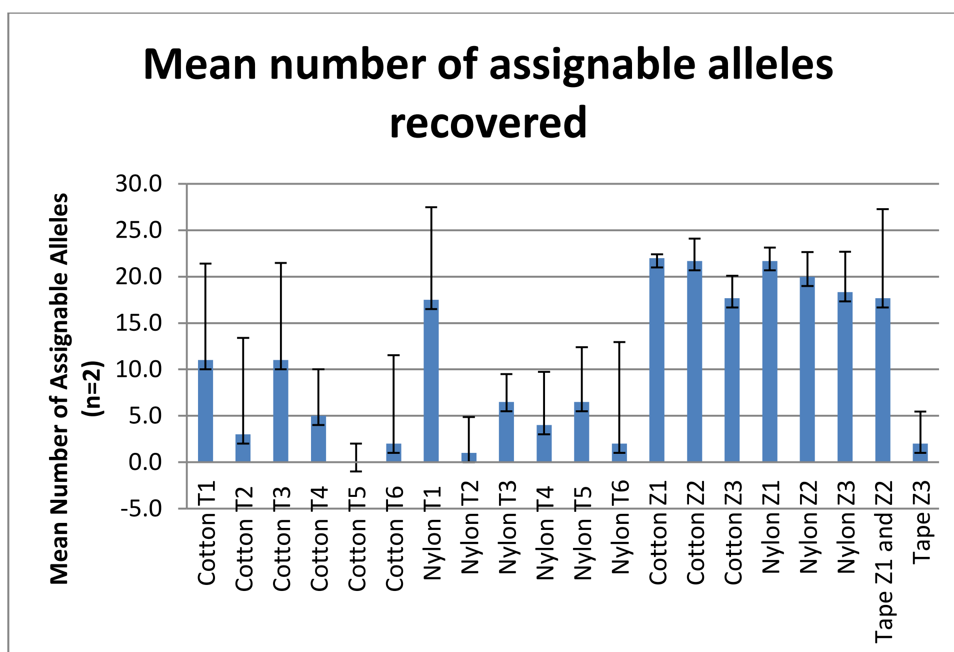


Figure 8.4 Mean number of assignable alleles recovered using nylon swabs, cotton swabs or tape lifts, comparing the zoned and targeted approach.

Figure 8.4 shows the mean number of assignable alleles recovered using cotton double swabbing, nylon flocked wet swabs and mini-tapes for both the targeted and zonal approaches. It must be noted, only the positive standard deviation readings have been included on the graph for this set of samples. The reasoning behind this was that for some of the samples, predominantly the cotton targeted samples, the standard deviation

values were very large. So much so in fact that when both positive and negative SDs were applied, the graph became unreadable, so for clarity only the positive SD values have been included in this instance.

As previously identified there doesn't appear to be any relationship between the quantification values and the potential for a successful DNA profile to be produced. For example, the nylon zoned samples had the three lowest concentrations according to the quantification results, but have consistently generated good quality profiles. Conversely, the cotton targeted samples had high DNA concentrations but have generated poor quality profiles. Figure 8.4 only includes the alleles recovered that were assignable to the handler, not any contaminating alleles, so this could offer some explanation for the lack of parity between the quantification and allele data. However, the incidence of contamination was very low across all of the samples so even with the inclusion of non-donor alleles, the lack of correlation was not a result of non-donor DNA being present. The quantification system is not human-DNA specific, so this could potentially explain the lack of a clear relationship between the two measures of DNA recovery.

Based on the DNA profiling results, it appears that the zonal approach for DNA recovery is optimal for generating full or almost complete DNA profiles. These findings are in agreement with Richert (2011) who observed that collective swabbing resulted in more complete profiles being recovered. The proportion of non-donor alleles is marginally greater in these samples (2.9% for cotton zonal when compared to 1.5% for cotton targeted, and 8.7% for nylon zonal when compared to 7.2% for nylon targeted), which was identified as a potential risk by Montpetit and O'Donnell (2015). However, the overall increase in profile quality offsets any concern about a slightly increased risk of contamination. Furthermore, the contaminating peaks were usually discernible from the donor alleles by examination of the peak height. Interestingly the tape lifts demonstrated the highest percentage of non-donor alleles in the samples at 27% as well as exhibiting the greatest standard deviation for the zoned approach samples. All non-donor alleles are likely to have originated from one of the other firearms officers, as he would have general responsibility for the weapons used. Comparison of these alleles with his DNA profile (not shown) confirmed this to be the case.

When considering variability in the effectiveness of the methods within the zoned approach grouping, the cotton swabs recovered the highest number of alleles overall and had a slightly smaller mean standard deviation. If a cost benefit analysis was carried out on the technique, this would result in a recommendation for the use of the cotton swab as cotton swabs cost approximately 17 pence per unit, as opposed to flocked nylon swabs which retail for GBP £2.77 per unit currently. In comparison, mini-tapes cost approximately 45 pence per unit. In evaluating the likelihood of a police force modifying their practices based on research findings, it is always pertinent to consider the cost implications of any changes to standard protocols. In the literature tape lifts are customarily recommended for porous surfaces (Verdon, Mitchell and van Oorschot, 2014) but are also used for recovery from other items of evidence (Murray, 2005; Barash, Reshef and Brauner, 2010). However, based on these results a zonal approach to double swabbing with cotton swabs and sterile water would be the recommendation to maximise DNA recovery.

In relation to the targeted swabs, both methods demonstrated significant variation in the number of assignable alleles recovered. However, it appears that a complete set of the cotton swabs failed to amplify so with such limited sample numbers these findings should be interpreted with caution. The profile quality for many of these samples was quite poor, as demonstrated by the electropherogram for one of the targeted nylon swabs presented in Appendix Seven, A7.2). Based on the alleles successfully amplified, it appears that the magazine has the lowest number of assignable alleles for both methods and the pistol grip generate the highest number of assignable alleles. This is to be expected, due to the extensive contact with the pistol grip throughout the handling process and conversely the very limited contact with the magazine during handling. The results are in agreement with those reported by Polley et al. (2006) and Ryan and Kelepecz (2008).

When considering areas for DNA recovery, it would be interesting to further examine the inside of the magazine, based on observations by Horsman-Hall et al. (2009) that DNA from handled cartridge cases was recovered from the barrel chamber of a weapon.

In terms of notable electropherogram phenomena, it was observed that heterozygote imbalance may have resulted in some of the alleles not being identified correctly (see

Appendix Seven, A7.3 for an exemplar electropherogram). Reconsideration of the baseline threshold value could be considered in the future, but this may cause issues surrounding an increased proportion of contaminating peaks being detected.

8.5 Conclusions

This chapter aimed to address several research questions in relation to the recovery of DNA from firearms. This research studied three different recovery methods (double cotton swabbing, nylon swabbing and mini-taping) using two different strategies (zonal and targeted). The results indicate that the zonal approach is optimal for enhanced recovery of DNA from firearms, and although nylon flocked swabs performed to an equivalent standard, double cotton swabbing would be recommended. This recommendation was based on an evaluation of performance as well as cost per unit. These recommendations should be viewed with some caution, as there was poor correlation between the quantity of DNA recovered and the subsequent quality of DNA profiles produced. For example, the zoned nylon swabs generated the lowest quantification results but the highest quality profiles. This may indicate that the quantification system used was not sufficiently accurate, especially with low yield samples. A further recommendation from this research would be the use of real-time PCR for quantification of low yield DNA samples.

Nine. General Discussion

9.1 Discussion of General DNA Interpretation

When analysing the DNA profiling data, there were specific limitations in relation to presenting the results in a transparent manner. For example, identifying multiple alleles at a locus in a tabular fashion gives no indication as to the respective heights of the peaks. As peak height can be used for separating mixtures, or for indicating stutter products, this information could be useful. However, peak height could be an unreliable method of identifying mixtures in this research, due to the low amount of DNA present resulting in stochastic effects such as allele drop out and heterozygote peak imbalance. Therefore, when alleles identified in the table are believed to be a result of stutter production or another specific phenomenon, this has been highlighted in the discussion of those samples.

In cases where a donor individual is homozygous at a locus, if that allele has been identified in a sample it has been counted twice in any number-of-allele calculations. This is to prevent an unfair numerical bias towards the other donor in contributing alleles to a mixed profile.

All interpreted data is presented in the preceding chapters, with all of the raw tabulated profiles or quantification data presented in the Appendices. The control SGM+ DNA profiles for all donors are tabulated in Appendix Eight.

With low level DNA analysis, contamination is a constant issue. Whilst every possible care was taken to reduce the potential for contamination, there was still evidence of contaminating peaks in the negative controls. The researcher wore appropriate personal protective clothing (lab coat, double gloves, hair net, and face mask) throughout the experiments. All pieces of equipment were autoclaved or exposed to UV light (where possible), wiped with 2% Virkon (bactericide, fungicide and virucide), 100% ethanol and double distilled water to remove any contaminating DNA from the surface. Although research has indicated that other techniques, such as DNA Away® (Molecular BioProducts, Inc.) may be able to effectively remove DNA present (Preuβe-Prange et al., 2009), there were practical issues with the use of these anti-contamination techniques in

this research. DNA Away® is not appropriate to use on metallic surfaces, which meant it was not appropriate for any of the firearms experiments. The equipment for exposing items to UV Light was not transportable and therefore this technique could not be used at any of the firing ranges. Similarly, it is not appropriate to autoclave live ammunition or blank firing firearms. For these reasons, the anti-contamination techniques outlined earlier were used consistently throughout the research. These are comparable to anti-contamination techniques used by other researchers (Raymond et al., 2004; van Hoofstat et al., 1999).

The predominant challenge with this research into the transfer and persistence of DNA was attempting to ensure that the amount of DNA deposited was constant when comparing variables. Several authors have eluded to this issue when designing their research methods, and most opt to set a defined period of time from hand washing to sampling (Alessandrini et al., 2003), the use of a body fluid in the place of 'touch DNA' samples (Goray, van Oorschot and Mitchell, 2012) and/or the rubbing of hands together to ensure even distribution of DNA. In this research the duration of two hours between hand washing and sample collection was chosen to enable replenishment of DNA on the hands (Lowe et al., 2002) and participants were asked to rub their hands together for a specified period of time prior to sample deposition.

The recovery method used throughout this research varied, based on results gained in preliminary studies. The use of taping, swabbing with cotton squares and swabbing with a buccal swab were used (as specified in the individual research chapters). The choice of recovery method may depend on the surface type being sampled and there are issues with all of the methods used. The processing of mini-tapes often involved cutting the tape into smaller pieces. This repeatedly resulted in sections of the tape being deposited onto the laboratory bench, which results in an increased potential for contamination. This issue has been raised by Raymond et al. (2004) therefore is not a problem solely encountered by this researcher. The cotton squares used have a relatively large surface area, which may result in an issue when trying to release the cellular material into the lysis buffer. This is an increased area of concern when the samples being swabbed contain very low levels of DNA. Similar issues have been raised with the potential issue of DNA being sufficiently exposed to the extraction reagents by Raymond et al. (2004).

The DNA extraction method used is also important in considering the validity of the results. Again, preliminary experiments indicated the optimal method of extraction for the different samples types. Balogh et al. (2003) reported large differences in the percentage of DNA recovered from fingerprints deposited on paper as a result of the extraction technique used. van Oorschot et al. (2003) reported that between 20% and 76% of DNA recovered is lost through the extraction stage. They suggested that this was a combination of the extraction process, the recovery technique used, the amount of starting material and the condition of the sample after collection (whether it was wet or dry). Goray, Mitchell and van Oorschot (2010) also suggest the quantity of DNA recovered may not be an accurate representation of the amount deposited as a result of loss of DNA during the extraction process.

In addition to considering the amount of DNA extracted, it is important to consider the purity of the extract. PCR may fail if the DNA present is highly degraded or if there is insufficient DNA present. However another common cause of PCR failure is if PCR inhibitors present in the original samples are co-extracted (Butler, 2009).

DNA quality was chosen to be studied as a measure of success in most of the research, as opposed to quantification, due to the fact the identifying information is housed in the DNA profile, not the quantification value, and also there is a recognised degree of variability with the reliability of quantification methods. This is supported by Verdon, Mitchell and van Oorschot (2014) who state that "Because the current best practice quantification method…..is only accurate for concentrations of 0.023 to 50 ng/µl, it is difficult to reach meaningful conclusions based on many of the low quantification results necessarily associated with trace DNA samples". They also identify the importance of DNA profiling as a metric for evaluating sampling techniques.

Some publications suggest the use of a concentration device to increase the yield of DNA but both Fridez and Coquoz (1996) and van Oorschot et al. (2003) report the loss of DNA as a result of using the Centricon device.

In this research, both SGM+ and Identifiler amplification kits have been used, as a function of the availability of specific kits at specific times in the laboratory. Forensic providers recommend using Identifiler with samples where the DNA yield is anticipated to

be low (Williams, 2012), DNA17 kits or chemistries where smaller amplicons are amplified.

During this research both standard and LCN amplification conditions were used. Beyond 40 cycles of amplification, reaction components within the PCR master mix may become limited thereby reducing the efficiency of the amplification, and the *Taq* polymerase enzyme becomes increasingly inefficient with increased cycles of amplification (Hughes and Moody, 2007). As indicated by Gill et al. (2001), the optimal LCN cycle number for SGM + is 34, therefore this is unlikely to be an issue with this type of analysis.

Given the types of samples encountered in this research, the majority of samples were mixtures which can make interpretation of the profiles very challenging. In this instance, the profiles of the donors were known, and so could be readily identified. This limitation must be considered when applying the outcomes of this research to a case scenario, where the donor is an unknown party.

In addition to the issue of mixed profiles when interpreting the results, there was also the challenge of shared alleles. In mixture interpretation when attempting to determine the degree to which an individual's profile is dominant, this may be misrepresented if you are looking solely at alleles that only that donor has contributed. This is because the other donor may have more shared alleles (through a higher number of homozygous alleles) and therefore they would not be able to contribute an equal amount. This was addressed to some degree in this research by considering both the individual donors contribution as well as the shared allele contribution. However, when measuring contribution to a mixed profile, alleles were only counted once, even when one of the donors was homozygous at that allele. This may have introduced some minor biases to the determination of degree of contribution in a mixed profile.

Finally, the quality of the electropherograms was very variable, as was anticipated with the profiling of LCN DNA samples. There was a tendency to see over-amplification of stutter products, as well as split peaks (due to non-template adenylation) and allelic drop-out. In some electropherograms pull up was evident, as well as broad peaks and OL (Off Ladder) alleles which suggested that there was an issue with the performance of the matrix or capillary (Gunn, 2006; Goodwin, Linacre and Hadi, 2007; Butler, 2004). All of

these phenomena are likely to be encountered when analysing samples containing poor quality or quantity DNA, such as the sample types analysed in this research.

9.2 Philosophical Discussion

When undertaking a body of research of this magnitude, there are inevitably wider philosophical discussions that should be considered alongside the technical findings of the primary research.

The discovery that there is the potential for DNA to be transferred has had a significant impact on the court room experiences of reporting forensic scientists. A decade ago, it was extremely unlikely that you would be summoned to court to be cross examined on your findings if your DNA sample was consistent to that of the suspect. With the desire for more activity level evidence to be incorporated into expert witnesses evaluations, it is increasingly likely you will now be asked how that DNA sample could have been deposited on to the evidential item in question, and what implications that would have in relation to the case circumstances. Without a clear understanding of the potential for DNA transfer occurring, a good defence lawyer suddenly had the ability to discredit the most seemingly unshakeable evidence type – DNA.

The field of forensic science centres on the impartiality of its experts. It is often quoted that the ‘evidence cannot lie’. It was therefore the cause of some moral deliberation that the subject of this research appeared to favour the defence proposition, rather than provide support for both sides of the adversarial system. Invariably, requests to find out more about the research findings outlined in this thesis have originated from defence teams looking for scientific support for their clients’ version of events. From a more holistic viewpoint, it should be understood that research drives scientific understanding and this is imperative if we wish to ensure justice is upheld. The potential to generate a body of research that could advance the understanding of DNA interpretation was extremely motivating.

The potential ramifications of transfer and persistence have a wider audience than solely those evaluating DNA based cases. The question of provenance is applicable to all traces

of evidence and furthermore, cross-discipline research efforts should be furthered to ensure a thorough evaluation of evidence is undertaken. For example, the Ipswich murders in 2006 clearly highlight the benefit of interpreting evidence using a combined approach. The presence of Steve Wrights' DNA on the bodies of the prostitutes would not have been sufficient evidence to charge him with murder. However, when combined with fibres evidence, more conclusions could be drawn from the forensic evidence present. The potential to develop a framework, perhaps using Bayesian models, to interpret DNA evidence transfer has been suggested by Biedermann and Taroni (2012), with Breathnach and Moore (2013) applying this approach to the interpretation of salivary amylase and DNA in cases of alleged oral intercourse. The application of multi-level models to assist in the prioritisation of variables governing transfer and persistence of evidence, such as logistical regression analysis to allow consideration of the combined influence of a number of predictors could also be worth exploring.

Taroni et al. (2013) recently published a journal article highlighting the gap between the judicial system and the scientific world, identifying the disparity between what a lawyer wants to know and the answers a scientist is willing and able to provide. Development of this field of research could go some way to bridging that gap, by involving lawyers in open and honest debates about the scientific principles underpinning the reliability of DNA evidence interpretation.

As DNA analysis is still in its relative infancy, the continual stream of developments to DNA techniques and related systems has been a constant concern when undertaking this research. To name a few of these changes; the introduction of DNA 17 and, more recently, DNA 21 chemistries; the significant reduction in the costs associated with the generation of DNA profiles; the introduction of rapid DNA analysis; the changes to the regulations on retention of DNA samples on the NDNAD based on human rights court cases; the use of DNA evidence to exonerate prisoners; the potential to discriminate between identical twins using DNA analysis; the possibility to predict facial features from a DNA sample, and so the list goes on. The impact those changes could have on this research and conversely, the impact this research could have on these developments, must be considered carefully. For example, if DNA transfer occurs so readily in environments such as mortuaries, where adherence to strict decontamination procedures is imperative, what is the likelihood that transfer may have also played a part in the

location of a key piece of cold case DNA evidence that has now been used to free a long serving prisoner? Lehmann et al (2013) has already suggested the potential for tertiary transfer of DNA evidence asking the question 'how far can it go?' in a very aptly named journal article about DNA transfer.

The ramifications of this type of research are likely to be far reaching so the dissemination of the findings to a wider audience must be approached with delicacy and with a sympathetic approach.

Ten. Conclusions, Further Work and Recommendations

10.1 Overall Conclusions and Future Developments for this Research

The overall aims of this body of research were to evaluate the potential for transfer and persistence of trace levels of DNA and to establish a protocol for optimising the retrieval of DNA evidence from firearms and related paraphernalia.

In order to achieve these aims it was first necessary to comprehend the mechanisms by which touch DNA is deposited onto a surface, and to attempt to understand the variables that can influence DNA transfer. This research has indicated that the traditionally accepted method for DNA transfer, the sloughing off of nucleated epithelial cells from the hands, is unlikely to explain the variability in the quality of DNA deposited. None of the variables that were examined in this research showed any correlation with the resulting degree of DNA deposition, and within-group results were not reproducible suggesting that the assumption that an individual can be classified according to his or her shedder status is misleading.

Due to the nature of touch DNA, generally deposited in very low amounts, in order to fully evaluate the variations in deposition, persistence and transfer it was imperative that the optimal methods for DNA recovery were utilised to reduce any variability from this aspect. In line with published research, the results gained suggested that cotton swabs performed well, and there was no significant difference in the DNA recovery rates of the swabbing and lifting methods utilised in this research. There was however, an indication that certain recovery methods would produce better quality profiles when collecting samples from a specific surface type. Glass samples favoured cotton double swabbing, polished metal surfaces responded well to both gel lifters and mini-tapes, and recovery of DNA from rusted metal surfaces was optimal when a wet cotton swab was used.

The main body of this research involved exploring the potential for DNA to be transferred onto surfaces and to determine the possibility that DNA could persist on a touched item even after subsequent handling.

The results gained in this research suggested that DNA from previous handlers could persist, but the degree to which the first handlers' DNA would dominate the recovered

profile could vary. The results have also indicated that the dominant donor present in a mixed profile is not always indicative of the final handler.

In relation to the potential for DNA transfer to occur, this research has indicated that DNA transfer could occur when the vector for transfer was another person and the final substrate was an object, but in transfer situations where the final substrate was another person (i.e. person to person to person transfer) secondary transfer of DNA was not detectable. The results also suggest that DNA transfer could occur when the vector for transfer was an object, but in this situation the first person's DNA would form the minor contribution to any profile recovered.

Given that one of the strategic objectives identified by the Serious and Organised Crime Agency (SOCA) was the threat of firearms, DNA recovery from firearms and associated evidence was examined to determine the potential for maximising DNA evidence retrieval. This research has indicated that although the potential for recovering DNA evidence from spent cartridge cases is very low, the ability to identify a shooter from DNA deposited on the weapon was much greater. This research has indicated that if a zonal strategy is applied when attempting to recover DNA from firearms, the potential for generating a successful DNA profile is very high. Several recovery methods were evaluated with results suggesting that double cotton swabbing should be recommended.

This thesis has examined a number of variables associated with the transfer and persistence of DNA, the findings of which have demonstrated that secondary transfer of DNA is a potential issue that should be considered when reporting DNA evidence originating from 'touch DNA' samples. In relation to recognising the variables that influence the incidence of secondary transfer, the findings reported here support the consideration of a case-by-case basis for interpretation of secondary transfer of DNA.

Although other variables are thought to potentially influence the degree of secondary transfer (e.g. duration and degree of contact, surface type, etc.) (Wickenheiser, 2002; Goray, Mitchell and van Oorschot, 2010) it may be beneficial to consider a scenario based approach to researching the potential for secondary transfer rather than isolating each individual variable. This approach may offer more of an insight into the degree to which secondary transfer should be considered in case work interpretation. Support for this approach has recently been presented in a publication by Goray, Mitchell and van

Oorschot (2012), where the authors suggest collation of data on transfer events to enable identification of the impact of specific variables.

A further consideration could be the identification of the source material from which the DNA profile has originated. Hanson et al. (2012) highlighted the impact that not being able to identify the biological source of a DNA sample could have on the evidential value of a resulting profile. In a situation where DNA from a second person is found on a neck tie used to hang a businessman, the ability to determine whether the DNA from the second person originated from a saliva sample (perhaps transferred through talking) or from skin cells could have a tangible impact on the significance of the DNA evidence. Further work into the determination of the biological source/origin of cellular material may provide additional intelligence information in situations where secondary transfer of the DNA is being purported. The application of mRNA characterisation, as suggested by Hanson et al. (2012), applied alongside laser capture micro-dissection, as outlined by Ballantyne, Hanson and Perlin (2013), could provide a very useful interpretational tool in the future.

In addition to the issue of transfer and persistence of DNA, this thesis has identified the challenges associated with interpreting profiles amplified from LCN samples. Graham and Rutty (2008) suggested a clearly defined set of guidelines need to be produced that inform how to interpret artefacts produced in LCN analysis. This could assist in the interpretation of DNA evidence, especially in instances where a sample may have originated as a result of transfer. Aitken, Taroni and Garbolino (2003) have proposed a model for the evaluation of DNA transfer evidence that could be adopted in certain circumstances.

Gill (2001) suggests there is an inevitable relationship between the quality of the DNA present and the relevance of the evidence. This is true to an extent; if the case scenario involves a transfer situation where the potential number of donors is limited, a DNA profile of poorer quality may still be relevant (e.g. sexual assault scenario). In support, Budowle, Eisenberg and van Daal (2009a) suggest that LCN should be considered as the sole form of identification in closed populations, and that “some errors in typing can be tolerated and yet proper identifications could still be made”.

This thesis has evaluated the potential for recovering DNA profiles from spent cartridge cases, and has determined that current protocols for the recovery of DNA from cartridge cases are not conducive to generating a usable DNA profile. The possible stages at which DNA could be damaged were identified, and experiments to determine the potential for reducing this damage were carried out. One possible cause of DNA damage was identified as being exposure to GSR. Contaminants present in the DNA sample could act as magnesium ion-collating agents. If the concentration of magnesium ions is too high or too low in an amplification reaction, the amplification efficiency and specificity of the reaction can be compromised (Gibb et al., 2009). However, this is only an issue if the exposure time to the heavy metals in GSR is having a significant effect or if the GSR is not completely removed during the extraction process. Studies where the amplification reaction mix was seeded with fingerprint powder indicated a complete inhibition of DNA amplification (van Oorschot et al., 2003). The results of this study, where the amplification reaction mix was seeded with GSR did not have such an inhibitory effect. Therefore the ability to remove GSR from a DNA sample, by way of a chelating substrate to bind heavy metals, was not required.

Recent publications have outlined the development of a new style of cartridge cases, designed to protect the DNA deposited (Sermon et al., 2012; Marks, 2012) and this will need further testing to determine the efficacy of the methods outlined.

Alternative methods not utilised in this research that could enhance the quality of profiles from low copy number samples in future could include an increased injection time for capillary electrophoresis [268], the use of WGA on samples prior to analysis (Giardina et al., 2009; Williams and Clarke, 2010) or the use of smaller amplicons (as these are more likely to be conserved in low yield samples) (Cotton et al., 2000).

10.2 Recommendations

The following recommendations have been devised, based on the research presented in this thesis:

- When attempting to recover low levels of DNA from a surface, it is recommended that the researcher reviews recent peer-reviewed publications to determine which recovery method is optimal for the specific substrate present.
- For those who wish to maximise DNA recovery from firearms, a zonal approach for swabbing should be used alongside double swabbing with a cotton swab.
- In cases where body fluids rich in cellular material (i.e. blood) are present on fired cartridge cases, DNA profiling of these samples should be attempted. In cases where low yields of DNA are anticipated, such as touch DNA, priority should be given to other evidential samples until methods are optimised such that DNA recovery is likely.
- Reporting forensic scientists must exercise caution when presenting activity level DNA evidence in court, and should have scientific research (published or defensible in some manner) to support their inferences in relation to how the DNA may have come to be recovered from that specific substrate.
- Scientists, law enforcement personnel and lawyers should not make assumptions on the likelihood of an individual being the final handler of an object based on the fact their profile is the major contributor.
- The term 'shedder status' should no longer be used when referring to DNA deposition or transfer; instead, more work should be undertaken on understanding the models for predicting the incidence of DNA transfer so this can be incorporated into a statistical framework.
- Cross-discipline discussions centred on the subject of transfer and persistence should take place, to ensure that all possible concepts for exploring the mechanisms and principles governing these processes are considered.

References

ABAZ, J., WALSH, S.J., CURRAN, J.M., MOSS, D.S., CULLEN, J., BRIGHT, J-A., CROWE, G.A., COCKERTON, S.L. and POWER, T.E.B. (2002) Comparison of the variables affecting the recovery of DNA from common drinking containers. *Forensic Sci Int*. 126. p. 233-240.

ACPO. (2005) ACPO Strategic Criminal Use of Firearms Group: Gun Crime Investigative Guidance. *Home Office Police Standards Unit* (Restricted).

ACPO. (2012*) Live-time Forensics: Harnessing Science and Innovation for Forensic Investigation in Policing* [Online]. Available from https://connect.innovateuk.org/web/forensics [Accessed: 06/10/2013].

ADITYA, S., SHARMA, A.K., BHATTACHARYYA, C.N. and CHAUDHURI, K. (2011) Generating STR profile from "Touch DNA". *Journal of Forensic and Legal Medicine*. 18(7). p. 295-298.

AITKEN, C., TARONI, F. and GARBOLINO, P. (2003) A graphical model for the evaluation of cross-transfer evidence in DNA profiles. *Theoretical Population Biology*. 63. p. 179-190.

ALAEDDINI, R., WALSH S.J. and ABBAS, A. (2010) Forensic implications of genetic analyses from degraded DNA – A review. *Forensic Sci Int: Genet*. 4. p. 148-157.

ALESSANDRINI, F., CECATI, M., PESARESI, M., TURCHI, C., CARLE, F. and TAGLIABRACCI, A. (2003) Fingerprints as evidence for a genetic profile: morphological study on fingerprints and analysis of exogenous and individual factors affecting DNA typing. *J Forensic Sci*. 48 (3). p. 1-7.

ALLEN, R.W., POGEMILLER, J., JOSLIN, J., GULICK, M. and PRITCHARD, J. (2008) Identification through Typing of DNA Recovered from Touch Transfer Evidence: Parameters Affecting Yield of Recovered Human DNA. *J Forensic Ident.* 58 (1). p. 33-41.

ANDRÈASSON, H., NILSSON, M., BUDOWLE, B., LUNDBERG, H. and ALLEN, M. (2006) Nuclear and mitochondrial DNA quantification of various forensic materials. *Forensic Sci Int*. 164. p. 56-64.

ANJOS, M.J., ANDRADE, L., CARVALHO, M., LOPES, V., SERRA, A., OLIVEIRA, C., BATISTA, L., BALSA, F., BRITO, P., CORTE-REAL, F. and VIDE, M.C. (2006) Low copy number: interpretation of evidence results. *International Congress Series.* 1288. p. 616-618.

APPLIED BIOSYSTEMS. (2014) *AmpFℓSTR® Identifiler® Plus PCR Amplification Kit* [Online]. Available from http://www.lifetechnologies.com/uk/en/home/industrial/human-identification/ampflstr-identifiler-plus-pcr-amplification-kit.html [Accessed: 20/01/2014].

APPLIED BIOSYSTEMS. (2012) *SNaPshot®Multiplex System for SNP genotyping: Product Bulletin* [Online]. Available from http://www.google.co.uk/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0CCwQFjAA&url=http%3A%2F%2Ftools.lifetechnologies.com%2Fcontent%2Fsfs%2Fbrochures%2Fcms_101014.pdf&ei=u1FKU8aHBqOP0AWK6oHIDQ&usg=AFQjCNFg0j-_RFsuWdhgMu9IE3N6fItTJA [Accessed: 20/01/2014].

BAECHTEL, F.S., PRESELY, K.W. and SMERICK, J.B. (1995) D1S80 typing on DNA from simulated forensic specimens. *J Forensic Sci.* 40(4). p. 536-545.

BALDING, D.J. and BUCKLETON, J. (2009) Interpreting low template DNA profiles. *Forensic Sci Int: Genet.* 4. p. 1-10.

BALOGH, M.K., BURGER, J., BENDER, K., SCHEIDER, P.M. and ALT, K.W. (2003a) Fingerprints from fingerprints. *International Congress Series*. 1239. p. 953-957.

BALOGH, M.K., BURGER, J., BENDER, K., SCHEIDER, P.M. and ALT, K.W. (2003b) STR genotyping and mtDNA sequencing of latent fingerprint on paper. *Forensic Sci Int.* 137. p. 188-195.

BALLANTYNE, J., HANSON, E.K. and PERLIN, M.W. (2013) DNA mixture genotyping by probabilistic computer interpretation of binomially-sampled laser captured cell populations: Combining quantitative data for greater identification information. *Science and Justice*. 53. p. 103-114.

BALLANTYNE, K.N., POY, A.L. and VAN OORSCHOT, R.A.H. (2013) Environmental DNA monitoring: beware of the transition to more sensitive typing methodologies. *Australian J Forensic Sci.* 45(3). p. 323-340.

BÄR, W. (2003) DNA profiling: evaluation of the evidentiary value. *Legal Medicine*. 5. p. 41-44.

BARASH, M., RESHEF, A. and BRAUNER, P. (2010) The use of adhesive tape for recovery of DNA from crime scene items. *J Forensic Sci.* 55(4). p. 1058-1064.

BARBARO, A., CORMACI, P. and BARBARO, A. (2006) LCN DNA typing from touched objects. *International Congress Series*. 1288. p. 553-555.

BARNES, H. (2012) *BBC News – DNA test jailed innocent man for murder* [Online]. Available from http://www.bbc.co.uk/news/science-environment-19412819 [Accessed: 31/08/2012].

BARNUM, C.A. and KLASEY, D.R. (1997) Factors affecting the recovery of latent prints on firearms. *J Forensic Ident*. 47 (2). p. 141-149.

BENSCHOP, C.C.G., WIEBOSCH, D.C., KLOOSTERMAN, A.D. and SIJEN, T. (2010) Post-coital vaginal sampling with nylon flocked swabs improves DNA typing. *Forensic Sci Int: Genet.* 4. p. 115-121.

BENTSEN, R.K., BROWN, J.K., DINSMORE, A., HARVEY, K.K. and KEE, T.G. (1996) Post firing visualisation of fingerprints on spent cartridge cases. *Science and Justice*. 36 (1). p. 3-8.

BENZINGER, E. (2005) *Memorandum to Jim Petro, State of Ohio Attorney General. Clarence Elkins DNA Reports* [Online]. Available from http://msnbcmedia.msn.com/i/msnbc/sections/tvnews/dateline%20nbc/dateline%20hidden/2007/march/dnaevidence.pdf [Accessed: 06/11/2009].

BERMAN, G. (2012) *Firearm Crime Statistics, House of Commons* [Online]. Available from www.parliament.**uk**/briefing-papers/SN01940.pdf [Accessed: 24/08/2012].

BERSELLINI, C., GAROFANO, L., GIANNETTO, M., LUSARDI, F. and MORI, G. (2001) Development of latent fingerprints on metallic surfaces using electropolymerization processes. *J Forensic Sci.* 46 (4). p. 871-877.

BERTI, A., BARNI, F., VIRGILI, A., COLOZZA, C., MAIORINO, F. and TOCCA, M. (2011) The recovery of DNA profiles from saliva and touch evidences after postal bomb explosion. *Forensic Sci Int: Genet Supplement Series.* 3. 471-472.

BILL, M., GILL, P., CURRAN, J., CLAYTON, T., PINCHIN, R., HEALY, M. and BUCKLETON, J. (2005) PENDULUM – a guideline-based approach to the interpretation of STR mixtures. *Forensic Sci Int.* 148. p. 181-189.

BILLE, T.W., CROMARTIE, C. and FARR, M. (2009) Effects of cyanoacrylate fuming, time after recovery, and location of biological material on the recovery and analysis of DNA from post-blast pipe bomb fragments. *J Forensic Sci.* 54 (5). p. 1059-1067.

BHOELAI, B. DE JONG, B. and SIJEN, T. (2013) Can mRNA markers distinguish traces generated by different types of contact? *Forensic Sci Int: Genet Supplement Series.* 4. p. 3-4.

BOHNERT, M., FALLER-MARQUARDT, M, LUTZ, S., AMBERG, R., WEISSER, H-J. and POLLAK, S. (2001) Transfer of biological traces in cases of hanging and ligature strangulation. *Forensic Sci Int.* 116. p. 107-115.

BOLTON-KING, R. (2012) *Firearms*. [E-mail]. Message to: Walton-Williams, L. 10th September 2012.

BOND, J.W. and PHIL, D. (2008) Visualization of latent fingerprint corrosion of metallic surfaces. *J Forensic Sci.* 53 (4). p. 812-822.

BOND, J.W. and PHIL, D. (2009) Visualization of latent fingerprint corrosion of brass. *J Forensic Sci.* 54 (4). p. 1034-1040.

BOND, J.W., PHIL, D. and HEIDEL, C. (2009) Visualization of latent fingerprint corrosion on a discharged brass shell casing. *J Forensic Sci*. 54 (4). p. 892-894.

BREATHNACH, M. and MOORE, E. (2013) Oral intercourse or secondary transfer? A Bayesian approach of salivary amylase and foreign DNA findings. *Forensic Sci Int.* 229. p. 52-59.

BRIGHT, J.A. and PETRICEVIC, S.F. (2004) Recovery of trace DNA and its applications to DNA profiling of shoe insoles. *Forensic Sci Int.* 145(1). p. 7-12.

BROŻEK-MUCHA, Z., ZADORA, G. and DANE, F. (2003) A comparative study of gunshot residue originating from a 9mm Luger ammunition from various producers. *Science and Justice.* 43 (4). p. 229-235.

BROWNLOW, R.J., DAGNALL, K.E. and AMES, C.E. (2012) A comparison of DNA collection and retrieval from two swab types (cotton and nylon flocked swab) when processed using three QIAGEN extraction methods. *J Forensic Sci*. 57(3). p. 713-717.

BUCKLETON, J. (2009) Validation issues around DNA typing of low level DNA. *Forensic Sci Int: Genet.* 3. p. 255-260.

BUCKLETON, J.S., CURRAN, J.M. and GILL, P. (2007) Towards understanding the effect of uncertainty in the number of contributors to DNA stains. *Forensic Sci Int: Genet*. 1. p. 20-28.

BUCKLETON, J. and GILL, P. (2011) Further comment on "Low copy number typing has yet to achieve "general acceptance"" by Budowle, B., et al. (2009). *Forensic Sci Int Genet*. 5(1). p. 7-11.

BUCKLETON, J., KELLY, H., BRIGHT, J-A., TAYLOR, D., TVEDEBRINK, T. and CURRAN, J.M. (2014) Utilising allelic dropout probabilities estimated by logistic regression in casework. *Forensic Sci Int: Genet.* 9. p. 9-11.

BUCKLETON, J., TRIGGS, C.M. and WALSH, S.J. (2005) *Forensic DNA Evidence Interpretation*. Boca Raton: CRC Press.

BUDOWLE, B., ALLARD, M.W., WILSON, M.R. and CHAKRABORTY, R. (2003) Forensics and mitochondrial DNA; applications, debates, and foundations. *Annu Rev Genomics Hum Genet*. 4. p. 119-41.

BUDOWLE, B., CHAKRABORTY, R. and VAN DAAL, A. (2010) Authors response to "Commentary on "Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework by Budowle, et al. (2009)"". *J Forensic Sci.* 55 (1). p. 269-272.

BUDOWLE, B., EISENBERG, A.J and VAN DAAL, A. (2009a) Validity of low copy number typing and applications to forensic science. *Croatian Medical Journal*. 50(3). p. 207-217.

BUDOWLE, B., EISENBERG, A.J and VAN DAAL, A. (2009b) Low copy number typing has yet to achieve "general acceptance". *Forensic Sci Int: Genet Supplement Series.* 2. p. 551-552.

BUDOWLE, B., EISENBERG, A.J and VAN DAAL, A. (2011) Response to comment on "Low copy number typing has yet to achieve "general acceptance" by Budowle, et al. (2009). *Forensic Sci Int: Genet*. 5(1). p. 5-7.

BUDOWLE, B., HOBSON, D.L., SMERICK, J.B. and SMITH, J.A.L. (2002) *Low Copy Number – Consideration and Caution* [Online]. Available from http://www.promega.com/~/media/files/resources/conference%20proceedings/ishi%2012/oral%20presentations/budowle.pdf?la=en [Accessed: 22/06/2010].

BUDOWLE, B., ONORATO, A.J., CALLAGHAN, T.F., MANNA, A.D., GROSS, A.M., GUERRIERI, R.A., LUTTMAN, J.C. and MCCLURE, D.L. (2009) Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. *J Forensic Sci*. 54 (4). p.810-821.

BURGER, M.F., SONG, E.Y. and SCHUMM, J.W. (2005) Buccal DNA samples for DNA typing: new collection and processing methods. *BioTechniques.* 39. p. 257-261.

BURGESS, D.J. (2013) Genomics: Finding Mr Anonymous. Nature Reviews Genetics. 14 (3). p. 152.

BUTLER, J.M. (2005) *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers, Second Edition*. London: Elsevier Academic Press.

BUTLER, J.M. (2010) *Fundamentals of Forensic DNA Typing*. London: Elsevier Academic Press.

BUTLER, J.M. (2012) *Advanced Topics in Forensic DNA Typing: Methodology*. London: Elsevier Academic Press.

BUTLER, J.M., BUEL, E., CRIVELLENTE, F. and MCCORD, B.R. (2004) Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. *Electrophoresis*. 25. p. 1397-1412.

CADDY, B., TAYLOR, G.R. and LINACRE, A.M.T. (2008) *A Review of the Science of Low Template DNA Analysis* [Online]. Available from https://www.gov.uk/government/publications/review-of-the-science-of-low-template-dna-analysis [Accessed: 17/01/2014].

CARAGINE, T. and PRINZ, M. (2011) Comment on "Low copy number typing has yet to achieve "general acceptance"" by Budowle, B., et al. (2009). *Forensic Sci Int: Genet.* 5(1). p.3-4.

CASTELLA, V., DIMO-SIMONIN, N., BRANDT-CASADEVALL, C. and MANGIN, P. (2006) Forensic evaluation of the QIAshredder/QIAamp DNA extraction procedure. *Forensic Sci Int.* 156. p. 70-73.

CASTELLA, V. and MANGIN, P. (2008) DNA profiling success and relevance of 1739 contact stains from caseworks. *Forensic Sci Int: Genet Supplement Series.* 1(1). p. 405-407.

CHAMPOD, C. (2013) DNA transfer: informed judgement or mere guesswork? *Frontiers in Genetics.* 4. p. 1-3.

CLAES, P., LIBERTON, D.K., DANIELS, K., ROSANA, K.M., QUILLEN, E.E., et al. (2014) Modeling 3D Facial Shape from DNA. *PLoS Genet*. 10 (3). e1004224.

CONTE, C., MUTTI, I., PUGLISI, P., FERRARINI, A., REGINA, G., MAESTRI, E. and MARMIROLI, N. (1998) DNA fingerprinting analysis by a PCR based method for monitoring the genotoxic effects of heavy metals pollution. *Chemosphere.* 37 (14-15). p.2739-2749.

COTTON, E.A., ALLSOP, R.F., GUEST, J.L., FRAZIER, R.R.E., KOUMI, P., CALLOW, I.P., SEAGER, A. and SPARKES, R.L. (2000) Validation of the AMP*Fl*STR® SGM Plus™ system for use in forensic casework. *Forensic Sci Int.* 112. p. 151-161.

COWELL, R.G., LAURITZEN, S.L. and MORTERA, J. (2007) Identification and separation of DNA mixtures using peak area information. *Forensic Sci Int.* 166 (1). p. 28-34.

COWELL, R.G., LAURITZEN, S.L. and MORTERA, J. (2011) Probabilistic expert systems for handling artifacts in complex DNA mixtures. *Forensic Sci Int Genet*. 5 (3). p. 202-9.

CROWN PROSECUTION SERVICE. (2008) *Review of the use of Low Copy Number DNA analysis in current cases: CPS Statement* [Online]. Available from http://www.cps.gov.uk/news/latest_news/101_08/index.html [Accessed: 05/02/2014].

DALBY, O., BUTLER, D. and BIRKETT, J.W. (2010) Analysis of gunshot residue and associated materials – a review. *J Forensic Sci.* 55 (4). p. 924-943.

DALY, D.J., MURPHY, C. and MCDERMOTT, S.D. (2012) The transfer of touch DNA from hands to glass, fabric and wood. *Forensic Sci Int: Genet.* 6 (1). p. 41-46.

DECORTE, R. (2010) Genetic identification in the 21st century – current status and future developments. *Forensic Sci Int*. 201. p. 160-164.

DEPARTMENT OF JUSTICE. (2013) *Justice Minister launches improved DNA Profiling Service* [Online]. Available from http://www.dojni.gov.uk/justice-minister-launches-improved-dna-profiling-service [Accessed: 05/02/2014].

DIELTJES, P., MIEREMET, R., ZUNIGA, S., KRAAIJENBRINK, T., PIJPE, J. and DE KNIJFF, P. (2011) A sensitive method to extract DNA from biological traces present on ammunition for the purpose of genetic profiling. *Int J Legal Med.* 125 (4). p. 597-602.

EDMINSTON, K.E. and JOHNSON, J. (2009) Determining an optimal sequence for chemical development of latent prints on cartridge cases and shotgun shells. *J Forensic Sci*. 54 (6). p. 1327-1331.

ESSLINGER, K.J., SIEGEL, J.A., SPILLANE, H. and STALLWORTH, S. (2004) Using STR analysis to detect human DNA from exploded pipe bomb devices. *J Forensic Sci.* 49 (3). p. 1-4.

EUROPEAN BIOINFORMATICS INSTITUTE (2015) *About IGSR and the 1000 Genomes Project* [Online]. Available from http://www.1000genomes.org/about [Accessed: 20/02/2016].

EUROPEAN COMMISSION (2013). *Prüm Decision* [Online]. Available from http://ec.europa.eu/dgs/home-affairs/what-we-do/policies/police-cooperation/prum-decision/index_en.htm [Accessed: 05/02/2014].

FARMEN., R.K.B., HAUKELI, I., RUOFF, P. and FRØYLAND, E.S. Assessing the presence of female DNA on post-coital penile swabs: Relevance to the investigation of sexual assault. *J Forensic Legal Medicine.* 19. p. 386-389.

FARMEN, R.K., JAGHØ, R., CORTEZ, P. and FRØYLAND, E.S. (2008) Assessment of individual shedder status and implication for secondary DNA transfer. *Forensic Sci Int: Genet Supplement Series*. 1 (1). p. 415-417.

FEIGELSON, H.S., RODRIGUEZ, C., ROBERTSON, A.S., JACOBS, E.J., CALLE, E.E., REID, Y.A. and THUN, M.J. (2001) Determinants of DNA yield and quality from buccal cell samples collected with Mouthwash. *Cancer Epidemiol Biomarkers Prev*. 10. p. 1005 – 1008.

FERREIRA, S.T.G., TRINDADE-FILHO, A., PAULA, K.A., MENDES, C.R., MAIA, F.A., PAK, H.I., NETO, R.P., JUNIOR, H.D. and MORAES, A.V. (2009) DNA analysis of biological material on perforating bullets and crime scene reconstruction. *Forensic Sci Int: Genet Supp Series*. 2. p. 213-214.

FINDLAY, I., TAYLOR, A., QUIRKE, P., FRAZIER, R. and URQUHART, A. (1997) DNA fingerprinting from single cells. *Nature*. 389. p. 555-556.

FINNEBRAATEN, M., GRANÈR, T. and HOFF-OLSEN, P. (2008) May a speaking individual contaminate the routine DNA laboratory? *For Science Int: Genet Supplement Series*. 1. p. 421-422.

FRANKE, N., AUGUSTIN, C. and PÜSCHEL, K. (2008) Optimization of DNA-extraction and typing from contact stains. *Forensic Sci Int: Genet Supplement Series*. 1. p. 423-425.

FREEMAN, H.N. (1999) Magnetic Fingerprint Powder on Firearms and Metal Cartridges. *J Forensic Ident*. 49 (5). p. 479-484.

FRIDEZ, F. and COQUOZ, R. (1996) PCR DNA typing of stamps: evaluation of the DNA extraction. *Forensic Sci Int*. 78. p. 103-110.

FORAN, D.R., GEHRING, M.E. and STALLWORTH, S.E. (2009) The recovery and analysis of mitochondrial DNA from exploded pipe bombs. *J Forensic Sci.* 54 (1). p. 90-94.

FORENSIC SCIENCE SPECIAL INTEREST GROUP. (2013) *Forensic Biology: UK Needs, Capabilities and Opportunities* [Online]. Available from https://connect.innovateuk.org/web/forensics [Accessed: 06/10/2013].

FUMAGALLI, L. and VANĚK, D. *Flocked Swabs, a New State of the Art for Forensic DNA applications* [Online]. Presented at Forensica 2008, International Conference Organised by Czechoslovak Society for Forensic Genetics. Available from http://www.copanswabs.com/studies/main.php [Accessed: 30/08/2011].

GASHI, B., EDWARDS, M.R., SERMON, P.A., COURTNEY, L., HARRISON, D. and XU, Y. (2010) Measurement of 9 mm cartridge case external temperatures and its forensic application. *Forensic Sci Int.* 200. p. 21-27.

GIARDINA, E., PIETRANGELI, I., MARTONE, C., ZAMPATTI, S., MARSALA, P., GABRIELE, L., RICCI, O., SOLLA, G., ASILI, P., ARCUDI, G., SPINELLA, A. and NOVELLI, G. (2009) Whole genome amplification and real-time PCR in forensic casework. *BMC Genomics*. 10. p. 159.

GIBB, A.J., HUELL, A-L., SIMMONS, M.C. and BROWN, R.M. (2009) Characterisation of forward stutter in the AmpF*l*STR® SGM Plus™ PCR. *Science and Justice*. 49. p. 24-31.

GILL, P. (2001) *Application of low copy number DNA profiling*. Croatian Medical Journal. 42(3). p. 229-232.

GILL, P. (2002) Role of short tandem repeat DNA in forensic casework in the UK – past, present and future perspectives. *BioTechniques*. 32. p. 366-385.

GILL, P., BRENNER, C.H., BUCKLETON, J.S., CARRACEDO, A., KRAWCZAK, M., MAYR, W.R., MORLING, N., PRINZ, M., SCHNEIDER, P.M. and WEIR, B.S. (2006) DNA commission of the International Society of Forensic Genetics: recommendations on the interpretation of mixtures. *Forensic Sci Int.* 160 (2-3). p. 90-101.

GILL, P. and BUCKLETON, J. (2010) Commentary on "Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework by Budowle et al. (2009)". *J Forensic Sci.* 55 (1). p. 265-268.

GILL, P., FEREDAY, L., MORLING, N. and SCHNEIDER, P.M. (2006a) The evolution of DNA databases – Recommendation for new European STR loci. *Forensic Sci Int*. 156 (2-3). p. 242-244.

GILL, P., FEREDAY, L., MORLING, N. and SCHNEIDER, P.M. (2006b) New multiplexes for Europe – Amendments and clarification of strategic development. *Forensic Sci Int*. 163 (1-2). p. 155-157.

GILL, P., KIRKHAM, A. and CURRAN, J. (2007) *LoComatioN*: a software tool for the analysis of low copy number DNA profiles. *Forensic Sci Int*. 166(2-3). p. 128-138.

GILL, P., SPARKES, R., PINCHIN, R., CLAYTON, T., WHITAKER, J and BUCKLETON, J. (1998) Interpreting simple STR mixtures using allele peak areas. *Forensic Sci Int*. 91. p. 41-53.

GILL, P., WHITAKER, J., FLAXMAN, C., BROWN, N. and BUCKLETON, J. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100pg of DNA. *Forensic Sci Int*. 112. p. 17-40.

GIVEN, B.W. (1976) Latent fingerprints on cartridges and expended cartridge casings. *J Forensic Sci.* 21 (3). p. 587-594.

GOLMAN, N., BERTONE, P., CHEN, S., DESSIMOZ, C., LEPROUST, E.M., SIPOS, B and BIRNEY, E. (2013) Towards practical, high-capacity, low-maintenance information storage in synthesized DNA. *Nature.* 494. p. 77-80.

GOODWIN, W., LINACRE, A. and HADI, S. (2007) *An Introduction to Forensic Genetics*. Chichester: John Wiley and Sons, Ltd.

GOODWIN, W.H. and PEEL, C. (2012) Theoretical value of the recommended expanded European Standard Set of STR loci for the identification of human remains. *Medicine, Science and the Law*. 52 (3). p. 162-168.

GORAY, M., EKEN, E., MITCHELL, R.J. and VAN OORSCHOT, R.A.H. (2010) Secondary DNA transfer of biological substances under varying test conditions. *Forensic Sci Int: Genet*. 4. p. 62-67.

GORAY, M., MITCHELL, J.R. and VAN OORSCHOT, R.A.H. (2010) Investigation of secondary DNA transfer of skin cells under controlled test conditions. *Legal Medicine*. 12. p. 117-120.

GORAY, M., MITCHELL, J.R. and VAN OORSCHOT, R.A.H. (2012) Evaluation of multiple transfer of DNA using mock scenarios. *Legal Medicine*. 14. p. 40-46.

GORAY, M. and VAN OORSCHOT, R.A.H. (2015) The complexities of DNA transfer during a social setting. *Legal Medicine*. 17. p. 82-91.

GORAY, M., VAN OORSCHOT, R.A.H. and MITCHELL, R.J. (2012) DNA transfer within forensic exhibit packaging: Potential for DNA loss and relocation. *Forensic Sci Int: Genet*. 6. p. 158-166.

GRAHAM, E.A.M. and RUTTY, G.N. (2008) Investigation into "normal" background DNA on adult necks: implications for DNA profiling of manual strangulation victims. J Forensic Sci. 53 (5). p. 1074-1082.

GRAHAM, E.A.M, WATKINS, W.J., DUNSTAN, F., MAGUIRE, S., NUTTALL, D., SWINFIELD, C.E., RUTTY, G.N. and KEMP, A.M. (2014) Defining background DNA levels found on the skin of children aged 0-5 years. *Int J Legal Med.* 128(2). p. 251-258.

GRŠKOVIĆ, B., MARKULIN, D., ZIDKOVA, A., CRNJAC, J., ANĐELINOVIĆ, Š., MARIJANOVIĆ, I., TOMAŠEVIĆ, L., POPOVIĆ, M., PRIMORAC, D. and MRŠIĆ, G. *Int J BioMedicine*. 4(3). p. 169-174.

GUNN, A. (2006) *Essential Forensic Biology*. Chichester: John Wiley and Sons, Ltd.

HAESLER, A. (N.D.) *DNA for Defence Lawyers* [Online]. Available from http://www.lawlink.nsw.gov.au/lawlink/pdo/ll_pdo.nsf/pages/PDO_dnadefencelawyers [Accessed: 25/08/2010].

HAINES, A.M., TOBE, S.S., KOBUS, H. and LINACRE, A. (2013) Detection of DNA within fingermarks. *Forensic Sci Int: Genet Supplement Series.* 4. p. 290-291.

HALL, D. and FAIRLEY, M. (2004) A single approach to the recovery of DNA and firearm discharge residue evidence. *Science and Justice.* 44 (1). p. 15-19.

HANED, H., PÈNE, L., SAUVAGE, F. and PONTIER, D. (2011) The predictive value of the maximum likelihood estimator of the number of contributors to a DNA mixture. *Forensic Sci Int: Genet*. 5 (4). p. 281-284.

HANNAM, A.G. (2010) Trends in converted firearms in England & Wales as identified by the National Firearms Forensic Intelligence Database (NFFID) between September 2003 and September 2008. *J Forensic Sci*. 55 (3). p. 757-766.

HANSON, E., HAAS, C., JUCKER. R. and BALLANTYNE, J. (2012) Specific and sensitive mRNA biomarkers for the identification of skin in 'touch DNA' evidence. *Forensic Sci Int: Genet.* 6. p. 548-558.

HANSSON, O., FINNEBRAATEN, M., HEITMANN, I.K., RAMSE, M. and BOUZGA, M. (2009) Trace DNA collection – Performance of minitape and three different swabs. *Forensic Sci Int: Genet Supplement Series.* 2. 189-190.

HEARD, B.J. (1997) *Handbook of Firearms and Ballistics: Examining and Interpreting Forensic Evidence.* Chichester: John Wiley and Sons Ltd.

HILL, J.B. (1995) *Weapons Law*. London: Sweet and Maxwell.

HOFFMANN, S.G., STALLWORTH, S.E. and FORAN, D.R. (2012) Investigative Studies into the Recovery of DNA from Improvised Explosive Device Containers. . *J Forensic Sci.* 57(3). p. 602-609.

HOLLAND, M.M., CAVE, C.A., HOLLAND, C.A. and BILLE, T.W. (2003) Development of a Quality, High Throughput DNA Analysis Procedure for Skeletal Samples to Assist with the Identification of Victims from the World Trade Center Attacks. *Croatian Medical Journal*. 44 (3). p. 264-272.

HOLLAND, M.M. and PARSONS, T.J. (1999) Mitochondrial DNA sequence analysis – validation and use for forensic casework. *Forensic Science Review*. 11. p. 21-50.

HOME OFFICE. (2013) *National DNA Database Strategy Board Annual Report 2012-13* [Online]. Available from https://www.gov.uk/government/publications/national-dna-database-annual-report-2012-to-2013 [Accessed: 06/03/2014].

HOME OFFICE. (2013a) National DNA Delivery Unit (NDU): The NDNAD Strategy Board Policy for Access and Use of DNA samples, Profiles and Associated Data [Online]. Available from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/254883/Access_And_Use_Of_DNA_Samples_Profiles.pdf [Accessed: 10/03/2014].

HORSMAN-HALL, K.M., KARCZYNSKI, S.L., ORIHUELA, Y., DAVIS, A.L., GREENSPOON, S.A. and BAN, J.D. (2008) *Developing STR profiles from fired cartridge cases using the AmpFlSTR® MiniFiler™ PCR amplification kit. Applied Biosystems Forensic News* [Online]. Available from http://marketing.appliedbiosystems.com/images/All_Newsletters/Forensic_Vol13/docs/53144_FN_Customer_Corner_b.pdf [Accessed: 05/11/2009].

HORSMAN-HALL, K.M., ORIHUELA, Y., KARCZYNSKI, S.L., DAVIS, A.L., BAN, J.D. and GREENSPOON, S.A. (2009) Development of STR profiles from firearms and fired cartridge cases. *Forensic Sci Int: Genet*. 3. p. 242-250.

HUGHES, S. and MOODY, A. (2007) *PCR*. Bloxham: Scion.

IBORRA, F.J., KIMURA, H. and COOK, P.R. (2004) The functional organization of mitochondrial genomes in human cells. *BMC Biology*. 2. p. 9-22.

INTERNATIONAL LABORATORY ACCREDITATION COOPERATION. (2002) *G19: Guidance for Forensic Science Laboratories* [Online]. Available from https://www.ilac.org/guidanceseries.html [Accessed: 17/11/2013].

JACKSON, A.R.W. and JACKSON, J.M. (2011) *Forensic Science, Third Edition.* Pearson Education Ltd.

JAMIESON, A. (2009) *Continuity and Contamination, The Forensic Institute* [Online]. Available from http://www.theforensicinstitute.com/PDF/Continuity%20and%20contamination.pdf [Accessed: 09/11/2009].

JAMIESON, A. (2012) *Comment on DNA Transfer. The Forensic Institute* [Online]. Available from http://www.theforensicinstitute.com/Articles/Comment%20on%20DNA%20transfer.htm [Accessed: 29/02/2012].

JAMIESON, A. and MEAKIN, G. (2010*) "Experience is the name that everyone gives to their mistakes" The Barrister Magazine* [Online]. Available from www.barristermagazine.com/archive-articles/issue-45/experience-is-the-name-that-everyone-gives-to-their-mistakes.html [Accessed: 20/02/2012].

JEFFREYS, A.J., WILSON, V. and THEIN, S.L. (1985) Hypervariable 'minisatellite' regions in human DNA. *Nature*. 314. P. 67-73.

JONES, H. (2007) Meeting Report: Advances in forensic DNA analysis. *Science and Justice.* 47. p. 174-177.

KARGER, B., MEYER, E. and DUCHESNE, A. (1997) STR analysis on perforating FMJ bullets and a new VWA variant allele. *Int J Legal Med.* 110. p. 101-103.

KASTELIC, V., POŚPIECH, E., DRAUS-BARINI, J., BRANICKI, W. and DROBNIČ, K. (2013) Prediction of eye color in the Slovenian population using the IrisPlex SNPs. *Croatian Medical Journal*. 54 (4). p. 381-386.

KELLEY, S. (2010) *Assessment of DNA Transfer Involving Routine Human Behaviour* [Online]. Available from http://www.google.co.uk/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0CDEQFjAA&url=http%3A%2F%2Fdigitalcommons.hsc.unt.edu%2Fcgi%2Fviewcontent.cgi%3Farticle%3D1098%26context%3Dtheses&ei=9iBHU67pF4qs7Qbh8YGQAg&usg=AFQjCNFBcOVk7IQcU4w5tuGwivPJqXFIew&bvm=bv.64507335,d.bGQ [Accessed 03/03/2014].

KENNA, J., SMYTH, M., MCKENNA, L., DOCKERY, C. and MCDERMOTT, S.D. (2011) The Recovery and Persistence of Salivary DNA on Human Skin. *J Forensic Sci*. 56 (1). p. 170-175.

KINDER, J.D. and NYS, B. (2000) Recording fingerprints on cartridge cases by 3D laser topography. *J Forensic Ident.* 50 (3). p. 271-275.

KIRBY, L.T. (1993) DNA Fingerprinting: An Introduction. New York: Oxford University Press.

KITA, T., YAMAGUCHI, H., YOKOYAMA, M., TANAKA, T. and TANAKA, N. (2008) Morphological study of fragmented DNA on touched objects. *Forensic Sci Int: Genet*. 3. p. 32-36.

KLASEY, D.R. and BARNUM, C.A. (2000) Development and enhancement of latent prints on firearms by vacuum and atmospheric cyanoacrylate fuming. *J Forensic Ident.* 50 (6). p. 572-580.

KLOOSTERMAN, A.D. and KERSBERGEN, P. (2003) Efficacy and limits of genotyping low copy number DNA samples by multiplex PCR of STR loci. *International Congress Series.* 1239. p. 795-798.

LADD, C., ADAMOWICZ, M.S., BOURKE, M.T., SCHERCZINGER, C.A and LEE, H.C. (1999) A systematic analysis of secondary DNA transfer. *J Forensic Sci*. 44 (6). p. 1270-1272.

LADD, C., LEE, H.C., YANG, N. and BIEBER, F.R. (2001) Interpretation of complex forensic DNA mixtures. *Croatian Medical Journal*. 42 (3). p. 244-246.

LEE, H.C. and LADD, C. (2001) Preservation and collection of biological evidence. *Croatian Medical Journal*. 42 (3). p. 225-228.

LEE, H.C., LADD, C., SCHERCZINGER, C.A. and BOURKE, M.T. (1998) Forensic applications of DNA typing, part 2: collection and preservation of DNA evidence. *The American Journal of Forensic Medicine and Pathology*. 19 (1). p. 10-18.

LEHMANN, V.J., MITCHELL, R.J., BALLANTYNE, K.N. and VAN OORSCHOT, R.A.H. (2013) Following the transfer of DNA: How far can it go? *Forensic Sci Int: Genet Supplement Series.* 4. 53-54.

LENZ, C. FLODGAARD, L.R., ERIKSEN, B. and MORLING, N. (2006) Retrieval of DNA and genetic profiles from swabs taken inside cars. *International Congress Series*. 1288. p. 595-597.

LÉVESQUE, S. and BOND, J.W. (2011) The effect of electrostatic fingerprint visualization on integrated ballistic identification systems. *J Forensic Sci.* 56 (5). p. 1283-1284.

LI, R. (2008) *Forensic Biology*. Boca Raton: CRC Press.

LI, R.C. and HARRIS, H.A. (2003) Using hydrophilic adhesive tape for collection of evidence for forensic DNA analysis. *J Forensic Sci.* 48(6). p. 1-4.

LINACRE, A., PEKAREK, V., SWARAN, Y.C. and TOBE, S.S. (2010) Generation of DNA profiles from fabrics without DNA extraction. *Forensic Sci Int: Genet*. 4. p. 137-141.

LINCOLN, P.J. and THOMSON, J. (1998) *Forensic DNA Profiling Protocols*. New Jersey: Humana Press Inc.

LIU, F., VAN DER LIJN, F., SCHURMANN, C., ZHU, G., CHAKRAVARTY, M.M., et al. (2012) A Genome-Wide Association Study Identifies Five Loci Influencing Facial Morphology in Europeans. *PLoS Genet*. 8 (9). e1002932.

LOWE, A., MURRAY, C., RICHARDSON, P., WIVELL, R., GILL, P., TULLY, G. and WHITAKER, J. (2003) Use of low copy number DNA in forensic inference. *International Congress Series*. 1239. p. 799-801.

LOWE, A., MURRAY, C., WHITAKER, J., TULLY, G. and GILL, P. (2002) The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *Forensic Sci Int*. 129. p. 25-34.

MARKS, P. Pollen-coated sticky bullets capture a gunman's DNA. *New Scientist*. 2669. p. 23.

MATTHEWS, R. (2002) *Armed Robbery*. Cullompton: Willan Publishing.

MEAKIN, G. and JAMIESON, A. (2013) DNA transfer: Review and implications for casework. *Forensic Sci Int*: *Genetics.* 7. p.434-443.

MELTON, T. and SENSABAUGH, G. (2000) *Mitochondrial Deoxyribonucleic Acid* In: Siegel, J.A., Saukko, P.J. and Knupfer, G.C. Encyclopaedia of Forensic Sciences. London: Academic Press.

MENG, H-H. and LEE, H-C. (2007) Elemental analysis of primer mixtures and gunshot residues from handgun cartridges commonly encountered in Taiwan. *Forensic Science Journal*. 6 (1). p. 39-54.

MICHIGAN DEPARTMENT OF NATURAL RESOURCES. (2011) *Basic Components of Ammunition* [Online]. Available from http://www.hunter-ed.com/mi/course/ch2_what_is_ammunition.htm [Accessed: 26/08/2012].

MIGRON, Y., HOCHERMAN, G., SPRINGER, E., ALMOG, J. and MANDLER, D. (1998) Visualisation of sebaceous fingerprints on fired cartridge cases: a laboratory study. *J Forensic Sci.* 43 (3). p. 543-548.

MORALES, E.B. and VÁZQUEZ, A.L.R. (2004) Simultaneous determination of inorganic and organic gunshot residues by capillary electrophoresis. *Journal of Chromatography A*. 1061. p. 225-233.

MONTPETIT, S. and O'DONNELL (2015) An optimised procedure for obtaining DNA from fired and unfired ammunition. *Forensic Sci IntL Genetics* http://dx.doi.org/10.1016/j.fsigen.2015.03.012

MOZAYANI, A., and NOZIGLIA, C. (2006) *The Forensic Laboratory Handbook: Procedures and Practice*. Totowa: Humana Press.

MULLIGAN, C.M., KAUFMAN, S.R. and QUARINO, L. (2011) The Utility of Polyester and Cotton as Swabbing Substrates for the Removal of Cellular Material from Surfaces. *J Forensic Sci.* 56 (2). p. 485-490.

MULOT, C., STÜCKER, I., CLAVEL, J., BEAUNE, P. and LORIOT, M-A. (2005) Collection of human genomic DNA from buccal cells for genetic studies: Comparison between cytobrush, mouthwash, and treated card. *Journal of Biomedicine and Biotechnology*. 2005 (3). p. 291-296.

MURRAY, J. (2005) Protocol for DNA Recovery Mini-Taping Method (Strathclyde Police, Forensic Support Department [Firearms]). Message to: L Walton. 4th November 2005.

MURRAY, C., LOWE, A., RICHARDSON, P., WIVELL, R., GILL, P., TULLY, G. and WHITAKER, J. (2002) Use of Low Copy Number (LCN) DNA in forensic inference. *Forensic Science Service Publication.*

NATIONAL CRIME AGENCY. (2014) National Strategic Assessment of Serious and Organised

Crime 2014 [Online]. Available from

http://www.nationalcrimeagency.gov.uk/publications/207-nca-strategic-assessment-of-serious-and-organised-crime/file [Accessed: 15/10/2014].

NATIONAL POLICING IMPROVEMENT AGENCY. (2011) *The National DNA Database* [Online]. Available from http://webarchive.nationalarchives.gov.uk/20121204155004/http://www.npia.police.uk/en/8934.htm [Accessed: 24/01/2014]

NATIONAL RESEARCH COUNCIL. (1992) *DNA Technology in Forensic Science*. Washington DC: National Academy Press.

NATIONAL RESEARCH COUNCIL. (1996) *The Evaluation of Forensic DNA Evidence*. Washington DC: National Academy Press.

NAUGHTON, M. and TAN, G. (2011) The need for caution in the use of DNA evidence to avoid convicting the innocent. *The International Journal of Evidence and Proof*. 15. p. 245-257.

NORLIN, S., NILSSON, M., HEDEN, P. and ALLEN, M. (2013) Evaluation of the Impact of Difference Visualization Techniques on DNA in Fingerprints. *J Forensic Ident*. 63(2). p. 189-204.

NUNN, S. (2013) Touch DNA Collection Versus Firearm Fingerprinting: Comparing Evidence Production and Identification Outcomes. *J Forensic Sci.* 58 (3). p. 601-608.

OUTHWAITE, M. (2012) Durham Firearms Unit. [E-mail]. Message to: Walton-Williams, L. 8th July 2012.

PANG, B.C.M and CHEUNG, B.K.K. (2007) Double swab technique for collecting touched evidence. *Legal Medicine.* 9. p. 181-184.

PEEL, C. and GILL, P. (2004) Attribution of DNA profiles to body fluid stains. *International Congress Series*. 1261. p. 53-55.

PERLIN, M.W. (2011) Combining DNA evidence for greater match information. (2011) *Forensic Sci Int: Genet Supplement Series.* 3. p. 510-511.

PERLIN, M.W., LEGLER, M.M., SPENCER, C.E., SMITH, J.L., ALLAN, W.P., BELROSE, J.L. and DUCEMAN, B.W. (2011) Validating TrueAllele® DNA Mixture Interpretation. *J Forensic Sci.* 56 (6). p. 1430-1447.

PESARESI, M., BUSCEMI, L., ALESSANDRINI, F., CECATI, M. and TAGLIABRACCI, A. (2003) Qualitative and quantitative analysis of DNA recovered from fingerprints. *International Congress Series*. 1239. p. 947-951.

PETRICEVIC, S.F., BRIGHT, J-A. and COCKERTON, S.L. (2006) DNA profiling of trace DNA recovered from bedding. *Forensic Sci Int*. 159. p. 21-26.

PHIPPS, M. and PETRICEVIC, S. (2007) The tendency of individuals to transfer DNA to handled items. *Forensic Sci Int*. 168. p.162-168.

PIZZAMIGLIO, M., MAMELI, A., MAUGERI, G and GAROFANO, L. (2004a) Identifying the culprit from LCN DNA obtained from saliva and sweat traces linked to two different robberies and use of a database. *International Congress Series*. 1262. p. 443-445.

PIZZAMIGLIO, M., MAMELI, A., MY, D. and GAROFANO, L. (2004b) Forensic identification of a murdered by LCN DNA collected from the inside of the victim's car. *International Congress Series.* 1261. p. 437-439.

POLLEY, D., MICKIEWICZ, P., VAUGHN, M., MILLER, T., WARBURTON, R., KOMONSKI, D., KANTAUTAS, C., REID, B., FRAPPIER, R. and NEWMAN, J. (2006) An Investigation of DNA recovery from firearms and cartridge cases. *Can Soc Forensic Sci J*. 39 (4). p. 217-228.

POPA, G., POTORAC, R. and PREDA, N. (2010) Method for fingerprints age determination. *Rom J Leg Med* 18. p. 149-154.

PORT, N.J., BOWYER, V.L., GRAHAM, E.A.M., BATUWANGALA, M.S. and RUTTY, G. (2006) How long does it take a static speaking individual to contaminate the immediate environment? *Forensic Sci, Med and Path*. 2 (3). p. 157-163.

POY, A and VAN OORSCHOT, R.A.H. (2006) Beware; gloves and equipment used during the examination of exhibits are potential vectors for transfer of DNA-containing material. *International Congress Series*. 1288. p. 556-558.

PREUβE-PRANGE, A., RENNEBERG, R., SCHWARK, T., POETSCH, M., SIMEONI, E. and VON WURMB-SCHWARK, N. (2009) The problem of DNA contamination in forensic case work – How to get rid of unwanted DNA? *Forensic Sci Int: Genet Supplement Series*. 2. p. 185-186.

PRINZ, M., SCHIFFNER, L., SEBESTYEN, J.A., BAJDA, E., TAMARIZ, J., SHALER, R.C., BAUM, H. and CARAGINE, T. (2006) Maximisation of STR DNA typing success for touched objects. *International Congress Series.* 1288. p. 651-653.

PROFF, C., SCHMITT, C., SCHNEIDER, P.M., FOERSTER, G. and ROTHSCHILD, M.A. (2006) Experiments on the DNA contamination risk via latent fingerprint brushes. *International Congress Series*. 1288. p. 601-603.

QUINONES, I. and DANIEL, B. (2012) Cell free DNA as a component of forensic evidence recovered from touched surfaces. *Forensic Sci Int: Genet*. 6 (1). p. 26-30.

QUINTÁNS, B., ALVAREZ-IGLESIAS, V., SALAS, A., PHILLIPS, C., LAREU, M.V. and CARRACEDO, A. (2004) Typing of mitochondrial DNA coding region SNPs of forensic and

anthropological interest using SNaPshot minisequencing. *Forensic Sci Int.* 140 (2-3). p. 510-511.

RAPLEY, R. and WHITEHOUSE, D. (2007) *Molecular Forensics.* Chichester: John Wiley and Sons, Ltd.

RAYMOND, J.J. (2010). *A Criminalistic Approach to Biological Evidence: Trace DNA and Volume Crime Offences.* A Thesis Submitted for the Degree of Doctor of Philosophy (Science). Sydney: University of Technology.

RAYMOND, J.J., ROUX, C., DU PASQUIER, E., SUTTON, J. and LENNARD, C. (2004) The effect of common fingerprint detection techniques on the DNA typing of fingerprints deposited on different surfaces. *J Forensic Ident*. 54(1). p. 22-36.

RAYMOND, J.J., VAN OORSCHOT, R.A.H., GUNN, P.R., WALSH, S.J. and ROUX, C. (2009a) Trace DNA success rates relating to volume crime offences. *Forensic Sci Int: Genet Supplement Series.* 2. 136-137.

RAYMOND, J.J., VAN OORSCHOT, R.A.H., GUNN, P.R., WALSH, S.J. and ROUX, C. (2009b) Trace evidence characteristics of DNA: A preliminary investigation of the persistence of DNA at crime scenes. *Forensic Sci Int: Genet.* 4. p. 26-33.

RAYMOND, J.J., WALSH, S.J., VAN OORSCHOT, R.A.H., GUNN, P.R., EVANS. L. and ROUX, C. (2008) Assessing trace DNA evidence from a residential burglary: Abundance, transfer and persistence. *Forensic Sci Int: Genet Supplement Series*. 1. p. 442-443.

RENNISON, A. (2012) Report into the circumstances of a complaint received from the Greater Manchester Police on 7 March 2012 regarding DNA evidence provided by LGC Forensics. Available from https://www.gov.uk/government/publications/forensic-science-regulators-report-on-the-dna-contamination-case-at-lgc-forensics [Accessed: 17/01/2014]

RICHERT, N.J. (2011) Swabbing Firearms for Handler's DNA. *J Forensic Sci.* 56 (4). p. 972-975.

RIVERA, M. (2009) Comparison of collection methods from touch samples on metal and wearer samples from simulated mixtures on clothing. Available from http://www.promega.com/~/media/files/resources/conference%20proceedings/ishi%2022/poster%20abstracts/082%20lee.pdf [Accessed: 17/03/2014]

ROMOLO, F.A. and MARGOT, P. (2001) Identification of gunshot residue: a critical review. *Forensic Sci Int*. 119. p. 195-211.

ROBERTSON, J., ROSS, A.M. and BURGOYNE, L.A. (1990) *DNA in Forensic Science: Theory, Techniques and Applications*. Chichester: Ellis Horwood Ltd.

ROTHERHAM, D. and HARBISON, S.A. (2011) Differentiation of drug and non-drug Cannabis using a single nucleotide polymorphism (SNP) assay. *Forensic Sci Int.* 207 (1-3). p. 193-197.

RUDIN, N. and INMAN, K. (2002) *An Introduction to Forensic DNA Analysis, Second Edition*. Boca Raton: CRC Press.

RUDIN, N. and INMAN, K. (2007) *The urban myths and conventional wisdom of transfer: DNA as trace evidence. News of the California Association of Criminalists 2007; 3rd Quarter; 26-29* [Online]. Available from http://www.cacnews.org/news/3rdq07a.pdf [Accessed: 10/03/2008].

RUTTY, G.N. (2000) Human DNA contamination of mortuaries: does it matter? *J Pathol.* 190. p. 410-411.

RUTTY, G.N. (2002) An investigation into the transference and survivability of human DNA following simulated manual strangulation with consideration of the problem of third party contamination. *Int J Legal Med*. 116. p. 170-173.

RUTTY, G.N., HOPWOOD, A. and TUCKER, V. (2003) The effectiveness of protective clothing in the reduction of potential DNA contamination of the scene of crime. *Int J Leg Med*. 117. p. 170-174.

RYAN, S. (2009) *Transfer theory in forensic DNA analysis: a review of primary, secondary and tertiary transfer. LawOfficer.com* [Online]. Available from http://www.lawofficer.com/news-and-articles/columns/ryan/transfer_theory_in_forensic_dna_analysis.html [Accessed: 16/01/2009].

RYAN, S.R. (2012) *Touch DNA. What is it? Where is it? How much can be found? And, how can it impact my case? A question and answer guide to all things touch DNA* [Online]. Available from
http://www.google.co.uk/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=

0CCwQFjAA&url=http%3A%2F%2Fwww.ryanforensicdna.com%2Fyahoo_site_admin%2Fassets%2Fdocs%2FTouch_DNA_article.59101908.pdf&ei=rBxHU-rZIufX7Ab_noDoDg&usg=AFQjCNF0GW19j-yGt_9OWp3FlrfsRu6tjg&bvm=bv.64507335,d.bGQ [Accessed: 03/03/2014].

RYAN, S. and KELEPECZ, B. (2008) *DNA on guns. How do you preserve the evidence?* [Online]. Available from http://www.lawofficer.com/article/magazine-feature/dna-guns [Accessed: 02/01/2014].

SASKOVA, L., GIAMBRA, A., POSPISEK, M. and VANĚK, D. (2008) *Novel testing system for monitoring of background contamination in DNA laboratories* [Online]. Presented at Forensica 2008, International Conference Organised by Czechoslovak Society for Forensic Genetics. Available from http://www.copanswabs.com/studies/main.php [Accessed: 30/08/2011].

SASKOVA, L., GIAMBRA, A. and VANĚK, D. (2008) *Validation Studies of the Sample Collection System for Forensic Use* [Online]. Presented at Forensica 2008, International Conference Organised by Czechoslovak Society for Forensic Genetics. Available from http://www.copanswabs.com/studies/main.php [Accessed: 30/08/2011].

SANDOVAL, M.P., JILLIAN, N.G., GREEN, W.M., PANACEK, E.A. and KANTHASWAMY, S. (2013) Yield of Male Contact DNA Evidence in an Assault Simulation Model. *J Forensic Res* T1: 002. doi: 10.4172/2157-7145. T1-002.

SAUNDERS, C.T., SANCHEZ, N., BALLANTYNE, J. and PETERSON, D.A. (2006) Laser microdissection of pure spermatozoa from epithelial cells for short tandem repeat analysis. *J Forensic Sci.* 51 (4). p. 748 – 757.

SAVINO, J.O. and TURVEY, B.E. (2011) Rape Investigation Handbook, Second Edition. London: Academic.

SCHNEIDER, P.M., FIMMERS, R., KEIL, W., MOLSBERGER, G., PATZELFT, D., PFLUG, W., ROTHÄMEL, T., SCHMITTER, H., SCHNEIDER, H. and BRINKMANN. B. (2009) The German Stain Commission: Recommendations for the interpretation of mixed stains. *International Journal Legal Medicine.* 123. p. 1-5.

SCHULZ, M.M. and REICHERT, W. (2000) A strategy for STR-analysis of cryptic epithelial cells on several textiles in practical casework. *Progress in Forensic Genetics*. 8. p. 514-516.

SCHULZ, M.M. and REICHERT, W. (2002) Archived or directly swabbed latent fingerprints as a DNA source for STR typing. *Forensic Sci Int.* 127. p. 128-130.

SCHWARK, T., Poetsch, M., Preusse-Prange, A., Kamphausen, T. and von Wurmb-Schwark, N. Phantoms in the mortuary – DNA transfer during autopsies. *Forensic Sci Int*. 216. p. 121-126.

SCHWOEBLE, A.J. and EXLINE, D.L. (2000) *Current Methods in Forensic Gunshot Residue Analysis*. Boca Raton: CRC Press.

SERMON, P.A., WORSLEY, M.P., CHENG, Y., COURTNEY, L., SHINAR-BUSH, V., RUZIMURADOV, O., HOPWOOD, A.J., EDWARDS, M.R., GASHI, B., HARRISON, D. and XU, Y. (2012) Deterring gun crime materially using forensic coatings. *Forensic Sci Int*. 221 (1-3). p. 131-136.

SEWELL, J., QUINONES, I., AMES, C., MULTANEY, B., CURTIS, S., SEEBORUTH, H., MOORE, S. and DANIEL, B. (2008) Recovery of DNA and fingerprints from touched documents. *Forensic Sci Int: Genet*. 2. p. 281-285.

SIJEN, T. (2014) Molecular Approaches for Forensic Cell Type Identification: On mRNA, miRNA, DNA Methylation and Microbial Markers. *Forensic Sci Int: Genet*. http://dx.doi.org/10.1016/j.fsgen.2014.11.015

SMITH, K., OSBORNE, S., LAU, I. and BRITTON, A. (2012) *Home Office Statistical Bulletin. Homicides, Firearm Offences and Intimate Violence 2010/11: Supplementary Volume 2 to Crime in England and Wales 2010/11* [Online]. Available from http://www.homeoffice.gov.uk/publications/science-research-statistics/research-statistics/crime-research/hosb0212/hosb0212?view=Binary [Accessed: 24/08/2012].

SNUSTAD, D.P. and SIMMONS, M.J. (2010) *Principles of Genetics, Fifth Edition.* Asia: John Wiley and Sons.

SOARES-VIEIRA, J.A., BILLERBECK, A.E.C., IWAMURA, E.S.M., CARDOSO, L.A. and MUÑOZ, D.R. (2000) Parentage testing on blood crusts from firearms projectiles by DNA typing settles an insurance fraud case. *J Forensic Sci.* 45 (5). p. 1142-1143.

SORENSON, K.J., TURTLETAUB, K., VRANKOVICH, G., WILLIAMS, J. and CHRISTIAN, A.T. (2004) Whole-genome amplification of DNA from residual cells left by incidental contact. *Analytical Biochemistry*. 324. p. 312-314.

SQUIRES, P. (2000) *Gun Culture or Gun Control: Firearms, Violence and Society*. London: Routledge.

STALEY, K. (2005) *The Police National DNA Database: Balancing Crime Detection, Human Rights and Privacy* [Online]. Available from http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/NationalDNADatabase.pdf [Accessed: 29/01/2010].

STOUDER, S.L., REUBUSH, K.J., HOBSON, D.L. and SMITH, J.L. (2002) Trace Evidence Scrapings: a valuable source of DNA? *Forensic Science Communications*. 4(4).

SWEET, D., LORENTE, M., LORENTE, J.A., VALENZUELA, A. and VILLANUEVA, E. (1997) An improved method to recover saliva from human skin: the double swab technique. *J Forensic Sci.* 42(2). p. 320-322.

SZIBOR, R., MICHAEL, M., PLATE, I. and KRAUSE, D. (2000) Efficiency of forensic mtDNA analysis: case examples demonstrating the identification of traces. *Forensic Sci Int.* 113. p. 71-78.

SZKUTA, B., HARVEY, M.L., BALLANTYNE, K.N. and VAN OORSCHOT, R.A.H. (2015) DNA transfer by examination tools – a risk for forensic casework? *Forensic Sci Int: Genet.* 16. p. 246-254.

TARONI, F., BIEDERMANN, A., VUILLE, J. and MORLING, N. (2013) Whose DNA is this? How relevant a question? (a note for forensic scientists). *Forensic Sci Int: Genet.* 7. p. 467-470.

TASKER, B. (2006) *Kovco inquiry hears DNA transfer 'unlikely'. The Age.com* [Online]. Available from http://www.theage.com.au/news/national/kovco-inquiry-hears-dna-transfer-unlikely/2006/08/28/1156617274979.html [Accessed: 05/11/2009].

TAUPIN, J.M. and CWIKLIK, C. (2011) *Scientific Protocols for Forensic Examination of Clothing*. London: CRC Press.

TAYLOR, M. (N.D.) *Study of DNA Transfer* [Online]. Available from http://www.bioforensics.com/conference04/Transfer/index.html [Accessed: 16/01/2009].

TAYLOR, M.S. and JOHNSON, E.A. (2001) *Summary Report TA Case # 1458, Commonwealth v. Dirk K. Greineder* [Online]. Available from http://www.bioforensics.com/conference04/Transfer/Taylor&Johnson%20Study.pdf [Accessed: 05/11/2009].

TECHNOLOGY STRATEGY BOARD NETWORK. (2011) *Forensic Challenges Catalogue* [Online]. Available from https://connect.innovateuk.org/web/forensics/research-challenges-catalogue [Accessed: 06/10/2013].

TEMPLETON, J., OTTENS, R., PARADISO, V., HANDT, O. and TAYLOR, D. (2013) Genetic profiling from challenging samples: Direct PCR of touch DNA. *Forensic Sci Int: Genet Supplement Series.* 4. p. 224-225.

THE BOSTON CHANNEL. (N.D) *Greineder witness casts doubt on DNA evidence* [Online]. Available from http://www.thebostonchannel.com/news/830906/detail.html [Accessed: 05/11/2009].

THE UNIVERSITY OF WESTERN CAPE. (1996) *Epithelial Tissue* [Online]. Available from: http://www.botany.uwc.ac.za/sci_ed/grade10/mammal/Epithelial.htm [Accessed 07/03/2008].

THOMASMA, S.M. and FORAN, D.R. (2013) The Influence of Swabbing Solutions on DNA Recovery from Touch Samples. *J Forensic Sci.* 58(2). p. 465-469.

THOMPSON, T. and BLACK, S. (2007) *Forensic Human Identification: An Introduction*. Boca Raton: CRC Press.

THOMPSON, W.C., FORD, S., DOOM, T.E., RAYMER, M.L. and KRANE, D.E. (2003*)* *Evaluating forensic DNA evidence, part 2. The Champion: National Association of Criminal Defense Lawyers* [Online]. Available from http://www.nasams.org/forensics/for_lib/Documents/1107456517.33/9c5f7c6d724a475185256e540074c156%3FOpenDocument&Highlight%3D0,forensic [Accessed: 05/11/2009].

TOLEDANO, T., QUARINO, L., LEUNG, S., BUFFOLINO, P., BAUM, H. and SHALER, R.C. (1997) An assessment of DNA contamination risks in New York City medical examiner facilities. *J Forensic Sci*. 42 (4). p. 721-724.

TOLLIVER, D.K. and SOBIERALSKI, C.A. (2008) Awareness of the potential of the EDD serving as a source for transfer of DNA. *Journal of the American Society of Questioned Document Examiners, Inc*. 11 (2). p. 37-40.

TOOTHMAN, M.H., KESTER, K.M., CHAMPAGNE, J., CRUZ, T.D., STREET, W.S. and BROWN, B.L. (2008) Characterization of human DNA in environmental samples. Forensic Sci Int. 178. p. 7-15.

TORRE, C. and GINO, S. (1996) Epidermal cells on stubs used for detection of GSR with SEM-EDX: Analysis of DNA polymorphisms. *J Forensic Sci.* 41 (4). p. 658-659.

TORRES, Y., FLORES, I., PRIETO, V., LÓPEZ-SOTO, M., FARFÁN, M.J., CARRACEDO, A. and SANZ, P. (2003) DNA mixtures in forensic casework: a 4-year retrospective study. *Forensic Sci Int*. 134. p. 180-186.

TVEDEBRINK, T., ERIKSEN, P.S., ASPLUND, M., MOGENSEN, H.S. and MORLING, N. (2012a) Statistical model for degraded DNA samples and adjusted probabilities for allelic drop-out. *Forensic Sci Int: Genet.* 6. p. 97-101.

TVEDEBRINK, T., ERIKSEN, P.S., ASPLUND, M., MOGENSEN, H.S. and MORLING, N. (2012b) Allelic drop-out probabilities estimated by logistic regression – Further considerations and practical implementation. *Forensic Sci Int: Genet.* 4. p. 263-267.

US NATIONAL LIBRARY OF MEDICINE®. (2011) *Genetics Home Reference* [Online]. Available from: http://ghr.nlm.nih.gov/glossary=heteroplasmy [Accessed: 30/08/2011].

VAN HOOFSTAT, D.E.O., DEFORCE, D.L.D, DE PAUW, I.P.H. and VAN DEN EECKHOUT, E.G. (1999) DNA typing of fingerprints using capillary electrophoresis: effect of dactyloscopic powders. *Electrophoresis*. 20. p. 2870-2876.

VAN OORSCHOT, R.A.H., BALLANTYNE, K.N. and MITCHELL, R.J. (2010) Forensic trace DNA: a review. *Investigative Genetics*. http://www.investigativegenetics.com/content/1/1/14

VAN OORSCHOT, R.A.H., GLAVICH, G. and MITCHELL., J. (2014) Persistence of DNA deposited by the original user on objects after subsequent use by a second person. *Forensic Sci Int: Genet.* 8. p. 219-225.

VAN OORSCHOT, R.A.H., GORAY, M., EKEN, E. and MITCHELL, R.J. (2009) Impact of relevant variables on the transfer of biological substances. *Forensic Sci Int: Genet Supplement Series*. 2. p. 547-548.

VAN OORSCHOT, R.A.H., GUTOWSKI, S.J., ROBINSON, S.L., HEDLEY, J.A. and ANDREW, I.R. (1996) HUMTHO1 validation studies: effect of substrate, environment and mixtures. *J Forensic Sci*. 41(1). p. 142-145.

VAN OORSCHOT, R.A.H. and JONES, M.K. (1997) DNA fingerprints from fingerprints. *Nature*. 387. p. 767.

VAN OORSCHOT, R.A.H., MCARDLE, R., GOODWIN, W.H. and BALLANTYNE, K.N. (2014) DNA transfer: The role of temperature and drying time. *Leg Med*. 16(3). p. 161-165.

VAN OORSCHOT, R.A.H., PHELAN, D.G., FURLONG, S., SCARFO, G.M., HOLDING, N.L. and CUMMINGS, M.J. (2003) Are you collecting all the available DNA from touched objects? *International Congress Series*. 2003. 1239. p. 803-807.

VAN OORSCHOT, R.A.H., TREADWELL, S., BEAUREPAIRE, J., HOLDING, N.L and MITCHELL, R.J. (2005) Beware of the possibility of fingerprinting techniques transferring DNA. *J Forensic Sci.* 50 (6). p. 1-6.

VERDON, T.J., MITCHELL, R.J. and VAN OORSCHOT, R.A.H. (2014) Evaluation of tapelifting as a collection method for touch DNA. *Forensic Sci Int: Genet.* 8. p. 179-186.

WALLACE, J.S. (2008) *Chemical Analysis of Firearms, Ammunition, and Gunshot Residue.* Boca Raton: CRC Press.

WALSH, P.S., ERLICH, H.A. and HIGUCHI, R. (1992) Preferential PCR amplification of alleles: mechanisms and solutions. *PCR Meth Appl*. 1. p. 240-251.

WARLOW, T.A. (1996) *Firearms, the Law and Forensic Ballistics*. London: Taylor and Francis Group.

WARLOW, T.A. (2007) The criminal use of improvised and re-activated firearms in Great Britain and Northern Ireland. *Science and Justice.* 47. p. 111-119.

WAY, D.M. (N.D) *Partial profiles and secondary transfer of DNA* [Online]. Available from: http://www.hgexperts.com/article.asp?id=7339 [Accessed: 05/11/2009].

WEBER-LEHMANN, J., SCHILLING, E., GRADL, G., RICHTER, D.C., WIEHLER, J. and ROLF, B. (2014) Finding the needle in the haystack: Differentiating "identical" twins in paternity testing and forensics by ultra-deep next generation sequencing. *Forensic Sci Int: Genet.* 9. p. 42-46.

WEI, W., AYUB, Q., XUE, Y. and TYLER-SMITH, C. (2013) A comparison of Y-chromosomal lineage using either resequencing or Y-SNP plus Y-STR genotyping. *Forensic Sci Int: Genet.* 7 (6). p. 568-572.

WEIR, B.S., TRIGGS, C.M., STARLING, L., STOWELL, L.I., WALSH, K.A.J. and BUCKLETON, J. (1997) Interpreting DNA mixtures. *J Forensic Sci*. 42 (2). p. 213-222.

WELCH, L.A., GILL, P., PHILLIPS, C., ANSELL, R., MORLINGE, N., PARSON W., PALOG, J.U. and BASTISCHH, I. (2012) European Network of Forensic Science Institutes (ENFSI): Evaluation of new commercial STR multiplexes that include the European Standard Set (ESS) of markers. *Forensic Sci Int: Genet.* 6. p. 819-826.

WELLCOME TRUST (2014) Available from http://www.wellcome.ac.uk/Funding/Biomedical-science/Funded-projects/Major-initiatives/SNP-Consortium-and-International-HapMap/index.htm# [Accessed: 02/04/2014]

WHITAKER, J.P., COTTON, E.A. and GILL, P. (2001) A comparison of the characteristics of profiles produced with the AMPF/STR® SGM Plus™ multiplex system for both standard and low copy number (LCN) STR DNA analysis. *Forensic Sci Int*. 123. p. 215-223.

WHITAKER, J., TULLY, G. and SULLIVAN, K. (2009) Response to low level DNA profiling. *Forensic Sci Med Pathol*. 5. p. 102-103.

WICKENHEISER, R.A. (1996) Evaluation of water soluble evidence collection adhesive tapes: Canadian Police Research Centre Technical Report. Available from http://publications.gc.ca/site/eng/327774/publication.html [Accessed 24/01/2010].

WICKENHEISER, R.A. (2002) Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. *J Forensic Sci.* 47 (3). p. 442-450.

WICKENHEISER, R. (2006) General guidelines for categorization and interpretation of mixed STR DNA profiles. *Can Soc Forensic Sci J*. 39 (4). p. 179-216.

WIEGAND, P., HEIMBOLD, C., KLEIN, R., IMMEL, U., STILLER, D. and KLINTSCHAR, M. (2011) Transfer of biological stains from different surfaces. *Int J Legal Med*. 125. p. 727-731.

WIEGAND, P. and KLEIBER, M. (1997) DNA typing of epithelial cells after strangulation. *Int J Leg Med.* 110(4). p. 181-183.

WIKIMEDIA COMMONS. (2005) *Blank Ammunition* [Online]. Available from http://upload.wikimedia.org/wikipedia/commons/6/6f/Platzpatronen762_9.jpg [Accessed: 12/06/2012].

WILLIAMS, S. (2012) *Key Identifiler Recommendations.* [E-mail]. Message to: Walton-Williams, L. 16th September 2012.

WILLIAMS, G. and CLARKE, D. (2010) *The use of multiple displacement amplification upon DNA recovered from discharged cartridge casings* [Online]. Presented at FORREST 2010. Available from

http://www.heacademy.ac.uk/assets/ps/documents/FORREST/2010/o4_williams.pdf [Accessed 30/08/2011].

WILLIAMS, R. and JOHNSON, P. (2008) *Genetic Policing: The use of DNA in criminal investigations.* Cullompton: Willan Publishing.

WILLIAMS, G., MCMURRAY, N. and WORSLEY, D.A. (2001) Latent fingerprint detection using a scanning Kelvin microprobe. *J Forensic Sci.* 46 (5). p. 1085-1092.

WILLIAMSON, A.L. (2012) Touch DNA: Forensic Collection and Application to Investigations. *J Assoc Crime Scene Reconstr.* 18 (1). p. 1-5.

XU, Y., HARRISON, D.J., HAY, G., HOPWOOD, A.J., GASHI, B., EDWARDS, M.R., COURTNEY, L. and SERMON, P.A. (2010) Design and manufacture of surface textures on gun cartridge cases to trap DNA material. *Proceedings of the Institute of Mechanical Engineers, Part B: Journal of Engineering Manufacture*. 224. p. 1229-1238.

YAMAMOTO, N. (2010) Optimisation of a real-time PCR assay to quantitate airborne fungi collected on a gelatin filter. *J BioSci BioEng*. 109(1). p.83-88.

ZAMIR, A., COHEN, Y. and AZOURY, M. (2007) DNA profiling from heroin street dose packages. *J Forensic Sci*. 52 (2). p.389-392.

ZAMIR, A., SPRINGER, E. and GLATTSTEIN, B. (2000) Fingerprints and DNA: STR typing of DNA extracted from adhesive tape after processing for fingerprints. *J Forensic Sci*. 45(3). p. 687-688.

ZOPPIS, S., MUCIACCIA, B., D'ALESSIO, A., ZIPARO, E., VECCHIOTTI, C. and FILIPPINI, A. (2014). DNA Fingerprinting Secondary Transfer From Different Skin Areas: Morphological And Genetic Studies. *Forensic Sci Int: Genet.* 11. P.137-143.

ZAMIR, A., OZ, C., WOLF, E., VINOKUROV, A. and GLATTSTEIN, B. (2004) A possible source of reference DNA from archived treated adhesive lifters. *J Forensic Sci.* 49 (1). p. 68-70.

ZECH, W-D., MALIK, N. and THALI, M. (2012) Applicability of DNA analysis on Adhesive Tape in Forensic Casework. *J Forensic Sci.* 57 (4). p. 1036-1041.

ZUPANIC, P.I., GORNJAK, P.B. and BALAZIC, J. (2010) Molecular genetic identification of skeletal remains from the Second World War Konfin I mass grave in Slovenia. *Int J Legal Med*. 124 (4). p. 307-317.

Appendices

Appendix One – Preparation of Mini-Tapes for the Use in Recovery of Trace Levels of DNA

In order to prepare the Mini-Tapes the following components were required:

- Acetate measuring 143 mm x 83 mm
- Double sided adhesive tape, 25 mm wide
- Sterile scissors
- Container, such as self seal polythene bags or plastic vials.

Preparation of the Mini-Tapes:

- A short length of the end of the tape was removed to reduce the potential for contamination.
- The double sided tape was placed along one edge of an acetate sheet, leaving a short overlap of approximately 10 mm either end, which was folded under.
- All bubbles were removed from between the tape and the acetate sheet by pressing with a finger.

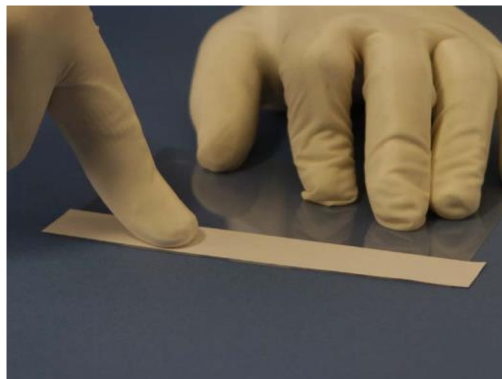


Figure A1.1 Preparation of Mini-Tape (Murray, 2005).

- The overlap at one end of the acetate sheet was cut off and discarded.

- From this acetate sheet five Mini-Tapes were produced. The remaining overlap was discarded.

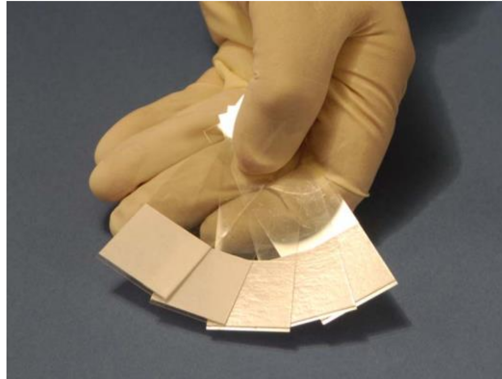
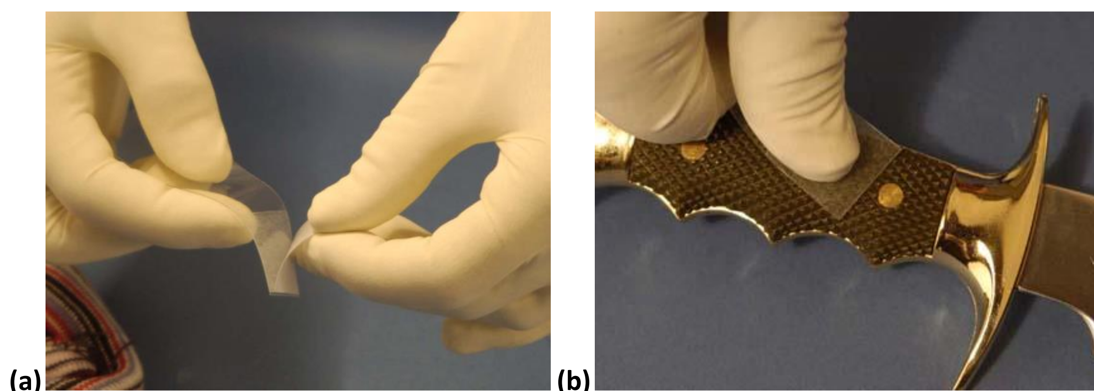


Figure A1.2 Finished Mini-Tapes (Murray, 2005).

- Once produced, the Mini-Tapes were stored in either plastic bags or small plastic vials.

The Mini-Tapes were used to recover skin cell DNA from several items including glass beakers, cartridge cases and weapons.

- The tape was repeatedly pressed against the surface of the item to transfer DNA from the item on to the tape.



(a) **(b)**

Figure A1.3 (a) and (b). (a) Removal of the back of the sticky tape ready for application onto the object. (b) The tape was repeatedly pressed against the item to collect DNA (Murray, 2005).

- The tape containing the DNA was then cut up into small pieces using sterile scissors and then transferred into an extraction tube ready for analysis.

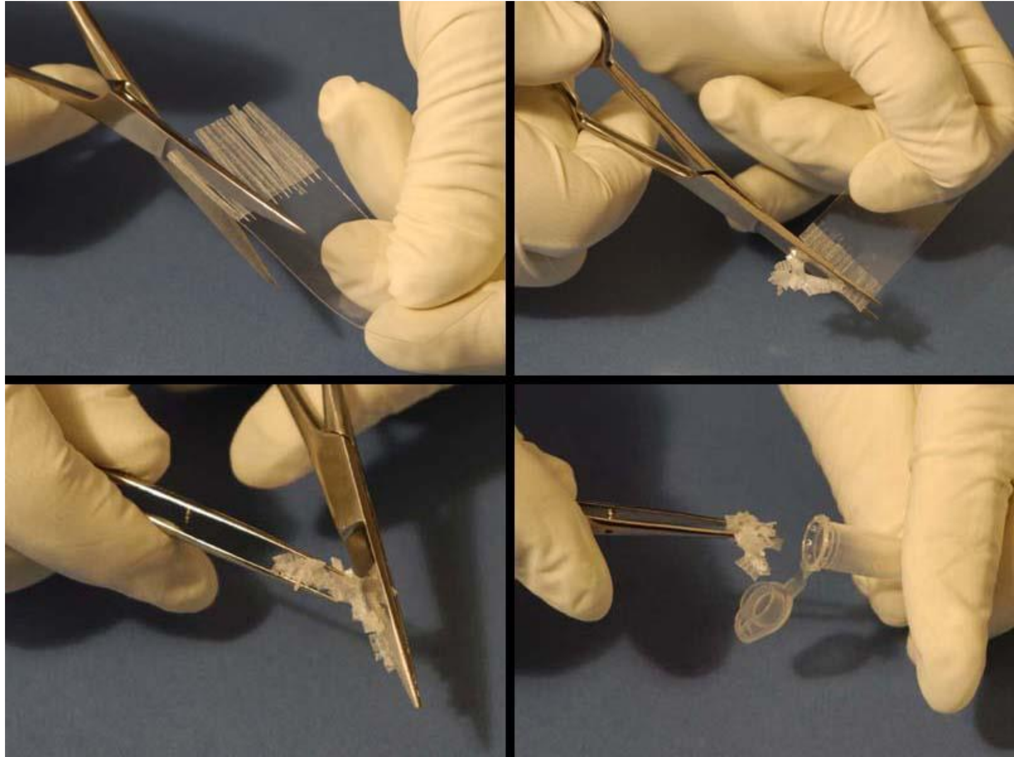


Figure A1.4 After collection of DNA the tape was then transferred into an eppendorf tube, ready for extraction (Murray, 2005).

Appendix Two – Preparation of 5% Chelex® Solution

Chelex® 100 Chelating Ion Exchange Resin was used for the Chelex® DNA extraction procedure.

Firstly a 20% w/v Chelex® solution was prepared by adding 5 gm Chelex® to 25 ml of Molecular Grade Water in a sterile 50 ml Duran bottle. The solution was gently mixed by repeatedly inverting for 30 seconds and then the resin was allowed to settle (which took approximately 2 minutes). The water supernatant was discarded, and then this Chelex® resin wash step was repeated for a total of three washes. After the final wash, Molecular Grade Water was added to a final volume of 25 ml to make a 20% w/v Chelex® solution.

From this 25 ml of 20% w/v Chelex® solution, 10 ml was removed and pipetted into a fresh duran bottle. To this 10 ml, 30 ml of Molecular Grade Water was added to make a 5% v/v Chelex® solution. This 5% v/v solution was used in the Chelex® DNA extraction procedure.

Appendix Three – DNA Deposition Studies

A3.1 Three Point Deposition Study

Key to the labelling of alleles in the following tables:

Bold Red are alleles consistent with the depositing subject. **Bold Black** are alleles not assignable to the depositing subject.

Table A3.1 Three Point Deposition Study (Donor 7)

Sample	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 7	10, 12	17, 20	15, 16	11, 13	10, 11	12, 13	8, 11	13, 13	12, 14	14, 14	30, 31.2	21, 23	6, 9	8, 11	17, 19	XX
Contact 1		18, 19, 23, 24			9	13		13	12, 17	13.2, 14	28, 30, 31.2		7		16, 18	XY
Contact 2		17, 20	14, 15, 16			9, 14		13		13, 14, 15	30, 31.2	19, 21	5.3, 6, 7, 8, 9, 9.3		15, 16, 17, 18	XY
Contact 3						10				13		23			16, 19	X

Table A3.2 Three Point Deposition Study (Donor 8)

Sample	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 8	11, 11	19, 19	16, 17	9, 13	13, 13	14, 14	11, 12	11, 13	12, 19	13, 14	27, 30	22, 23	6, 9.3	8, 10	17, 18	XY
Contact 1		23	15, 16			10			16	14	30				17, 18	XY
Contact 2			17			12	11	9								X
Contact 3			16	13	13	11					27, 30	22	9.3	8, 10	18	XY

Table A3.3 Three Point Deposition Study (Donor 9)

Sample	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 9	11, 11	17, 23	15, 16	12, 13	10, 11	11, 13	11, 12	12, 13	13, 17	15, 15	30, 31.2	21, 22	8, 9	9, 12	15, 15	XY
Contact 1	11		15		11	13, 14	11	13		14	28	21	8, 9		16, 18	XY
Contact 2			15	13		11, 13	11, 12	12, 13	17	14, 15	30	21	8, 9	9, 12	14, 15	XY
Contact 3	11	17, 23	15, 16	12, 13	10, 11	11, 13	11, 12	12, 13	13, 17	15	30, 31.2	21, 22	8, 9	9, 12	15	XY

Table A3.4 Three Point Deposition Study (Donor 10)

Sample	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 10	11, 12	20, 23	14, 17	11, 11	11, 13	12, 14	10, 12	12, 13	17, 20	14, 14	30, 30	20, 22	9, 9.3	9, 11	16, 18	XX
Contact 1	11	19, 20	14, 16, 17	11	11, 13	13, 14		13	17, 20	14, 15	30	20, 22	7, 9, 9.3	9	18	XY
Contact 2	10, 11	19, 20	15, 16, 18	11	11, 13	12, 13	11	11, 13	15	14	31.2	19, 20, 22	9.3, 10	8, 9	16, 17, 18, 19	XY
Contact 3	11, 12	20, 23	13, 14, 17	12	11	12, 13, 14	8, 10, 12	12, 13	17, 20	14	29, 30, 31.2	20, 22	9, 9.3	9	16, 18	XY

Table A3.5Three Point Deposition Study (Donor 11)

Sample	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 11	12, 12	17, 18	15, 18	11, 11	9, 9	13, 14	9, 11	11, 13	10, 16	14, 15	28, 31.2	20, 20	6, 9	8, 8	17, 17	XX
Contact 1	12	18	15, 16, 18	11	9	8, 10, 12, 13	9, 11	13	10, 16	13, 16	30.2, 31.2	20, 22	6, 7, 9	7, 8	17	X
Contact 2	12	17, 18, 20	15, 18	11	9	11, 13	9, 11	11, 12, 13	10, 13	14, 16	28, 31.2	20, 22	6, 9	8	14, 17	XY
Contact 3			14, 15	11	9	13, 14		13	10	14, 15		18.2, 20, 22	9			XY

A3.2 Three Point Deposition Study – Partial Electropherogram from Donor 7, Contact 1

See Attached Disc

A3.3 Three Point Deposition Study – Partial Electropherogram from Donor 9, Contact 3

See Attached Disc

A3.4 Five Point Deposition Study

Key to the labelling of alleles in the following tables:

Bold Black are alleles consistent with the depositing subject

Black are alleles not assignable to the depositing subject

Red are alleles that are consistent with contaminating peaks from the negative control swab on the beaker.

Bold Red are alleles that could originate from the depositing subject or from contamination originating from the beaker.

Table A3.6 DNA Profiles deposited by Donor 12 at five consecutive deposition contacts, with both left and right hand.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 12	**14, 15**	**17, 17**	**10, 11**	**17, 20**	**XY**	**10, 13**	**30, 30**	**11, 20**	**13, 15**	**8, 9.3**	**19, 22**
12negR	17, 19	18	13		**X**	7, **13**, 15		16, 17	14	9, **9.3**	**22**
12RH1	**14, 15**, 16	16	9, 13	23		**13**, 14	29	17, 18	9.2, 12, 14	5.3, 6, 8.3, **9.3**	21
12RH2	**14**	16			**X**	**13,** 14	29, 31.2, 32.2		9.2, 12, 14	6, **9.3**	**19**, 21
12RH3	17, 18	16		**17**, 26					14		
12RH4	16				**XY**	14			12	6	
12RH5	**14,** 16	15	9, **10**, 13		**X**	**13**, 14			9.2, 12, 14	6	21, **22**
12negL	**14**, 17	**17**, 18			**X**	**13**, 14	25, **30**		9.2, 14	9, **9.3**	
12LH1	**14**, 16	16	13		**XY**		29, **30**	18	12	6, 7, **8**, 9, **9.3**	
12LH2	**14, 15**, 16,	16		**17**	**XY**	**10, 13**	**30**	**11, 20**	14, **15**	7, **8**, **9.3**	

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
	17										
12LH3	**14, 15**, 16		9		**X**	**13,** 14	29, **30**		12, **13**, 14	6	20, 21, **22**
12LH4	**14, 15**, 16	16, **17**	9, **11**, 13	22, 23	**XY**	**10**, 14	29, **30**, 32.2	**11**, 18	9.2, 12, **13**	6, **8, 9.3**	21, **22**
12LH5	**14, 15,** 16	16, **17**	**11**, 13		**XY**	**10, 13**, 14	29	**11**	12, **13**, 14, **15**	6**, 8, 9.3**	21, **22**

Table A3.7 DNA Profiles deposited by Donor 5 at five consecutive deposition contacts, with both left and right hand.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 5	**14, 15**	**18, 19**	**12, 13**	**16, 23**	**XX**	**11, 12**	**27, 33.2**	**13, 14**	**13, 14**	**6, 9.3**	**20, 21**
5negR	13, **14**, 17		16, 18		**X**	15	30		9.2, 12	10	**20**
5RH1	**14, 15**, 16, 17	16, **18**	9, **13**		**X**	8, 14	28, 29, 32.2	15, 18	9.2, 10.2, 12, 13.2, **14**	**6, 9.3**	**21**
5RH2	12, 13, **14, 15**, 16, 17, 17.2, 18, 19, 20	15, 16, **18, 19**	9, **12, 13**	20, **23**	**XY**	15	28, 29, 30, 31.2, 32.2, **33.2**	16, 18	9.2, 10.2, 11.2, 12, **14**, 17.2	5.3, **6, 9.3**	**21,** 22
5RH3					**X**						
5RH4	16				**X**	14	**27**, 31.2		9.2, 12, **14**	**6**	
5RH5	**14, 15**, 16	16, **19**			**X**		32.2	16	9.2, 12, **14**	**6, 9.3**	**21**, 22
5negL	**14**, 17	16, **18**	**12**		**X**	14	29, 30		9.2, **14**		**20**
5LH1	**14**, 16	16, **19**	**12**		**X**	13, 14	**27**, 28, 31.2, 32.2		9.2, **13, 14**	**9.3**	**21**, 22

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
5LH2	**14**, 16				**X**	**12**, 13		17	9.2, 12	8.3, **9.3**	
5LH3	16	16	**13**	**16**	**X**Y	**12,** 14	29, 32.2		9.2, 10.2, 12, **14**	**6**	**21**
5LH4	**14**, 16	**19**	**13**		**X**	9, 14	29, 32.2		9.2, 12, **14**	5.3, **6, 9.3**	
5LH5	**14**		**12**		**X**		29, 32.2, **33.2**		12, **13, 14**	**9.3**	

Table A3.8 DNA Profiles deposited by Donor 8 at five consecutive deposition contacts, with both left and right hand.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 8	**16, 17**	**17, 18**	**11, 13**	**19, 19**	**XY**	**14, 14**	**27, 30**	**12, 19**	**13, 14**	**6, 9.3**	**22, 23**
8negR	14, **16, 17,** 17.2	16, **17, 18**	9, **13**		**X**	12, 13, **14,** 15	32.2	16	9.2, 12, **14**	**6,** 9, **9.3**	21
8RH1	13, 14, **16, 17**	15, 16, **17, 18**, 19	9, **11, 13**		**X**	7, 8, 10, **14,** 15	29, **30**, 31.2, 30.2		9.2, 11, 12, **13, 14**	**6, 9.3**	
8RH2	14, **16**				**X**	13, **14**			12, **14**	**9.3**	
8RH3	12, 13, 14, 15, **16,** 16.2, **17,** 17.2, 18.2, 20	16, **18,** 19	9, 12, **13**	**19**, 20, 23	**XY**	**14,** 15, 20	28, 29, **30**, 31.2, 32, 32.2, 33.2	15, 16	9.2, 10.2, 11, 11.2, 12, **14**, 15, 16.2, 17.2	5.3, **6**, 6.3, **9.3**	20, 21, **22**
8RH4	14, **16**	16	8, 9, **13**			8, 13, **14**	**27**	16	9.2, 12, **14**	**6, 9.3**	21
8RH5	18, 19				**XY**	13	29		**14**	**9.3**	
8negL	14, **17**	16	**13**		**X**	10, 13, **14,** 15	**30**		**13, 14**	**6, 9.3**	
8LH1		16			**X**	15			**14**	**9.3**	20

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
8LH2	15, **16**				**X**		28, 29		**14**		
8LH3	14, **16**			20, 23	**X**	15	29, 31.2, 32.2	18	9.2, **14**	**6, 9.3**	21
8LH4	14, **16, 17**	**18**			**X**						
8LH5	13, 14, 15, **16**, **17**				**X**	**14**, 15			12	**9.3**	21

Table A3.9 DNA Profiles deposited by Donor 13 at five consecutive deposition contacts, with both left and right hand.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 13	**15, 16**	**17, 17**	**9, 13**	**21, 23**	**XY**	**12, 14**	**28, 29.2**	**14, 16**	**12, 14**	**7, 7**	**22, 22**
13negR	14, 17	15, 16, 18			**X**	13, 15			9.2, **14**		**22**
13RH1	14	18			**X**		30		**14**	9.3	
13RH2	**16**, 16.2	15, 16			**X**						
13RH3	14, **16**				**X**				**14**	6	**22**
13RH4	17	16	**13**		**X**	13, 15	30				
13RH5	14, **16**	17	**9**, 12, 13		**X**	**14**	**28**, 30			**7**	
13negL	14, 17	15, 16, **17**, 18	**9**		**X**	13, 15			9.2, **12, 14**	9.3	
13LH1	14	16			**X**					9.3	
13LH2	14	16			**X**		**28**			**7**	
13LH3		**17**, 18			**X**						20

Sample Name	BLUE				GREEN				YELLOW		
	D3	**vWA**	**D16**	**D2**	**Amelogenin**	**D8**	**D21**	**D18**	**D19**	**THO1**	**FGA**
13LH4	14		7		**XY**		30		**14**	**7**	**22**
13LH5	14	18			**X**	13, 15					

Table A3.10 DNA Profiles deposited by Donor 14 at five consecutive deposition contacts, with both left and right hand.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 14	**16, 16**	**16, 18**	**11, 12**	**17, 17**	**XX**	**14, 15**	**28, 30**	**13, 16**	**14, 14**	**7, 9.3**	**21, 22**
14negR	14, **16**, 17		9	20	**X**	13, **15**	29, **30,** 32.2	**16**	13, **14**		
14RH1	14, 17	**16**			**X**	13, **15**	**30**	20	**14**	9	
14RH2	14, 17		**12,** 13		**X**	8, 12, **15**	29, **30**		**14**	**9.3**	**22**
14RH3	12, 14, 17, 19	**16, 18**			**X**	13, **14, 15**	29, **30**		9.2, 10.2, 13, **14**	**9.3**	20
14RH4	14	15, **16, 18,** 19			**X**Y	13, **14, 15**	**30**		12, **14**		
14RH5	14, **16,** 17, 19	15, **16**	9, **12**, 13	20	**X**	13, **14, 15**	29, **30,** 32.2	**16**	9.2, 12, **14**	6, 9**, 9.3**	20, **21, 22**
14negL	13, 14, 15, **16**, 17	**16**, 17, **18,** 19			**X**	13, **14, 15**	29		**14**	6, **9.3**	
14LH1	13, 14,	**18**			**X**	12, 13, **15**	**30**		9.2, **14**	6	

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
	16, 17, 19										
14LH2		**16, 18**			**X**	13, **15**	**30**		9.2, **14**	9	
14LH3	14, 19	**16, 18**			**X**	13, **15**	**30**, 31		9.2, **14**		**22**
14LH4	14, 17	15, **16**		23	**X**	8, **15**	**30**		12, **14**, 15.2		20, **22**
14LH5	14, 17			19	**X**	13, **14, 15**	**30**, 38.2	16.2	13, **14**	**9.3**	20

Table A3.11 DNA Profiles deposited by Donor 15 at five consecutive deposition contacts, with both left and right hand.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 15	**13, 17**	**16, 16**	**10, 13**	**18, 25**	**XY**	**14, 16**	**30, 31**	**13, 17**	**12, 16**	**9.3, 9.3**	**20, 22**
15negR	14, 16, **17**	**16**, 18			**X**	13, 15	28, 29, **30**		14		19.2
15RH1	12, 14, 16, **17**, 19	**16**			**X**	13, 15	29, **30**		9.2, 14	6, **9.3**	
15RH2	14, 16, **17**	**16**, 17, 18			**X**	13, **14**, 15	28, 29		9.2, 14	9	21
15RH3	14, 16, **17**	**16**, 18	**13**		**X**	13, 15			13, 14	6, 9, **9.3**	**22**
15RH4	16, **17**	13	9		**X**	12, 13, 15	**30**		14		
15RH5	**13,** 14, 16, **17,** 19		**13**		**X**	12, 13, 15	**30**		9.2, 14		**20, 22**
15negL	14, 16, **17**	**16**, 17			**X**	13, **14**, 15	**30**	16	14	**9.3**	**20, 22**
15LH1	14, **17**, 19	18	**13**		**X**	13, **14**, 15	**30**		9.2, 14	9	
15LH2	14, 16, **17,** 19	**16**	12, **13**	17	**X**	13, 15	**30**		9.2, 14	9, **9.3**	
15LH3	**13**, 14, 16, **17**	18	**10**		**X**	8, 12, 13, 15, **16**	**30**		9.2, 14		**20, 22**

Sample Name	BLUE				GREEN				YELLOW		
	D3	**vWA**	**D16**	**D2**	**Amelogenin**	**D8**	**D21**	**D18**	**D19**	**THO1**	**FGA**
15LH4	15, 16, **17**	**16**			**X**	12, 13, 15	**30**		14	**9.3**	**20, 22**
15LH5											

A3.5 Five Point Deposition Study – Allele Comparison Charts

In relation to the results, the maximum number of assignable alleles would be 32 and the maximum number of non-assignable alleles cannot be defined (as theoretically there can be as many non-assignable alleles as there are potential alleles at these loci in the population). For ease of comparison, the scale for the y-axis (number of alleles) was set at a constant for all samples. The maximum value for the scale was set at 32 to represent the maximum number of assignable alleles possible.

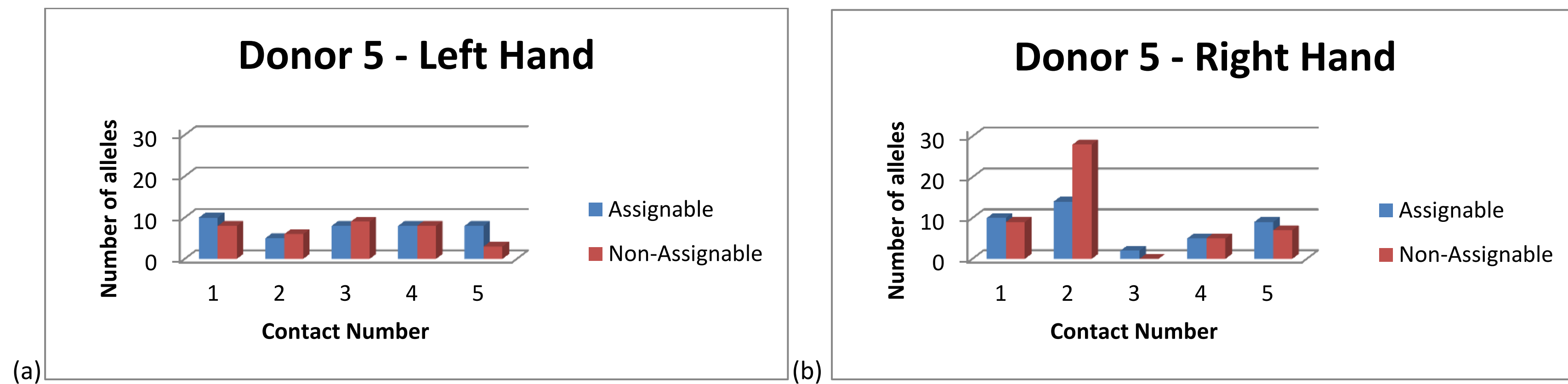
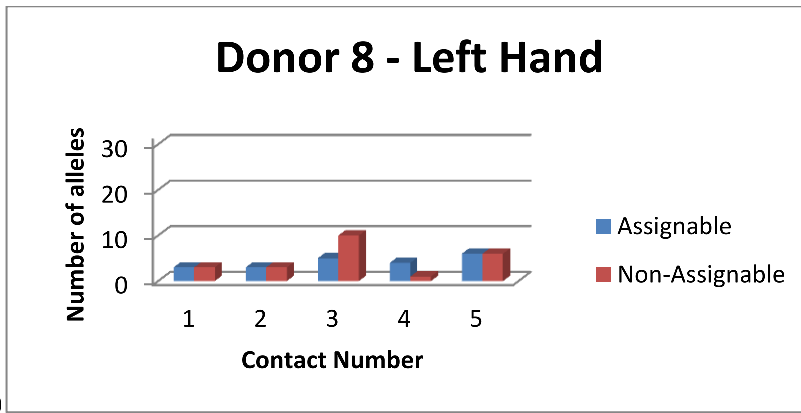


Figure A3.1 (a) and (b). Number of alleles recovered from consecutive contacts with (a) Donor 5's left hand and (b) Donor 5's right hand.

(a) 

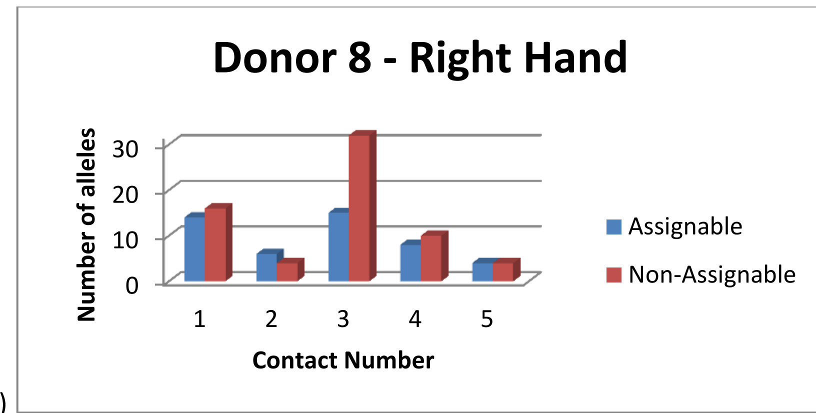
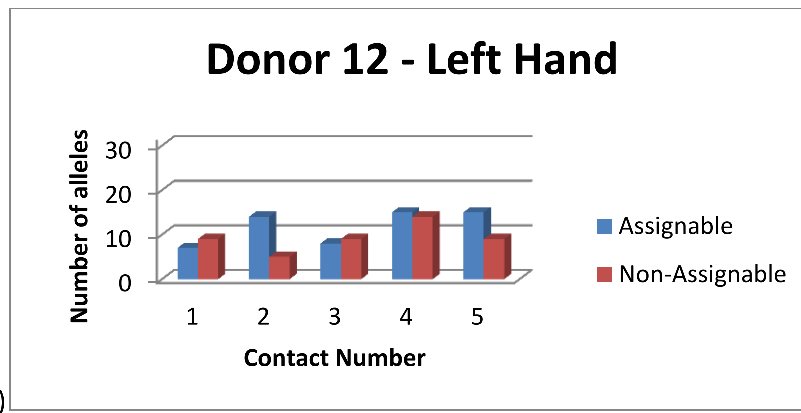
(b) 

Figure A3.2 (a) and (b). Number of alleles recovered from consecutive contacts with (a) Donor 8's left hand and (b) Donor 8's right hand. (NB the number of non-assignable alleles recovered from contact 3 for the right hand exceeds the scale used).

(a) 

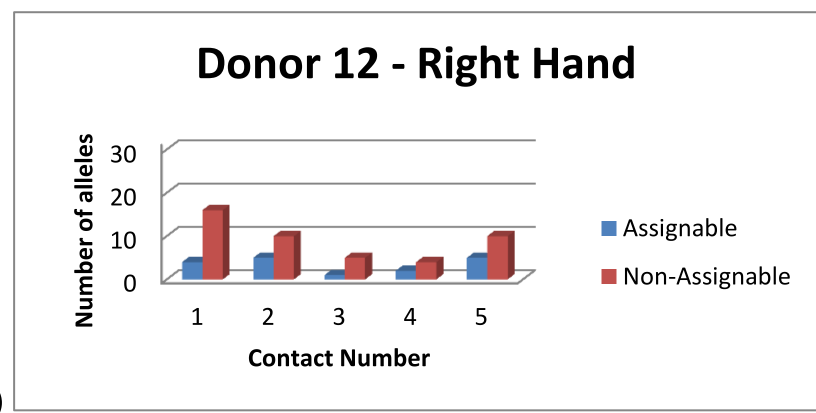
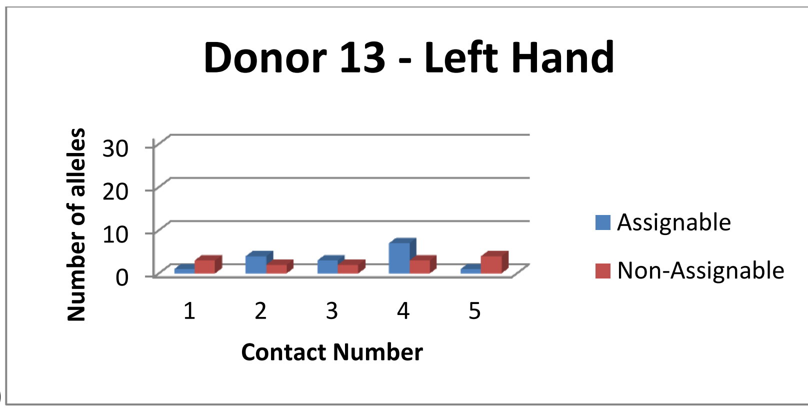
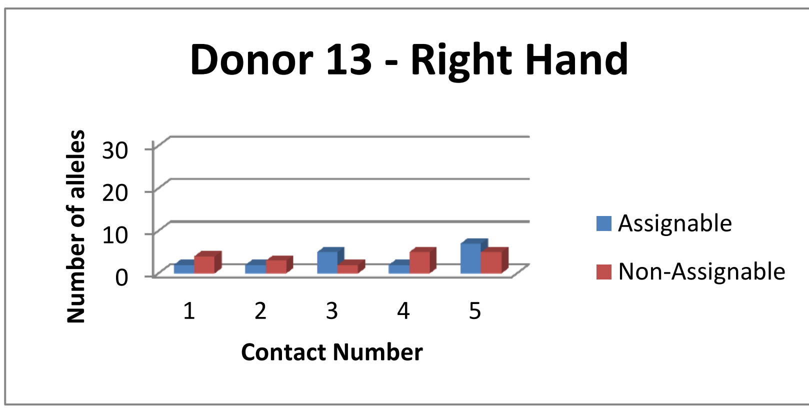
(b) 

Figure A3.3 (a) and (b). Number of alleles recovered from consecutive contacts with (a) Donor 12's left hand and (b) Donor 12's right hand.

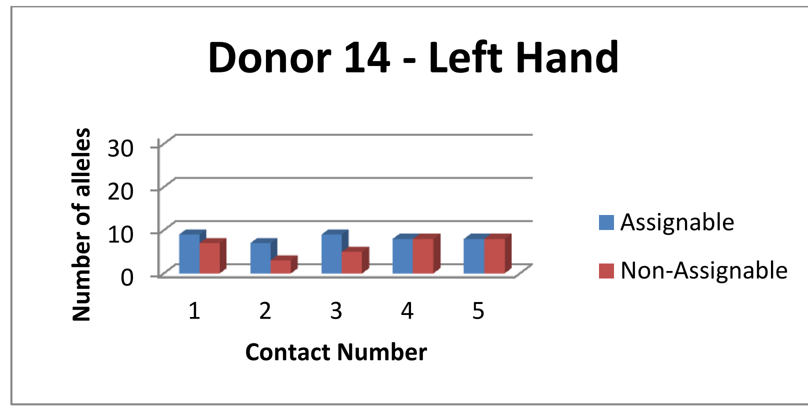


(a)

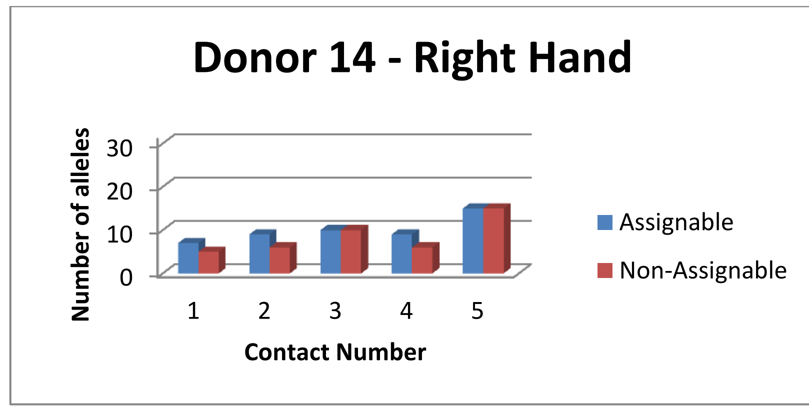


(b)

Figure A3.4 (a) and (b). Number of alleles recovered from consecutive contacts with (a) Donor 13's left hand and (b) Donor 13's right hand.



(a)



(b)

Figure A3.5 (a) and (b). Number of alleles recovered from consecutive contacts with (a) Donor 14's left hand and (b) Donor 14's right hand.

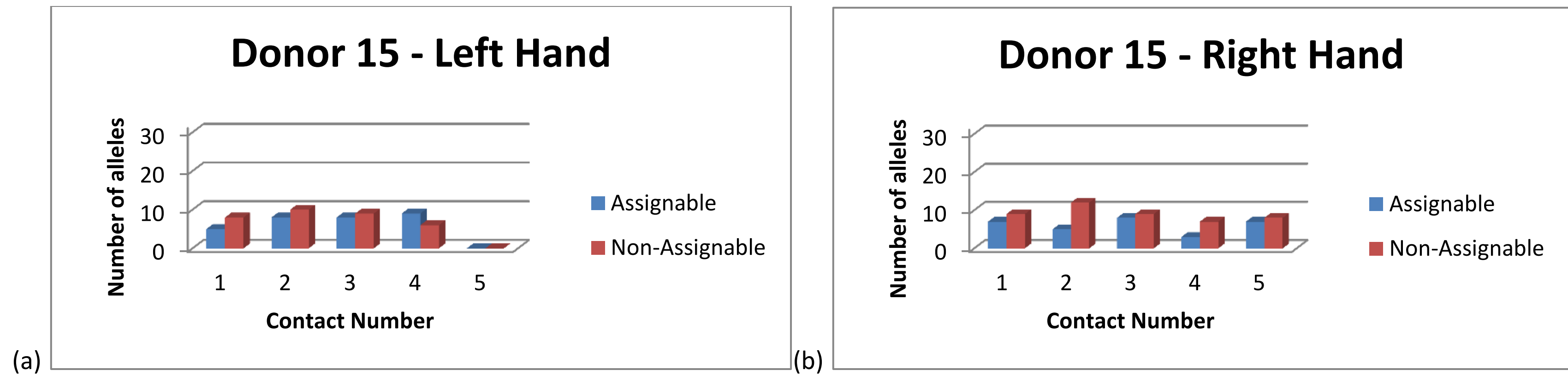


Figure A3.6 (a) and (b). Number of alleles recovered from consecutive contacts with (a) Donor 15's left hand and (b) Donor 15's right hand.

A3.6 Five Point Deposition Study – Partial Electropherogram from Donor 8, Contact 3, Left Hand

See Attached Disc

10.3 A3.7 Five Point Deposition Study – Electropherogram from Donor 13, Contact 4, Right Hand

See Attached Disc

A3.8 Primary Transfer (Subject A ↔Subject B)

The following tables show the results from the hand swabs for three pairings of subjects (where the ARTB profile refers to the hand swab from Subject A in the pairing and the BRTB profile refers to the hand swab from Subject B). So for example, in Table A3.12, ARTB7 refers to the hand swab from Subject A's right hand (Donor 7), and BRTB6 refers to the hand swab from Subject B's right hand (Donor 6), and so on.

In each of the tables, alleles originating from Subject A in the pairing are highlighted in red, alleles originating from Subject B in the pairing are highlighted in blue and alleles that could have originated from Subject A or B in the pairing (i.e. common alleles between the two subjects) are highlighted in green. Alleles in black are non-assignable alleles.

Table A3.12 DNA Profiles recovered from hand swabs from Donor 6 and Donor 7.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 7	**10, 12**	**17, 20**	**15, 16**	**11, 13**	**10, 11**	**12, 13**	**8, 11**	**13, 13**	**12, 14**	**14, 14**	**30, 31.2**	**21, 23**	**6, 9**	**8, 11**	**17, 19**	**XX**
ARTB7	9, 10, 12	17, 20	14, 16, 18, 20	11, 13	10, 11	11, 12, 13, 14	8, 11	13, 13	12, 14	14, 15	30, 31.2, 32.2	21, 23	6, 9, 9.3	8, 11	17, 18, 19	XY
BRTB6			15, 18	10, 11	9, 10	8, 12, 13, 14	11, 11			12, 15	28, 28		9.3		17, 18	XY
Donor 6	**10, 10**	**17, 24**	**16, 18**	**11, 13**	**10, 10**	**11, 12**	**11, 14**	**12, 12**	**14, 14**	**12, 14**	**28, 28**	**20, 21**	**6, 9.3**	**11, 12**	**17, 18**	**XX**

Table A3.13 DNA Profiles recovered from hand swabs from Donor 4 and Donor16.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 16	**11, 12**	**18, 20**	**15, 17**	**13, 13**	**10, 11**	**14, 14**	**12, 12**	**11, 13**	**11, 16**	**14, 15.2**	**28, 30**	**22.2, 23**	**9.3, 9.3**	**8, 11**	**15, 16**	**XY**
ARTB16	11		14, 15, 16, 17	13	10, 11	10, 12, 13, 14	12	11, 13	11, 16	13, 14, 15.2	27, 28, 29, 30	22.2, 23	6, 9.3	8, 11	15, 16	XY
BRTB4	11, 11		16, 16	11, 13	8, 12	10, 13	9, 12	9, 11	12, 20	11, 14	27, 30	23, 26	6, 9	8, 11	15, 17	XY
Donor 4	**10, 11**	**19, 24**	**16, 16**	**11, 13**	**8, 12**	**13, 13**	**9, 12**	**9, 11**	**15, 20**	**11, 14**	**27, 30**	**23, 26**	**6, 9**	**8, 11**	**15, 17**	**XX**

Table A3.14 DNA Profiles recovered from hand swabs from Donor 17 and Donor 13.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 17	**11, 12**	**20, 23**	**14, 17**	**11, 11**	**11, 13**	**12, 14**	**10, 12**	**12, 13**	**17, 20**	**14, 14**	**30, 30**	**20, 22**	**9, 9.3**	**9, 11**	**16, 18**	**XX**
ARTB17	12	23	14, 15, 16, 17	10, 11, 12	9, 11	12, 13, 14	11, 11	13	16	14, 14	31.2, 31.2	20, 22	6, 7	8, 8	14, 16, 17	XY
BRTB13		21	15, 16	11, 12	9, 11	12, 14	12	9, 13	14	12, 14, 15	28	20, 21	6, 7	8, 8	17, 18	XY
Donor 13	**12, 13**	**21, 23**	**15, 16**	**12, 12**	**9, 11**	**12, 14**	**12, 12**	**9, 13**	**14, 16**	**12, 14**	**28, 29.2**	**20, 22**	**7, 7**	**8, 8**	**17, 17**	**XY**

A3.9 Primary Transfer (Subject A ↔Subject B) Pie Charts

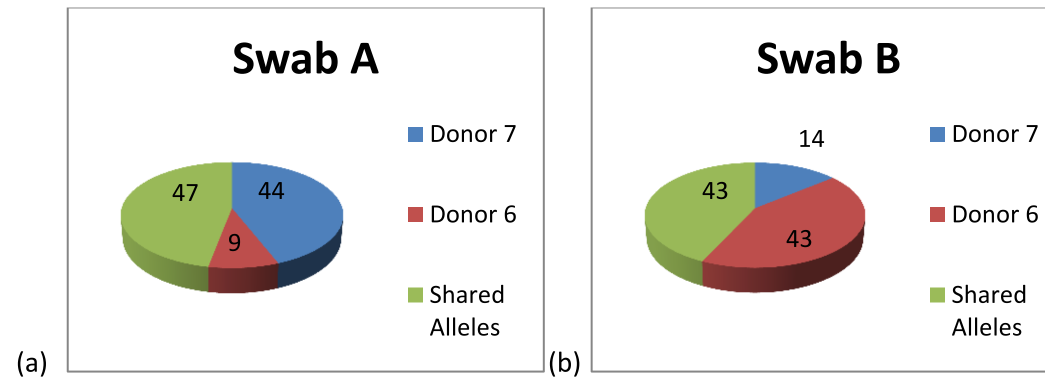


Figure 3.7 (a) and (b) Proportion of alleles recovered from (a)Hand Swab A [Hand of Donor 7] and (b) Hand Swab B (Hand of Donor 6], collected after contact between the two donors.

Figure 3.7 (a) shows the percentage of assignable alleles from the swab taken from the hand of Donor 7, after shaking hands with Donor 6, that could be identified as originating from Donor 7 (44% of all assignable alleles), Donor 6 (9% of all assignable alleles) or from either Donor 7 or 6 (47% of all assignable alleles).

Figure 3.7 (b) shows the percentage of assignable alleles from the swab taken from the hand of Donor 6, after shaking hands with Donor 7, that could be identified as originating from Donor 6 (43% of all assignable alleles), Donor 7 (14% of all assignable alleles) or from either Donor 7 or 6 (43% of all assignable alleles).

These results suggest that the majority of alleles recovered from an individual's hands after contact with another person can be assigned to that individual.

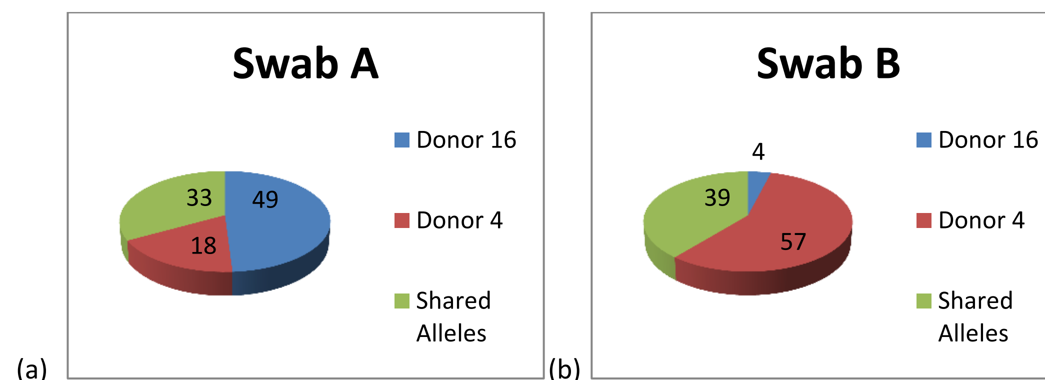


Figure 3.8 (a) and (b) Proportion of alleles recovered from (a)Hand Swab A [Hand of Donor 16] and (b) Hand Swab B (Hand of Donor 4], collected after contact between the two donors.

Figure 3.8 (a) shows the percentage of assignable alleles from the swab taken from the hand of Donor 16, after shaking hands with Donor 4, that could be identified as originating from Donor 16 (49% of all assignable alleles), Donor 4 (18% of all assignable alleles) or from either Donor 16 or 4 (33% of all assignable alleles).

Figure 3.8 (b) shows the percentage of assignable alleles from the swab taken from the hand of Donor 4, after shaking hands with Donor 16, that could be identified as originating from Donor 4 (57% of all assignable alleles), Donor 16 (4% of all assignable alleles) or from either Donor 16 or 4 (39% of all assignable alleles).

These results suggest that the majority of alleles recovered from an individual's hands after contact with another person can be assigned to that individual.

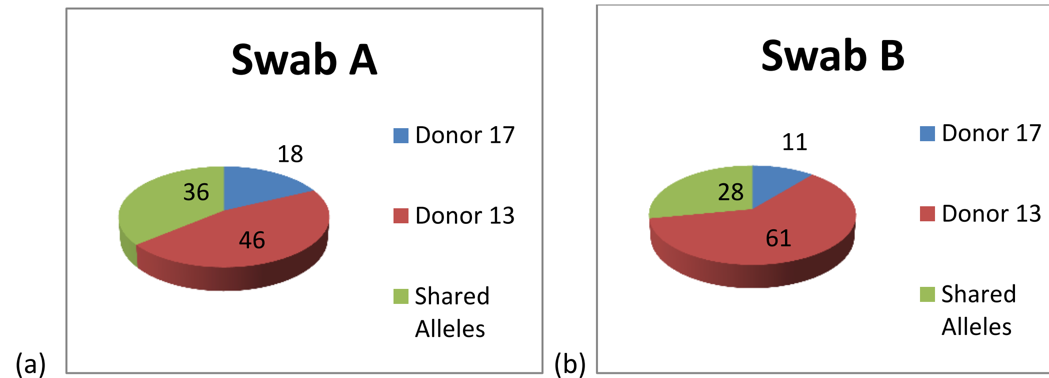


Figure 3.9 (a) and (b) Proportion of alleles recovered from (a)Hand Swab A [Hand of Donor 17] and (b) Hand Swab B (Hand of Donor 13], collected after contact between the two donors.

Figure 3.9 (a) shows the percentage of assignable alleles from the swab taken from the hand of Donor 17, after shaking hands with Donor 13, that could be identified as originating from Donor 17 (18% of all assignable alleles), Donor 13 (46% of all assignable alleles) or from either Donor 17 or 13 (36% of all assignable alleles).

Figure 3.9 (b) shows the percentage of assignable alleles from the swab taken from the hand of Donor 13, after shaking hands with Donor 17, that could be identified as originating from Donor 13 (61% of all assignable alleles), Donor 17 (11% of all assignable alleles) or from either Donor 17 or 13 (28% of all assignable alleles).

These results suggest that the majority of alleles recovered from an individual’s hands after contact with another person do not necessarily originate from that individual.

A3.10 Primary Transfer of DNA with Time Delay (Subject A ↔Subject B)

Key to labelling of samples in table:

Left Hand Primary Transfer (Subject A ↔Subject B; 30 min delay then swab hands)

2AL30Ha – Subject A Hand swab after 30 min, Repeat (a).

2BL30Ha – Subject B Hand swab after 30 min, Repeat (a).

Alleles highlighted in Blue are those from Subject A.

Alleles highlighted in Red are those from Subject B.

Alleles highlighted in Green are those that could be from either Subject A or B.

Table A3.15 DNA Profiles of Time Delay Primary Transfer hand swabs (Repeat a)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
2AL30Ha		14, 15, 16, 17			XY	12, 13, 14, 15			12, 13, 14	6, 7	
2BL30Ha	12, 15, 16	14, 17			XY	9, 12			12, 13, 14	6, 7	
Subject A	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22
Subject B	15, 15	14, 15	11, 11	18, 23	XX	13, 15	29, 32.2	15, 16	14, 14	6, 6	19, 21

Table A3.16 DNA Profiles of Time Delay Primary Transfer hand swabs (Repeat b)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
2AL30Hb	15	14, 15, 16, 17			XY	12, 13, 14, 15			12, 13, 14	6, 7	
2BL30Hb		14, 15, 17			XY	12, 13, 14			12, 13, 14	6, 7	
Subject A	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22
Subject B	15, 15	14, 15	11, 11	18, 23	XX	13, 15	29, 32.2	15, 16	14, 14	6, 6	19, 21

Table A3.17 DNA Profiles of Time Delay Primary Transfer hand swabs (Repeat c)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
2AL30Hc	15	16, 17			XY	12, 14			12, 14	6, 7	
2BL30Hc											
Subject A	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22
Subject B	15, 15	14, 15	11, 11	18, 23	XX	13, 15	29, 32.2	15, 16	14, 14	6, 6	19, 21

A3.10 Stochastic Effect Study

Key to labelling of samples in table:

TA1 signifies first pooled replicate of sample 9RTBOA; TA2 signifies the second pooled replicate, and so on.

TC1 signifies first pooled replicate of sample 9RTBOC; TC2 signifies the second pooled replicate, and so on.

Alleles highlighted in Red are those from Subject A.

Alleles highlighted in Blue are those from Subject B.

Alleles highlighted in Green are those that could be from either Subject A or B.

Table A3.18 Stochastic Effect (Sample 9RTBOA)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Original Sample (9RTBOA)		16			XY	16		14	14	7	
TA1	14, 16	16, 19	9, 13		X	14, 15	29, 31.2, 32.2		11, 12, 13, 14	7, 9.3	
TA2	16	17, 18	12			13	29, 30		14		18, 22
TA3					X		28, 29, 30			6, 7, 9	22
TA4	17	16	13		X	14, 15	28, 30	16.2	14		
Subject A	14, 17	16, 18	12, 13	20, 23	XX	12, 14	30, 30	17, 20	14, 14	9, 9.3	20, 22
Subject B	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22

Table A3.19 Stochastic Effect (Sample 9RTBOC)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Original Sample (9RTBOC)	16, 17, 19	17			X	11, 14	28, 34		15.2	8	20
TC1		17			X	14	28, 29.2		12, 14		22
TC2	15, 16		9		X		28, 30		14	7	
TC3	16	17	13		X		28		14	7	
TC4			9		X		28, 32.2		14	7, 9.3	
Subject A	14, 17	16, 18	12, 13	20, 23	XX	12, 14	30, 30	17, 20	14, 14	9, 9.3	20, 22
Subject B	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22

Table A3.20 Stochastic Effect (Sample 9RTBOE)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Original Sample (9RTBOE)	14		9			9, 15, 16			12, 13.2		
TE1	14, 16	16, 17	9		XY	14	29.2		12	9	20
TE2	16	18	13		X		29.2, 30		14		20
TE3					Y						
TE4	13, 14, 16	19	13		X		29, 32.2	16	14	5.3, 6, 9.3	
Subject A	14, 17	16, 18	12, 13	20, 23	XX	12, 14	30, 30	17, 20	14, 14	9, 9.3	20, 22
Subject B	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22

Table A3.21 Stochastic Effect (Sample 9LTOBB)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Original Sample (9LTOBB)	14, 15, 16, 19	15, 16, 17, 18, 24	9		XY	10, 11, 14, 16	28, 29.2, 34, 37	14, 16, 20	12, 14, 14.2, 15.2	7, 8, 9	20, 29
TB1	15, 16	16, 17			XY	13, 15	28, 29.2	14, 15.2	14, 15.2	6.3, 7	22
TB2	15, 16	17	9, 12, 13	17	XY	13, 15	28, 29.2, 30	14, 15.2	12, 14	7	20, 22
TB3	15	17	9, 13		X		29.2	15.2	12	7	
TB4	15, 16	17	8, 12, 13		XY	15	28, 29, 29.2	14, 15.2	11, 12, 14	7	22
Subject A	14, 17	16, 18	12, 13	20, 23	XX	12, 14	30, 30	17, 20	14, 14	9, 9.3	20, 22
Subject B	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22

Table A3.22 Stochastic Effect (Sample 9LTOBD)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Original Sample (9LTOBD)	14, 15, 17, 18, 19	16, 18, 19	12		X	9, 10, 12, 13, 14, 16	24, 24.2, 30, 31, 34, 36	17, 20	13.2, 14, 16.2	7, 9, 9.3	20, 22, 28, 29, 46.2
TD1	14	16, 18			X	13, 15	30		12, 14	9.3	
TD2	14, 17, 19	14, 16, 17, 18	12, 13		X	12, 13, 14, 15	30, 31	16.2, 18	9.2, 12, 14	8, 9, 9.3	20, 22
TD3	14, 16, 17	16, 17, 18	8, 12, 13		XY	12, 13, 14, 15	28, 30, 31	16.2	10.2, 11, 11.2, 12, 14	6, 7, 9, 9.3	20, 21, 22
TD4	14, 17	16, 18	12, 12.2	19	XY	13, 15	28, 30	16, 18	11, 14	7, 9, 9.3	20.2, 22.2
Subject A	14, 17	16, 18	12, 13	20, 23	XX	12, 14	30, 30	17, 20	14, 14	9, 9.3	20, 22
Subject B	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22

Table A3.23 Stochastic Effect (Sample 9LTOBF)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Original Sample (9LTOBF)	14, 15, 17, 19	16, 17, 18	12		X	9, 12, 14, 15, 16	30		12, 13.2, 14, 16.2	7, 8, 9, 9.3	
TF1	14, 16, 17	15, 16, 17, 18	12	20, 23	XY	15	30	16, 17, 20	14	7, 9, 9.3	20, 22
TF2	14, 15, 17	16, 17, 18	13		XY	13, 15	30	16, 17, 20	12, 14	9, 9.3	20
TF3	14, 17, 19	16, 17, 18	12, 13	20, 21, 23	XY	13, 15	28, 30, 31	16, 17, 20	12, 14	9, 9.3	20, 22
TF4	14, 17	16, 17, 18	9, 12, 13	23	X	13, 15	29.2, 30	17, 20	14	6.3, 7, 9, 9.3	20, 22
Subject A	14, 17	16, 18	12, 13	20, 23	XX	12, 14	30, 30	17, 20	14, 14	9, 9.3	20, 22
Subject B	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22

Appendix Four – DNA Recovery Studies

A4.1 Quantification Results

Table A4.1 DNA Recovery Quantification Results

Recovery Method	DNA Concentration (ng/ml)		
	Glass	Metal	Rusted Metal
Cotton Wet 1	996	1088	836
Cotton Wet 2	1034	96	670
Cotton Wet 3	1058	1084	460
Cotton Double 1	1014	786	980
Cotton Double 2	460	1128	780
Cotton Double 3	312	1134	682
Viscose 1	932	416	432
Viscose 2	974	780	406
Viscose 3	766	165.4	428
Nylon Flocked 1	702	954	704

Nylon Flocked 2	516	880	534
Nylon Flocked 3	536	930	444
Mini-tape 1	226	188.8	352
Mini-tape 2	120.4	185.2	244
Mini-tape 3	96.6	189.8	248
Gel lift 1	109.8	270	119.8
Gel lift 2	230	228	54.2
Gel lift 3	216	368	288
Buccal Swab Control	20.8 µg/ml		

A4.2 DNA Profile Results

Table A4.2 DNA Recovery Control Profiles

Sample	D3S1358	vWA	D16S539	D2S1338	Amel	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
Buccal	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28, 30	14, 18	13, 14	7, 9	20, 21
Positive control	15, 16	14, 16	9, 10	20, 23	X, Y	12, 13	28, 31	12, 15	14, 15	7, 9.3	24, 26
Negative control	-	-	-	-	-	-	-	-	-	-	-

Table A4.3 DNA Recovery Profiles recovered from glass

Sample	D3S1358	vWA	D16S539	D2S1338	Amel	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
Cotton double 1 G	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28, 30	14, 18	13, 14	9	20, 21
Cotton double 2 G	14, 14	18, 19	12	-	X, X	13, 15	28, 30	18	13, 14	7	20, 21, 22
Cotton double 3 G	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28, 30	14, 18	13, 14	7, 9	21
Cotton wet 1 G	14, 14	18	11, 12	-	X, X	-	-	14, 18	13, 14	7, 9	-
Cotton wet 2 G	-	19	-	-	X, X	13, 15	28	-	14	7	-
Cotton wet 3 G	14, 14	18	11	18	X, X	13	28	14	14	7, 9	-
Gel lift 1 G	14, 14	18	-	-	-	-	28	14	13	7, 9	20
Gel lift 2 G	14, 14	18	11	18	X, X	13	-	18	13, 14	9	20
Gel lift 3 G	14, 14	19	11, 12	18	-	13	28, 30	14	-	-	20, 21
Nylon flocked 1 G	-	-	-	-	-	-	-	-	14	-	-

Nylon flocked 2 G	-	-	-	-	-	-	-	-	-	-	-
Nylon flocked 3 G	-	-	-	-	-	-	-	-	-	-	-
Tape lift 1 G	14, 14	18	-	-	-	13	30	-	-	9	20, 21
Tape lift 2 G	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28, 30	18	13, 14	7, 9	20, 21
Tape lift 3 G	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	30	14	13, 14	7, 9	20, 21
Viscose 1 G	-	-	-	-	-	-	-	14	-	-	-
Viscose 2 G	-	-	-	-	-	-	30	18	-	-	-
Viscose 3 G	-	-	-	-	-	-	-	-	-	-	-

Table A4.4 DNA Recovery Profiles recovered from metal

Sample	D3S1358	vWA	D16S539	D2S1338	Amel	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
Cotton double 1 M	14, 14	18	11, 12	17, 18	X, X	13, 15	28, 30	14, 18	13	7, 9	-
Cotton double 2 M	14, 14	-	11	-	X, X	13	28	18	13, 14	9	-
Cotton double 3 M	14, 14	-	11	17, 18	-	13	28, 30	14	13	7	20
Cotton wet 1 M	14, 14	19	12	17	X, X	14,15	30	14, 18	13, 14	7, 9	20, 21
Cotton wet 2 M	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28, 30	14, 18	13, 14	7, 9	20, 21
Cotton wet 3 M	14, 14	18, 19	-	17, 18	X, X	13, 15	28, 30	14, 18	13, 14	7, 9	21
Gel lift 1 M	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28, 30	14, 18	13, 14	7, 9	20, 21
Gel lift 2 M	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28	14, 18	13, 14	7, 9	20, 21
Gel lift 3 M	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28, 30	14, 18	13, 14	7, 9	20, 21
Nylon flocked 1 M	14, 14	18, 19	11	17, 18	X, X	13	28, 30	18	13, 14	7, 9	21
Nylon flocked 2 M	-	-	-	-	-	-	-	18	-	-	20

Nylon flocked 3 M	14, 14	-	-	-	-	-	-	18	-	-	-
Tape lift 1 M	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28, 30	14, 18	13, 14	7, 9	20, 21
Tape lift 2 M	14, 14	18, 19	11, 12	17, 18	-	13, 15	28, 30	14	13, 14	7, 9	20, 21
Tape lift 3 M	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28, 30	14, 18	13, 14	7, 9	20, 21
Viscose 1 M	-	-	-	-	-	13	-	18	-	-	-
Viscose 2 M	-	-	-	-	-	13, 17	-	-	-	7	-
Viscose 3 M	14, 14	18, 19	11, 12	18, 18	X, X	13, 15	28, 30	14, 18	14, 14	6, 7, 9	20

Table A4.5 DNA Recovery Profiles recovered from rusted metal

Sample	D3S1358	vWA	D16S539	D2S1338	Amel	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
Cotton double 1 RM	14, 14	18, 19	11, 12	18	X, X	13, 15	28	14, 18	13, 14	9	21
Cotton double 2 RM	-	-	-	-	X, X	-	-	14	14	7, 9	20
Cotton double 3 RM	-	-	-	-	-	-	-	-	13	-	-
Cotton wet 1 RM	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28,30	14, 18	13	7, 9	20, 21
Cotton wet 2 RM	14, 14	18, 19	11, 12	-	X, X	13, 15	30	14, 18	13, 14	7	20, 21
Cotton wet 3 RM	14, 14	18	11	-	X, X	13, 15	28	14	13, 14	7	20, 21
Gel lift 1 RM	-	-	12	-	-	-	-	-	-	-	-
Gel lift 2 RM	-	-	12	17	-	-	-	-	-	-	-
Gel lift 3 RM	-	-	-	-	X, X	-	-	-	-	-	-
Nylon flocked 1 RM	14, 14	18	12	-	X, X	-	30	14	13	-	20, 21

Nylon flocked 2 RM	-	-	11	17	-	-	-	-	14	-	-
Nylon flocked 3 RM	-	-	-	-	-	-	-	-	-	-	-
Tape lift 1 RM	14, 14	18, 19	12	17, 18, 20	X, X	13, 15	-	14, 18	13, 14	9	20, 21
Tape lift 2 RM	-	-	11	17	X, X	13	28, 30	14	14	7	21
Tape lift 3 RM	14, 14	18, 19	11, 12	17, 18	X, X	13, 15	28, 30	14, 18	13, 14	7, 9	20, 21
Viscose 1 RM	-	-	-	-	-	-	-	-	-	-	-
Viscose 2 RM	-	-	-	-	-	-	-	-	-	-	-
Viscose 3 RM	14, 14	-	-	18	-	-	28	-	-	9	-

A4.3 Percentage Profile Rankings

Table A4.6 DNA Recovery Percentage Profile Sample Rankings

Sample	No. of common alleles	% profile		Sample	No. of common alleles	% profile
Buccal reference	22	100		Nylon flocked 1 RM	11	50.00
Cotton wet 2 M	22	100.00		Tape lift 2 RM	11	50.00
Gel lift 1 M	22	100.00		Cotton double 2 M	10	45.45
Gel lift 3 M	22	100.00		Gel lift 1 G	9	40.91
Tape lift 1 M	22	100.00		Cotton wet 2 G	8	36.36
Tape lift 3 M	22	100.00		Tape lift 1 G	8	36.36
Tape lift 3 RM	22	100.00		Cotton double 2 RM	7	31.82
Cotton double 1 G	21	95.45		Viscose 3 RM	5	22.73
Cotton double 3 G	21	95.45		Positive control	5	22.73
Cotton wet 1 RM	21	95.45		Nylon flocked 2 RM	3	13.64
Gel lift 2 M	21	95.45		Nylon flocked 3 M	3	13.64
Tape lift 2 G	21	95.45		Gel lift 2 RM	2	9.09
Tape lift 3 G	20	90.91		Gel lift 3 RM	2	9.09
Cotton wet 3 M	19	86.36		Nylon flocked 2 M	2	9.09

Tape lift 2 M	19	86.36		Viscose 1 M	2	9.09
Viscose 3 M	19	86.36		Viscose 2 G	2	9.09
Cotton wet 1 M	18	81.82		Viscose 2 M	2	9.09
Cotton wet 2 RM	18	81.82		Cotton double 3 RM	1	4.55
Nylon flocked 1 M	18	81.82		Gel lift 1 RM	1	4.55
Tape lift 1 RM	18	81.82		Nylon flocked 1 G	1	4.55
Cotton double 1 M	17	77.27		Viscose 1 G	1	4.55
Cotton double 1 RM	17	77.27		Nylon flocked 2 G	0	0.00
Cotton double 2 G	16	72.73		Nylon flocked 3 G	0	0.00
Cotton wet 3 RM	15	68.18		Nylon flocked 3 RM	0	0.00
Cotton wet 1 G	13	59.09		Viscose 1 RM	0	0.00
Cotton wet 3 G	13	59.09		Viscose 2 RM	0	0.00
Gel lift 2 G	13	59.09		Viscose 3 G	0	0.00
Cotton double 3 M	12	54.55		Negative control	0	0.00
Gel lift 3 G	12	54.55				

A4.4 Heterozygote Balance Calculations Table

Table A4.7 DNA Recovery Heterozygote Balance Calculations

Sample	Marker	Allele (max)	Allele (min)	φ min	φ max	HBX	Heterozygote balance (%)
Buccal reference	vWA	19	18	6309	6993	0.902188	90.22
	D16S539	12	11	5650	5978	0.945132	94.51
	D2S1338	17	18	4089	4317	0.947186	94.72
	D8S1179	15	13	6327	7646	0.827491	82.75
	D21S11	28	30	5071	5779	0.877487	87.75
	D18S51	14	18	4646	5266	0.882264	88.23
	D19S433	14	13	7772	9753	0.796883	79.69
	TH01	9	7	8296	8795	0.943263	94.33
	FGA	21	20	3999	5526	0.72367	72.37
Positive control	D3S1358	15	16	2476	2637	0.938946	93.89
	vWA	16	14	1682	1701	0.98883	98.88
	D16S539	10	9	1730	1931	0.895909	89.59
	D2S1338	20	23	1303	1450	0.898621	89.86
	AMEL	X	Y	1524	1698	0.897527	89.75
	D8S1179	13	12	1339	1982	0.67558	67.56

	D21S11	31	28	1213	1226	0.989396	98.94
	D18S51	15	12	811	1149	0.705831	70.58
	D19S433	15	14	1749	1819	0.961517	96.15
	TH01	9.3	7	2508	2617	0.958349	95.83
	FGA	24	26	1560	2001	0.77961	77.96
Cotton double 1 Glass	vWA	18	19	67	80	0.8375	83.75
	D16S539	12	11	108	190	0.568421	56.84
	D2S1338	17	18	61	79	0.772152	77.22
	D8S1179	13	15	80	261	0.306513	30.65
	D21S11	30	28	76	223	0.340807	34.08
	D18S51	18	14	157	238	0.659664	65.97
	D19S433	13	14	69	87	0.793103	79.31
	FGA	20	21	183	313	0.584665	58.47
Cotton double 2 Glass	vWA	18	19	189	237	0.797468	79.75
	D8S1179	15	13	154	223	0.690583	69.06

Sample	Marker	Allele (max)	Allele (min)	φ min	φ max	HBX	Heterozygote balance (%)
Cotton double 2 Glass cont.	D21S11	30	28	105	131	0.801527	80.15
	D19S433	14	13	152	267	0.569288	56.93
	FGA	21	20	117	180	0.65	65.00
Cotton double 3 Glass	vWA	19	18	66	251	0.262948	26.29
	D16S539	12	11	90	325	0.276923	27.69
	D2S1338	18	17	122	153	0.797386	79.74
	D8S1179	15	13	127	211	0.601896	60.19
	D21S11	28	30	50	198	0.252525	25.25
	D18S51	14	18	146	292	0.5	50.00
	D19S433	13	14	233	299	0.779264	77.93
	TH01	7	9	318	394	0.807107	80.71
Cotton double 1 Metal	D16S539	11	12	209	242	0.863636	86.36
	D2S1338	18	17	63	116	0.543103	54.31
	D8S1179	15	13	78	358	0.217877	21.79
	D21S11	28	30	95	172	0.552326	55.23
	D18S51	14	18	51	78	0.653846	65.38

	TH01	9	7	242	341	0.709677	70.97
Cotton double 2 Metal	D19S433	13	14	122	201	0.606965	60.70
Cotton double 3 Metal	D2S1338	17	18	175	184	0.951087	95.11
	D21S11	30	28	115	116	0.991379	99.14
Cotton double 1 Rusted metal	vWA	19	18	63	140	0.45	45.00
	D16S539	11	12	125	176	0.710227	71.02
	D8S1179	13	15	70	146	0.479452	47.95
	D18S51	18	14	96	253	0.379447	37.94
	D19S433	13	14	152	193	0.787565	78.76
Cotton double 2 Rusted metal	TH01	7	9	183	251	0.729084	72.91
Cottonn wet 1 Glass	D16S539	11	12	102	203	0.502463	50.25
	D18S51	18	14	76	99	0.767677	76.77
	D19S433	13	14	92	107	0.859813	85.98
	TH01	7	9	63	225	0.28	28.00
Cotton wet 2 Glass	D8S1179	15	13	78	96	0.8125	81.25

Sample	Marker	Allele (max)	Allele (min)	φ min	φ max	HBX	Heterozygote balance (%)
Cotton wet 3 Glass	TH01	7	9	146	184	0.793478	79.35
Cotton wet 1 Metal	D8S1179	14	15	51	277	0.184116	18.41
	D18S51	14	18	111	127	0.874016	87.40
	D19S433	14	13	121	169	0.715976	71.60
	TH01	9	7	111	138	0.804348	80.43
	FGA	21	20	128	204	0.627451	62.75
Cotton wet 2 Metal	vWA	19	18	801	864	0.927083	92.71
	D16S539	12	11	792	822	0.963504	96.35
	D2S1338	17	18	488	551	0.885662	88.57
	D8S1179	13	15	654	1347	0.485523	48.55
	D21S11	30	28	556	855	0.650292	65.03
	D18S51	14	18	456	615	0.741463	74.15
	D19S433	14	13	964	1780	0.541573	54.16
	TH01	9	7	939	1462	0.642271	64.23
	FGA	21	20	1025	1172	0.874573	87.46
Cotton wet 3 Metal	vWA	19	18	96	183	0.52459	52.46

	D2S1338	17	18	84	87	0.965517	96.55
	D8S1179	15	13	106	222	0.477477	47.75
	D21S11	28	30	132	292	0.452055	45.21
	D18S51	18	14	83	91	0.912088	91.21
	D19S433	14	13	59	184	0.320652	32.07
	TH01	7	9	56	134	0.41791	41.79
Cotton wet 1 Rusted metal	vWA	19	18	159	238	0.668067	66.81
	D16S539	11	12	113	183	0.617486	61.75
	D2S1338	18	17	90	124	0.725806	72.58
	D8S1179	13	15	165	209	0.789474	78.95
	D21S11	30	28	157	179	0.877095	87.71
	D18S51	18	14	112	184	0.608696	60.87
	TH01	9	7	62	81	0.765432	76.54
	FGA	21	20	86	194	0.443299	44.33
Cotton wet 2 Rusted metal	vWA	19	18	162	318	0.509434	50.94

Sample	Marker	Allele (max)	Allele (min)	φ min	φ max	HBX	Heterozygote balance (%)
Cotton wet 2 Rusted metal cont.	D16S539	11	12	61	119	0.512605	51.26
	D8S1179	13	15	105	142	0.739437	73.94
	D18S51	14	18	99	175	0.565714	56.57
	D19S433	13	14	119	239	0.497908	49.79
	FGA	21	20	148	153	0.96732	96.73
Cotton wet 3 Rusted metal	D8S1179	13	15	88	253	0.347826	34.78
	D19S433	14	13	201	250	0.804	80.40
	FGA	20	21	208	297	0.700337	70.03
Gel lift 1 Glass	TH01	9	7	166	213	0.779343	77.93
Gel lift 2 Glass	D19S433	14	13	112	114	0.982456	98.25
Gel lift 3 Glass	D16S539	11	12	196	215	0.911628	91.16
	D21S11	28	30	88	97	0.907216	90.72
	FGA	20	21	81	110	0.736364	73.64
Gel lift 1 Metal	vWA	18	19	1063	1136	0.935739	93.57
	D16S539	12	11	755	965	0.782383	78.24
	D2S1338	18	17	964	1086	0.887661	88.77

	D8S1179	13	15	1460	1818	0.80308	80.31
	D21S11	28	30	983	1016	0.96752	96.75
	D18S51	18	14	900	1285	0.700389	70.04
	D19S433	14	13	1295	1938	0.668215	66.82
	TH01	9	7	1212	1858	0.652314	65.23
	FGA	21	20	782	2066	0.378509	37.85
Gel lift 2 Metal	vWA	19	18	101	441	0.229025	22.90
	D16S539	12	11	317	438	0.723744	72.37
	D2S1338	18	17	371	594	0.624579	62.46
	D8S1179	15	13	293	657	0.445967	44.60
	D18S51	14	18	228	399	0.571429	57.14
	D19S433	13	14	541	988	0.547571	54.76
	TH01	9	7	149	412	0.36165	36.17
	FGA	21	20	400	432	0.925926	92.59
Gel lift 3 Metal	vWA	18	19	2800	2802	0.999286	99.93

Sample	Marker	Allele (max)	Allele (min)	φ min	φ max	HBX	Heterozygote balance (%)
Gel lift 3 Metal cont.	D16S539	12	11	3102	3131	0.990738	99.07
	D2S1338	18	17	1223	2324	0.526248	52.62
	D8S1179	13	15	2171	2521	0.861166	86.12
	D21S11	28	30	2102	3685	0.570421	57.04
	D18S51	18	14	1856	1901	0.976328	97.63
	D19S433	14	13	2214	2758	0.802756	80.28
	TH01	7	9	2065	2547	0.810758	81.08
	FGA	20	21	2084	2256	0.923759	92.38
Nylon flocked 1 Metal	vWA	18	19	108	261	0.413793	41.38
	D2S1338	18	17	53	54	0.981481	98.15
	D21S11	30	28	226	399	0.566416	56.64
	D19S433	13	14	155	210	0.738095	73.81
	TH01	9	7	99	115	0.86087	86.09
Nylon flocked 1 Rusted metal	FGA	20	21	104	114	0.912281	91.23
Tape lift 1 Glass	FGA	21	20	105	120	0.875	87.50
Tape lift 2 Glass	vWA	18	19	59	65	0.907692	90.77

	D16S539	12	11	214	333	0.642643	64.26
	D2S1338	17	18	127	279	0.455197	45.52
	D8S1179	15	13	180	280	0.642857	64.29
	D21S11	30	28	115	118	0.974576	97.46
	D19S433	13	14	131	210	0.62381	62.38
	TH01	9	7	100	313	0.319489	31.95
	FGA	21	20	406	430	0.944186	94.42
Tape lift 3 Glass	vWA	18	19	260	460	0.565217	56.52
	D16S539	11	12	81	213	0.380282	38.03
	D2S1338	17	18	264	279	0.946237	94.62
	D8S1179	15	13	101	422	0.239336	23.93
	D19S433	13	14	112	239	0.468619	46.86
	TH01	9	7	230	306	0.751634	75.16
	FGA	20	21	259	523	0.49522	49.52
Tape lift 1 Metal	vWA	19	18	541	613	0.882545	88.25

Sample	Marker	Allele (max)	Allele (min)	φ min	φ max	HBX	Heterozygote balance (%)
Tape lift 1 Metal cont.	D16S539	12	11	299	702	0.425926	42.59
	D2S1338	17	18	322	507	0.635108	63.51
	D8S1179	15	13	438	903	0.48505	48.50
	D21S11	30	28	347	366	0.948087	94.81
	D18S51	18	14	321	360	0.891667	89.17
	D19S433	14	13	397	719	0.552156	55.22
	TH01	9	7	202	552	0.365942	36.59
	FGA	21	20	510	631	0.808241	80.82
Tape lift 2 Metal	vWA	18	19	123	284	0.433099	43.31
	D16S539	12	11	122	489	0.249489	24.95
	D2S1338	17	18	116	217	0.534562	53.46
	D8S1179	15	13	74	182	0.406593	40.66
	D21S11	28	30	194	222	0.873874	87.39
	D19S433	14	13	130	298	0.436242	43.62
	TH01	9	7	141	349	0.404011	40.40
	FGA	21	20	195	214	0.911215	91.12

Tape lift 3 Metal	vWA	18	19	553	599	0.923205	92.32
	D16S539	12	11	473	518	0.913127	91.31
	D2S1338	17	18	298	497	0.599598	59.96
	D8S1179	15	13	645	899	0.717464	71.75
	D21S11	28	30	445	560	0.794643	79.46
	D18S51	18	14	306	565	0.541593	54.16
	D19S433	13	14	648	722	0.897507	89.75
	TH01	7	9	509	628	0.81051	81.05
	FGA	21	20	536	551	0.972777	97.28
Tape lift 1 Rusted metal	vWA	19	18	112	135	0.82963	82.96
	D2S1338	18	17	75	150	0.5	50.00
	D8S1179	15	13	372	524	0.709924	70.99
	D18S51	18	14	110	241	0.456432	45.64
	D19S433	14	13	257	418	0.614833	61.48
	FGA	20	21	133	268	0.496269	49.63

Sample	Marker	Allele (max)	Allele (min)	φ min	φ max	HBX	Heterozygote balance (%)
Tape lift 2 Rusted metal	D21S11	30	28	105	199	0.527638	52.76
Tape lift 3 Rusted metal	vWA	19	18	794	1014	0.783037	78.30
	D16S539	12	11	751	795	0.944654	94.47
	D2S1338	17	18	540	742	0.727763	72.78
	D8S1179	15	13	494	627	0.787879	78.79
	D21S11	30	28	672	816	0.823529	82.35
	D18S51	14	18	263	551	0.477314	47.73
	D19S433	14	13	640	1665	0.384384	38.44
	TH01	9	7	388	1228	0.315961	31.60
	FGA	21	20	557	1240	0.449194	44.92
Viscose 3 Metal	vWA	18	19	98	249	0.393574	39.36
	D16S539	12	11	81	237	0.341772	34.18
	D8S1179	15	13	178	247	0.720648	72.06
	D21S11	28	30	157	313	0.501597	50.16
	D18S51	18	14	156	250	0.624	62.40
	TH01	7	9	69	73	0.945205	94.52

	D21S11	28	30	2102	3685	0.570421	57.04
	D18S51	18	14	1856	1901	0.976328	97.63
	D19S433	14	13	2214	2758	0.802756	80.28
	TH01	7	9	2065	2547	0.810758	81.08
	FGA	20	21	2084	2256	0.923759	92.38

A4.5 DNA Recovery - Electropherogram from Metal Slide using mini-taping

See Attached Disc

A4.6 DNA Recovery - Electropherogram from Metal Slide using gel-lifter

See Attached Disc

A4.7 DNA Recovery – Electropherogram from Glass Slide using viscose swab

See Attached Disc

A4.8 DNA Recovery – Electropherogram from Glass Slide using nylon flocked swab

See Attached Disc

A4.9 DNA Recovery – Electropherogram from Rusted Metal Slide using gel-lifter

See Attached Disc

A4.10 DNA Recovery – Electropherogram from Rusted Metal Slide using mini-taping

See Attached Disc

A4.11 DNA Recovery – Electropherogram from Glass Slide using cotton double swabbing (1)

See Attached Disc

A4.12 DNA Recovery – Electropherogram from Glass Slide using cotton double swabbing (3)

See Attached Disc

Appendix Five – DNA Persistence and Transfer Studies

A5.1 Persistence and Secondary Transfer of DNA with Person as Vector for Transfer and Final Substrate (Subject A ↔Subject B ↔Subject C)

The following tables show the results from the hand swabs for three groupings of subjects (where the ALTB profile refers to the hand swab from Subject A in the grouping, the BLTB profile refers to the hand swab from Subject B and the CLTB profile refers to the hand swab from Subject C). So for example, in Table 9.4, ALTBC7 refers to the hand swab from Subject A's left hand (Donor 7), BLTBC6 refers to the hand swab from Subject B's left hand (Donor 6), and CLTBC8 refers to the hand swab from Subject C' left hand (Donor 8).

In each of the tables, alleles originating from Subject A in the grouping are highlighted in red, alleles originating from Subject B in the grouping are highlighted in blue, and alleles originating from Subject C in the grouping are highlighted in purple. Any alleles that could have originated from Subject A or B in the grouping (i.e. common alleles between the two subjects) are highlighted in green, any alleles shared between Subject B and C in the grouping are highlighted in pink and any alleles shared between Subject A and C in the grouping are highlighted in grey. Alleles common between all three subjects were highlighted in brown.

Table A5.1 DNA Profiles recovered from hand swabs from Donor 6, Donor 7 and Donor 8.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 7	**10, 12**	**17, 20**	**15, 16**	**11, 13**	**10, 11**	**12, 13**	**8, 11**	**13, 13**	**12, 14**	**14, 14**	**30, 31.2**	**21, 23**	**6, 9**	**8, 11**	**17, 19**	**XX**
Donor 6	10, 10	17, 24	16, 18	11, 13	10, 10	11, 12	11, 14	12, 12	14, 14	12, 14	28, 28	20, 21	6, 9.3	11, 12	17, 18	XX
ALTBC7		16, 17, 20	14, 16, 18	11, 13		12, 13, 14		13, 13	12, 12	14, 15	28, 29.2, 30		6, 9.3	8, 11	16, 17, 18	XY
BLTBC6		17, 19	16, 18	11, 13		11, 14				10, 11, 13	28, 30	20	9.3, 9.3	8, 11	16, 17, 18, 22	XY
CLTBC8				13, 13		14		12, 12		13, 13			9.3, 9.3	8, 10	16, 16	XY
Donor 8	11, 11	19, 19	16, 17	9, 13	13, 13	14, 14	11, 12	11, 13	12, 19	13, 14	27, 30	22, 23	6, 9.3	8, 10	17, 18	XY

Table A5.2 DNA Profiles recovered from hand swabs from Donor 4, Donor 9 and Donor 16.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 16	11, 12	18, 20	15, 17	13, 13	10, 11	14, 14	12, 12	11, 13	11, 16	14, 15.2	28, 30	22.2, 23	9.3, 9.3	8, 11	15, 16	XY
Donor 4	10, 11	19, 24	16, 16	11, 13	8, 12	13, 13	9, 12	9, 11	15, 20	11, 14	27, 30	23, 26	6, 9	8, 11	15, 17	XX
ALTBC16	11, 12	16, 18, 19, 20	15, 17	13, 16	10, 10	13, 14	12, 12	11, 13	11, 16	14, 15.2	28, 29, 30, 32.2	22.2, 23	9.3, 9.3	8, 11	15, 16, 18	XY
BLTBC4	11, 11		13, 16	11	10, 10	11, 12, 13, 14	12, 12	13, 13		9, 11, 14, 15	27, 31 31.2	23, 26	6, 8, 9	8, 8	15, 16, 17	XY
CLTBC9	11, 11	17, 23	15, 16	12, 13	10, 11	11, 13	11, 12	12, 13	13, 17	15, 15	30, 31.2	21	8, 9	9, 12	15, 15	XY
Donor 9	11, 11	17, 23	15, 16	12, 13	10, 11	11, 13	11, 12	12, 13	13, 17	15, 15	30, 31.2	21, 22	8, 9	9, 12	15, 15	XY

Table A5.3 DNA Profiles recovered from hand swabs from Donor 11, Donor 13 and Donor 17.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 17	11, 12	20, 23	14, 17	11, 11	11, 13	12, 14	10, 12	12, 13	17, 20	14, 14	30, 30	20, 22	9, 9.3	9, 11	16, 18	XX
Donor 13	12, 13	21, 23	15, 16	12, 12	9, 11	12, 14	12, 12	9, 13	14, 16	12, 14	28, 29.2	20, 22	7, 7	8, 8	17, 17	XY
ALTBC17		20	15, 15	12, 12		12, 14		9, 13		14, 14		25, 25	6, 9.3		16, 17, 18	XX
BLTBC13	12, 12	18	15, 16	12	9, 11	12, 13, 14	12, 12	9, 13		12, 14, 16, 17	28, 29.2	22	6, 7, 9	8, 8	12, 15, 17	XY
CLTBC11				11, 11		13, 14								8, 8	17, 17	XY
Donor 11	12, 12	17, 18	15, 18	11, 11	9, 9	13, 14	9, 11	11, 13	10, 16	14, 15	28, 31.2	20, 20	6, 9	8, 8	17, 17	XX

This experiment was designed to determine the potential for an individual to act as a vector for DNA transfer. This would mimic a situation where a suspect has claimed their DNA profile has been recovered from a victim's body through mutual contact with an intermediary person. In this experiment Subject A shook hands with Subject B. Subject B then went on to shake hands with Subject C. All three subjects' hands were swabbed after their final contact. The results are summarised in Figures A5.1 – A5.3.

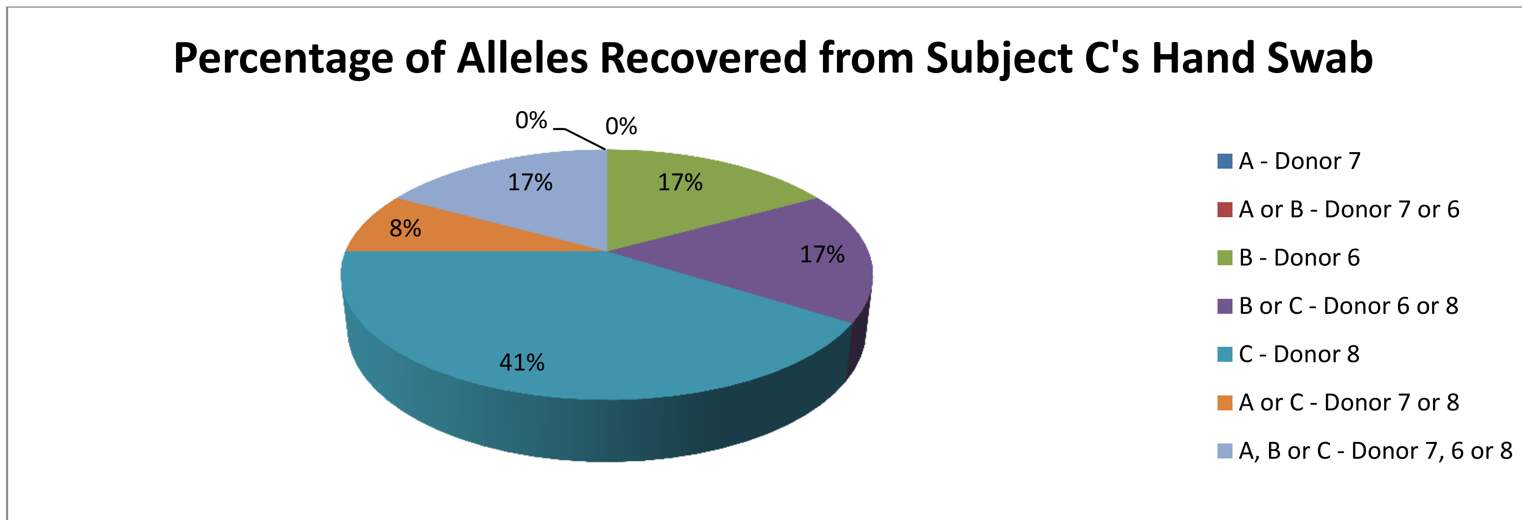


Figure A5.1 Percentage of alleles recovered from the hand swab of Subject C (grouping of Donors 7, 6 and 8).

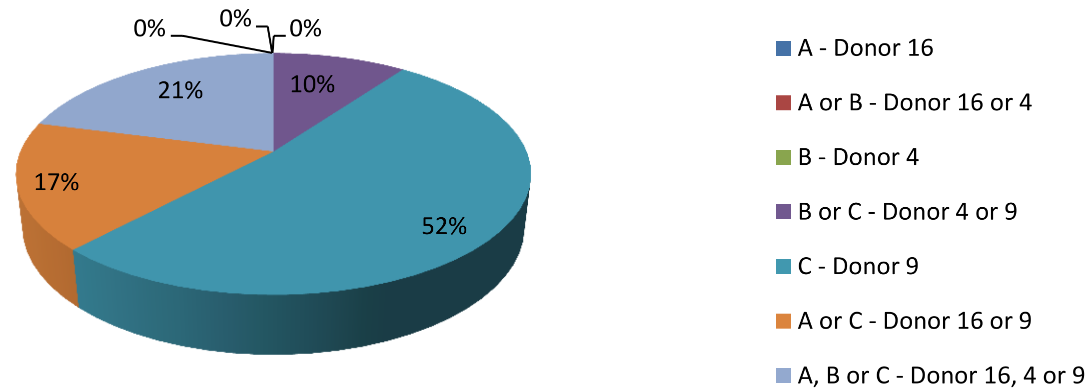


Figure A5.2 Percentage of alleles recovered from the hand swab of Subject C (grouping of Donors 16, 4 and 9).

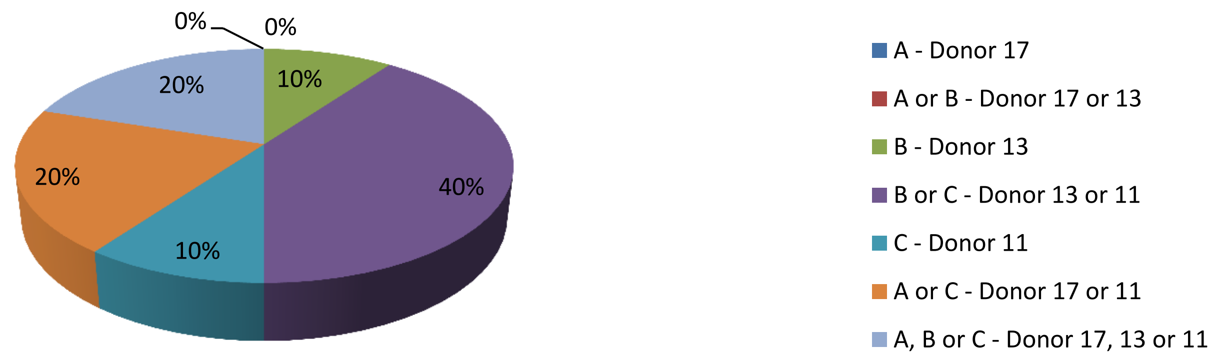


Figure A5.3 Percentage of alleles recovered from the hand swab of Subject C (grouping of Donors 17, 13 and 11).

It was also possible to determine the potential for persistence of DNA from other sources on an individual's hand by examining the percentage of alleles present on Subject B's hand, after contact with both Subject A and then Subject C.

These results are summarised in Figures A5.4 – A5.6.

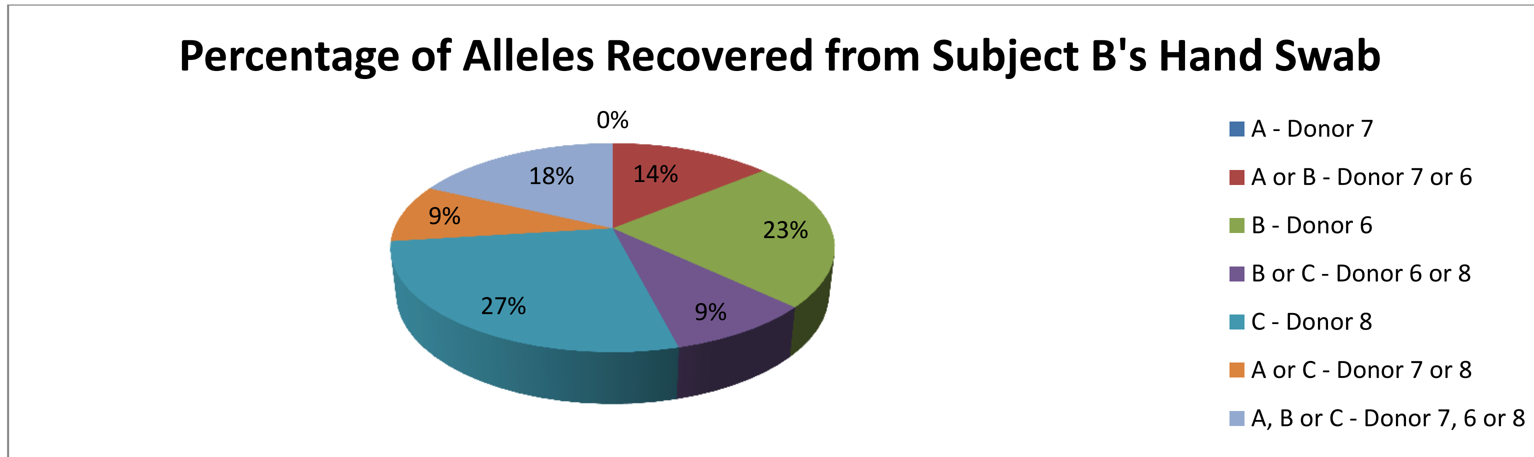


Figure A5.4 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 7, 6 and 8).

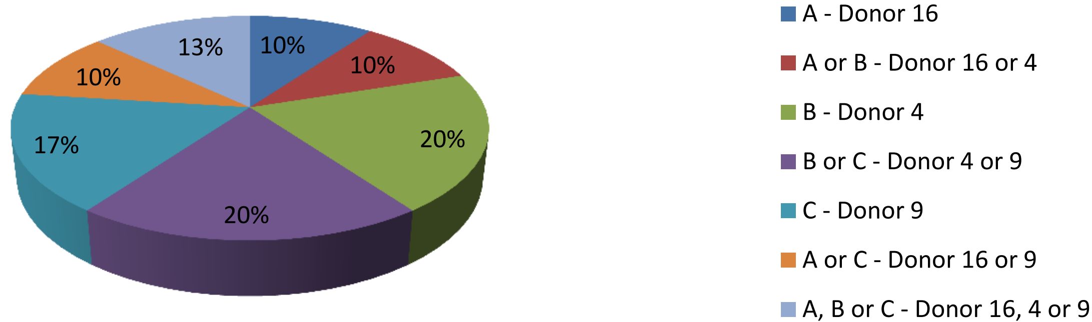


Figure A5.5 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 16, 4 and 9).

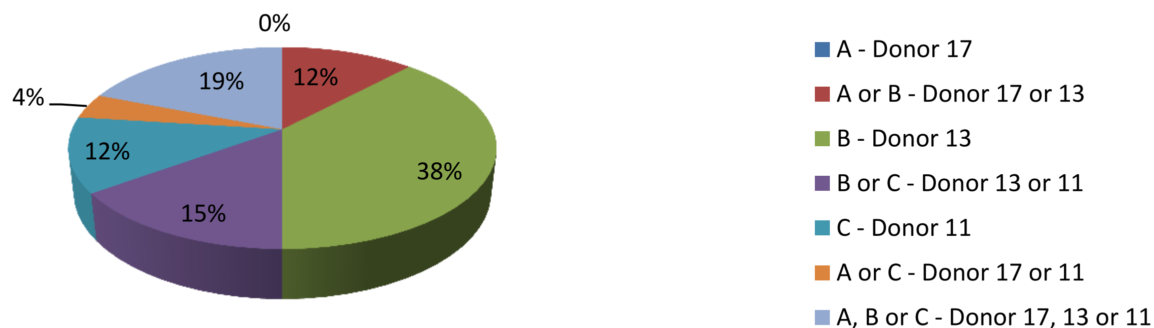


Figure A5.6 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 17, 13 and 11).

A5.2 Persistence and Secondary Transfer of DNA with Person as Vector for Transfer and Object as Final Substrate (Subject A↔ Subject B → Object)

In each of the tables, alleles originating from Subject A in the pairing are highlighted in red, alleles originating from Subject B in the pairing are highlighted in blue and alleles that could have originated from Subject A or B in the pairing (i.e. common alleles between the two subjects) are highlighted in green.

Table A5.4 DNA Profiles recovered from hand swabs from Donor 6, Donor 7 and Object.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 7	**10, 12**	**17, 20**	**15, 16**	**11, 13**	**10, 11**	**12, 13**	**8, 11**	**13, 13**	**12, 14**	**14, 14**	**30, 31.2**	**21, 23**	**6, 9**	**8, 11**	**17, 19**	**XX**
Donor 6	**10, 10**	**17, 24**	**16, 18**	**11, 13**	**10, 10**	**11, 12**	**11, 14**	**12, 12**	**14, 14**	**12, 14**	**28, 28**	**20, 21**	**6, 9.3**	**11, 12**	**17, 18**	**XX**
Subject A Hand	10, 10	17, 20	15, 16	11, 11		9, 12, 14	8, 11	13, 13	14, 15	12, 14		22, 22	6, 6	8, 11	14, 17, 19	XY
Subject B Hand					10, 10	11, 12, 13				11, 14				8	17, 17	XY
Object		24	15, 18	11, 11	10, 10	11	11, 11					20, 22	9		17, 17	XX

Table A5.5 DNA Profiles recovered from hand swabs from Donor 4, Donor 16 and Object.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 16	11, 12	18, 20	15, 17	13, 13	10, 11	14, 14	12, 12	11, 13	11, 16	14, 15.2	28, 30	22.2, 23	9.3, 9.3	8, 11	15, 16	XY
Donor 4	10, 11	19, 24	16, 16	11, 13	8, 12	13, 13	9, 12	9, 11	15, 20	11, 14	27, 30	23, 26	6, 9	8, 11	15, 17	XX
Subject A Hand	11, 12	18, 20	14, 15, 17	13	10	14, 14	11, 12	11, 13	11, 16	14, 15.2	28, 30	23, 23	9.3, 9.3	8, 11	15, 16	XY
Subject B Hand		18, 19	15, 16	11, 13	11	13, 14		9, 11, 13		11, 14, 15.2	27, 30	22.2, 23, 26	9.3, 9.3	8, 11	15, 16, 17	XY
Object	12	17, 17	16	12, 13	10, 11	9, 13, 14	12, 12		16, 20	13, 14 15, 15.2	29, 30 31.2	23, 23	6, 9.3	8, 11	15, 16	XY

Table A5.6 DNA Profiles recovered from hand swabs from Donor 13, Donor 17 and Object.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 17	11, 12	20, 23	14, 17	11, 11	11, 13	12, 14	10, 12	12, 13	17, 20	14, 14	30, 30	20, 22	9, 9.3	9, 11	16, 18	XX
Donor 13	12, 13	21, 23	15, 16	12, 12	9, 11	12, 14	12, 12	9, 13	14, 16	12, 14	28, 29.2	20, 22	7, 7	8, 8	17, 17	XY
Subject A Hand		23, 24		12, 12		10, 12, 13		9, 13		11, 14	30, 30		6, 7, 8	8, 11	16, 17, 18	XY
Subject B Hand			15, 17	12, 12		12, 14		9, 13		12, 14, 15	28, 30		7, 7	8, 8	17, 17	XY
Object				12, 12		12, 14	12, 12	9	14, 14	9	29.2, 29.2	23, 23	6, 7, 9, 9.3	8, 8	16, 17	XX

In this experiment Subject A shook hands with Subject B. Subject B then went on to handle an object (glass beaker). Subject A's hand was swabbed after contact with Subject B, and Subject B's hand and the object were swabbed after their contact. The results of the DNA recovered from the object swabs are summarised in Figures A5.7 – A5.9.

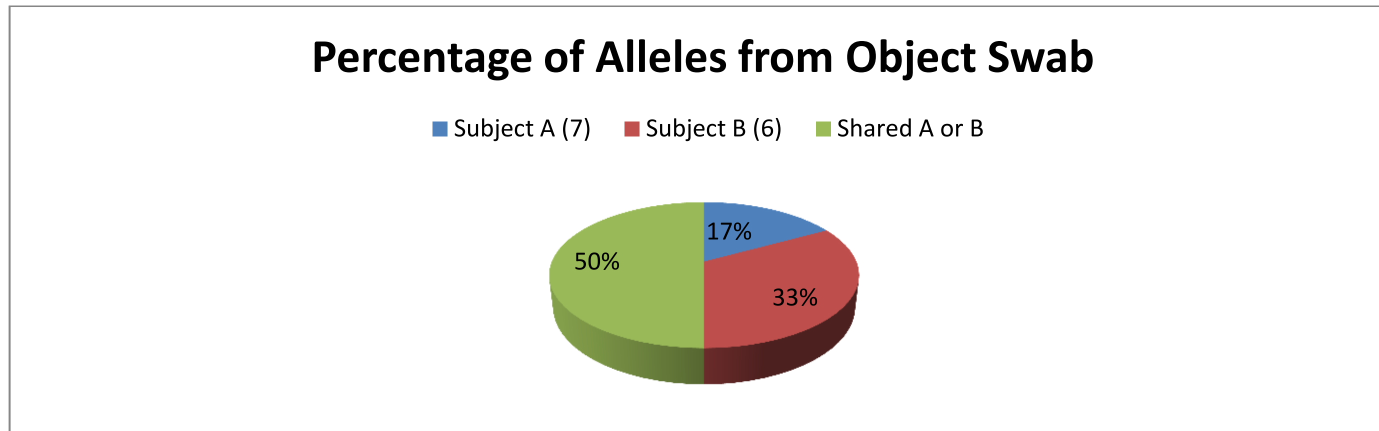


Figure A5.7 Percentage of alleles originating from Subject A or B recovered from Object swab (Grouping of Donor 7 and 6).

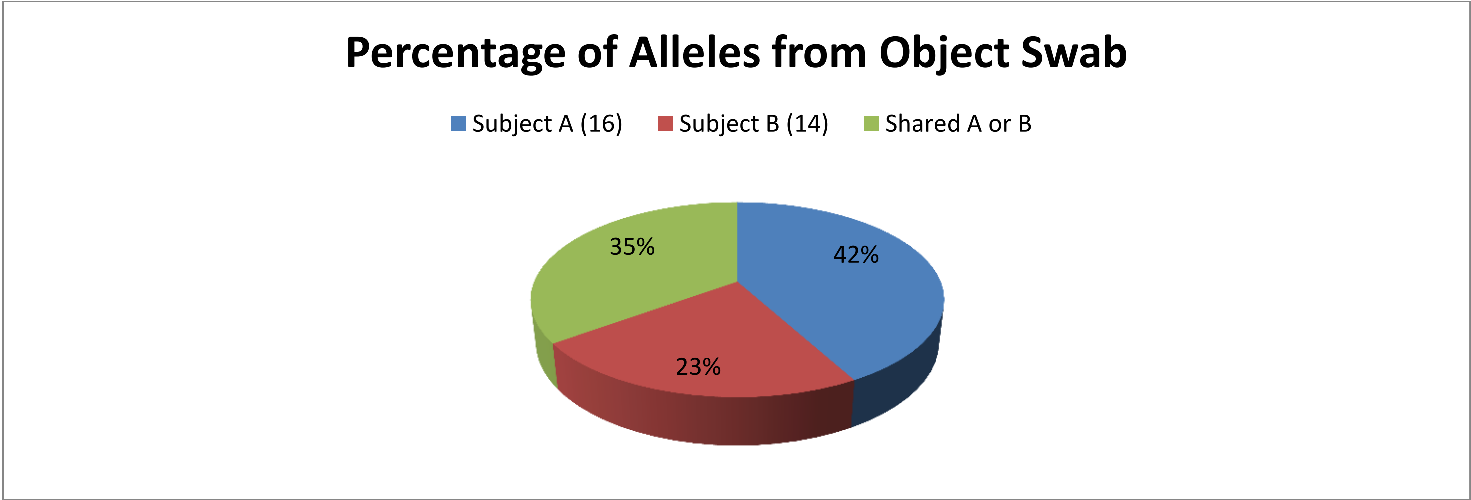


Figure A5.8 Percentage of alleles originating from Subject A or B recovered from Object swab (Grouping of Donor 16 and 4).

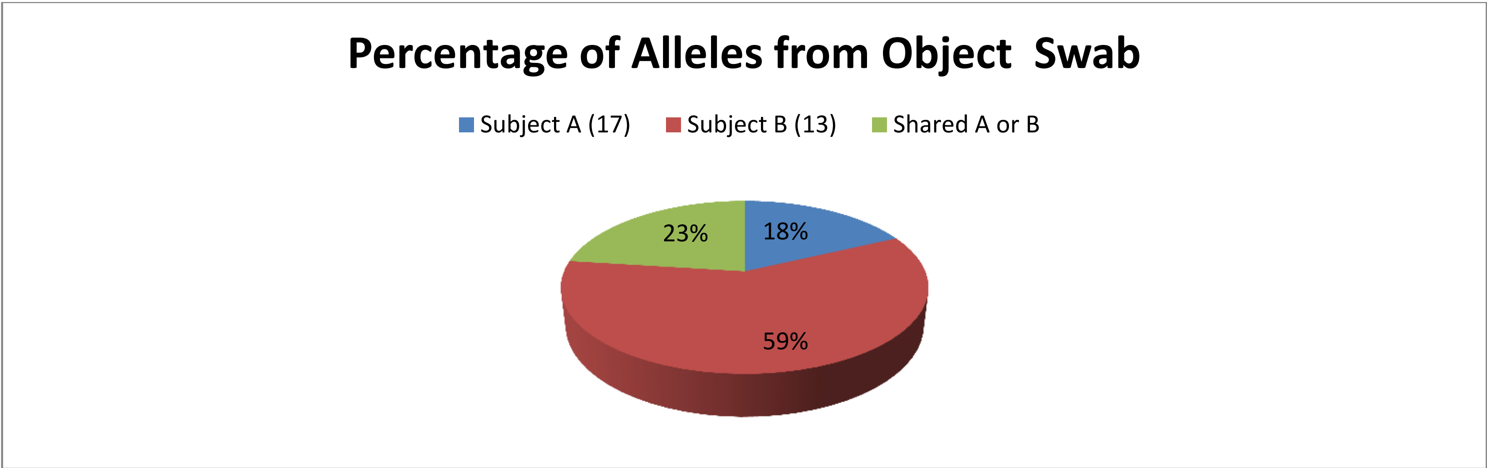


Figure A5.9 Percentage of alleles originating from Subject A or B recovered from Object swab (Grouping of Donor 17 and 13).

It was also possible to determine the potential for the persistence of DNA from other sources on an individual's hand after subsequent contact with an object by examining the percentage of alleles present on Subject B's hand, after contact with both Subject A and then the object. These results are summarised in Figures A5.10 – A5.12.

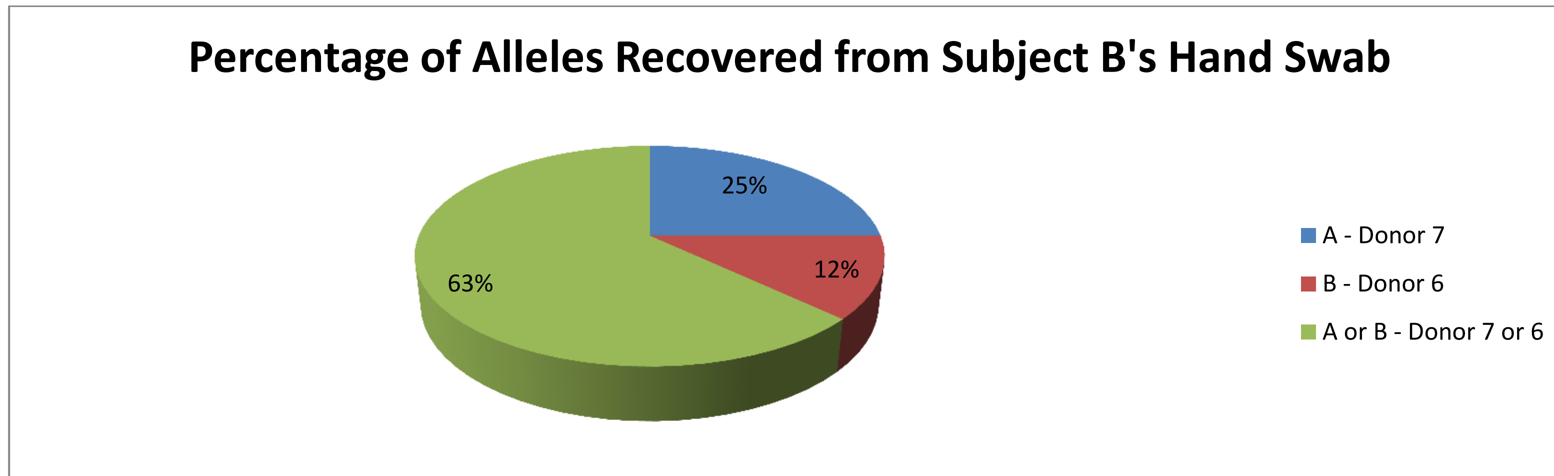


Figure A5.10 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 7 and 6).

Figure A5.11 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 16 and 4).

Figure A5.12 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 17 and 13).

A5.3 Daily Repetitions of Persistence and Secondary Transfer of DNA with Person as Vector for Transfer and Object as Final Substrate (Subject A↔ Subject B → Object)

Key to labelling of samples in table:

A signifies samples taken on day 1; B signifies samples taken on day 2, and so on.

9RTBO – Object Swab from Object handled with Right Hand; Subject A → Subject B → Object

Alleles highlighted in Red are those from Subject A. Alleles highlighted in Blue are those from Subject B. Alleles highlighted in Green are those that could be from either Subject A or B.

Table A5.7 Object Swabs from daily repeats of Secondary Transfer (Subject A↔ Subject B → Object)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
9RTBOA		16			XY	16		14	14	7	
9RTBOB	19					14				7	
9RTBOC	16, 17, 19	17			X	11, 14	28, 34		15.2	8	20
9RTBOD	17					12	30		14, 16.2		
9RTBOE	14		9			9, 15, 16			12, 13.2		
9RTBOF	18	17, 24				13	28, 29.2, 30, 31.2	14	12, 17.2	7, 8	
Subject A	14, 17	16, 18	12, 13	20, 23	XX	12, 14	30, 30	17, 20	14, 14	9, 9.3	20, 22
Subject B	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22

A5.4 Persistence and Secondary Transfer of DNA with Person as Vector for Transfer and Object as Final Substrate (Object 1 ←Subject A↔ Subject B → Object 2)

Alleles highlighted in Red are those from Subject A. Alleles highlighted in Blue are those from Subject B. Alleles highlighted in Green are those that could be from either Subject A or B.

Table A5.8 DNA Profiles recovered from hand swabs from Donor 11 and Donor 12 and Object 1 and 2 swabs.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 12	**14, 15**	**17, 17**	**10, 11**	**17, 20**	**XY**	**10, 13**	**30, 30**	**11, 20**	**13, 15**	**8, 9.3**	**19, 22**
Donor 11	**15, 18**	**17, 17**	**11, 13**	**17, 18**	**XX**	**13, 14**	**28, 31.2**	**10, 16**	**14, 15**	**6, 9**	**20, 20**
Donor 12 Hand Swab					Y					8, 9.3	
Object 1	-										
Donor 11 Hand Swab	15						30				21
Object 2	-										

Table A5.9 DNA Profiles recovered from hand swabs from Donor 5 and Donor 15 and Object 1 and 2 swabs.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 5	**14, 15**	**18, 19**	**12, 13**	**16, 23**	**XX**	**11, 12**	**27, 33.2**	**13, 14**	**13, 14**	**6, 9.3**	**20, 21**
Donor 15	**13, 17**	**16, 16**	**10, 13**	**18, 25**	**XY**	**14, 16**	**30, 31**	**13, 17**	**12, 16**	**9.3, 9.3**	**20, 22**
Donor 5 Hand Swab		18, 19			XX		33.2		13	6	21
Object 1	-										
Donor 15 Hand Swab					XY		27, 31.2		13		
Object 2							24, 29			5, 7, 8	

Table A5.10 DNA Profiles recovered from hand swabs from Donor 13 and Donor 14 and Object 1 and 2 swabs.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 14	**16, 16**	**16, 18**	**11, 12**	**17, 17**	**XX**	**14, 15**	**28, 30**	**13, 16**	**14, 14**	**7, 9.3**	**21, 22**
Donor 13	**15, 16**	**17, 17**	**9, 13**	**21, 23**	**XY**	**12, 14**	**28, 29.2**	**14, 16**	**12, 14**	**7, 7**	**20, 22**
Donor 14 Hand Swab	16, 18				XX		28		12, 14	6	
Object 1		17	12, 13	16, 21, 23	XX	14		14, 16			20
Donor 13 Hand Swab	16, 18	15, 16, 17	9, 13	21, 23		14	28, 29.2	14, 16	14	7	20, 22
Object 2					X						

A5.5 Persistence and Secondary Transfer of DNA with Object as Vector for Transfer (Subject A→ Object → Subject B)

In each of the tables, alleles originating from Subject A in the pairing are highlighted in red, alleles originating from Subject B in the pairing are highlighted in blue and alleles that could have originated from Subject A or B in the pairing (i.e. common alleles between the two subjects) are highlighted in green.

Table A5.11 DNA Profiles recovered from hand swab from Donor 6, Object Swab and hand swab from Donor 7.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 7	**10, 12**	**17, 20**	**15, 16**	**11, 13**	**10, 11**	**12, 13**	**8, 11**	**13, 13**	**12, 14**	**14, 14**	**30, 31.2**	**21, 23**	**6, 9**	**8, 11**	**17, 19**	**XX**
Subject A Hand	12, 12	17, 20	14, 15	11, 13	11, 11	13, 14	8, 11	12, 13, 14	12, 14	14, 14	30, 31.2	18, 19	6, 7	8, 11	16, 16	XX
Object	10, 10	16, 16	14, 18	11, 11		11, 13, 14	14, 14			13, 14	28, 28		9.3, 9.3		15, 16	XY
Subject B Hand	10, 10		16, 16			11, 12, 13		12, 12			30, 30			11, 12		XX
Donor 6	**10, 10**	**17, 24**	**16, 18**	**11, 13**	**10, 10**	**11, 12**	**11, 14**	**12, 12**	**14, 14**	**12, 14**	**28, 28**	**20, 21**	**6, 9.3**	**11, 12**	**17, 18**	**XX**

Table A5.12 DNA Profiles recovered from hand swab from Donor 4, Object Swab and hand swab from Donor 16.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 16	**11, 12**	**18, 20**	**15, 17**	**13, 13**	**10, 11**	**14, 14**	**12, 12**	**11, 13**	**11, 16**	**14, 15.2**	**28, 30**	**22.2, 23**	**9.3, 9.3**	**8, 11**	**15, 16**	**XY**
Subject A Hand	11, 12	18, 20	15, 17	13	10, 11	13, 14	12	11, 13	11, 16	14, 15.2	28, 30	22.2, 23	9.3	8, 11	15, 16	XY
Object		20, 20	13, 15	12, 13		14, 14	12, 12		11, 13, 16	14, 15	30, 30	22.2, 23	9.3, 9.3		14, 15 16	XY
Subject B Hand	10, 10	19, 19	15, 16	11, 11	12, 12		9, 12	9, 11	12, 15, 20	11, 13, 14	27, 27	23, 26	9, 9	11, 11	15, 17	XX
Donor 4	**10, 11**	**19, 24**	**16, 16**	**11, 13**	**8, 12**	**13, 13**	**9, 12**	**9, 11**	**15, 20**	**11, 14**	**27, 30**	**23, 26**	**6, 9**	**8, 11**	**15, 17**	**XX**

Table A5.13 DNA Profiles recovered from hand swab from Donor 4, Object Swab and hand swab from Donor 17.

Samples	CSF1P0	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	XY
Donor 17	**11, 12**	**20, 23**	**14, 17**	**11, 11**	**11, 13**	**12, 14**	**10, 12**	**12, 13**	**17, 20**	**14, 14**	**30, 30**	**20, 22**	**9, 9.3**	**9, 11**	**16, 18**	**XX**
Subject A Hand	12, 12		13, 14, 15	11, 11		8, 13, 14		9, 9	20, 20	10, 11, 15				11, 11	15, 16, 18	XY
Object	12, 13	21, 22, 23	14, 15 16, 18	11, 12	9, 11	12, 13	12, 12	13, 13	16, 16	12, 14	28, 29.2	20, 22 24		8, 8	17, 17	XY
Subject B Hand	12, 13	21, 23	14, 15 16, 17	12, 12	9, 11	12, 14	12, 12	9, 11, 13	14, 16	12, 14	28, 29.2	19, 20 22	7, 7	8, 8	17, 17	XY
Donor 13	**12, 13**	**21, 23**	**15, 16**	**12, 12**	**9, 11**	**12, 14**	**12, 12**	**9, 13**	**14, 16**	**12, 14**	**28, 29.2**	**20, 22**	**7, 7**	**8, 8**	**17, 17**	**XY**

Table A5.14 DNA Profiles recovered from hand swab from Donor 11, Object Swab and hand swab from Donor 12.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 12	**14, 15**	**17, 17**	**10, 11**	**17, 20**	**XY**	**10, 13**	**30, 30**	**11, 20**	**13, 15**	**8, 9.3**	**19, 22**
Donor 11	**15, 18**	**17, 17**	**11, 13**	**17, 18**	**XX**	**13, 14**	**28, 31.2**		**14, 15**	**6, 9**	**20, 20**
Donor 12 Hand Swab	-										
Object					Y						
Donor 11 Hand Swab	15, 18		13		XX	14					

Table A5.15 DNA Profiles recovered from hand swab from Donor 11, Object Swab and hand swab from Donor 12.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 5	**14, 15**	**18, 19**	**12, 13**	**16, 23**	**XX**	**11, 12**	**27, 33.2**	**13, 14**	**13, 14**	**6, 9.3**	**20, 21**
Donor 15	**13, 17**	**16, 16**	**10, 13**	**18, 25**	**XY**	**14, 16**	**30, 31**	**13, 17**	**12, 16**	**9.3, 9.3**	**20, 22**
Donor 5 Hand Swab	14, 15	18, 19	11, 12, 13	16, 23	XY	11, 12	27, 33.2	13, 14	13, 14	6, 9.3	20, 21
Object	-										
Donor 15 Hand Swab	13, 17	16	10	18		12, 13, 15	30, 31	13	15, 16		20, 22

Table A5.16 DNA Profiles recovered from hand swab from Donor 13, Object Swab and hand swab from Donor 14.

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 14	**16, 16**	**16, 18**	**11, 12**	**17, 17**	**XX**	**14, 15**	**28, 30**	**13, 16**	**14, 14**	**7, 9.3**	**21, 22**
Donor 13	**15, 16**	**17, 17**	**9, 13**	**21, 23**	**XY**	**12, 14**	**28, 29.2**	**14, 16**	**12, 14**	**7, 7**	**20, 22**
Donor 14 Hand Swab	16	18			XX	12	30		13, 14	9.3	
Object	16, 18				XX	14	28, 30				
Donor 13 Hand Swab	14, 15, 16	17, 17	9, 13	21	XXY	12, 13, 14	28, 29.2	14, 16	12, 14	7, 7, 8	20, 22

In this experiment Subject A held an object (glass beaker) followed by Subject B then handling the same object. Subject A's hand was swabbed after contact with the object, Subject B's hand was swabbed after contact with the object and the object was swabbed after contact with Subject B. The results of the DNA recovered from Subject B's hand swabs are summarised in Figures A5.13 – A5.18.

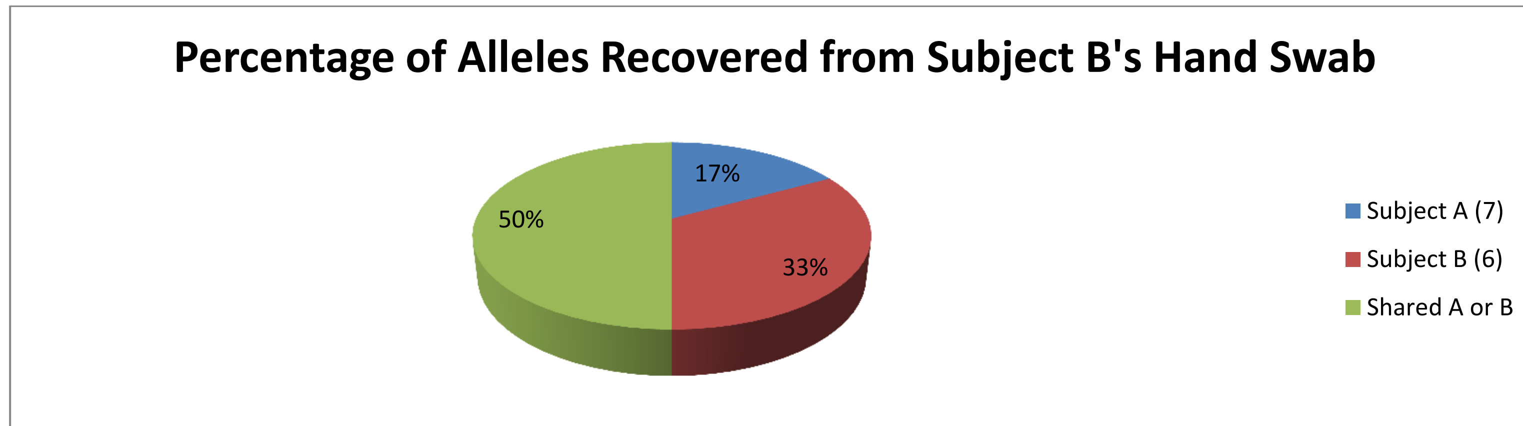


Figure A5.13 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 7 and 6).

Figure A5.14 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 16 and 4).

Figure A5.15 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 17 and 13).

Figure A5.16 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 11 and 12).

Figure A5.17 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 5 and 15).

Figure A5.18 Percentage of alleles recovered from the hand swab of Subject B (grouping of Donors 14 and 13).

It was also possible to determine the potential for persistence of DNA on an object by examining the percentage of alleles present on the object swab, after contact with both Subject A and then Subject B. These results are summarised in Figures A5.19 –A5.22.

Figure A5.19 Percentage of alleles recovered from the object swab after contact with Subject A and B (grouping of Donors 7 and 6).

Figure A5.20 Percentage of alleles recovered from the object swab after contact with Subject A and B (grouping of Donors 16 and 4).

Figure A5.21 Percentage of alleles recovered from the object swab after contact with Subject A and B (grouping of Donors 17 and 13).

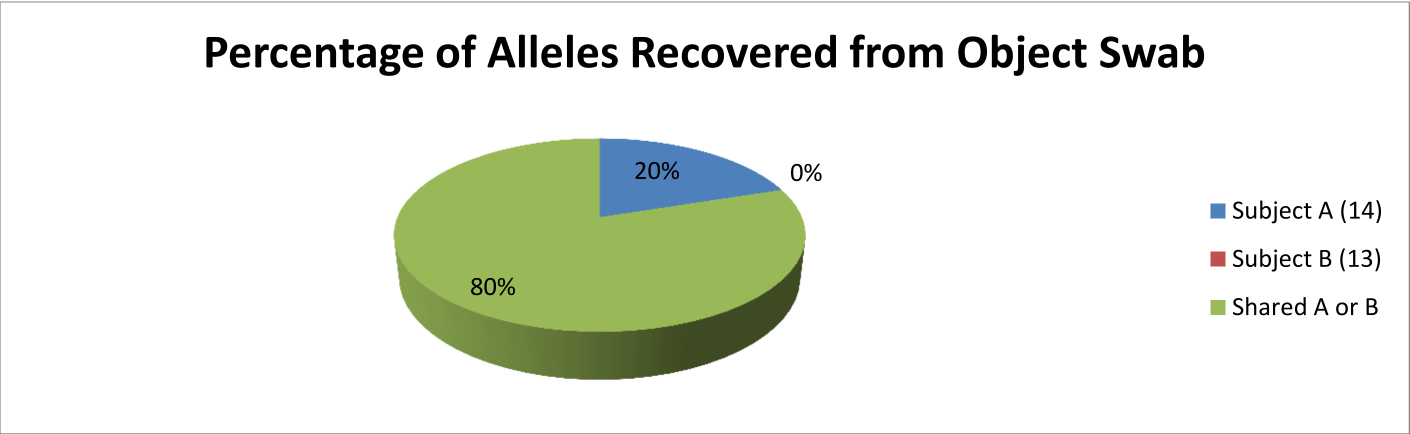


Figure A5.22 Percentage of alleles recovered from the object swab after contact with Subject A and B (grouping of Donors 14 and 13).

A5.6 Daily Repetitions of Persistence and Secondary Transfer of DNA with Object as Vector for Transfer (Subject A→ Object → Subject B)

Key to labelling of samples in table:

A signifies samples taken on day 1; B signifies samples taken on day 2, and so on.

9LTOB – Object Swab from Object handled with Left Hand,

Left Hand, Subject A → Object → Subject B

Alleles highlighted in Red are those from Subject A.

Alleles highlighted in Blue are those from Subject B.

Alleles highlighted in Green are those that could be from either Subject A or B.

Table A5.16 Object Swabs from daily repeats of Secondary Transfer (Subject A→ Object → Subject B)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
9LTOBA											
9LTOBB	14, 15, 16, 19	15, 16, 17, 18, 24	9		XY	10, 11, 14, 16	28, 29.2, 34, 37	14, 16, 20	12, 14, 14.2, 15.2	7, 8, 9	20, 29
9LTOBC	16	17	9		XY	11, 16	27, 28, 34, 36	16	11, 14, 15.2	7, 8	20, 22, 29
9LTOBD	14, 15, 17, 18, 19	16, 18, 19	12		X	9, 10, 12, 13, 14, 16	24, 24.2, 30, 31, 34, 36	17, 20	13.2, 14, 16.2	7, 9, 9.3	20, 22, 28, 29, 46.2
9LTOBE	16, 17, 18				XY	11, 12, 16			11, 14, 15.2, 16.2	9.3	
9LTOBF	14, 15, 17, 19	16, 17, 18	12		X	9, 12, 14, 15, 16	30		12, 13.2, 14, 16.2	7, 8, 9, 9.3	
Subject A	14, 17	16, 18	12, 13	20, 23	XX	12, 14	30, 30	17, 20	14, 14	9, 9.3	20, 22
Subject B	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22

A5.7 Secondary Transfer of DNA with Time Delay

Key to labelling of samples in table:

Right Hand Secondary Transfer (Subject A ↔Subject B; 30 min delay then both Subject A and B hold object)

1AR30Ba – Subject A Object swab after 30 min, Repeat (a).

1AR30Ha – Subject A Hand swab after 30 min, Repeat (a).

1BR30Ba – Subject B Object swab after 30 min, Repeat (a).

1BR30Ha – Subject B Hand swab after 30 min, Repeat (a).

Alleles highlighted in Blue are those from Subject A.

Alleles highlighted in Red are those from Subject B.

Alleles highlighted in Green are those that could be from either Subject A or B.

Table A5.17 DNA Profiles of Time Delay Secondary Transfer hand swabs (Repeat a)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
-ve ABa					XY						
1AR30Ba		14, 16	9, 10	20, 23	XY						
1AR30Ha	15	14, 15, 17			XY	12, 13, 14, 15			12, 13, 14	6, 7	
-ve BBa											
1BR30Ba	15	14, 15, 16, 17			XY	11, 12, 13, 14, 15			12, 13, 14	6, 7	
1BR30Ha	15, 16	14, 17			XY	13					
Subject A	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22
Subject B	15, 15	14, 15	11, 11	18, 23	XX	13, 15	29, 32.2	15, 16	14, 14	6, 6	19, 21

Table A5.18 DNA Profiles of Time Delay Secondary Transfer hand swabs (Repeat b)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
-ve ABb											
1AR30Bb	15	14, 15, 16, 17	9		XY	11, 12, 13, 14, 15	28		12, 13, 14	6, 7	
1AR30Hb	14, 15, 16	15, 16, 17	9, 11	23	XY	12, 13, 14, 15	28, 29.2, 32.2		12, 13, 14	6, 7, 9.3	
-ve BBb											
1BR30Bb	15	14, 15, 17			XY	12, 13, 14, 15			12, 13, 14	6, 7	
1BR30Hb	15	14, 15, 16, 17	9, 11		XY	12, 14	28		12, 13, 14	6, 7	
Subject A	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22
Subject B	15, 15	14, 15	11, 11	18, 23	XX	13, 15	29, 32.2	15, 16	14, 14	6, 6	19, 21

Table A5.19 DNA Profiles of Time Delay Secondary Transfer hand swabs (Repeat c)

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
-ve ABc											
1AR30Bc	14, 15	14, 15, 16, 17	9		XY	13, 15			12, 13, 14, 15	6, 7	
1AR30Hc	13, 15	13, 14, 15, 16, 17			XY	12, 13, 14, 15			12, 13, 14	6, 7	
-ve BBc		15, 18								6	
1BR30Bc	15	14, 15, 16, 17	9		XY		28		12, 13, 14	6, 7	
1BR30Hc	12, 13, 14, 15	14, 15, 17	11		XY	13, 15			13, 14	6, 7	
Subject A	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	20, 22
Subject B	15, 15	14, 15	11, 11	18, 23	XX	13, 15	29, 32.2	15, 16	14, 14	6, 6	19, 21

In this experiment Subject A shook hands with Subject B. After 30 minutes of 'regular' activity, during which the participants were asked not to contact any other individuals or wash their hands, both Subject A and B handled an object. After this contact, both the objects and the Subjects' hands were swabbed. The results of the DNA recovered from Subject A and B's hand swabs and object swabs are summarised in Figures A5.23 and A5.24.

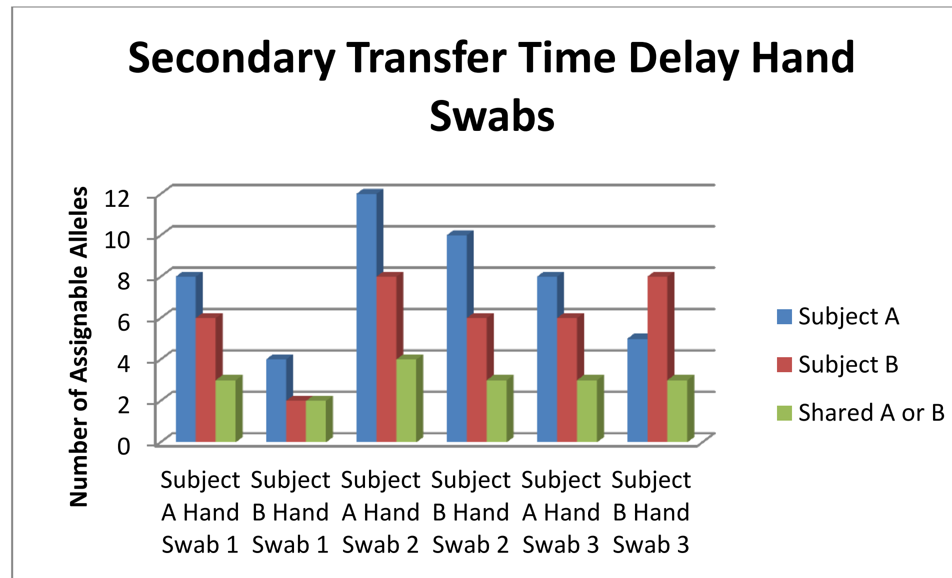


Figure A5.23 Number of alleles originating from Subject A or B recovered from Hand Swabs 30 minutes after initial contact and immediately after handling an object.

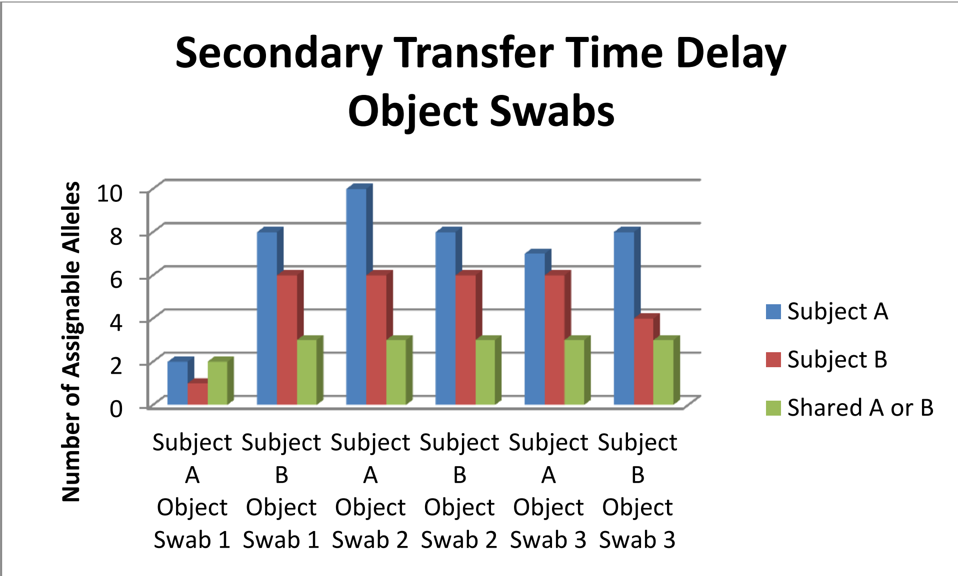


Figure A5.24 Number of alleles originating from Subject A or B recovered from Object Swabs where object was handled 30 minutes after initial contact.

Appendix Six – DNA Recovery from Cartridge Cases

A6.1 Firearms and Ammunition Information

Staffordshire Police

Table A6.1 Staffordshire Police Firearms Unit: Weapons and Ammunition.

Weapon	Ammunition
Sig Sauer Handgun Model P228	Radway Green 9mm Centre Fire 95 Grain
Sig Sauer Rifle Model 551	Remington Express .223 Centre Fire 55 Grain
Blaser Rifle Model R93 LRS 2	Remington Express .308 Centre Fire 180 Grain

Figure A6.1 (a), (b) and (c). (a) Sig Sauer Handgun Model P228. (b) Blaser Rifle Model R93 LRS 2. (c) Sig Sauer Rifle Model 551.

Nottinghamshire Police

Table A6.2 Nottinghamshire Police Firearms Unit: Weapons and Ammunition.

Weapon	**Ammunition**
Walther P990 Self-loading Pistol	Magtech 9mm Luger Centre Fire 95 Grain
Remington 870 Wingmaster Shotgun 12 Gauge	Interstate Shotgun Cartridges 00 Buckshot 9 Ball (8.43mm Diameter)
Accuracy International (A.I) 7.62mm Rifle	Lapua .308 Win Scenar 167 Grain

(a)

(b)

(c)

Figure A6.2 (a), (b) and (c). (a) Walther P990 Pistol. (b) Remington 870 Wingmaster Shotgun. (c) Accuracy International (A.I) 7.62mm Rifle.

Strathclyde Police

Table A6.3 Strathclyde Police Firearms Unit: Weapons and Ammunition.

Weapon	**Ammunition**
Taurus .22 Revolver	ELEY .22RF Long Rifle
Beretta .32 Self Loading Pistol Model 81	Winchester .32 ACP
Beretta 9mm SLP Model 92FS	Samson 9mm Luger Para CF
Smith & Wesson 357 Magnum Revolver	Winchester Western .38 Special Pistol-Revolver

Figure A6.3 (a), (b), (c) and (d). (a) Taurus .22 Revolver. (b) Beretta .32 Self Loading Pistol Model 81. (c) Smith & Wesson 357 Magnum Revolver. (d) Beretta 9mm SLP Model 92FS.

Imitation Firearms and Blank Ammunition

Staffordshire University

All firing of non-live ammunition and weapons was carried out in the laboratory facilities at Staffordshire University.

Table A6.4 Staffordshire University: Weapons and Ammunition.

Weapon	Ammunition
Revolver	NC-KNALL Kal. 9mmx17/.380
Semi-Automatic	NC-KNALL Kal. 9mmx17/.380

Figure A6.4 (a) and (b). (a) 9mm.k Webley Cal 380 Olympic 38 Revolver. (b) 9mm Weihrauch Sportwaffenfabrik Semi-Automatic weapon and magazine.

Cartridge Storage

Storage devices for holding spent cartridge cases were designed in order to maximise the potential for retrieving the optimal amount of DNA from the collected cartridge case. The storage device was designed to suspend the cartridge case so it was not in contact with any other surface, making it less likely that DNA would be transferred onto the surrounding surfaces.

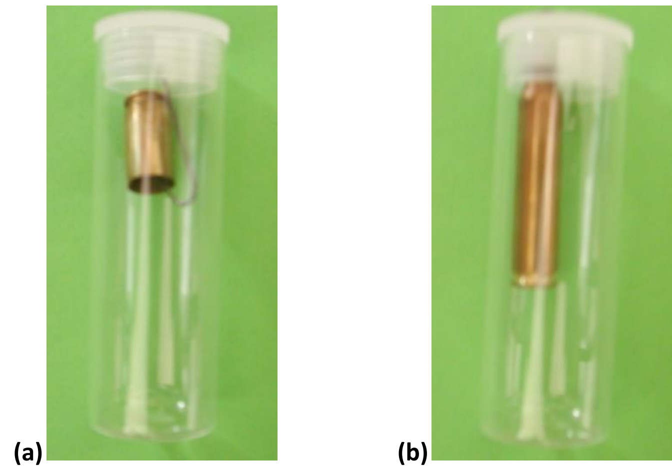


Figure A6.5 Storage Devices for Spent Cartridge Cases. (a) Mechanism for storing smaller calibre cartridge cases. (b) Mechanism for storing larger calibre cartridge cases.

A6.2 Saliva Seeded Unfired Cartridge Cases

Table A6.5 DNA profiles recovered from 9mm cartridge cases seeded with saliva (unfired) – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
9mm.1	15, 16, 17, 19	14, 15, 16, 17	9, 11	18, 19, 24	XY	14, 15, 16	26, 28, 29, 30	15, 16	11, 14.2, 15, 15.2	6, 7, 8, 9, 9.3	21, 22, 23, 27
9mm.2	15, 16, 19	15, 17	11	19	XY	14, 15	28, 35, 36	15, 16	14, 15, 15.2	6, 7, 8, 9.3	21, 22, 23
9mm.3	15, 16	17	11, 13	19	XY	14, 15	26, 28	15, 16	14, 15	7, 9, 9.3	23
9mm.4	14, 15, 16	16, 17	9, 11	18, 19, 24	XY	14, 15, 16	26, 28, 29, 30	15, 16	15	6, 7, 8, 9, 9.3	21, 22, 23, 27
9mm.5	15, 16	15, 17	11, 12, 13	19	XY	14, 15	28	15, 16	14, 15	6, 7, 9.3	21
9mm.6	15, 16	17	9, 11, 13	19	XY	14	28, 30	15, 16	14	7	21, 22
9mm.7	15, 16, 17, 19	16, 17	9, 11	18, 19, 24	XY	14, 15, 16	26, 28, 29, 30	15, 16	15, 15.2	6, 7, 8, 9, 9.3	21, 22, 23, 27
9mm.8	15, 16, 19	15, 17	11	19	XY	14, 15	28, 35, 36	15, 16	14, 15, 15.2	7, 9.3	21, 22, 23
9mm.9	15, 16, 17	16, 17	11, 13	18, 19	X	14, 15, 16	28, 30, 26	15, 16	15.2	7, 8, 9	21
9mm.10	15, 16, 17	14, 15, 16, 17	9, 11	18, 19, 24	XY	14, 15, 16	26, 28, 29, 30	15, 16	14.2, 15, 15.2	6, 7, 9, 9.3	21, 22, 23, 27

Table A6.6 DNA profiles recovered from Rem 223 cartridge cases seeded with saliva (unfired) – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
Rem223.1	15, 16	17	11, 13	19, 24	XY	14, 15	28, 30, 36	15, 16, 18	14, 15	6, 7, 8, 9.3	21, 22, 27
Rem223.2	15, 16, 19	15, 17	11, 13	16, 19, 24	XY	11, 14, 15	28, 30, 35, 36	15, 16	11, 14, 15, 15.2	7, 8, 9.3	21, 22, 23
Rem223.3	15, 16	16, 17	11, 13	19, 24	XY	11, 14, 15	28, 30	14, 15, 16	14, 15	7, 9.3	21, 22
Rem223.4	15, 16, 17	17	11, 13	19, 23, 24	XY	14, 15	28, 30, 35, 36	15, 16	14, 15	7, 9, 9.3	21, 22
Rem223.5	15, 16	17	11, 13	19	XY	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22, 23
Rem223.6	15, 16	17	11, 13	19, 24	XY	14, 15	28, 30	15, 16	12, 14, 15	7, 8, 9.3	21, 22
Rem223.7	15, 16, 19	15, 16, 17	11, 13	19, 24	XY	14, 15	28, 30, 36	14, 15, 16, 17	13, 14, 15	6, 7, 8, 9.3	21, 22, 23
Rem223.8	15, 16, 19	17	11, 13	16, 19, 24	XY	14, 15	28, 30	15, 16	14, 15	7, 8, 9.3	22
Rem223.9	15, 16	17	11, 13	19, 24	XY	14, 15	28	15, 16	14, 15, 15.2	7, 9.3	21, 22
Rem223.10	15, 16	17	11, 13	19	XY	14, 15	28, 35, 36	15, 16	14, 15	7, 9, 9.3	21, 22

Table A6.7 DNA profiles recovered from Rem 308 cartridge cases seeded with saliva (unfired) – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
Rem308.1	15, 16	17	13		XY	11, 14, 15	28, 30, 35, 36	16	11, 14, 14.2, 15	7, 8, 9, 9.3	16, 21, 22
Rem308.2	15, 16	17	9, 11, 13	19, 24	XY	11, 14, 15	28, 30, 35	16, 18, 23	11, 14, 14.2, 15, 15.2	7, 8, 9.3	21, 22
Rem308.3	15, 16	17	11, 13	19, 24	XY	11, 14, 15,	28, 30, 35	15, 16	14, 15	7, 9, 9.3	21, 22
Rem308.4	15, 16	17	11, 13	19, 24	XY	14, 15	28, 30	15, 16	11, 14, 15	7, 9.3	21, 22
Rem308.5	15, 16	15, 16, 17	9, 11, 12, 13	19, 24	XY	14, 15	28, 30	15	14, 14.2, 15	7, 8, 9, 9.3	21
Rem308.6	15, 16	17	9, 11, 12, 13	19, 24	XY	13, 14, 15	28, 29, 30	16	14, 15	7, 9.3	21, 22
Rem308.7	15, 16	17	11, 13	19, 24	XY	14, 15	28, 30	15, 16	14, 15	7, 9.3	16, 22
Rem308.8	15, 16	15, 17	9, 11, 12, 13	19, 24	XY	14, 15, 16	28, 30, 35, 36	15, 16	14, 14.2, 15, 15.2	7, 8, 9, 9.3	21, 22
Rem308.9	15, 16	17	11, 13	19, 24	XY	14, 15	28, 30	15, 16	14, 15	7, 9.3	22
Rem308.10	15, 16	17	11, 13	19, 24	XY	14, 15	28, 30	15, 16	14, 15	7, 8, 9.3	22

Table A6.8 DNA profiles recovered from 762mm cartridge cases seeded with saliva (unfired) – Donor 2.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 2	16, 16	17, 17	9, 13	22, 23	X, Y	14, 15	29, 31.2	14, 16	12, 14	6, 9.3	20, 21
762mmA.1	16	17	9, 13	22, 23	XY	14, 15	28, 29, 30, 31.2	14, 15, 16	12, 14	6, 9.3	20, 21
762mmA.2	15, 16	16, 17	9, 11, 13	22, 23	XY	14, 15	29, 30, 31.2	14, 15, 16	11, 12, 14	6, 8, 9, 9.3	20, 21, 22
762mmA.3	16, 19	17	9, 13	22, 23	XY	14, 15	29, 31.2	14, 16	12, 14	6, 9, 9.3	20, 21
762mmA.4	16	17	9, 11, 12, 13	22, 23	XY	14, 15	29, 31.2	14, 16	12, 14	6, 8, 9, 9.3	20, 21
762mmA.5	16	17	9, 11, 13	22, 23	XY	14, 15	29, 30, 31.2	14, 16	12, 14	6, 9.3	20, 21
762mmA.6	16, 19	17	9, 11, 13	22	XY	14, 15	28, 29, 31.2	14, 16	12, 14	6, 8, 9.3	20, 21
762mmA.7	16	17	9, 13	22, 23	XY	14, 15	28, 29, 30, 31.2	14, 15, 16	12, 14	6, 9.3	20, 21, 22
762mmA.8	16	17	9, 11, 13	22, 23	XY	14, 15	28, 29, 31.2	14, 16	12, 14	6, 9, 9.3	21
762mmA.9	16	17	9, 13	22, 23	XY	14, 15	29, 31.2	14, 16	12, 14	6, 9.3	20, 21
762mmA.10	16	17	9, 13	22, 23	X	14, 15	29, 31.2	14, 16	12, 14	6, 9.3	20, 22

Table A6.9 DNA profiles recovered from P990 cartridge cases seeded with saliva (unfired) – Donor 2.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 2	**16, 16**	**17, 17**	**9, 13**	**22, 23**	**X, Y**	**14, 15**	**29, 31.2**	**14, 16**	**12, 14**	**6, 9.3**	**20, 21**
P990A.1	**16**	15, **17**	**9,** 11, **13**	21, **22, 23**	**XY**	**14, 15**	**29, 31.2**	**14, 16**	**12, 14**	**6,** 8, 9, **9.3**	**20, 21**
P990A.2	**16**	**17**	**9, 13**	**22, 23**	**XY**	**14, 15**	**29, 31.2**	**14, 16**	11, **12, 14**	**6,** 8, **9.3**	**20, 21**
P990A.3	**16**	**17**	**9, 13**	**22**	**XY**	**14, 15**	**29, 31.2**	**14, 16**	**12, 14**	**6, 9.3**	**20, 21,** 22
P990A.4	**16**	**17**	**9, 13**	18, **22, 23**	**XY**	**14, 15**	**29, 31.2**	**14, 16**	**12, 14**	**6,** 8, **9.3**	**20, 21**
P990A.5	15, **16**	15, 16, **17**	**9,** 11, **13**	21, **22, 23**	**XY**	**14, 15,** 16	**29,** 30.2, **31.2**	**14,** 15, **16,** 18	11, **12,** 13, **14**	5.3, **6,** 9, **9.3**	19, **20, 21,** 22
P990A.6	**16**	**17**	**9, 13**	**22, 23**	**XY**	**14, 15**	**29, 31.2**	**14, 16**	**12, 14**	**6, 9.3**	**20, 21**
P990A.7	**16**	15, **17**	**9, 13**	**22, 23**	**XY**	**14, 15**	**29, 31.2**	**14, 16**	**12, 14**	**6,** 7, **9.3**	**20,** 22
P990A.8	**16**	**17**	**9,** 11	18, **22**	**XY**	**14, 15**	**29, 31.2**	**14, 16**	**12, 14**	**6, 9.3**	**20**
P990A.9	**16**	**17**	**9, 13**	**22, 23**	**XY**	**14, 15**	**29, 31.2**	**14, 16**	**12, 14**	**6, 9.3**	**20, 21**
P990A.10	**16**	**17**	**9, 13**	**22, 23**	**XY**	**14, 15**	**29, 31.2**	**14, 16**	**12, 14**	**6,** 8, 9, **9.3**	19, **20, 21,** 22

Table A6.10 DNA profiles recovered from 762mm cartridge cases seeded with saliva (unfired) – Donor 3.

Calibre. Repeat number	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 3	14, 15	14, 16	11, 12	22, 24	X, Y	16, 16	28, 30	20, 20	14, 14	8, 8	22.2, 22.2
762MMB.1	14, 15	14, 16	11, 12	22, 24	XY	15, 16	28, 30	20	14	8, 9.3	22.2
762MMB.2	14, 15, 16	14, 15, 16	9, 11, 12	22	XY	16	28, 29, 30	18, 20	14	8	22
762MMB.3	14, 15	14, 16	11, 12	22, 24	XY	16	28, 30	20	14	8	22.2
762MMB.4	14, 15	14, 16	11, 12	22, 24	XY	16	28, 30	20	14	8	22.2
762MMB.5	14, 15, 19	14, 16	11, 12, 13	22, 24	XY	14, 15, 16	28, 29, 30, 32	16, 18, 20	11, 14	8, 9.3	20, 22.2
762MMB.6	14, 15	14, 15, 16	11, 12, 13	22, 24	XY	16	28, 30	20	14	8	22, 22.2
762MMB.7	14, 15	14, 16	11, 12	22, 24	XY	15, 16	28, 30, 36	20	14	8	22.2
762MMB.8	14, 15	14, 16	11, 12	22, 24	XY	16	28, 30	20	14	8	22.2
762MMB.9	14, 15	14, 16	11, 12	22, 24	XY	16	28, 30	20	14	8, 9	22.2
762MMB.10	14, 15	14, 16	11, 12	22, 24	XY	16	28, 30	20	14	8	22.2

Table A6.11 DNA profiles recovered from 762mm cartridge cases seeded with saliva (unfired) – Donor 3.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 3	14, 15	14, 16	11, 12	22, 24	X, Y	16, 16	28, 30	20, 20	14, 14	8, 8	22.2, 22.2
P990B.1	14, 15	14, 15	11, 12	22, 24	XY	15, 16, 18	28, 29, 30	20	14	7, 8, 9	20, 22, 22.2
P990B.2	14, 15	14, 16	11, 13	20, 22, 24	XY	15, 16	28, 30	20	14	8, 9	22.2
P990B.3	14, 15	14, 15, 16	11, 12	22, 24	X	16	28, 30	20	14	8, 9.3	22.2
P990B.4	14, 15	14, 16	11, 12	22	X	16	28, 30	20	14	8	22
P990B.5	14, 15	14, 16	11, 12, 13	22, 24	XY	15, 16	28, 30	20	14	8	20, 22.2
P990B.6	14, 15	14, 16	12	22, 23	XY	16, 18	28, 30	20	14	8	22.2
P990B.7	14, 15	14	11, 12	22, 24	XY	16	28, 30	20	14	8	22.2
P990B.8	14, 15	15, 16	11, 12, 13	20, 22	X	16	28, 29, 30		14	8	22.2
P990B.9	14, 15	14, 16	12		XY	14, 16	28		14	8	
P990B.10	14, 15	14, 16	11, 12	22, 24	XY	16	28, 30	20	14	8	22.2

A6.3 Saliva Seeded Fired Cartridge Cases

Table A6.12 DNA profiles recovered from 9mm cartridge cases seeded with saliva and then fired – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
9mm.1	14, 15, 16, 17	15, 17			X	12, 15	26, 27, 30			9, 9.3	26
9mm.2	12, 17					15					
9mm.3	15, 16, 17				XY						
9mm.4	14					14, 15			13, 14, 15	9.3	
9mm.5	15, 16	17	11		XY						
9mm.6	14, 15, 16	17				14			14		
9mm.7	15, 16	17								9.3	
9mm.8					X	14, 15			13		
9mm.9					XY				14	9.3	
9mm.10	14, 15	15, 17			X	12, 14, 15			14, 15	9	

Table A6.13 DNA profiles recovered from Rem223 cartridge cases seeded with saliva and then fired – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
Rem223.1											
Rem223.2		17	12, 13		X	14	23.2			9	
Rem223.3					X						
Rem223.4	15				X						
Rem223.5	15, 16	17	13		XY	14			14	9.3	
Rem223.6					X	14					
Rem223.7	15, 16, 17	17	11, 13		XY	14, 15	28		14, 15	9.3	
Rem223.8	12	16, 17	11		X					9.3	
Rem223.9	14, 15, 16	17	11, 13		X	14			14	7, 9.3	
Rem223.10	15	17			XY	15			14		

Table A6.14 DNA profiles recovered from Rem308 cartridge cases seeded with saliva and then fired – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
Rem308.1									11	9	
Rem308.2	16				X	11, 15					
Rem308.3									11	9	
Rem308.4	15, 16	17	11		XY	15				7	
Rem308.5	15	17	11		X				11, 14	9, 9.3	
Rem308.6	16		11		XY	14, 15			14	7, 9.3	
Rem308.7	15, 16	17	11, 13		XY	14			14, 15	7, 9, 9.3	
Rem308.8	16	17			X	12			14		
Rem308.9	15				XY						
Rem308.10		17			X				11, 14		

Table A6.15 DNA profiles recovered from 762mm cartridge cases seeded with saliva and then fired – Donor 2.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 2	**16, 16**	**17, 17**	**9, 13**	**22, 23**	**X, Y**	**14, 15**	**29, 31.2**	**14, 16**	**12, 14**	**6, 9.3**	**20, 21**
762mmA.1	**16**	**17**	**9**		**XY**	**14**			**12**	9, **9.3**	
762mmA.2					**X**	**14**			**12, 14**	**9.3**	
762mmA.3	**16**	**17**	**9**		**X**						
762mmA.4	**16**	**17**			**XY**	**14, 15**			**12,** 13, **14**	9, **9.3**	
762mmA.5		**17**			**X**						
762mmA.6					**XY**	11					
762mmA.7	**16**	16, **17**			**XY**	13, **14, 15**					
762mmA.8					**X**	**14**			**12,** 13	**6,** 9**, 9.3**	
762mmA.9	15, **16**	16									
762mmA.10		**17**			**XY**				**12**		

Table A6.16 DNA profiles recovered from P990 cartridge cases seeded with saliva and then fired – Donor 2.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 2	16, 16	17, 17	9, 13	22, 23	X, Y	14, 15	29, 31.2	14, 16	12, 14	6, 9.3	20, 21
P990A.1	16				XY	14			12		
P990A.2	16	17	9, 11		X				12, 14	9, 9.3	
P990A.3					X	14					
P990A.4	16	17							12	9.3	
P990A.5	16	17			XY	14			11, 12, 14	6, 9.3	
P990A.6	16	17			X						
P990A.7						14			12	9	
P990A.8					X	14					
P990A.9	16				X	14					
P990A.10	16	17	9		XY	14			12, 14	6	

Table A6.17 DNA profiles recovered from 762mm cartridge cases seeded with saliva and then fired – Donor 3.

Calibre. Repeat number	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 3	14, 15	14, 16	11, 12	22, 24	X, Y	16, 16	28, 30	20, 20	14, 14	8, 8	22.2, 22.2
762MMB.1	14	14, 16			X	16			14	9	
762MMB.2	14, 15	14, 15, 16	11		XY	16				9.3	
762MMB.3	14	14			X				14		
762MMB.4	14	16			XY	16	28				
762MMB.5	14, 15								14		
762MMB.6		15, 16			X	16					
762MMB.7	14				XY	16					
762MMB.8	14, 15	14, 16	11		XY	16	28, 30		14		
762MMB.9	14	14, 16	11		XY	16				8, 9	
762MMB.10	14, 15				X				14		

Table A6.18 DNA profiles recovered from 762mm cartridge cases seeded with saliva and then fired – Donor 3.

Calibre. Repeat number	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 3	14, 15	14, 16	11, 12	22, 24	X, Y	16, 16	28, 30	20, 20	14, 14	8, 8	22.2, 22.2
P990B.1	14, 15	14, 16	11		XY	16			14		
P990B.2	14	14, 15	11		XY	16	29		14		
P990B.3	14	14			XY				14		
P990B.4						16			14		
P990B.5	14, 15		11								
P990B.6					X				14	9	
P990B.7		14				16					
P990B.8			11		X				14	9.3	
P990B.9	14, 15	14	11, 12		X	16				8	
P990B.10					XY	16					

A6.4Handled Unfired Cartridge Cases

Table A6.19 DNA profiles recovered from handled 9mm cartridge cases (unfired) – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
9mm.1	14, 15, 16, 17	16, 17	11, 13	19, 22, 24	XY	12, 14, 15, 16	28, 30	14, 15	12, 14, 15	7, 9, 9.3	21, 22
9mm.2	14, 15, 16	14, 15, 17	11, 12		XY	14, 15, 16	28	14, 15, 16	12, 13, 14, 15	7, 8, 9.3	20, 22
9mm.3	15, 16	17	11	19, 22	XY	14, 15	28	15	14, 15	7, 9	22
9mm.4	15, 16, 17	15, 17	11, 13		X	14, 15, 16	28, 29, 30	15	14, 15	7, 9.3	21, 22
9mm.5	12, 15	17		19, 22	XY	14, 15	30	15, 16	14, 15	9, 9.3	20
9mm.6	15	17	11		X	14, 15	28, 29, 30, 31.2	14, 15	14, 15	9.3	22

Table A6.20 DNA profiles recovered from handled Rem223 cartridge cases (unfired) – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
Rem223.1	15, 16	15, 17	9, 11	19	XY	14, 15	30	15, 16, 17	12, 13, 14, 15	7, 9.3	20, 21
Rem223.2	15, 16	17	11, 13	19, 22, 24	X	14, 15	28, 30, 30.2	15	14	9.3	
Rem223.3	14, 15, 16	15, 16, 17	9, 10, 11, 13	20, 24	XY	15	29, 30	16	12, 15	7, 9	22
Rem223.4	15, 16	17	11, 13		XY	14, 15	28, 30	20	14, 15	7, 9.3	20, 21, 22
Rem223.5	15, 16, 17	14, 16	13	19, 20	XY	14, 15	27, 28		13, 14	6, 9.3	21, 22
Rem223.6	15, 16	17	11, 13	19	XY	14	28	15, 17	14, 15	9.3	

Table A6.21 DNA profiles recovered from handled Rem308 cartridge cases (unfired) – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
Rem308.1	14, 15, 16	14, 17	10, 13	19, 22	XY	14, 15, 16	28	15	13, 14	9.3	21
Rem308.2	15	17	11, 13	24	XY	15	29, 30.2	15, 20	12, 14	9	
Rem308.3	15, 16	17	9, 11, 13	19, 20	XY	14, 15	28, 29	15	14	9.3	
Rem308.4	15, 17	16, 17	11	19, 24	XY	14, 15	28, 30	15, 16	14, 15	7, 8, 9	
Rem308.5	15, 16	17	11, 13	20, 24	XY	14, 15, 16	30	15, 16, 17	12, 14, 15	7, 9.3	22.2
Rem308.6	15, 16	15, 17	13		XY	15	28, 30	16	14, 15	7	22

Table A6.22 DNA profiles recovered from handled 762mm cartridge cases (unfired) – Donor 2.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 2	16, 16	17, 17	9, 13	22, 23	X, Y	14, 15	29, 31.2	14, 16	12, 14	6, 9.3	20, 21
762mmA.1	16	17	9, 13	23	X	14, 15	30.2 31.2		12	9.3	20
762mmA.2	16	17	9, 11, 13	19, 22, 23, 24	XY	14, 15	29	14	12	9.3	20, 21
762mmA.3	16	17	9, 11	22, 24	X	14, 15, 16	29, 30.2, 31. 2	14, 16	14	6, 9	
762mmA.4	16	17	9, 12	22	XY	14, 15	30	14	12	6	22
762mmA.5	16	17	9, 13	22, 23	XY	14, 15, 16	29, 31.2	16	12, 14		20
762mmA.6	16	17	9	19, 22	XY	14, 15	29		12	8	20

Table A6.23 DNA profiles recovered from handled P990 cartridge cases (unfired) – Donor 2.

Calibre. Repeat number	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 2	16, 16	17, 17	9, 13	22, 23	X, Y	14, 15	29, 31.2	14, 16	12, 14	6, 9.3	20, 21
P990A.1	16	17	9	22	XY	14	29	14, 20	12, 14	9.3	21
P990A.2	16	17	11, 13	20, 22, 23	XY	14, 15, 16	30.2	15, 16	12	9.3	20, 22
P990A.3	16	17	9, 13	22	XY	14, 15	29, 30		12	6	20
P990A.4	16	17	9, 11	20	XY	14, 15	29	14	12		20
P990A.5	16	17	9, 13	22, 23	XY	14, 15	29	14, 20	14		22
P990A.6	16	17	9, 13		XY	14, 15	29, 30		12	8, 9.3	

Table A6.24 DNA profiles recovered from handled 762mm cartridge cases (unfired) – Donor 3.

Calibre. Repeat number	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 3	14, 15	14, 16	11, 12	22, 24	X, Y	16, 16	28, 30	20, 20	14, 14	8, 8	22.2, 22.2
762MMB.1	14, 15	14	11		XY	16	29, 30	20	14	8	20, 22
762MMB.2	14, 15, 16	14, 15, 16, 17	11, 12	22, 23, 24	XY	14, 16	28, 29,30	16, 20	12, 14	8, 9.3	22.2
762MMB.3	16	14, 16	10, 11	22	X	16	28, 30	20	12, 14	7, 8	20, 22.2
762MMB.4	14, 15	14, 15	11	20	XY	16	28, 30	20	14	8	22.2
762MMB.5	14, 15	14, 15, 16	11, 12	22, 23, 24	XY	16	28	20	14	8	22, 22.2
762MMB.6	14, 15	14	10, 12	22	XY	16	28, 29		14		

Table A6.25 DNA profiles recovered from handled 762mm cartridge cases (unfired) – Donor 3.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 3	14, 15	14, 16	11, 12	22, 24	X, Y	16, 16	28, 30	20, 20	14, 14	8, 8	22.2, 22.2
P990B.1	14, 15	14, 16	11	22	XY	16	28	20	14	6, 7, 8	22, 22.2
P990B.2	14, 15, 16	14, 15, 16	11, 12, 13	23, 24	XY	14, 15, 16	28, 29.2	20	12, 14, 15	6, 8	20, 22.2
P990B.3	14, 15	14	11, 13	22, 23, 24	XY	16	28		14	8	22.2
P990B.4	14, 15	14	11, 12	19, 22, 24	XY	16	28, 30, 30.2	20	14	8, 9.3	
P990B.5	14, 15	14, 16			XY	15, 16	28, 29, 30		14	8	22
P990B.6	14, 15	14, 16	11, 12	19, 22	XY	16	28, 30	20	14	8, 9.3	22.2

A6.5 Handled Fired Cartridge Cases

Table A6.26 DNA profiles recovered from 9mm cartridge cases handled and then fired – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
9mm.1	15, 17		11		X					9	
9mm.2			11		XY				12		
9mm.3	15, 16					14, 16					
9mm.4	15	17			XY				14	9.3	
9mm.5						16					
9mm.6					X				14		

Table A6.27 DNA profiles recovered from Rem223 cartridge cases handled and then fired – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
Rem223.1											22
Rem223.2					X	12		20		7	
Rem223.3											
Rem223.4	14, 15										
Rem223.5											
Rem223.6		17	11		XY						

Table A6.28 DNA profiles recovered from Rem308 cartridge cases handled and then fired – Donor 1.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	X, Y	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
Rem308.1					X						
Rem308.2	15	17							14		
Rem308.3		17			X					9.3	
Rem308.4									15.2		
Rem308.5	14					12				6	
Rem308.6											

Table A6.29 DNA profiles recovered from 762mm cartridge cases handled and then fired – Donor 2.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 2	16, 16	17, 17	9, 13	22, 23	X, Y	14, 15	29, 31.2	14, 16	12, 14	6, 9.3	20, 21
762mmA.1						12			12		
762mmA.2	16				XY						22
762mmA.3	16	15							12	9.3	
762mmA.4							30.2				
762mmA.5											
762mmA.6	16				X						

Table A6.30 DNA profiles recovered from P990 cartridge cases handled and then fired – Donor 2.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 2	16, 16	17, 17	9, 13	22, 23	X, Y	14, 15	29, 31.2	14, 16	12, 14	6, 9.3	20, 21
P990A.1	16	17			X					9.3	
P990A.2	16	17									
P990A.3										9	
P990A.4			11		XY						20
P990A.5											
P990A.6					X						22

Table A6.31 DNA profiles recovered from 762mm cartridge cases handled and then fired – Donor 3.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 3	14, 15	14, 16	11, 12	22, 24	X, Y	16, 16	28, 30	20, 20	14, 14	8, 8	22.2, 22.2
762MMB.1	12, 14				X	16		20	14	9	
762MMB.2											
762MMB.3	16			20	X				14		22
762MMB.4										8	
762MMB.5						16					
762MMB.6											

Table A6.31 DNA profiles recovered from 762mm cartridge cases handled and then fired – Donor 3.

Calibre.	BLUE				GREEN				YELLOW		
Repeat number	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 3	14, 15	14, 16	11, 12	22, 24	X, Y	16, 16	28, 30	20, 20	14, 14	8, 8	22.2, 22.2
P990B.1		15			X	16					
P990B.2	14									7	
P990B.3	15				X	16			14		
P990B.4									14		
P990B.5					XY			20			
P990B.6											

A6.6 Inhibition of DNA Profiling by GSR

Variables as labelled in Tables A6.32 and A6.33:

1. Control: Non-seeded saliva was applied to the clean cartridge case, and the DNA was collected, extracted, amplified and profiled.

2. Extract: GSR-seeded saliva was applied to the clean cartridge case, and the DNA was collected, extracted, amplified and profiled.

3. Amplify: Non-seeded saliva was applied to the clean cartridge case, the DNA was collected, extracted, and the DNA extract was seeded with GSR suspension. The sample was then amplified and profiled.

4. Separate: Non-seeded saliva was applied to the clean cartridge case, and the DNA was collected, extracted, and amplified. The PCR product was then seeded with GSR suspension and placed on the 310 Genetic Analyser for capillary electrophoresis.

Table A6.32 Chelex Extracted Samples spiked with GSR

Variable & Cartridge Calibre	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 4	16, 16	15, 17	9, 11	19, 24	XX	14, 14	27, 30	15, 20	11, 14	6, 9	23, 26
1 9mm											
2 9mm											
3 9mm					X				14		
4 9mm					X						
1 .308	16	15, 17			X	14	27, 30		11, 14	6, 9	
2 .308	16	15			X	14			11, 14		
3 .308					X						
4 .308					X						
1 .223	16	15			X	14			11, 14		
2 .223	16				X				11, 14		
3 .223	16				X	14			11		
4 .223											

Table A6.33 Qiagen Extracted Samples spiked with GSR

Variable & Cartridge Calibre	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 4	16, 16	15, 17	9, 11	19, 24	XX	14, 14	27, 30	15, 20	11, 14	6, 9	23, 26
1 9mm	16	15, 17, 22	9, 11		X	13	27, 30		11, 14	6, 8, 9	
2 9mm	16	15, 17, 22	9, 11		X	13	27, 30		11, 14, 15.2	6, 8, 9	
3 9mm	16	15, 17	9, 11		X	13	27, 30		11, 14	6, 8, 9	
4 9mm	16	15, 17	9, 11		X	13	27, 30		11, 14, 15.2	6, 8, 9	
1 .308	16	15, 17, 22	9, 11		X	13	27, 30		11, 14	6, 8, 9	
2 .308	16	15, 17	9, 11		X	9, 13	27, 30		11, 14	6, 8, 9	
3 .308	16	15, 17	9, 11		X	13	27, 30		11, 14, 15.2	6, 8, 9	
4 .308	16	15, 17	9, 11		X	13	27, 30		11, 14, 15.2	6, 8, 9	
1 .223	16	15, 17	9, 11		X	14	27, 30		11, 14	6, 8, 9	
2 .223	16	15, 17	9, 11		X	14	27, 30		11, 14	6, 8, 9	
3 .223	16	15, 17	9, 11		X	14	27, 30		11, 14	6, 8, 9	
4 .223	16	15, 17	9, 11		X	14	27, 30		11, 14	6, 8, 9	

A6.7 Short Term Exposure to GSR Study

Table A6.34 Short Term GSR Study – No GSR Control

Sample (Incubation period)	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 4	16, 16	15, 17	9, 11	19, 24	XX	14, 14	27, 30	15, 20	11, 14	6, 9	23, 26
1 (24 hours)											
2 (24 hours)				19	X	14	27		11		
3 (24 hours)		15		19		14					
4 (24 hours)		17			XY	14					
5 (1 week)	15, 16				X	14					26
6 (1 week)				19	X	14			11, 14	6	
7 (1 week)	16				X				11		26
8 (1 week)											

Table A6.35 Short Term GSR Study – GSR Seeded Samples

Sample (Incubation period)	**BLUE**				**GREEN**				**YELLOW**		
	D3	**vWA**	**D16**	**D2**	**Amelogenin**	**D8**	**D21**	**D18**	**D19**	**THO1**	**FGA**
Donor 4	**16, 16**	**15, 17**	**9, 11**	**19, 24**	**XX**	**14, 14**	**27, 30**	**15, 20**	**11, 14**	**6, 9**	**23, 26**
1 (24 hours)					**X**						
2 (24 hours)					**X**						
3 (24 hours)											
4 (24 hours)					**X**				**11**		
5 (1 week)	**16**	**15,** 16, 18			**X**	**14**				8	19
6 (1 week)		**15**									
7 (1 week)								**15**	**14**	**9**	
8 (1 week)					Y						

A6.8 Exposure of DNA to Discharged Levels of GSR

Table A6.36 Donor 5 Semi-Automatic Weapon (DNA Collection Method – Taping)

Sample		**BLUE**				**GREEN**				**YELLOW**		
		D3	**vWA**	**D16**	**D2**	**Amelogenin**	**D8**	**D21**	**D18**	**D19**	**THO1**	**FGA**
Donor 5		**14, 15**	**18, 19**	**12, 13**	**16, 23**	**XX**	**11, 12**	**27, 33.2**	**13, 14**	**13, 14**	**6, 9.3**	**20, 21**
Left Hand (Samples taken prior to firing)	**Fingerprint**	**14, 15**	**19**			**XX**		**27**			**9.3**	
	Hand Swab	**14**, **15**	17, **19**	**12, 13**	**16**	**XX**		**27**	**13**	**13, 14**	**6,** 9, **9.3**	**20**
	Fingerprint (1 week)	**14**				**XX**						
Right Hand (Samples taken after firing)	**Fingerprint**	**14, 15**				**XX**		30			**6, 9.3**	
	Hand Swab		16	**12**		**XX**				**14**	**9.3**	
	Fingerprint (1 week)	13, **14**									**9.3**	
Negative Weapon Swab												
Weapon Swab			**18, 19**	11		**XX**		**33.2**	16			

Table A6.37 Donor 5 Semi-Automatic Weapon (DNA Collection Method – Swabbing)

Sample		BLUE				GREEN				YELLOW		
		D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 5		**14, 15**	**18, 19**	**12, 13**	**16, 23**	**XX**	**11, 12**	**27, 33.2**	**13, 14**	**13, 14**	**6, 9.3**	**20, 21**
Left Hand (Samples taken prior to firing)	**Fingerprint**	13, **14**	17, **19**			**X**Y	**11**			12, **13, 14**	9, **9.3**	
	Hand Swab	**14, 15**, 16	17, **18**			**X**	**11**			**13, 14**	**9.3**	**20**
	Fingerprint (1 week)	**14, 15**	16, 17	12	17	**X**Y	8, 9, **11**, 14	28, 31, 32	**13,** 15	**13, 14**, 14.2	**6**, 9	**20**
Right Hand (Samples taken after firing)	**Fingerprint**	**14, 15**	16, **18**			**X**				**13**	**9.3**	
	Hand Swab	**14, 15,** 19	16, 17, **18, 19**	**12, 13**	**16, 23**	**XX**	8, **12,** 13	**27**, 28, **33.2**	**13,** 16	**13, 14**	**6, 9.3**	**20, 21**
	Fingerprint (1 week)	13, **14**				**X**						
Negative Weapon Swab												
Weapon Swab		**14**			**16, 23**	**XX**		32.2, **33.2**	**13**	**13**	**6**	**20**

Table 6.38 Donor 6 Semi-Automatic Weapon (DNA Collection Method – Taping)

Sample		BLUE				GREEN				YELLOW		
		D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 6		**16, 18**	**17, 18**	**12, 12**	**17, 24**	**XX**	**11, 12**	**28, 28**	**14, 14**	**12, 14**	**6, 9.3**	**20, 21**
Left Hand (Samples taken prior to firing)	Fingerprint	**16,** 17, **18**				**X**	**11**				**6, 9.3**	
	Hand Swab	13, 14, **16, 18**	**17, 18**	10, **12**	**17, 24**	**XX**	**11, 12**	**28,** 29	**14**	**12, 14**	**6, 9.3**	**20, 21**
	Fingerprint (1 week)		**18**				10, **11**			15		
Right Hand (Samples taken after firing)	Fingerprint					**X**Y			**14**			
	Hand Swab		**17, 18**	**12**	**17, 24**	**XX**Y	**12,** 13, 15	**28,** 30	13, **14**	**12, 14**, 15	**6**, 9, **9.3**	**20, 21**
	Fingerprint (1 week)	**16**		**12**								**21**
Negative Weapon Swab												
Weapon Swab			**17, 18**	**12**	**24**	**XX**	**11, 12**	**28**	**14**	**12, 14**		

Table A6.39 Donor 6 Semi-Automatic Weapon (DNA Collection Method – Swabbing)

Sample		BLUE				GREEN				YELLOW		
		D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 6		**16, 18**	**17, 18**	**12, 12**	**17, 24**	**XX**	**11, 12**	**28, 28**	**14, 14**	**12, 14**	**6, 9.3**	**20, 21**
Left Hand (Samples taken prior to firing)	Fingerprint	15, **16, 18**		**12**		**X**	**11**	**28**	**14**		**9.3**	
	Hand Swab	**16, 18**	**17, 18**	**12**	**17, 24**	**XX**	9, **11, 12**	19, **28**	**14**	**12, 14**	**6, 9.3**	**20, 21**
	Fingerprint (1 week)	**16**		**12**		**X**	**11**					
Right Hand (Samples taken after firing)	Fingerprint	**16,** 19					9, **11**	**28**				**20**
	Hand Swab	**16, 18,** 19	**17, 18**	**12**	**17, 24**	**XX**	9, 10, **11, 12**	**28**	**14**	**12, 14**	**6, 9.3**	**20, 21**
	Fingerprint (1 week)	15, **16**				**X**				**12**		
Negative Weapon Swab						**X**						
Weapon Swab		**16,** 17, **18**		**12**		**X**		**28,** 30	**14**	**14**	**9.3**	**20,** 22

Table A6.40 Donor 5 Revolver Weapon (DNA Collection Method – Taping)

Sample		BLUE				GREEN				YELLOW		
		D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 5		**14, 15**	**18, 19**	**12, 13**	**16, 23**	**XX**	**11, 12**	**27, 33.2**	**13, 14**	**13, 14**	**6, 9.3**	**20, 21**
Left Hand (Samples taken prior to firing)	Fingerprint	**14**				**X**				**13, 14**	**9.3**	
	Hand Swab	**14, 15**, 16	**18, 19**	**12, 13**	**16, 23**	**X**Y	**11, 12**, 13	**27**, 28, **33.2**	**13, 14**, 15	**13, 14**	**6**, 8, **9.3**	19, **20, 21**
	Fingerprint (1 week)	**14**, 16	15, 16			**XX**				**13, 14**	**6, 9.3**	
Right Hand (Samples taken after firing)	Fingerprint							**27**		**14**		
	Hand Swab	**14, 15**, 17	**19**	**12, 13**	**16**	**X**Y	**11, 12**, 14	**27, 33.2**	**13, 14**, 17	**13**, 13.2, **14**, 15	**6**, 7, **9.3**	**20, 21**
	Fingerprint (1 week)			9		**XX**	13			**14**	**6**, 9	
Negative Weapon Swab		18				**XX**	13				**6**	
Weapon Swab		16, 17	**19**	**12**		**X**Y	**11**, 13	31, 32.2		**13, 14**	**9.3**	**21**, 24

Table A6.41 Donor 5 Revolver Weapon (DNA Collection Method – Swabbing)

Sample		**BLUE**				**GREEN**				**YELLOW**		
		D3	**vWA**	**D16**	**D2**	**Amelogenin**	**D8**	**D21**	**D18**	**D19**	**THO1**	**FGA**
Donor 5		**14, 15**	**18, 19**	**12, 13**	**16, 23**	**XX**	**11, 12**	**27, 33.2**	**13, 14**	**13, 14**	**6, 9.3**	**20, 21**
Left Hand (Samples taken prior to firing)	**Fingerprint**	**14, 15,** 16				**X**	**11**				**6**	
	Hand Swab	**14, 15,** 18	17, **18, 19**	**12, 13**	**16, 23**	**XX**	10, **11, 12**	19, 23.2, **27, 33.2**	**13, 14**	**13,** 17.2	**6, 9.3**	**20, 21**
	Fingerprint (1 week)		**18**								**9.3**	
Right Hand (Samples taken after firing)	**Fingerprint**	**14**	17			**X**	**11**			12,**13**	**6, 9.3**	
	Hand Swab	**15**	**19**	**12, 13**	**16, 23**	**XX**	**11**	19, **27**	**13, 14**	**13, 14**	**6, 9.3**	**20, 21**
	Fingerprint (1 week)					**X**	**11**					
Negative Weapon Swab												
Weapon Swab		**14, 15**	17, **18**			**X**					9, **9.3**	**20**

Table A6.42 Donor 6 Revolver Weapon (DNA Collection Method – Taping)

Sample		BLUE				GREEN				YELLOW		
		D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 6		**16, 18**	**17, 18**	**12, 12**	**17, 24**	**XX**	**11, 12**	**28, 28**	**14, 14**	**12, 14**	**6, 9.3**	**20, 21**
Left Hand (Samples taken prior to firing)	Fingerprint	**16**	**17, 18**	**12**		**X**				**14**		
	Hand Swab	**16, 18**	**17, 18**	**12, 12**	**17**	**XX**	**12,** 13	**28, 28**	**14**	**12, 14**	**6**, 9, **9.3**	**20, 21**
	Fingerprint (1 week)					**X**		30.2			**9.3**	
Right Hand (Samples taken after firing)	Fingerprint	**16**		**12**		**X**						
	Hand Swab	**16, 18**	16, **17, 18**	**12**		**X**Y	**12**, 14	**28**, 30.2	**14**		**9.3**	**20,** 22
	Fingerprint (1 week)		**18**	**12**			14				**6**	
Negative Weapon Swab			15			Y			13	15	9, **9.3**	
Weapon Swab		**18**		11		**XX**				**12**	**9.3**	

Table A6.43 Donor 6 Revolver Weapon (DNA Collection Method –Swabbing)

Sample		BLUE				GREEN				YELLOW		
		D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 6		**16, 18**	**17, 18**	**12, 12**	**17, 24**	**XX**	**11, 12**	**28, 28**	**14, 14**	**12, 14**	**6, 9.3**	**20, 21**
Left Hand (Samples taken prior to firing)	**Fingerprint**	**16,** 17		**12**		**X**						
	Hand Swab	**16, 18**	**18**, 19	**12**		**X**	13	27	13	13		**21**
	Fingerprint (1 week)			**12**				27, **28**				
Right Hand (Samples taken after firing)	**Fingerprint**	**16**				**X**						
	Hand Swab	**16**, 17, **18**	**17, 18**		**17, 24**	**XX**	**11, 12**, 13	**28**	11, 12, **14**	**12, 14**	**6, 9.3**	**20, 21,** 22, 25
	Fingerprint (1 week)						13			**12**		
Negative Weapon Swab												
Weapon Swab			16		23	**XX**	14	30		**14**	9, **9.3**	

Appendix Seven – DNA Recovery from Firearms

A7.1 DNA Recovery from Firearms Study – DNA Profiles

Table A7.1 DNA Recovery Control Profiles

Sample	D3S1358	vWA	D16S539	D2S1338	Amel	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
Buccal	14, 15	17, 17	9, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 9.3	21, 23
Positive control	15, 16	14, 16	9, 10	20, 23	X, Y	12, 13	28, 31	12, 15	14, 15	7, 9.3	24, 26
Negative control	-	-	-	-	-	-	-	-	-	-	-

Table A7.2 DNA Recovery Profiles recovered using cotton swabs and a targeted approach

Sample	D3S1358	vWA	D16S539	D2S1338	Amel	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
Cotton T1 A	14, 15	17, 17	9, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 9.3	21, 23
Cotton T2 A	14, 15	17, 17		20					14		
Cotton T3 A	14, 15	17, 17	9, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 9.3	21, 23
Cotton T4 A	14, 15, 16	17, 17		20	X, Y			16		7, 9.3	23
Cotton T5 A											
Cotton T6 A	14, 15				X, Y						
Cotton T1 B											
Cotton T2 B											
Cotton T3 B											
Cotton T4 B											
Cotton T5 B											
Cotton T6 B											

Table A7.3 DNA Recovery Profiles recovered using nylon flocked swabs and a targeted approach

Sample	D3S1358	vWA	D16S539	D2S1338	Amel	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
Nylon T1 A	14, 15, 16	17, 17	9, 14	20	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 7, 9.3	22, 23
Nylon T2 A											
Nylon T3 A	14, 15, 16				X, Y						
Nylon T4 A	14, 15				X, Y				14, 15		
Nylon T5 A	14, 15	17, 17			X, Y	15, 16	30		14, 15	6, 9.3	
Nylon T6 A					X, Y						
Nylon T1 B	14, 15	17, 17			X, Y	15, 16	30	16	14, 15	9.3	23
Nylon T2 B					X, Y						
Nylon T3 B	14	17, 17		20	X, Y			16	14, 15		
Nylon T4 B					X, Y						
Nylon T5 B											
Nylon T6 B								16			22

Table A7.4 DNA Recovery Profiles recovered using cotton swabs and a zoned approach

Sample	D3S1358	vWA	D16S539	D2S1338	Amel	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
Cotton Z1 A	14, 15	17, 17	9, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 9.3	21, 23
Cotton Z2 A	14, 15	17, 17	9, 14	20	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 9.3	21, 23
Cotton Z3 A	14, 15, 16	17, 17	9, 14	20	X, Y	14	30, 31.2		14, 15	9.3	23
Cotton Z1 B	14, 15	17, 17	9, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 7, 9.3	21, 23
Cotton Z2 B	14, 15	17, 17	9, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 9.3	21, 23
Cotton Z3 B	14, 15	17, 17	9, 10	20	X, Y	15, 16	30	16	14, 15	6, 9.3	23
Cotton Z1 C	14, 15	17, 17	9, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 9.3	21, 23
Cotton Z2 C	14, 15	17, 17	9, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 7, 9.3	21, 23
Cotton Z3 C	14, 15	17, 17	9, 14	20	X, Y	15, 16	30, 31.2	16	14, 15	6, 9.3	23

Table A7.5 DNA Recovery Profiles recovered using nylon flocked swabs and a zoned approach

Sample	D3S1358	vWA	D16S539	D2S1338	Amel	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
Nylon Z1 A	14, 15	17, 17	9, 10, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 7, 9.3	21, 23
Nylon Z2 A	14, 15	17, 17	9, 14	20	X, Y	15, 16	30, 31.2	16	14, 15	6, 9.3	23
Nylon Z3 A	14, 15	17, 17	9, 14	20	X, Y	16			14, 15	6, 7	21, 23
Nylon Z1 B	14, 15	17, 17	9, 14	20	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 9.3	21, 23
Nylon Z2 B	14, 15, 18	17, 17	9, 10, 14	20	X, Y	14, 15, 16	30, 31.2	13, 16	14, 15	6, 7, 9.3	
Nylon Z3 B	14, 15	17, 17	9, 10, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 7, 9.3	21, 23
Nylon Z1 C	14, 15	17, 17	9, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 7, 9.3	21, 23
Nylon Z2 C	14, 15	17, 17	9, 14	20, 23	X, Y	15, 16	30, 31.2	13, 16	14, 15	6, 9.3	21, 23
Nylon Z3 C	14, 15	17, 17	9, 14	20	X, Y	15, 16	30, 31.2		14, 15	6, 9.3	23

Table A7.6 DNA Recovery Profiles recovered using mini-tapes and a zoned approach

Sample	D3S1358	vWA	D16S539	D2S1338	Amel	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
Taping Z1 and Z2 A	14, 15, 18	17, 17, 18	9, 10, 14	20, 23	X, Y	13, 15, 16	30, 31.2	13, 16	14, 15	6, 7, 9.3	21, 23
Taping Z3 A											
Taping Z1 and Z2 B	14, 15, 18	17, 17		20, 23	X, Y	13, 15, 16	30, 31.2, 32	13, 14, 16	14, 15	6, 7	21, 22, 23
Taping Z3 B	15, 16		10		X, Y	13	30			6	21
Taping Z1 and Z2 C	14, 15	17, 17	9, 13, 14	23	X, Y		32	13, 16		7	
Taping Z3 C											

A7.2 DNA Recovery from Firearms Study –Electropherogram from Nylon swab 6, targeted approach.

See Attached Disc

A7.3 DNA Recovery from Firearms Study – Partial Electropherogram from Nylon swab 6, zoned approach.

See Attached Disc

Appendix Eight – Summary Table of Donor SGM+ Profiles

Sample Name	BLUE				GREEN				YELLOW		
	D3	vWA	D16	D2	Amelogenin	D8	D21	D18	D19	THO1	FGA
Donor 1	15, 16	17, 17	11, 13	19, 24	XY	14, 15	28, 30	15, 16	14, 15	7, 9.3	21, 22
Donor 2	16, 16	17, 17	9, 13	22, 23	XY	14, 15	29, 31.2	14, 16	12, 14	6, 9.3	20, 21
Donor 3	14, 15	14, 16	11, 12	22, 24	XY	16, 16	28, 30	20, 20	14, 14	8, 8	22.2, 22.2
Donor 4	16, 16	15, 17	9, 11	19, 24	XX	14, 14	27, 30	15, 20	11, 14	6, 9	23, 26
Donor 5	14, 15	18, 19	12, 13	16, 23	XX	11, 12	27, 33.2	13, 14	13, 14	6, 9.3	20, 21
Donor 6	16, 18	17, 18	12, 12	17, 24	XX	11, 12	28, 28	14, 14	12, 14	6, 9.3	20, 21
Donor 7	15, 16	17, 19	13, 13	17, 20	XX	12, 13	30, 31.2	12, 14	14, 14	6, 9	21, 23
Donor 8	16, 17	17, 18	11, 13	19, 19	XY	14, 14	27, 30	12, 19	13, 14	6, 9.3	22, 23
Donor 9	15, 16	15, 15	12, 13	17, 23	XY	11, 13	30, 31.2	13, 17	15, 15	8, 9	21, 22
Donor 10	14, 17	16, 18	12, 13	20, 23	XX	12, 14	30, 30	17, 20	14, 14	9, 9.3	20, 22
Donor 11	15, 18	17, 17	11, 13	17, 18	XX	13, 14	28, 31.2	10, 16	14, 15	6, 9	20, 20
Donor 12	14, 15	17, 17	10, 11	17, 20	XY	10, 13	30, 30	11, 20	13, 15	8, 9.3	19, 20
Donor 13	15, 16	17, 17	9, 13	21, 23	XY	12, 14	28, 29.2	14, 16	12, 14	7, 7	22, 22
Donor 14	16, 16	16, 18	11, 12	17, 17	XX	14, 15	28, 30	13, 16	14, 14	7, 9.3	21, 22
Donor 15	13, 17	16, 16	10, 13	18, 25	XY	14, 16	30, 31	13, 17	12, 16	9.3, 9.3	20, 22
Donor 16	15, 17	15, 16	11, 13	18, 20	XY	14, 14	28, 30	11, 16	14, 15.2	9.3, 9.3	22.2, 23
Donor 17	14, 17	16, 18	12, 13	20, 23	XX	12, 14	30, 30	17, 20	14, 14	9, 9.3	20, 22