ANALYSIS OF TOUCH DNA

By

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DECLARATION

I declare that the work contained in this thesis has not been previously submitted for any other award from an academic institution. To the best of my knowledge and belief, the thesis contains no materials previously published or written by another person except where due reference is made.

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ABSTRACT

Over the last three decades, forensic DNA analysis has significantly advanced in terms of the power discrimination, speed, and sensitivity of DNA profiling methods, as well as the capability to deal with increasingly challenging samples. However, the validity of Touch or trace DNA collected from used objects is associated with some issues related to the quantities, deposition, collection, and materials or techniques used. The quality and quantity of Touch DNA deposited on an object affects DNA recovery and the amount of DNA collected is often not constant due to the nature of the surface, the time between DNA deposition and recovery, environmental influence, collection type, and extraction. Therefore, this study aimed to examine the factors affecting the recovery of Touch DNA, in a bid to propose novel methodologies and techniques that can improve Touch DNA analysis in forensic casework.

Collection, extraction, quantification, and amplification techniques for DNA profiling were chosen based on their common use in published research in the field of Touch DNA analysis, and their popularity among the forensic laboratories. The duration of time over which the deposit on the surface and the area over which the touch occurs were standardised among the various experiments in this study, to more effectively evaluate the factors examined.

When using cotton swab, wetting techniques and swab conditions before extraction have had a significant ($p < 0.001$) impact on DNA quantities recovered. The spray bottle technique used to moisten the swab prior to collection, and freezing the swab following DNA recovery while it is moist, rather than drying it before extraction, should be considered for better Touch DNA recovery when using cotton swab as a collection method.

By examining Copan cotton swab (150C), Copan nylon flocked swab (4N6FLOQSwabs®), and SceneSafe Fast™ minitape (MT) on collecting Touch DNA from various types of common surfaces encountered in crime scenes, it was observed that that the amount of DNA collected was significantly affected by the type of surface ($p < 0.05$), as well as the extraction method ($p < 0.05$), when manufacturers recommendations were followed. However, when the amount of lysis buffer was increased to 460 µL with PrepFiler

Express BTA™ Extraction Kit, results were equally efficient to using the QIAamp® DNA Investigator Kit. Nonetheless, the Express BTA™ Extraction Kit was more effective for extracting DNA from samples collected by MT than the QIAamp® DNA Investigator Kit.

By investigating the collection methods further, the quantity of DNA collected from non-porous surfaces (e.g., glass), and from porous surfaces (e.g., copier paper) was affected by collection type ($p < 0.05$). Cotton and nylon swabs were successful in collecting biological material from non-porous surfaces, nevertheless, nylon swabs were more efficient for collecting Touch DNA from rough non-porous surfaces (e.g., textured plastic) than cotton swabs. MT were the best option for recovering trace DNA from porous surfaces such as fabric, however fabric type ($p < 0.05$), area size ($p < 0.05$), and the influence of deposition area and time ($p < 0.05$) can impact DNA quantities recovered from fabric. Furthermore, MT can allow dual recovery of DNA and fingerprints from visible fingerprints deposited on the smooth non-porous surfaces.

When common surfaces encountered in crime scenes were examined under different environmental conditions, such as high/low temperature and high/low humidity, to replicate weather conditions in UAE and UK, it was noted that the amount of recovered DNA was significantly affected by the conditions the surfaces were exposed to ($p < 0.05$), the interaction between each surface type and the conditions ($p < 0.05$), the interaction between the conditions and time ($p < 0.05$), as well as sand found on the surfaces ($p < 0.05$).

By investigating different collection techniques with cotton and nylon swabs on Touch DNA collected from human skin following a strangulation scenario, there was a significant difference between the three recovery techniques used ($p < 0.05$). However, the average number of alleles observed was more consistent when the skin was moistened before collection with dry cotton or nylon swab. In addition, trace DNA collected from the neck was influenced by time ($p < 0.05$): when the receiver (victim) did not wash the neck, full mixture DNA profiles were obtained up to 48 h later, but when the receiver (victim) washed the neck within the 6 h period after deposition, there were no alleles observed of the donor's (perpetrator) DNA in the samples collected.

The performance of six collection types with direct amplification on the collected Touch samples was examined. The number of alleles observed in the DNA profiles was impacted by collection type ($p < 0.001$), as microFLOQ™ Direct swab (MF) and SceneSafe Fast™ Minitape (MT) recovered a higher percentage of alleles and were more effective than the other swabs used. However, DNA profiles generated by direct amplification produced some artifacts, mostly split and shoulder peaks. When comparing direct PCR to extraction on the same set of collected trace samples, direct amplification generated higher peak heights than DNA extracted from a small surface area ($p < 0.001$), in contrast to samples collected from a larger surface area ($p < 0.001$) which generated higher peak heights when compared to direct amplification.

This study proposed an innovative solution (CS+MF), which is a combination of using cotton swab (CS) and MicroFLOQ™ Direct swab (MF) to maximise DNA recovery from the tested surface. Touch DNA was first collected by CS to concentrate the biological materials in a small area, to be then collected with MF. However, when comparing the number of alleles obtained from direct amplification to the amount of DNA collected by cotton swabs (CS), it was seen that the number of alleles obtained by direct amplification was impacted by the amount of DNA collected by CS ($p < 0.001$).

During the last six years, the Biology and DNA Section of the General Department of Forensic Science and Criminology of Dubai Police Force has received an average of two thousand cases per calendar year. From the total number of samples processed for DNA profiling between 2019 and 2021, 5488 (63%) were trace samples recovered from various touched or used items, and the success rate of trace samples was 64%, with only 3489 producing usable DNA profiles.

By implementing the recommended procedures and using multiple collection methods developed in this study to improve Touch DNA recovery for forensic casework over three months, the recovery rate of 156 trace samples from various types of surfaces improved by 17%. Freezing cotton swabs while moist prior to extraction when samples collected from smooth non-porous surfaces ($p < 0.05$), using nylon swab on textured non-porous surfaces ($p < 0.05$), and using minitapes on porous surfaces ($p < 0.05$) helped increase DNA quantities, and samples produced more usable DNA profiles with much higher peak heights.

By using direct amplification on case work trace samples (n= 100), the innovative solution (cotton swab (CS) and MicroFLOQ™ Direct (MF)) produced 70% usable DNA results, which was much better than using MF alone, which produced 55% usable DNA results.

The findings of this research should be considered when establishing protocols for Touch or trace DNA profiling, to improve recovery methods and techniques, enhance extraction with PrepFiler Express BTA™ Extraction Kit, as well as consider the use of the innovative solution for direct amplification and dual recovery of DNA and fingerprints with minitapes.

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ABBREVIATIONS

ASCLD	American Society of Crime Laboratory Directors
BS	Banana skin
CODIS	Combined DNA Index System
CP	Copier paper
CS	Copan cotton swab
DNA	Deoxyribonucleic Acid
DTC	Direct-to-consumer genetic tests
EVCs	Externally visible characteristics
EXT1	PrepFiler Express BTA™ extraction Kit (Thermo Fisher Scientific Inc.)
EXT2	QIAamp® DNA Investigator extraction Kit (QIAGEN)
FB1	Fabric composed of 65% polyester and 35% cotton
FB2	Fabric composed of 100% woven cotton
FDP	Forensic DNA phenotyping
FM	Full mixture DNA profile
FS	Full single DNA profile
FSS	Britain's Forensic Science Service
G	Glass
HT	High temperature with moderate humidity (40 °C/50%)
ISFG	The International Society for Forensic Genetics
IHGSC	International Human Genome Sequencing Consortium
ISO	International Organization for Standardization
LCN	Low copy number
LT	Low temperature with high humidity (5 °C/78%)
LTDNA	Low template DNA
MCS	Fenshine mini cotton swab
MF	Copan microFLOQ™ swab
MPS	Massively parallel sequencing
MT	SceneSafe Fast™ minitape
mtDNA	Mitochondrial DNA
N	Negative DNA profile with alleles in less than nine loci (< 9)

NDNAD	United Kingdom national DNA database
NGS	Next-generation sequencing
NDIS	National DNA Index System
NHGRI	National Human Genome Research Institute
NIH	National Library of Medicine
NS	Copan nylon flocked swab (4N6 FLOQSwabs®)
PCR	Polymerase chain reaction
PM	Partial mixture DNA profile with alleles in nine loci or more (≥ 9)
PS	Partial single DNA profile with alleles in nine loci or more (≥ 9)
qPCR	Quantitative real-time PCR
RFLP	Restriction fragment length polymorphisms
RT	Room temperature with moderate humidity (20 to 25 °C/50%)
SBE	Single base extension
SNP	Single Nucleotide Polymorphism
SOP	Standard operating procedures
SS	Stainless steel
SZ1	Fabric piece cut into 5 x 7 cm
SZ2	Fabric piece cut into 10 x 14 cm
STR	Short tandem repeat
SWGDAM	Scientific Working Group on DNA Analysis Methods
TP	Textured plastic
TW	Textured wood
UV	Ultraviolet Radiation
VNTRs	Variable number of tandem repeats

CHAPTER ONE

1. INTRODUCTION

Forensic genetics uses molecular tools and related scientific applications to solve criminal and civil lawsuits (Editorial, 2007). Over the last three decades, Forensic DNA analysis has made significant advancements in terms of the power discrimination, speed and sensitivity of DNA profiling methods, as well as the capability to deal with increasingly challenging samples.

This chapter discussed forensic genetics and its importance as a forensic tool, the historical improvement of DNA analysis techniques to date, and new developments related to forensic genetics, focusing on Touch DNA and the factors affecting its recovery. Finally, the research aims and objectives were provided.

1.1 FORENSIC GENETICS

The origin of the word 'forensic' comes from the Latin word 'forensis' which means 'of or before the forum'. The history of the term arises from marketplaces within ancient Rome where public affairs, criminal cases and actions by courts of law were conducted. The term 'forensic genetics' usually refers to the use and application of human deoxyribonucleic acid (DNA) in the investigation of crime (Goodwin *et al.,* 2011).

Like many other scientific fields, forensic genetics progressed through the long-term social practice. After the discovery of the ABO blood groups by Landsteiner in 1900 (Yamamoto & Hakomori, 1990), human blood type was used as an identification tool, and forensic genetics entered the scientific age. The foundation for modern forensic science was laid in 1910 when the French criminologist Edmond Locard proposed the Locard's exchange principle (Byard *et al.,* 2016) stating that "every contact leaves a trace". In 1926, Thomas Hunt Morgan (Altshuler *et al.,* 2002) proposed the theory of genes, which provided the foundation for the development of forensic genetics.

The discovery of the double-helical structure of DNA in 1953 initiated forensic genetics research at the molecular level (Altshuler *et al.,* 2002). In 1984, "DNA fingerprinting" or DNA typing was discovered by Alec Jeffreys (Jeffreys, 2013). He specified that the fragments represented different combinations of DNA repetitive elements, which are

unique to each individual and could be applied to better identify individuals or kinship lineages (Jeffreys *et al.,* 1985b). Initially, this technology was used in several paternity, immigration, and forensic genetics cases (Gill *et al.,* 1985; Jeffreys *et al.,* 1985a; Evans, 2007), which was just the start of a whole new era in forensic DNA typing. Today, forensic genetics lies at the interchange between law and science, two forms of institutionalised pursuit characterised as the most important contemporaneous sources and guardians of social order (Williams, 2015).

1.1.1 AN OVERVIEW OF DNA

The double helix structure of DNA enables it to carry biological information through the generations (Figure 1.1). DNA is found inside the cell nucleus in organisms called eukaryotes, and because organisms have numerous DNA molecules per cell, each DNA molecule is tightly packaged (NHGRI, 2016). This packaged form of the DNA is called a chromosome, of which, 23 pairs from each parent are passed to their offspring during reproduction (NHGRI, 2016). A small amount of DNA can also be present in the mitochondria known as mitochondrial DNA (mtDNA), structures within cells that transform the energy from food into a form that cells can use (NIH, 2021).

Like fingerprints, each person has a distinctive DNA signature that remains unchanged throughout their lives. DNA testing or DNA typing, generally known as DNA profiling in forensics, takes advantage of the fact that the genetic material of every person is unique and that we leave a DNA trail wherever we go, except for homozygous twins (Butler, 2015).

The Human Genome Project has completely sequenced the DNA instruction book or human genome, and today, it is recognised to contain about 3 billion bases and between 20,000 and 25,000 protein-coding genes on 23 pairs of chromosomes (IHGSC, 2004). This confirmed what scientists had formerly known, that the non-coding regions of the genome carry, among others, tracts of repetitive sequences (Hildebrand, 2011). The single-locus satellites are centralised at a specific site of a given human chromosome, while the multilocus satellite elements or short tandem repeats (STR) are distributed around the entire genome (Panneerchelvam *et al.,* 2003).

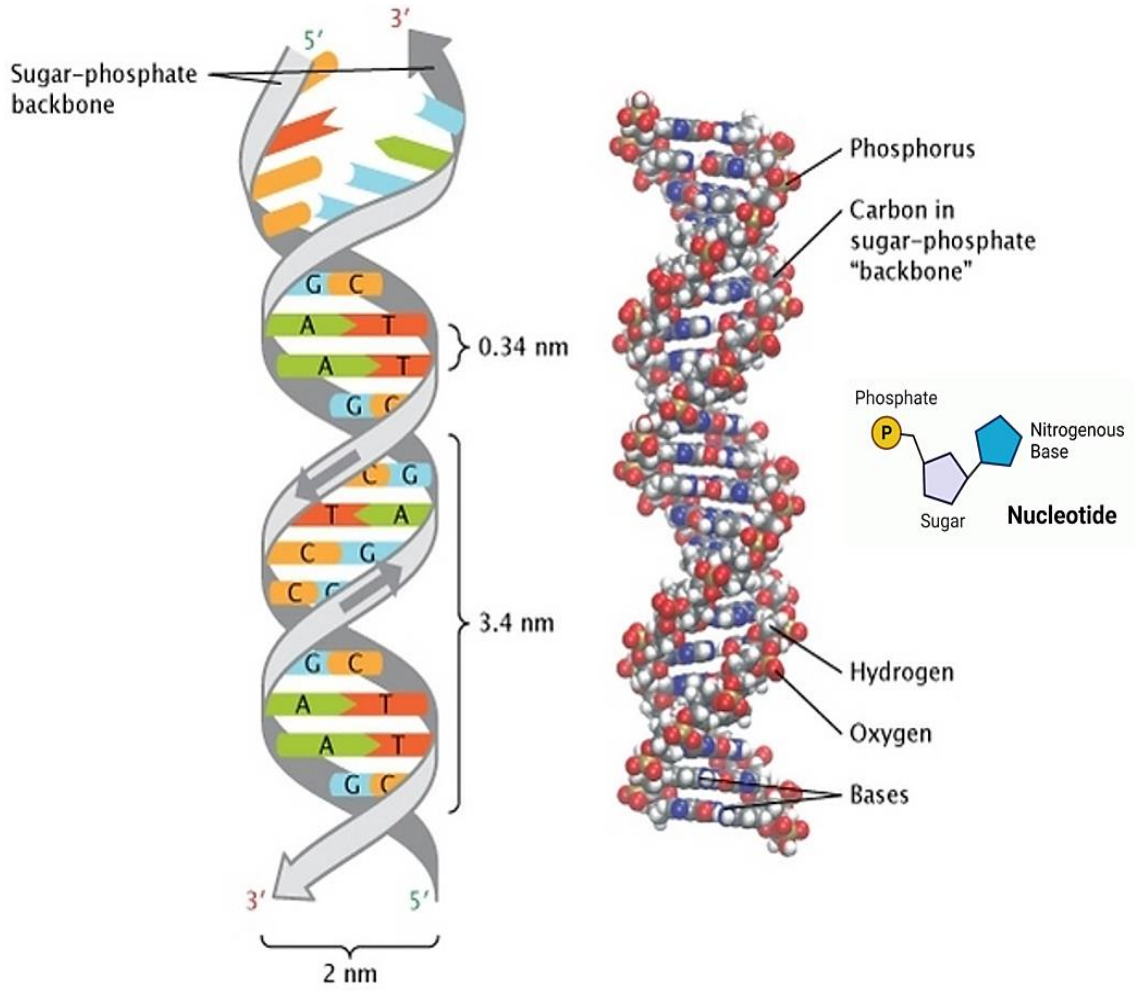


Figure 1.1: The double helix structure of DNA comprising complementary bases bound as a pair by hydrogen bonds (Pray, 2008). Nucleotides are the chemical building blocks of DNA and are made of a phosphate group, a sugar group and one of four types of nitrogen bases. The phosphate and sugar groups alternate, linked into chains with nucleotides to form a strand of DNA. Nucleotides consists of four types of nitrogen bases, adenine (A), thymine (T), guanine (G) and cytosine (C). They determine what biological instructions are contained in a strand of DNA when ordered in a specific way, for instance, the sequence ATCGCT could instruct for brown eyes, while ATCGTT might instruct for blue.

1.1.2 REPETITIVE DNA

Repetitive DNA sequences can be moderately or highly repetitive, tandemly or dispersedly categorised within the eukaryotic genome. Repetitive DNA can be categorised into two classes, the tandem repetitive sequences known as satellite DNA and the interspersed repeats. The term satellite refers to the DNA sequences that

consist of short head-to-tail tandem repeats that include specific motifs, and they make up one-third of DNA repeats (Trent, 2012). These are exemplified by minisatellites and microsatellites which form highly repetitive tandem sequences or a variable number of tandem repeats (VNTRs) (Biscotti *et al.,* 2015).

The mutation rate in the VNTR region is considerably high, 10 to 100,000 times higher than the average rate at other genomic sites and the variations in the number of repeat units also contribute to polymorphisms in the loci (López-Flores & Garrido-Ramos, 2012). Minisatellites are distinguished from microsatellites in structure and function, having a heterogeneous arrangement of 10–100 bp tandem repeats that extends to 1–15 kb (Vijg, 2007). Microsatellites or STRs are a homogeneous range of short tandem repeats 2–7 bp (Goodwin *et al.,* 2011), with a repeat size of less than or around 1 kb (Abdurakhmonov, 2016). Though minisatellite polymorphisms have been explored, the profusion of STR markers along with its polymerase chain reaction (PCR) compatibility made it the method of choice for forensic investigations.

1.1.3 DNA AS FORENSIC EVIDENCE

Forensic crime investigation owes its existence to Locard's exchange rule that 'every contact leaves a trace', meaning that there is an exchange of material between two objects during contact leaving a trace. Traces found at the crime scene often exist with multiple evidence and intermixing between the remains of victims, or severe fragmentation of the bodies, makes it challenging for the traditional identification based on anthropological and physical characteristics of the victim inefficient or inconclusive. Therefore, DNA profiling is the gold standard in resolving forensic cases because it provides victim identification and helps to link suspects to the crime (Ziętkiewicz *et al*., 2011; Van Oorschot *et al*., 2019).

It has been reported that 99.9% of the DNA sequence is the same in all humans, with only around 0.1% variation, and the odds of two individuals not related by blood having the same DNA sequence is about 1 in 594.1 trillion persons (Nizami *et al.,* 2018). This makes DNA testing a powerful tool to exonerate the innocent and convict the guilty (Pyrek, 2007), thus forensic science has adapted to DNA molecular biology tools more

than any other scientific field (Budowle & Daal, 2009). Nowadays, forensic DNA analysis routinely deals with materials related to crime scenes, paternity testing and the identification of human remains (Jobling & Gill, 2004).

1.1.4 THE PAST, PRESENT AND FUTURE OF DNA TYPING

Forensic science has developed through the decades due to many discoveries and technological advancements. The basis of differences, advantages, and disadvantages of the past and the current technologies are summarised in Table 1.1 and Figure 1.2 provides a timeline of the developments in DNA typing technologies from 1900 to the present.

ABO blood typing is considered the first form of human identification because of Landsteiner's research in 1900 on body fluids to improve blood transfusions (Yamamoto & Hakomori, 1990; Farhud & Yeganeh, 2013). Since then, the human ABO blood grouping was used in court in 1915 to resolve a paternity case in Italy based on different blood groups (Gaensslen, 1983), which makes it the first genetic tool used. Even though it uses a limited technique from a forensic point of view due to the high biological materials required for the analysis, the application of ABO blood grouping was believed to be revolutionary by law enforcement agencies. Moreover, even though a positive identification was not imaginable during that period since the ABO system results in only a few phenotypes (1 in 10) (Bruns *et al.,* 2007), it was considered a significant achievement in forensic biology (Jobling & Gill, 2004).

One of the main aspects of forensic genetics is the employment of genetic markers, which are the common identifiable phenotypes of genotypes. They are useful because they have features such as vigorous polymorphisms, codominant expression and ease of observation and recording. The use of genetic markers has also developed gradually, with the advancements in genetics. The development of genetic markers has gone through four major phases described by the use of morphological markers, cytological markers, biochemical markers and molecular markers.

Table 1.1: The basis of the differences, advantages, and disadvantages of the past and the current technologies in DNA profiling.

Analysis technique	Basis of differentiation	Advantages	Disadvantages
Restriction Fragment Length Polymorphism (RFLP)	Restriction site sequence and fragment length	– High power of discrimination – Reproducible – No prior sequence information required – Can differentiate between homozygotes and heterozygotes	– Time-consuming – Partial digests – Need at least 10–25 ng of DNA – Genetic mutations only identified at restriction cut sites – Not ideal for whole genome variation identification – requires radioisotopes
Short Tandem Repeat (STR)	STR fragment length	– Fast – Highly reproducible – High level of discrimination, codominant alleles – Standardized across forensic laboratories – Uses low DNA amounts for amplification – Database of genetic profiles and allelic frequencies for statistical comparisons	– Mixture deconvolution not easy – PCR artifacts can complicate results – Challenges with highly degraded or low template DNA
Sanger Sequencing	Sequences every base	– Gold standard for sequence analyses – Uses capillary electrophoresis techniques	– Low throughput – Only 500–700 bases sequenced at a time – Cannot sequence mixtures without cloning
SNaPshot™	Single base changes	– Detects bi-allelic and multi-allelic SNP markers – Able to distinguish between heterozygotes and homozygotes – Human SNP database for statistical comparisons	– Time-consuming – Need to know SNP sequence in advance to design – primers – Multiple markers required for high level of discrimination
Next-Generation Sequencing (NGS)	Massive parallel sequencing using various technologies	– High throughput – Deconvolve mixtures – Sequence entire genomes/metagenomes – Simultaneous detection of STR amplicon lengths – and SNPs within the amplicon – Used for any DNA (human, non-human, viral, microbes)	– Massive data output that may be challenging to analyse – Analysis algorithms not standardised – Difficult with some technologies to analyse metagenomes to species level

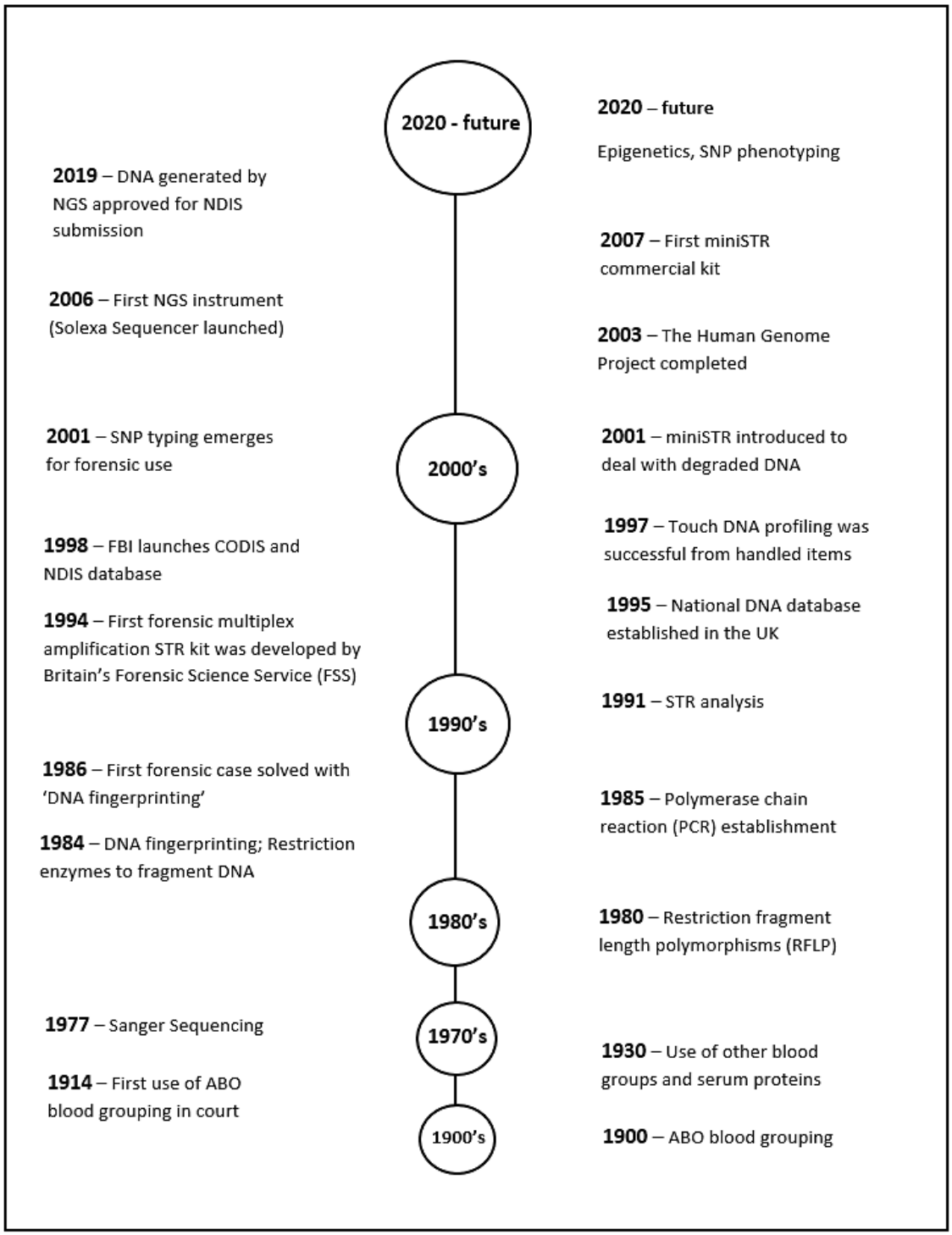


Figure 1.2: Timeline of the developments in DNA profiling technologies from the 1900's to the present.

1.1.4.1 RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

DNA fingerprinting initiated forensic genetics when Jeffreys first found that patterns in some regions of a person's DNA could be used to identify one person from another (Jeffreys, 1985b). He developed a novel hybridisation technique to examine these regions using restriction enzymes to fragment DNA, a procedure in which restriction endonucleases (RE) enzymes segment the genomic DNA making restriction fragment length polymorphisms (RFLP) patterns (Jeffreys, 2013). After this discovery, numerous DNA analysis techniques involving electrophoretic fragment separation were developed based on the RFLP concept (Botstein *et al.,* 1980). DNA fingerprinting was first used in forensic science in 1986, when police requested Dr Alec J. Jeffreys to produce a DNA profile of a suspect in a rape and murder case related to 15-year-old Dawn Ashworth in Leicestershire, UK (Gill & Werrett, 1987). While Jeffrey's DNA fingerprinting method allowed a very high power of discrimination of 1×10^{11} (Jeffreys, 1985b), it was very time consuming and demanded at least 10–25 ng of DNA to be successful (Wyman & White, 1980). The technique was limited to mostly fresh samples available in large quantities, like blood or semen to be tested successfully (Evett & Gill, 1991). With these limitations, the RFLP technique was not always practical for forensic cases, especially with challenging samples (degraded or minute) (Butler, 2006).

1.1.4.2 STR TYPING

Just after the discovery of DNA fingerprinting, PCR was discovered by Kary Mullis in 1985, which helped transform all DNA analyses with its ability to amplify DNA (Mullis *et al.,* 1986). After a few improvements to the PCR-based technology, the forensic community decided on the use of STR analysis (Butler, 2005; McCord *et al.,* 2019). This procedure amplifies highly polymorphic, repetitive DNA regions by PCR and then separates them by amplicon length using capillary electrophoresis. These inheritable markers are a series of short repeated sequences (2–7 bp) at a particular locus, often in non-coding genetic regions, and they are commonly tetranucleotide repeats (Goodwin *et al.,* 2011). They account for approximately 3% of the human genome (Lander *et al.,* 2001), and the number of repeat units is highly variable between individuals, which offers a high power of discrimination when used for identification purposes (Kim *et al.,*

2015). For these reasons, STRs are the current standard for human DNA typing (McCord *et al.*, 2019).

In 1994, the first forensic multiplex amplification STR kit was developed by the Forensic Science Service (FSS) in Britain, and it included four genetic loci - TH01, vWA, FES/FPS and F13A1 (Kimpton *et al.,* 1994). However, there are some difficulties and challenges when analysing highly degraded or low template DNA samples, and to overcome these limitations, regulated mini-STR kits have been developed which use shorter versions of STRs core and can be used in the same manner for forensic cases (Butler *et al.,* 2007; Constantinescu *et al.,* 2012). Today, commercial STR kits can normally detect 15–20 STR loci at one time, and with the development of fluorescent marker techniques, six-colour fluorescent marker STR kits have appeared on the market which allow 25–30 STR loci to be detected. The International Society for Forensic Genetics (ISFG) has published a guide for the forensic validation of STR kits to be used in forensic labs, which lays out the high standards and guidance for the forensic application of STR kits.

Forensic DNA processes may vary somewhat between laboratories around the world, but it will generally start at the crime scene where trace materials or biological samples, such as blood, semen and saliva etc., are examined, identified, collected, and transferred to the forensic laboratory for further examination and analyses. The nature of forensics requires high-quality assurance, hence standards such as ISO 17025 have been designed to ensure that standard operating procedures (SOPs) of forensic DNA analysis are followed to a high standard, and to avoid sample contamination during the process. The chain of custody to document all the analysis processes must be put in place to ensure that the aforementioned standards are maintained. Failing to follow correct procedures, the court could reject the evidence being put forward, possibly resulting in the perpetrator/s getting away (Wecht & Rago, 2005). The Scientific Working Group on DNA Analysis Methods (SWGDAM) has also published many guidelines to regulate and improve forensic DNA analysis.

Analysis of STR typing usually follows a general methodology of DNA profiling but depends on the SOPs provided by the makers of the commercial kit for use in a forensic laboratory. Typically, DNA profiling steps are performed in the following order: DNA

extraction or the isolation of DNA, quantification of the DNA yield in the sample, amplification of STR loci using PCR technology, DNA electrophoresis which is the separation of the PCR amplicons on a genetic analyser with the use of bioinformatics to analyse the resulting data, and finally comparing the sample data to reference DNA profiles or database housing previously generated STR sets (Linacre & Templeton, 2014; Nims *et al.,* 2010) (Figure 1.3). Statistical analysis will be performed to determine the probability of this match for the court, and with the type of standard technology used today, this likelihood is in the magnitude of one in billions for a random match.

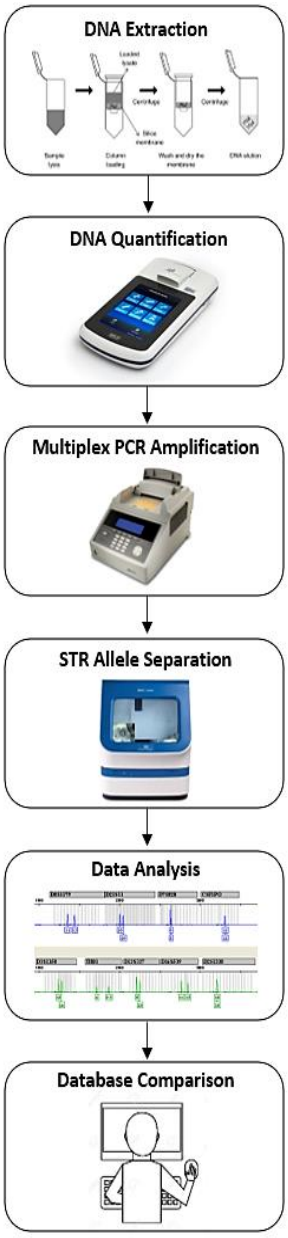


Figure 1.3: Workflow of DNA profiling.

The development of STR multiplex kits led to forensic DNA databases being established, that is, computer databases containing records of suspects' DNA profiles or DNA profiles of crime scene samples, and it shapes an important investigative tool in contemporary criminal justice systems (Jakovski *et al.,* 2017; Santos *et al.,* 2013). The National DNA Database (NDNAD), the world's first DNA database, was established in April of 1995 in the United Kingdom (Shrode, 2014), and contains both personal DNA profiles together with results obtained from crime scene samples (Carracedo *et al.,* 2008). Soon after, the FBI introduced the Combined DNA Index System (CODIS) national DNA database in 1998. The primary function of a criminal DNA database is to generate hits to the inserted STR profiles between stored DNA profiles of suspects, convicted criminals, victims and DNA evidence found at the crime scenes as allowed by the legislation of each country. Nowadays, it is estimated that around 69 countries currently operate national forensic DNA databases, with others being expanded or developed in at least 34 additional countries (Machado & Silva, 2019).

Interpretation of DNA profiles is not straightforward, especially from minute samples such as trace or Touch DNA. Samples collected from crime scenes can produce a single DNA profile which refers to a single source of DNA or a mixture DNA profile which refers to multiple sources of DNA. These DNA profiles can be full or partial, full means all the alleles are detected at the used loci, whereas partial means an allele or few alleles are missing. In some cases, a DNA-free profile can be obtained which means there is no human DNA detected. There are features or artifacts to be considered when interpreting electropherograms of DNA profiles: allele drop-out where one allele in a heterozygote locus is not visible (Li, 2008; Butler, 2005), heterozygote imbalance at the locus when the two peaks of a heterozygote are not close in height as expected in standard DNA profiles which is usually 60-90% (Gill *et al.,* 2000; Walsh *et al.*, 1992; Li, 2008), and allele drop-in when there is a non-specific generation of extra alleles, often much smaller in peak height (Buckelton, 2009). Stutter products are the most common, occurring as a result of strand slippage during the extension stage of PCR amplification, and are visible in the form of a small peak, usually one repeat unit smaller than the true (parent allele) peak (Goodwin *et al.,* 2011). Low levels of DNA often produce these stochastic effects, and it can be difficult to determine if the DNA present has an evidential value without

comparison to a suspect reference DNA sample because sometimes it can be a result of DNA contamination (Ballantyne, *et al.,* 2013). Individuals may share many alleles in a DNA profile, in which a mixture of DNA from two persons may contain two, three or four peaks at each locus. This makes the task of interpreting profiles and deciding the number of contributors to a mixed profile very complex, particularly when there are more than two contributors (Naughton & Tan, 2011). With the advancement in new software technologies such as STRmix™, DNA data interpretation has become much easier to process and upload for DNA databases (Bright *et al.,* 2019).

1.1.4.3 ALTERNATIVE FORENSIC DNA ANALYSIS METHODS

In addition to the standard STR profiling protocols used, there is an abundance of alternative or supportive DNA analysis methods which have been established with forensic applications in mind, and which may be more suited to generating usable identification information in specific situations, for instance, in cases where the sample DNA is extremely degraded.

One such method employs the Y-chromosome, unique to males, it consists of roughly 60 million bps including over 400 STRs (Li, 2008; Gunn, 2006). The main developers of autosomal multiplex kits have always included AMEL as an indicator of sex but are now adding more markers, usually Y-STRs to avoid incidences of AMELY null in casework, such as Yindel, DYS391, DYS570 and DYS576. Today, two manufacturers produce the leading Y-STR multiplex kits used in forensic casework using up to 23 and 27 loci, respectively (Court, 2021). Using Y-STRs profiling is a particularly beneficial technique for samples of a mixture of male and female components, such as in cases of sexual assault because it allows separation of the male-specific information from the mixture (Thompson & Black, 2007; Rudin & Inman, 2002; Savino & Turvey, 2011). It is more useful in cases where the male individual does not produce spermatozoa, as a DNA profile can be extracted from the male epithelial cells present, which usually in traditional STR analysis is dominated by the female epithelial cells (Gunn, 2006). They are also useful in the identification of human remains, as well as for cases of paternity investigations (Rapley & Whitehouse, 2007).

Another example of the more recently explored alternative to STRs is the analysis of single nucleotide polymorphisms (SNPs). SNPs are sequence variations at particular locations within the human genome and occur as a result of a single base pair mutation. These mutations can be a result of the deletion, insertion or substitution of a nucleotide (Li, 2008; Decorte, 2010). Compared with STR loci, SNP sites have a lower mutation rate, approximately 10^{-8}, and the amplification products of individual SNP sites could be very short, making SNPs convenient for the analysis of highly degraded forensic samples (Kidd *et al.,* 2006; Budowle & van Daal, 2008; Goodwin *et al.,* 2011), thus a useful forensic tool for the identification of individuals after mass disasters. Furthermore, SNPs can be used within four forensically relevant SNP classes, identity-testing, phenotype informative, ancestry informative, and lineage informative. Currently, commercially available SNP kits such as SNaPshot™ Multiplex can help identify known SNPs using single base extension (SBE) technology (Daniel *et al.,* 2015; Fondevila *et al.,* 2017). Since forensic polymorphic STR loci are limited in number, SNPs could replace STRs in the future but this process might take considerable time.

Other examples of possible forensic applications for SNP technology incorporate analysis of mitochondrial coding regions for haplotyping (Quintán *et al.,* 2004). Mitochondrial DNA (mtDNA) is found in the mitochondria organelles contained within the cell cytoplasm and is different from autosomal and Y-chromosome DNA which is located within the cell nucleus. Another distinction between nuclear and mtDNA is the number of copies in which they are present within each cell. There is solely one copy of the nuclear genome per cell, whereas each cell contains a few hundred to 1,000 mitochondria, with each mitochondrion having 2 to 10 copies of mtDNA, and each copy is identical for that individual, except for any mutations (Iborra *et al.*, 2004; Zhang *et al.,* 2015). Human mtDNA is a 16,569 bp in length, double-stranded, closed-circular DNA molecule, and encodes 13 polypeptides, of which, two rRNAs, and one set of 22 tRNAs are needed for protein synthesis in mitochondria (Wallace, 2012). Within the D-loop of the mtDNA, there are two polymorphic regions useful for forensic exploitation. These regions are known as Hypervariable regions I and II (HVI and HVII), which have a mutation rate 5-10 times that of nuclear genes (Budowle *et al.,* 2003). The main beneficial forensic use of mtDNA is its prevalence in very old or extremally degraded

samples (Holland & Parsons, 1999; Holland *et al.,* 2003). However, the major disadvantage of mtDNA analysis is that the mtDNA sequence is not unique to an individual because mtDNA is maternally inherited, and due to the mechanism of inheritance, except for mutations, all maternally related individuals will have the same mtDNA sequence (Decorte, 2010).

1.1.4.4 MASSIVELY PARALLEL SEQUENCING (MPS)

Massively parallel sequencing (MPS) or next-generation sequencing (NGS) has attracted widespread attention among forensic genetics researchers. This technology enables the sequencing of thousands of genomic regions simultaneously, which allows whole genome sequencing, metagenomic sequencing, or even targeted amplicon sequencing (Gettings *et al.,* 2016). STR typing methodology based on capillary electrophoresis can only show differences in length but NGS can also detect differences in the internal sequence and flanking structure of STRs. This increases the obtainable genetic information and provides new possible methods to deal with troublesome cases involving complex kinship identification and the resolution of mixtures which is one of the main challenges in DNA profile interpretation. NGS technology provides an increased power of discrimination of STR alleles by using the intrinsic SNPs genetic microhaplotypes, which is a combination of 2–4 closely related SNPs within an allele (Kidd *et al.,* 2014; Pang *et al.,* 2020). However, the approval of analysis programmes to deconvolve mixtures has not been regulated to the same level as it has for STRs.

Today, diverse NGS technologies are accessible, each using somewhat different technologies to sequence DNA (Heather & Chain, 2016). For human forensic genomics use, Verogen has developed kits using Illumina's MiSeq FGx system (Guo *et al.,* 2017; Moreno *et al.,* 2018). In 2019, DNA profiles generated by Verogen forensic technology were approved to be uploaded into the National DNA Index System (NDIS) (SWGDAM, 2019), which makes it the first NGS technology approved for a forensic database.

1.1.4.5 OTHER DEVELOPMENTS RELATED TO FORENSIC GENETICS

Forensic DNA typing has advanced quickly within a short timeframe, which can be attributed to the many developments in molecular biology technologies. As these techniques progress, forensic scientists will analyse more uncommon forms of evidence to answer questions considered unresolvable with traditional DNA analysis, generating new information about the donor of a biological sample (McCord *et al.,* 2019; Butler & Willis, 2020). Some examples of these new advancements are epigenetics and DNA methylation analysis, body fluid identification, forensic DNA phenotyping and genetic genealogy.

Epigenetics and DNA methylation markers have been suggested to estimate the age of the person, decide a tissue type, and even distinguish between monozygotic twins (Vidaki & Kayser, 2018). However, since epigenetic patterns are also affected by environmental factors, they can change, so several factors have the potency to influence predictions and should be considered when designing prediction models (i.e., age estimation) (Jung & Lee, 2017). The ability to identify the existence of a specific human body fluid can be quite useful to an investigation, providing valuable information on the activities involved in an incident, especially if it means that a DNA profile can be linked to a particular biological source. The common presumptive/confirmatory tests used to identify some (but not all e.g., vaginal material, menstrual blood) body fluids have limitations, which include a lack of high sensitivity and specificity, and sometimes require multiple tests to be performed that might lead to the destruction of limited samples (An *et al.,* 2012). This led to some interest in the analysis of RNA for the use of body fluid identification from stains, in particular, as RNA can be co-extracted with DNA, thus opening the possibility of having a DNA profile besides body fluid testing (Cooper *et al.,* 2015). The initial test assay concentrated on identifying body fluid-specific messenger RNA (mRNA) markers, and advancement of multiplexes indicating the existence of single or multiple body fluid types, the latter is more useful when analysing mixed samples (Albani & Fleming, 2018). In instances where standard STR profiling cannot advance an investigation because no match to a known offender or DNA database is found, any relevant information that can help in identifying the donor of the DNA sample would be very valuable. This has led to the development of forensic DNA

phenotyping (FDP), which predicts externally visible characteristics (EVCs) from DNA samples, bio-geographic ancestry, and age using epigenetic markers (Parson, 2018). This can generate new information to help narrow the pool of possible suspects and can also be useful in missing person cases and body identification in mass disasters (Kayser, 2015). FDP techniques have advanced from many decades of research identifying relevant SNPs that are statistically associated with specific characteristics through genome-wide association research (Wray *et al.,* 2015). From these studies, small sets of SNPs have been specified that can be typed in PCR multiplexes and analysed using a set of statistical models that can predict EVCs of interest with high accuracy. This, by far, is considered the most advanced and effective use of these approaches to predict human pigmentation traits (Kayser, 2015). Familial searching of forensic DNA databases has been successfully used to identify close (generally first/second degree) relatives of suspects through the observation of allele sharing in STR profiles between the related individuals. Genealogists with the use of genetic genealogy can identify more distant relatives (generally third to the ninth degree) by looking at the stretches of DNA in the genome that are identical by origin, indicating common ancestry (Mateen *et al.,* 2021). This can be achieved by utilising huge genetic datasets accumulated by individuals taking Direct-to-consumer (DTC) genetic tests for the goal of genealogical research. These tests often screen hundreds of thousands of autosomal SNP variants, the results of which are then published on large public platforms such as GEDmatch, that permit testers to identify potential relatives (Kennett, 2019; Kling *et al.,* 2021). Searching online platforms using profiles produced from biological samples recovered in cases related to criminal investigations may help identify relatives of the potential offender, and additional genealogical research could lead to the identification of a suspect whose DNA can then be collected and compared to crime samples (Greytak *et al.,* 2019). Nevertheless, this approach has raised concerns regarding data privacy and ethics (Greytak *et al.,* 2018). Even though these developments related to forensic genetics can be useful, DNA profiling with the use of multiplex STRs has long been the gold standard for human forensic analysis due to the standardisation of DNA markers, databases and statistical analyses. It has laid the basis for these promising technologies that will help enhance intelligence gathering and improve human identification in forensic cases, but it will take considerable time to be established for reasons related to time and cost.

1.2 TOUCH DNA

Recovering DNA from crime scenes and matching it to someone who may have been involved is now a common practice in forensics. Advancements in scientific techniques have increased the sensitivity of DNA profiling to allow better recovery rates and examination of not only body fluids such as blood and semen but also DNA deposited through handling items (Wickenheiser, 2002). Methods of DNA collection, extraction, amplification, and detection continue to improve and be optimised. This has led to results being produced from highly limited amounts of DNA, often from multiple individuals (Van Oorschot *et al.,* 2010), and has proved to be useful in the investigation of many serious crimes, such as homicides, burglaries, sexual assault etc. (Raymond *et al.,* 2009a; Williamson, 2012; Quinones & Daniel, 2012; Martin *et al.,* 2018). Despite the current increased sensitivity of DNA profiling technology, there is a need for improved methods for trace DNA analysis to enhance the recovery methods and techniques, as well as extraction of Touch DNA collected from the different surfaces under examination (Van Oorschot *et al.,* 2010; Verdon *et al.*, 2014a; Alketbi & Goodwin, 2019a). Generally, samples collected from handled items contribute to more than half the overall number of samples being processed for DNA profiling (Van Oorschot *et al.*, 2019), and the overall level of DNA recovered from these trace samples can be quite low which often leads to a DNA-free profile being produced (Raymond *et al.,* 2009a).

Trace DNA or Touch DNA is a suitable term when talking about the collection of minute amounts of DNA from biological samples. Previously, it was referred to as low copy number (LCN) (Gill *et al.,* 2000; Gill*,* 2001) or low template DNA (LTDNA) (Budowle *et al.,* 2009) but LTDNA is used to describe the amplification phase where the use of tiny amounts of materials can generate stochastic effects, while LCN is also used to describe the method of cycles in PCR rather than the amount of DNA present. This can be misleading therefore, in this study, the term trace or Touch DNA will be used to refer to the DNA collected from the touched samples.

1.2.1 SOURCE OF TOUCH DNA (SKIN DNA)

Skin is the largest organ of the body accounting for 7-15% of the total body weight, and it consists of cells with a density of 500,000 cells for each square centimetre of the skin (Marieb & Hoehn, 2007). Those cells are subject to continual regeneration i.e., a shedding process (Butler *et al.*, 2004) and individuals are estimated on average to shed 400,000 cells per person every day (Wickenheiser, 2002). Epithelial cells coat the whole outer surface of the body and epithelial skin tissue is called stratified epithelium (Marieb & Hoehn, 2007). The epidermal layer (the outer layer of skin – Figure 1.4) regenerates continually as the basal cells are subjected to mitotic division and slow maturation (Kita *et al.,* 2008). The stratum corneum (upper epidermal layer) is made up of 8–13 µm thickness of flattened, fully differentiated keratinocytes called corneocytes or anucleate corneocytes (Balogh *et al.,* 2003a) because they lose their nuclei and organelles during keratinisation which is related to apoptosis (programmed cell death) (Kierszenbaum & Tres, 2015; Bragulla & Homberger, 2009).

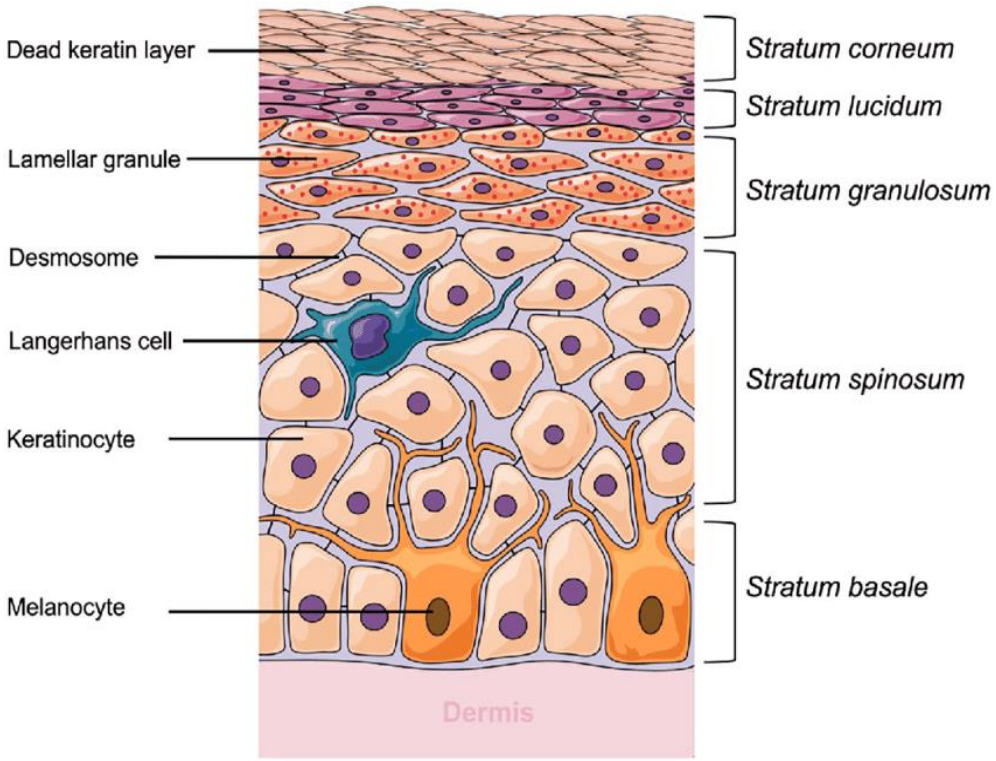


Figure 1.4: Epidermal skin layers of the hands represented by cell type (Ramadon *et al.,* 2021). The process of terminal differentiation happens as cells move up to the upper layer of skin, producing an outer layer made up of flattened keratinocytes without nuclei (corneocytes).

Historically, forensic scientists presumed that DNA deposited by touch came from sloughed off external skin cells (Wiegand & Kleiber, 1997), and that statement continues to be used in courtroom testimony and publications today regardless of limited research to support it (Bright & Petricevic, 2004; Djuric *et al.,* 2008; Helmus *et al.,* 2016; Huo & Zhang, 2016; Kanokwongnuwut *et al.,* 2018a). Often data in studies related to touch deposits are regularly in the form of DNA typing results rather than any cellular characterisation, therefore indicating only the existence of DNA and not its origin.

Burrill *et al.* (2019) proposed that Touch DNA can originate from various places, sloughed keratinocytes or their component parts from the outermost layers of an individual's skin on the hands, nucleated epithelial cells of other fluids such as saliva, nasal fluids, eyes etc., or of body parts that come in contact with one's hands, it can be also from cell-free DNA that either originates from hands such as sweat or relocated onto the hands from the above-mentioned fluids (Figure 1.5). Keratinocytes deposited by touching have been reported to stain positive for DNA with the use of multiple nucleic acid dyes and produced detectable profiles, even though they have been presumed to be fully keratinised and inactive (Kanokwongnuwut *et al.*, 2018a). An experiment performed by Kita *et al.* (2008) showed that tiny amounts of the DNA are present on the skin surface, and they considered that these fragments of DNA could be sloughed off the keratinised cornified layer of the skin from the epidermis, and that sweat could also contain fragmented DNA. Another study by Quinones and Daniel (2012) theorised that the presence of sweat deposited by the skin helps to contribute to the DNA profile generated from Touch DNA samples.

Nucleated epithelial cells are considered to be a rich source of DNA, and although they may not be generated in great numbers from the skin on the hands, they can still account for a considerable source of Touch DNA deposited if they are relocated onto the hands from elsewhere on the body. In summary, Touch DNA refers to the mechanism by which DNA is deposited on the surface but does not mean the cells containing the DNA originate only from the hand.

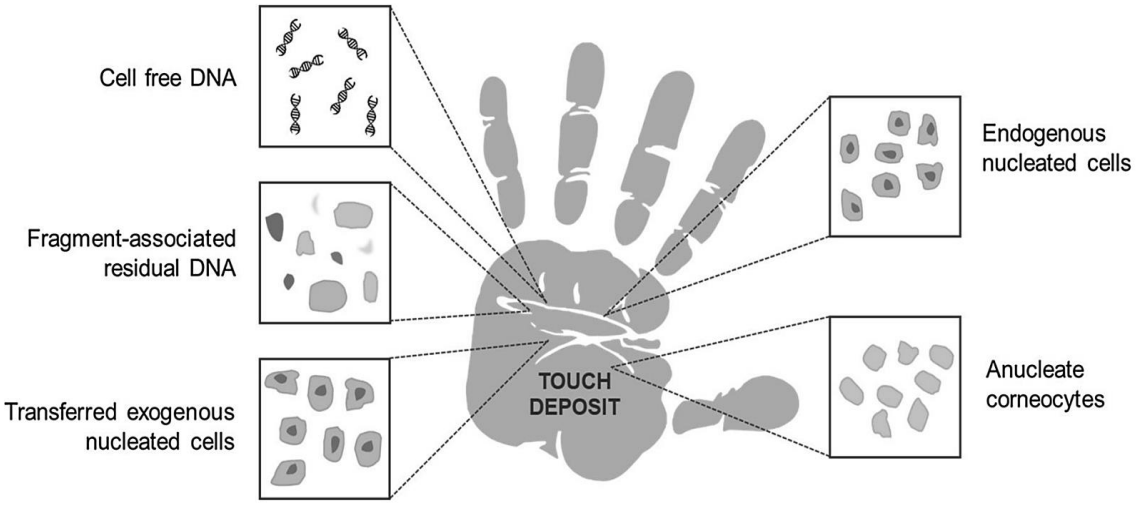


Figure 1.5: Possible source of DNA deposited by touch/handling. DNA collected from handled items in forensic cases typically comes from nucleated or anucleate cells of hands, nucleated cells relocated onto hands from elsewhere by coming in contact with hands, residual cell fragments originated from hands including free nuclei, or cell free DNA in form of sweat on hands or transferred residual of body fluids (Burrill *et al.* 2019).

1.2.2 AMOUNT OF TOUCH DNA

The amounts of DNA collected from Touch DNA usually vary because it is affected by many variables, and the quantity of DNA deposited by touching wearing/handling items is vastly different among and within individuals, which makes predictions about the amounts of DNA on these items generally unrealistic (Meakin & Jamieson, 2013). However, the information summarised in Table 1.2 which has been collected from previous studies can provide an estimate regarding how much DNA can be possibly recovered from touched items.

Even though worn clothing in general and headgear are reported to retain more DNA than handled items (Mapes *et al.,* 2016a), the variation in DNA deposition can range from 0 ng to nearly 170 ng of DNA measured (see Table 1.2). Nevertheless, the studies do differ considerably in their deposition process and quantification methodologies, which make direct comparisons complicated and predictive conclusions impractical.

Table 1.2: Summary of the studies reporting published DNA quantities recovered from diverse touched or handled items (adapted from table produced by Meakin & Jamieson, 2013 and Burrill *et al*. 2019)

Surface	Length and Nature of contact		Quantity (ng)	Publication	Year
Swabbing of hand	-	-	2-150	van Oorschot and Jones	1997
plastic knife handle, mug, glass	15 min	holding	7-34	van Oorschot and Jones	1997
Swabbing of hand	-	-	0.1-6.4	Bright and Petricevic	2004
New lower bed sheet	1 night	sleeping	0-8	Petricevic *et al.*	2006
Glass slides	5 sec	pressure	0-2	Allen *et al.*	2008
Paper	30 sec	pressure	0-110	Sewell *et al.*	2008
Door frame	1 min	grabbing	0- > 0.2	Raymond *et al.*	2008
Cartridge casing	30 sec	handling	0.3-0.7	Horsman-Hall *et al.*	2009
Cotton	10-15 sec	rubbing	6-12	Goray *et al.*	2010
Plastic	10-15 sec	rubbing	0.4-0.5	Goray *et al.*	2010
Melamine-coated Board	10 sec	pressure	0-160	Kamphausen *et al.*	2012
Glass	1 min	holding	0-5	Daly *et al.*	2012
Fabric	1 min	holding	0-15	Daly *et al.*	2012
Wood	1 min	holding	0-169	Daly *et al.*	2012
Infant's clothing	1 min	rubbing	0.3-9	Goray *et al.*	2012
Plastic block	1 min	rubbing	0-2.5	Goray *et al.*	2012
Plastic syringe	10 sec	holding	0-80	Poetsch *et al.*	2013
Glass slides	brief	fingerprint pressure	0-17.6	Thomasma and Foran	2013
Knife handle	1 min x 4	simulated regular use	~ 1-10	Meakin *et al.*	2015
Glass slides	15 sec	fingerprint pressure	0-1.5	Oleiwi *et al.*	2015
Glass	10 sec	pressure	0-5	Goray *et al.*	2016
Knife handle	brief	grip/stabbing	0-4.8	Samie *et al.*	2016
Non-porous cables	brief	fingerprint pressure	0-3	Lim *et al.*	2016
Plastic tubes	10 sec	holding	0.04-3.8	Fonnelop *et al.*	2017
Car steering wheel	2-60 min	holding	0.21-134	Kirgiz and Calloway	2017
Plastic cable ties	brief	used to bind objects	0-39.8	Steensma *et al.*	2017
Polycarbonate board	brief	fingerprint pressure	0-3.5	Tobias *et al.*	2017

Generally, people who leave more or less DNA upon contact are called “good shedders” and “bad shedders”, respectively (Lowe *et al.,* 2002; Lowe *et al.,* 2003). This categorisation is considered to be overly simplified because of the wide intra-individual variations observed (Phipps & Petricevic, 2007), but the terminology to describe different shedders has continued in research and testimony (Djuric *et al.,* 2008; Bright & Petricevic, 2004; Horsman-Hall *et al., 2009*). It is still unclear what exactly is being “shed” to deposit DNA and where, among the component parts of the touch deposit, detectable DNA originates. Knowing what the deposited material on surfaces could be would allow researchers to understand the shedding differences between people, foretell DNA deposition levels and improve DNA recovery from these sample types.

The amount of collected DNA from Touch deposits can be very low but STR typing is relatively sensitive to detect 0.200 ng or less, which is tantamount to approximately 30 nucleated cells (Butler, 2011), and the current DNA profiling methods can generate profiles from nearly single-cell levels (0.008–0.010 ng) (Butler, 2015; Alfonse *et al.,* 2017; Geng & Mathies, 2014).

1.2.3 DIRECT AND INDIRECT TRANSFER OF TOUCH DNA

Touch DNA is non-visible biological material left on a surface simply by touching it by hand, and one contact event can simultaneously include both direct/primary and indirect/secondary transfer events (Van Oorschot & Jones, 1997; Van Oorschot *et al.,* 2019). A self-DNA deposited through the handprint may be considered a direct deposit, whereas a non-self-component is considered an indirect deposit (Raymond *et al.,* 2009b; Mapes *et al.,* 2016b). In most case scenarios when multiple DNA transfers are unknown, it is preferable to use the term 'indirect transfer' rather than 'secondary transfer'.

Direct and indirect transfers are related to the ways by which DNA can be transferred, as shown in Figure 1.6, and are not only related to non-visible biological materials but it body fluids such as blood, saliva or semen, and usually contain more DNA materials than Touch DNA. An example of direct/primary transfer is the transfer of DNA when handling an object/surface with the bare hand, shaking another person's bare hand, or when wearing clothes in contact with the skin. An example of indirect transfer is DNA from person A deposited on a knife handle when using it with bare hands, then DNA from person A collected by person B when they handled the knife with bare hands (Buckingham *et al.,* 2016). This can lead to person A being detected on an object handled by person B after they handled the knife that was used by person A.

DNA transfer, as described above, and DNA contamination have the same dynamics of DNA movement from one surface/object/location to another. The timing of this DNA movement clarifies whether DNA transfer is linked to a crime related activity before securing a crime scene (which can be pre-, during, or post-crime), or a non-crime related DNA contamination event happened during or post-, or even pre-securing of the crime

scene (Rudin & Inman, 2001). Consequently, it is important to consider all these different scenarios of Touch DNA movements when dealing with mixed DNA profiles.

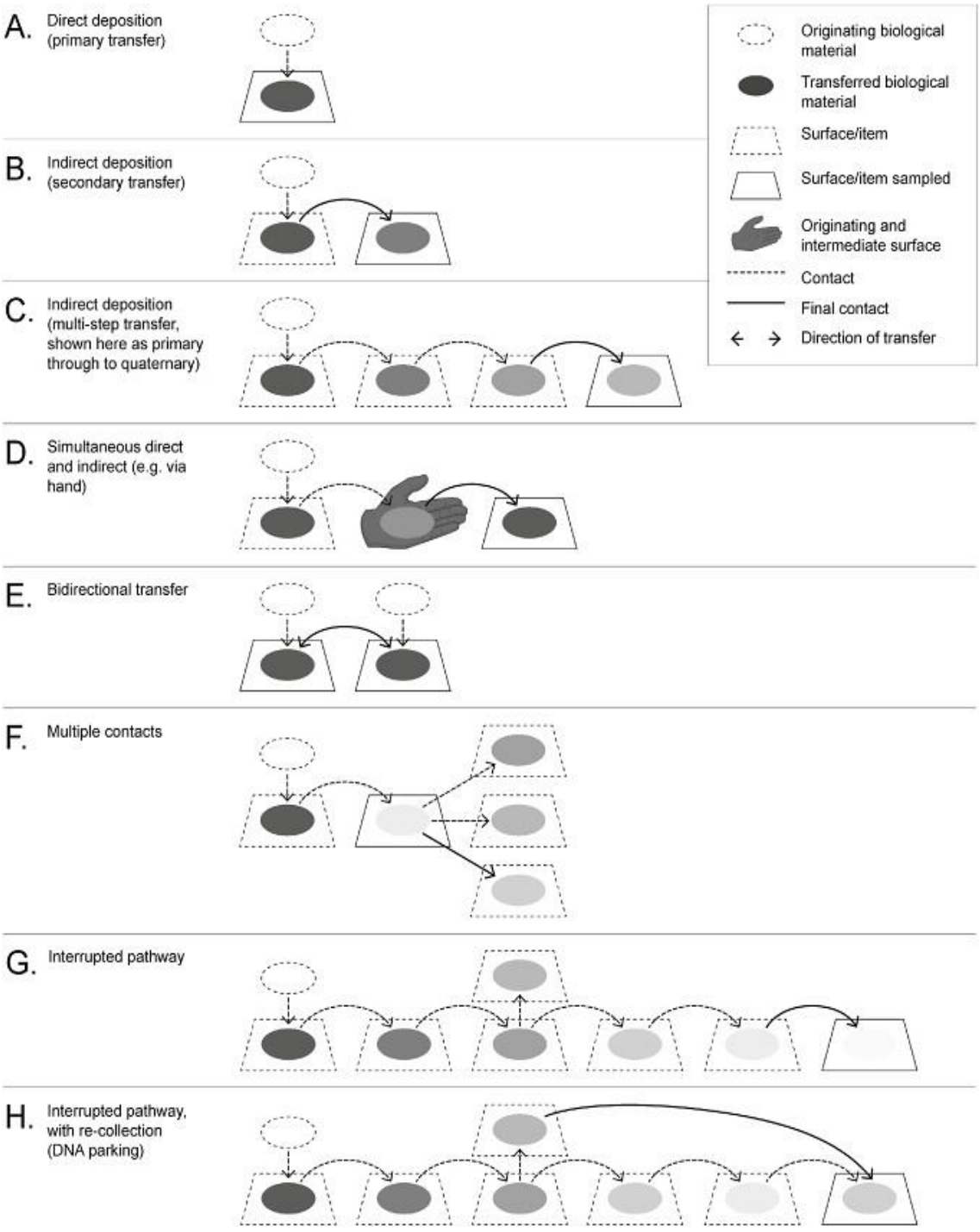


Figure 1.6: Diagram illustrating different types of DNA transfer (Oorschot *et al.,* 2019). **(A)** Primary transfer (direct deposition), **(B)** secondary transfer (indirect deposition), and **(C-H)** different types of indirect transfer.

1.3 FACTORS AFFECTING TOUCH DNA

Touch DNA can be collected from numerous items found at crime scenes and many published studies have explored the DNA recovered from handled items such as handbags, clothing, jewellery, weapons, knives, tools, car steering wheels, etc. (Findlay *et al.,* 1997; Schulz & Reichert, 2000; Pizzamiglio *et al.,* 2004; Barbaro *et al.,* 2006; Petricevic *et al.,* 2006; Zamir *et al.,* 2007; Franke *et al.,* 2008; Sewell *et al.,* 2008; Aditya *et al.,* 2011; Taupin & Cwilklik, 2011). However, Touch DNA collected from used objects is associated with some issues related to the quantities, deposition, collection and materials or techniques used (Van Oorschot *et al.,* 2003). The quality and quantity of Touch DNA deposited on an object affects the DNA recovery and often the amount of DNA collected is not constant due to various reasons, as discussed in detail below.

1.3.1 SHEDDING STATUS

The quantity of DNA deposited and its convenience for analysis is determined by the DNA deposition process and it can be impacted by various factors including the propensity of the individuals shedding the DNA, the activities of the individual prior to DNA deposition, the type of the surface from which the DNA was collected, as well as the nature of the physical contact of the DNA deposition (Lowe *et al.,* 2002; Raymond *et al.,* 2004; Phipps & Petricevic, 2007; Allen *et al.,* 2008; Cowell, 2011). Several studies have noted differences in the propensity of individuals to deposit their DNA when contacting an item or surface (Ladd *et al.,* 1999; Lowe *et al.,* 2002; Alessandrini *et al.,* 2003; Phipps & Petricevic, 2007; Farmen *et al.,* 2008; Meakin Graham *&* Rutty, 2008; Oleiwi *et al.,* 2015; Goray *et al.,* 2016; Meakin *et al.,* 2017; Szkuta *et al.,* 2017; Fonneløp *et al.,* 2017; Kanokwongnuwut *et al.,* 2018b).

As discussed previously, some referred to the shedding status of individuals as ‘good’ or ‘bad’ (Lowe *et al.,* 2002; Lowe *et al.,* 2003). Lowe *et al.* (2002) categorised people with a tendency to slough their skin cells easily as compared to other individuals as “good shedders”. This was however conflicted with a study by Phipps and Petricevic (2007), which stated that it was not possible to decide whether an individual was a good or bad shedder because different shedding tests were used on the same individuals under different scenarios and conditions yielded different results.

While there is proof that some individuals shed more than others, the factors impacting this are to some extent still uncertain. Warshauer *et al.* (2012) and Oleiwi *et al*. (2015) reported that the amount of DNA sloughed from the hand palm surface is considerably less than from fingers, which may be linked to the projection of sweat and sebum secretion from sebaceous glands (Linacre *et al.,* 2010; Quinones & Daniel, 2012). Furthermore, some studies demonstrated that younger individuals can deposit more DNA compared to older people (Poetsch *et al.,* 2013; Manoli *et al.,* 2016), while other studies suggested that males are more likely to be categorised as heavy shedders compared to females (Allen *et al.,* 2008; Lacerenza *et al.,* 2016; Manoli *et al.,* 2016).

Other factors that influence the shedding status of an individual involve the habits of a person. It has been argued that individuals with behaviours of touching their face (eyes, nose, hairs, etc.), body, or eating their fingernails, are more likely to collect DNA from those areas and transfer it to other objects or surfaces through touch (Phipps & Petricevic, 2007). This process can be characterised as "loading" the fingers with DNA (Wickenheiser, 2002).

1.3.2 CONDITION OF SKIN

Deposited Touch DNA from various types of shedders can vary due to environmental, behavioural and health conditions. For example, psoriasis is a skin condition that leads to an increased number of nucleated cells produced in the outer layers of the epidermis, and in normal epidermis, DNA accounts for 0.1% of weight compared to 0.55% in the psoriatic epidermis (De Bersaques, 1966). Moreover, individuals with comparatively dry hands are suggested to be more likely to shed or peel more than others, which increases the amount of DNA recovered (Bright & Petricevic, 2004). Another study by Kamphausen *et al.* (2012) supported this hypothesis, finding that individuals with flaky skin due to skin conditions on their hands such as neurodermatitis, atopic dermatitis and psoriasis shed more DNA, which resulted in better quality DNA profiles than those without such disorders. However, more research is required to understand how hand skin conditions can influence shedding status.

1.3.3 ACTIVITIES PRIOR TO TOUCHING

The type of activity before deposition can influence the amount of DNA recovered. Lowe *et al*. (2002) were the first to suggest that handwashing before deposition may impact DNA transfer, and a study by Phipps and Petricevic (2007) noted a general increase in the numbers of alleles observed when handwashing was uncontrolled. Quinones (2011) proposed that there is a statistically considerable relationship in the duration between handwashing, sample deposition and repeated DNA deposits but over 1 ng of DNA was recovered in samples taken immediately 5 mins after handwashing and over 2 hours.

In contrast, Zoppis *et al.* (2014) reported that no DNA profiles were generated after 10 min of deep handwashing with antiseptic soap and air drying, while full profiles were obtained with no handwashing, but there was some source of contamination observed. Similarly, Szkuta *et al.* (2017) detected no direct connection between the duration of handwashing and the DNA deposition collected from handprints on glass plates which were deposited after a handshake, even though the time between handwashing and deposition ranged from 5 minutes to 6 hours. Based on these mixed results, further systematic studies about the influence of handwashing on the quantity and quality of DNA deposited by hands are required (Van Oorschot *et al.,* 2019).

1.3.4 TYPE OF SURFACE ON WHICH DNA IS DEPOSITED

An important factor in the inconstancy of trace DNA is the surface type from and onto which the Touch DNA is deposited. DNA has been recovered successfully from a vast range of surfaces including fingerprints (Wiegand *et al.,* 1993; Van Renterghem *et al.,* 2000), metal cables (Lim *et al.,* 2016), lipstick (Webb *et al.,* 2001), banana skin (Alketbi, 2020), shoes (Bright *et al.,* 2004; Hillier *et al.,* 2005), car interiors (Pizzamiglio *et al.,* 2004), plastic bags (Hellerud *et al.,* 2008; Helmus *et al.,* 2016), sheets (Petricevic *et al.,* 2006), firearms and ammunition (Polley *et al.,* 2006; Horsman-Hall *et al.,* 2009) and paper (Balogh *et al.,* 2003a; Sewell *et al.,* 2008). In general, rough and porous surfaces retain more DNA than smooth ones, with wood being preferable to fabrics followed by glass (Daly *et al.,* 2012), and cotton being better than plastic upon frequent handling (Goray *et al.,* 2010). However, the success of collecting trace DNA from various surfaces may also depend on the collection method (Alketbi & Goodwin, 2019a). Most studies

were based on different deposition processes and collection techniques that can influence the amount of DNA retrieved from these surfaces.

According to Wickenheiser (2002), a rough, porous surface has more potential to retain DNA than smooth, nonporous surfaces due to the abrasive nature of a rough surface which is likely to extricate cells and therefore, increasing the possibility of DNA retention (Sutherland *et al.,* 2003). Contrary to this, a study by Pesaresi *et al.* (2003) indicated that smooth and nonporous surfaces like glasses have increased chances of retaining more DNA when compared to rough, porous surfaces like untreated wood possibly because smooth and nonporous surfaces can increase the rate of perspiration during the interaction with the surface, thereby increasing the amount of DNA deposited but that can be dependent on the duration of the deposition and other environmental factors such as humidity (Alketbi, 2018; Alketbi & Goodwin, 2019b). Nonetheless, Goray *et al.* (2010) reported that the amount of DNA retrieved from a cotton substrate (rough porous surface) on average is 11.68 ng, which was notably higher than the amount of DNA (0.4 ng) collected from plastic (smooth nonporous surface). This suggests that it is likely that more DNA will be deposited on porous compared to nonporous surfaces because they can retain more DNA during a longer duration of deposition.

Wickenheiser (2002) argued that although more DNA is expected to be deposited on a rough and porous surface, the quantity of DNA that can be recovered from such surfaces was lower, and this could be related to the inefficient recovery technique. Similarly, a study by Alketbi and Goodwin (2019a) of a selection of six surfaces ranging from smooth nonporous, smooth porous, rough nonporous and rough porous reported that the quantity of DNA was significantly affected by the type of surface, as well as the interaction between collection type and surface type. It is important to differentiate between the retention capability of the surface and recovery, as an inefficient DNA collection technique will reduce the amount of DNA recovered regardless of the surface (Alketbi, 2018). Regarding crime casework, Williams and Johnson (2008) reported that around 18% of samples retrieved from watch straps were successfully profiled by the FSS, therefore, further work is needed to improve Touch DNA profiling.

1.3.5 NATURE OF CONTACT

The nature of that contact such as pressure or frequency can influence the amount of DNA deposited. A common proposition is that more DNA will be deposited on most surfaces when the time and pressure applied on the surface are increased. However, Van Oorschot and Jones (1997) noted that the length of touch during deposition had no impact as the DNA transferred at the first contact. Another study by Balogh *et al.,* (2003a) confirmed these findings, showing that the average amount of DNA collected from different items was the same regardless of the length of time it was held; four donors deposited DNA on white office paper, the handling time was from 1 to 60 seconds and full STR profiles were obtained during 1, 2, 50, 60 sec handling time periods. In contrast, Tobias *et al.* (2017) argued that the increase in pressure of the direct skin on a surface during contact can increase the quantity of DNA deposited despite the DNA deposition ability of an individual.

The handling of many items in advance of touching the tested object decreases the amount of deposited DNA, therefore low DNA recovery could suggest either limited contact or contact after prior handling of other items, with no distinctions in the data observed (Van Oorschot *et al.,* 2003; Buckingham *et al.,* 2017). Furthermore, it has also been observed that repeated touching of pieces of plastic resulted in reducing the amount of DNA deposited (Farmen *et al.,* 2008). In the same study, one person classified as a poor shedder showed a steady DNA deposition after three repeated handlings of the same glass beaker compared to medium and good shedders whose DNA deposits reduced with repeated contacts (Farmen *et al.,* 2008), indicating that shedder status can have a significant influence in each scenario.

1.3.6 THE TIME BETWEEN DEPOSITION AND RECOVERY

Time is crucial when collecting Touch DNA from crime scenes, but its influence is still not fully known. Fregeau *et al.* (2010) reported that the quantity of DNA recovered from a fresh touch deposit was higher than the amount of DNA collected from a deposit that has been stored over a long period of time. In contrast, another study found that it was possible to collect a full DNA profile from a plastic tube after a ten-second contact of deposition by a good shedder after four months when kept at room temperature, yet

there was a notable decrease in the quantity of DNA that was recovered from touch deposits made by a poor shedder (Murray *et al.,* 2001). As such, the time between deposition and recovery of DNA can be a substantial factor in the amount of DNA recovered (Alketbi, 2018).

Similarly, Bille *et al.* (2009) concluded that there was a decrease in the quantity of DNA recovered between samples collected and analysed within a frame time of seven days (average 0.34 ng/µL) compared to a sample collected and analysed within ninety days (average 0.038 ng/µL). Furthermore, another study also pointed out that the amount of DNA deteriorated with the advance in time (Raymond *et al.,* 2009b). However, it is important to consider that the rate of deterioration significantly depends on the environmental conditions that the touched object is exposed to (Alketbi & Goodwin, 2019b). Another substantial factor to consider with the impact of time is the issue of DNA contamination, which can be minimised by a short time frame between deposition and collection (Li & Harris, 2003). It has been observed that recovered DNA from worn clothes increases over time when unwashed and often consists of mixtures (Mapes *et al.,* 2016), which can include the wearer's DNA, as well as individuals touching the surface of the clothes and unrelated background DNA that can be picked up by coming in contact with other surfaces.

1.3.7 ENVIRONMENTAL FACTORS

Once DNA has been deposited on the surface, the inquiry arises of how long it will stay on the surface before it gets collected and analysed, and what factors impact that persistence. A longer time between original deposition and recovery may lower DNA yield but mostly in combination with the environmental conditions (Raymond *et al.,* 2009b). Storage in a laboratory environment at room temperature resulted in no considerable decrease in DNA quantity from either "wearer DNA" on underpants after 12 weeks (Breathnach *et al.,* 2016), or from DNA deposited on slides after 6 weeks (Raymond *et al.,* 2008). However, there are few systematic studies to understand the impact of environmental conditions on touch deposit samples, which are usually hard to control in certain outdoor crime scene scenarios.

Exposure to potentially degrading outdoor environments such as UV light from the sun can affect the persistence of DNA deposition. Raymond *et al.* (2009b) observed some degradation of recovered DNA from window frames collected two weeks after deposition. UV irradiation of the DNA often leads to cross-linking of the adjacent thymine nucleotides, preventing the action of the DNA polymerase during PCR (Lindahl, 1993). A high temperature can also cause DNA degradation (Baptista *et al.,* 2015), with increases in heat and humidity usually leading to a rise in the rate of hydrolytic cleavage which affects the direct cleavage of the DNA strands due to drying (Poinar, 2003). Similarly, oxidation can damage DNA and cause the degradation of samples (Lindahl, 1993; Tasker *et al.,* 2020). Another factor is sand or dust found on objects in an outdoor environment, which affects the extraction process (Alketbi & Goodwin, 2019c).

Alketbi and Goodwin (2019b) showed that time does not affect Touch DNA samples when collected at room temperature but conditions such as low/high temperature and humidity can influence the amount of DNA collected by interacting differently with various types of surfaces. Therefore, the persistence of deposited Touch DNA found in outdoor crime scene items in countries that have low/high humidity and temperature variables can be directly affected (Alketbi & Goodwin, 2019b).

1.3.8 TYPE OF SAMPLING METHOD EMPLOYED

The collection method or sampling technique is one of the most crucial steps in Touch DNA profiling. Often, it requires choosing a collection method, the amount of wetting reagent, as well as the technique used in the process to help improve Touch DNA recovery (Alketbi, 2018). Verdon *et al.* (2014b) noted that the type of sampling device used can determine the success of the DNA collection and extraction. Nowadays, laboratories use different processes to collect Touch DNA from similar items but the main common methodologies used are swabbing, tape lifting or cutting out the area of interest for direct extraction or amplification (Van Oorschot *et al.,* 2010; Van Oorschot *et al.,* 2016). However, there are various types of swabs and tape-lifts, and means of their use, with considerable differences in the rates of recovery (Van Oorschot *et al.,* 2010; Verdon *et al.,* 2014b; Plaza *et al.,* 2016).

Other examples of less common methods such as a wet-vacuum system (Hedman *et al.,* 2015) or direct sampling of individual skin flakes (Schneider *et al.,* 2011). In some special cases, Touch DNA is collected by soaking the item in solutions, such as fired cartridges (Dieltjes *et al.,* 2011). Usually, the method is specified by the nature of the surface from which the DNA is to be recovered (Williamson, 2012).

DNA collection processes from solid and smooth surfaces are commonly done using wet and dry swabs (Sweet *et al.,* 1996), for example, recovering DNA from a knife handle (Goray *et al.,* 2010). However, Van Oorschot *et al.* (2003) indicated that a significant amount of DNA is wasted when using cotton swabs as it retains some DNA (24% and 52% of 100 ng in the 100 µL extract was recovered when using dry and wet swabs). Typically, cotton swabs are commonly used to obtain DNA from crime scenes. However, little is known about the suitability of nylon flocked swabs as a tool for collecting evidence at a crime scene (Alketbi, 2018) because nylon flocked swabs are not commonly used in many forensic DNA laboratories. Nylon flocked swabs, such as 4N6FLOQSwabs® (COPAN Diagnostics Inc), are designed to help increase DNA collection and improve efficiency. Some studies have shown that 4N6FLOQSwabs™ outperform traditional fibre swabs (e.g. cotton swabs) regarding DNA recovery (Dadhania *et al.,* 2013; Verdon *et al.,* 2014a; Brownlow *et al.,* 2012). However, techniques may vary between cotton and nylon swabs, such as the amount of wetting reagent used on the swab before collection which can influence DNA recovery. Also, cotton swabs typically absorb more wetting solution than nylon swabs but it can be more challenging to extract the collected Touch DNA (Van Oorschot *et al.,* 2003; Alketbi & Goodwin, 2019d).

Another technique that can be used to collect biological material from porous substrates for forensic analysis is tape lifting (Barash *et al.,* 2010). Daly *et al.* (2012) proposed that mini tapes can be useful for surfaces such as glass and wood. However, the efficiency when compared to swabbing should be investigated. The use of adhesive tapes for DNA collection and then swabbing off the collected material is useless and may result in a loss of DNA due to incomplete recovery from the tapes, as such, a direct extraction or direct amplification of the collection tapes is favourable (Stoop *et al*., 2017). Recently, the SceneSafe FAST™ minitape (Scene safe, UK) has gained popularity among several Swiss police corps due to its ease of use and it has already been characterised in some

other studies (Verdon *et al.,* 2014b; Verdon *et al.,* 2015; Hansson *et al.,* 2009). Hansson *et al.* (2009) compared Scene safe FAST™ minitape and three swab types (cotton, flocked and foam), reporting that the use of tape was more efficient (concentration of recovered DNA; 0.1–0.48 ng/µL) than the other three swabs (concentration of the recovered DNA; 0.0–0.075 ng/µL) when DNA was collected from a single type of cotton shirt material.

Based on the studies above, it would be useful to extend the existing knowledge on the influence of sampling methods in recovering Touch DNA from various surfaces by conducting a comparative study of different collection methods on the same set of surfaces.

1.3.9 THE EFFICIENCY OF EXTRACTION AND AMPLIFICATION

Usually, after the collection of the biological materials deposited on the surfaces, the process starts with extracting the DNA from the device used. Phenol-chloroform is a common type of extraction method used in laboratories for many years. Despite being convenient to perform extraction of high molecular weight DNA, phenol-chloroform is toxic, prompting forensic laboratories to look for new less hazardous methodologies (Ip *et al.,* 2015). Currently, there is a wide range of methodologies for DNA extraction from collection devices or directly amplifying from the sample substrate with diverse degrees of competency (Butler, 2011; Ip *et al.,* 2015). Some of the most used extraction methods include 5% Chelex, which has been reported to outperform organic methods in recovering DNA from samples collected from heels and toes (Bright & Petricevic, 2004). In addition, the DNeasy® plant mini kit, when compared with the QIAamp® mini kit, improved DNA recovery from paper by over 150% (Sewell *et al.,* 2008). In a study comparing the performance of five extraction kits, Ip *et al.* (2015) reported that the QIAamp® DNA Investigator Kit, QIAsymphony® DNA Investigator® Kit and DNA IQ™ produced extracts with a higher success rate for subsequent DNA typing analysis compared to Chelex®100 and the QIAamp® DNA Blood Mini Kit, even with the use Microcon to increase their concentration. The use of manual extraction methods such as the QIAamp® DNA Investigator Kit or automated once such as AutoMate Express™ Forensic DNA Extraction System are preferred in forensic DNA laboratories (Tasker *et*

al., 2019). However, the nature of the device used for DNA recovery could influence DNA extraction (Daly *et al.,* 2012; Alketbi & Goodwin, 2019a).

Furthermore, it has been reported the DNA extraction process could result in a loss of about 20% to 90% of the initial template amount depending on the extraction process used, as well as the accuracy of the quantification method (Ottens *et al.,* 2013; Balogh, *et al.,* 2003b). Also, the purification step used in forensic DNA casework can be time consuming and labour-intensive. Additionally, the column-based methods often used in the purification process can result in a loss of DNA (Barta *et al.,* 2014; Doran & Foran, 2014). Some studies demonstrated that direct amplification of trace quantities of DNA from swabs or small items and even fabrics can generate profiles as good as or better than traditional methods (Linacre *et al.,* 2010; Templeton *et al.,* 2015; Swaran & Welch*,* 2012; Ambers *et al.,* 2018; Cavanaugh & Bathrick*,* 2018; Martin *et al.,* 2018). Direct amplification means omitting the extraction, quantification, and concentration steps with maximal DNA recovered if laboratory personnel error and DNA contamination are minimised, thereby reducing the overall sample processing time and costs (Van Oorschot *et al.,* 2010; Linacre *et al.,* 2010). Nonetheless, direct amplification of Touch DNA is relatively new and requires further investigation. Ambers *et al.* (2018) reported that microFLOQ® Direct swabs obtained full DNA profiles from a variety of surfaces such as computer keyboards, computer mousses, cell phones, door handles and a necklace. However, the amount of biological material deposited on the item and the type of surface could affect the likelihood of success.

The Applied Biosystems™ GlobalFiler™ PCR Amplification kit is one of the popular amplification kits used in forensic laboratories because of its ability to produce good quality DNA profiles from trace samples. The kit is a 6-dye, short tandem repeat (STR) multiplex assay and amplifies 21 autosomal STR loci including 10 mini-STR loci, which can be useful for low quantity or degraded samples. Moreover, the kit is validated for use with 1.0 ng DNA (15 µL input volume) for 29 cycles, and 0.5 ng DNA (15 µL input volume) for 30 cycles for increased sensitivity for low-concentration samples (Thermo Fisher Scientific Inc., 2016). It has been reported that full DNA profiles can be even obtained with quantities as low as 125 pg when using the GlobalFiler™ PCR Amplification kit (Ludeman *et al.,* 2018).

1.4 RESEARCH AIMS AND OBJECTIVES

1.4.1 SIGNIFICANCE OF THE STUDY

Despite DNA recovery and extraction remaining the most critical steps in Touch DNA analysis, nearly all assays require a sufficient amount of DNA both quantitatively and qualitatively. Therefore, the success of DNA typing relies on the availability of existing DNA templates. Improved methods are needed to recover DNA, predominately when addressing the most challenging samples such as Touch DNA.

Although previous studies investigated the effectiveness of different DNA recovery methods from other types of DNA samples such as body fluids (Plaza *et al.,* 2016; Verdon *et al.,* 2014a), there is a lack of published data regarding trace DNA (Brownlow *et al.,* 2012; Hefetz *et al.,* 2019). Improving DNA recovery as well as extraction efficiencies are vital steps in enhancing Touch DNA profiling (Taylor *et al.,* 2017; Burrill *et al.,* 2019). There are many methodologies for collecting trace DNA, with most forensic laboratories developing their own protocols (Hansson *et al.,* 2009; Van Oorschot *et al.,* 2010; Verdon *et al.,* 2014b; Plaza *et al.,* 2016; Verdon *et al.,* 2015), but there is lack of data regarding Touch DNA recovery rates from various objects which will impact the development of new methodologies or techniques (Dziak *et al.,* 2018; Van Oorschot *et al*., 2019). Various reports by Bond and Hammond, (2010), Mapes *et al.* (2015), and Baechler (2015) have indicated the importance of the success of data collection and comparisons.

Most DNA is collected from crime scenes by cotton swabs and in most cases, the use of cotton swabs for all types of items results in a loss of DNA (Van Oorschot *et al*., 2003) due to the nature of the surface and extraction efficiency. Raymond *et al.* (2009a) noted that out of 252 trace casework samples collected from surfaces touched by hands, 44% did not generate a DNA profile. Additionally, Castella and Mangin (2008) reported that out of 1739 contact traces from crime scenes casework samples, only 26% had a DNA profile acceptable for entry into the Swiss DNA database.

Some of the study findings were published to assist in improving Touch DNA analysis in forensic casework, see Appendix A9 for the full list of presentations and publications.

1.4.2 AIMS AND OBJECTIVES

The findings from the literature review reflect the natural variability of people to shed DNA in different amounts, as well as the factors impacting the Touch DNA profiling process, such as the nature of the surface, the time between DNA deposition and recovery, environmental influence, collection type and extraction methods (Prinz *et al.,* 2006; Sewell *et al.,* 2008; Goray *et al.*, 2010; Raymond *et al.,* 2009b; Van Oorschot *et al.,* 2010; Verdon *et al.,* 2014a; Ip *et al.,* 2015; Mapes *et al.,* 2016b; Tobias *et al.,* 2017; Hefetz *et al.,* 2019). Therefore, this study aims to examine the factors affecting the recovery of Touch DNA in a bid to propose novel methodologies and techniques that can improve Touch DNA analysis in forensic casework.

The null hypothesis is "*Touch DNA is not affected by the type of surface, the time between deposition and recovery, environmental conditions, as well as collection and extraction methods*".

The study objectives are as follows:

- To examine the effect of the collection process using cotton swabs (e.g., wetting techniques, and drying or freezing prior to extraction) on Touch DNA.
- To examine the effect of surface type, collection and extraction methods on Touch DNA deposited on a range of surfaces.
- Simulate outdoor crime scene casework scenarios to examine the effect of environmental conditions (e.g., temperature, humidity and sand) and time on Touch DNA deposited on a range of surfaces.
- Simulate sexual harassment casework scenarios to examine the effect of different collection methods or techniques on Touch DNA deposited on human skin and fabric, and investigate the influence of time after deposition.
- To examine the effect of direct PCR amplification on the type of collection method used and the amount of Touch DNA collected.
- To implement the finding from previous objectives on forensic casework and report the data at the Biology and DNA Section of the Dubai Police General Department of Forensic Science and Criminology.

1.5 ETHICAL APPROVAL

This study has obtained ethical approval from the Ethics Committee of the University of Central Lancashire, UK (*Unique Reference Number: STEMH 912* - Appendix A1).

CHAPTER TWO

2. MATERIALS AND METHODS

The methods summarised in this chapter were used to perform the research experiments, and any changes to these methods will be mentioned in the related chapters. Collection, extraction, quantification and amplification techniques for DNA profiling were chosen based on their common use in published research in the field of Touch DNA analysis and their popularity among the forensic laboratories. A summary of the research objectives and methodologies is provided in Appendix A2.1.

2.1 ANTI-CONTAMINATION PROCEDURES

During laboratory work, the necessary personal protective equipment (PPE) (laboratory coat, gloves, hair net, and face mask) were worn, which were changed routinely during all experiments. All surfaces and materials were sterilised with 2% Virkon (disinfectant virucidal), 96-100% ethanol and molecular grade water to remove any external source of DNA. Aerosol barrier pipette tips were changed between samples or solutions to prevent cross-contamination. Blanks were taken from materials/surfaces after sterilisation and negative controls for the DNA profiling process during collection, extraction, quantification and amplification to monitor background contamination; all of which were negative for DNA when quantified (DNA-free).

2.2 TOUCH DNA DEPOSITION

The duration of time over which the deposit on the surface and the area over which the touch occurs is important as it helps in the evaluation of the effectiveness of sampling Touch DNA. As such, participants were asked to wash their hands with antibacterial/antimicrobial soap (LabGUARD) for 45 seconds to remove any source of contamination and refrain from undertaking any activity for 10 minutes, including using their mobile phones or touching any other surfaces. Then, they were requested to charge the fingers of both hands with eccrine sweat by touching behind their ears or forehead to load them with enough DNA to help improve the quality and quantity of DNA deposit.

The participants were then asked to deposit the DNA by using their index, middle, and ring fingers of both hands separately while applying medium pressure on a 5 x 7 cm area of the surface for 1 minute (Figure 2.1).

Figure 2.1: Demonstration of the DNA deposition process: the fingers of both hands were charged with eccrine sweat from the forehead or behind the ears to increase the quantity of DNA present on the fingers before the deposition, then used to touch the surfaces with the index, middle, and ring fingers of both hands separately by applying medium pressure on a 5 x 7 cm area of the surface for 1 minute.

The same procedure of handwashing and deposition was repeated pre any deposition process for standardisation and a better evaluation of the results. Any changes made to this DNA deposition process will be detailed in the relevant chapters.

2.3 DNA COLLECTION

The main methods used to recover Touch DNA in this study were Copan cotton swab (150C) (CS), Copan nylon flocked swab (4N6FLOQSwabs®) (NS), SceneSafe Fast™ minitape (K545) (MT), and microFLOQ™ Direct swab (MF) (co-developed by the French Gendarmerie Forensic Research Institute, IRCGN™ and Copan) which was used mainly for direct amplification (Figure 2.2).

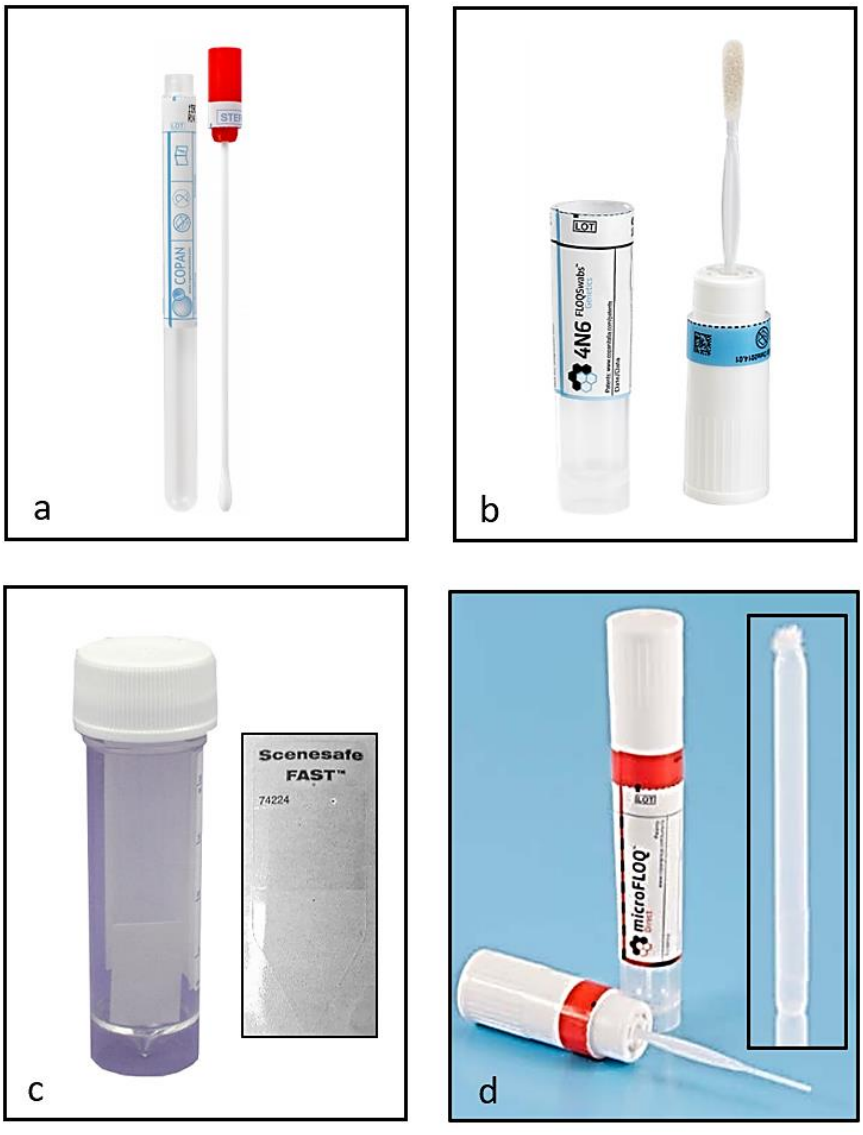


Figure 2.2: Main methods used to recover Touch DNA: **(a)** 150C cotton swab (Copan, Brescia, Italy) (CS), **(b)** 4N6FLOQSwabs® (Copan, Brescia, Italy) (NS), **(c)** K545 minitape (SceneSafe Fast™, UK) (MT), and **(d)** microFLOQ™ Direct swab (Copan, Brescia, Italy) (MF).

Only molecular biology-grade water (Thermo Scientific™) was used as a wetting solution for the swabs. Before collection, approximately 100 µL of water was applied to moisten the CS using a plastic spray bottle (Figure 2.3). This technique was used to minimise water contamination and to avoid soaking the swabs with too much water which may affect the amount of DNA collected. For NS, 30 µL of water was applied to moisten the swab using a pipette as recommended by the manufacturer. No water was added to MT but to increase the amount of Touch DNA collected, each minitape was applied 16 times to the area as recommended by Verdon *et al.* (2014b). For MF, 1 µL of water was applied to moisten the swab using a pipette as recommended by the manufacturer.

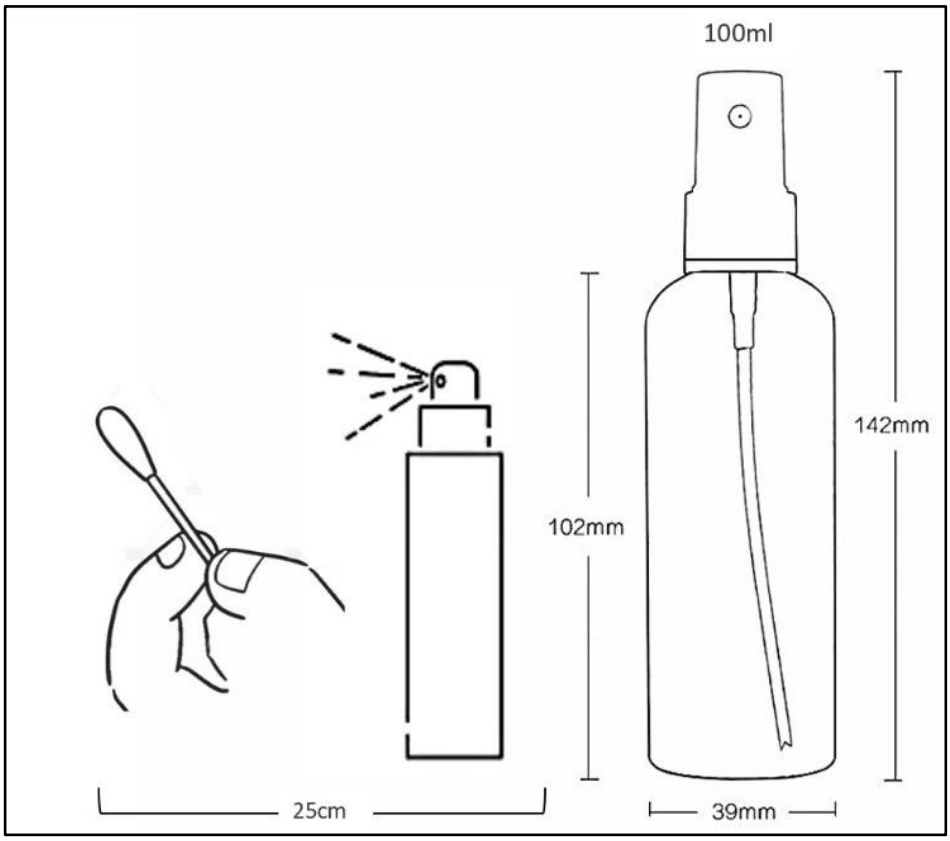


Figure 2.3: The plastic spray bottle technique was developed in the Biology and DNA Section of the Dubai Police General Department of Forensic Science and Criminology. The bottle was sterilised with 2% Virkon and ultraviolet radiation (UV) for 15 min, then filled with molecular biology-grade water (DNA-free). For DNA collection, the swab was held approximately 25 cm from the bottle and then sprayed with every single spray containing approximately 50 µL of solution (3 to 4 sprays should be applied while rotating the swab tip).

2.4 DNA EXTRACTION

The main extraction methods used to extract DNA from the collected samples were with PrepFiler Express BTA™ kit using an AutoMate Express Forensic DNA Extraction System (EXT1) (Thermo Fisher Scientific Inc., 2018a) and manually using the QIAamp® DNA Investigator Kit (EXT2) (QIAGEN, 2012) (Figure 2.4). DNA extractions were performed according to the manufacturer's recommendations (for full extraction SOPs, see Appendix A2.2 for QIAamp DNA Investigator protocol, and Appendix A2.3 for PrepFiler Express BTA Protocol). After collection, the CSs were cut into the extraction tubes using sterilised (DNA-free) scissors, NSs were self-broken directly into the extraction tube, while MTs were cut into small pieces into the extraction tube using sterilised (DNA-free) scissors. Full swab heads and the lower sticky part of the minitape were extracted, with a final sample elution of 50 µL.

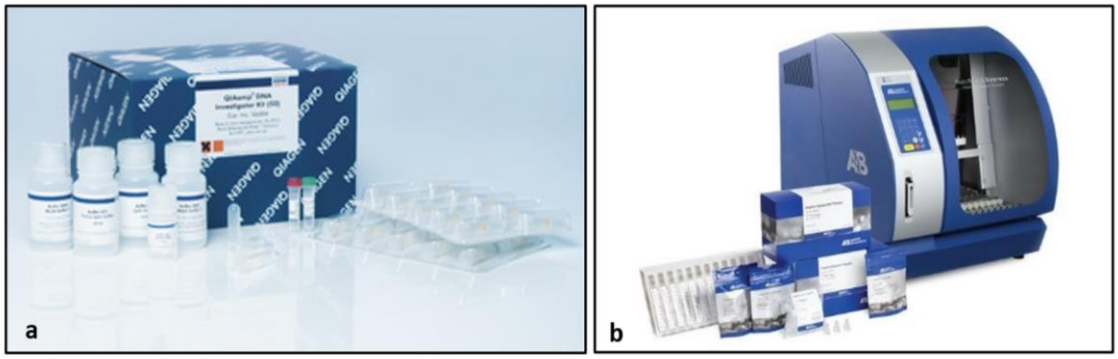


Figure 2.4: Extraction methods used: **(a)** manually using the QIAamp® DNA Investigator Kit (EXT2) and **(b)** automated using the PrepFiler Express BTA™ kit with AutoMate Express Forensic DNA Extraction System (EXT1).

2.4.1 QIAamp® DNA INVESTIGATOR KIT

Briefly, 400 µL of Tissue Lysis Buffer (ATL) and 20 µL of proteinase K were added to each sample and mixed in a thermo-mixer at 56 °C for 1 h with shaking at 900 rpm. Then, 400 µL of Lysis Buffer (AL) with 1 µL Dissolve carrier RNA was added, vortexed for 15 s and the tube was incubated at 70 °C for 10 min before the addition of 200 µL of absolute ethanol and vortexed for 15 s. After briefly centrifuging, the entire supernatant was transferred to the QIAamp® MinElute column placed in a 2 ml collection tube and centrifuged for 1 min at 6000*g*. The column was washed with 500 µL of diluted Wash

Buffer 1 (AW1) and centrifuged at 6000*g* for 1 min. The MinElute column was then transferred to a new 2 ml tube and washed twice with 700 µL of Wash Buffer 2 (AW2) and centrifuged at 6000*g* for 1 min. The MinElute column was transferred to a new 2 ml tube and centrifuged at full speed for 3 min to dry the membrane completely. Finally, the MinElute column was transferred to a clean 1.5 ml tube and incubated at 56 °C for 3 min, then 50 µL of Buffer ATE was added and incubated at room temperature for 5 min before centrifugation at maximum speed for 1 min to collect the purified DNA.

2.4.2 AUTOMATE EXPRESS™ SYSTEM

Briefly, the master mix for lysis was prepared by mixing 220 µL of PrepFiler BTA™ Lysis buffer, 3 µL DTT and 7 µL of proteinase K. The master mix was then added to the PrepFiler® LySep column provided containing the sample and inserted into a PrepFiler® Sample tube. The tube containing the column was then placed in a thermal shaker for 1-h at 56 °C with shaking at 750 rpm. The lysate was transferred to the sample tube by centrifuging the LySep column for 2 min at maximum speed, then processed on the Automate Express™ Forensic DNA extraction instrument according to the PrepFiler Express BTA™ instrument protocol.

2.5 DNA QUANTIFICATION

The DNA was quantified using the following methods, Quantifiler™ Human (Thermo Fisher Scientific Inc., 2018b), and Quantifiler™ Trio DNA Quantification Kits (Thermo Fisher Scientific Inc., 2018c) using the QuantStudio 5 Real-Time PCR (qPCR) system (Figure 2.5) with HID Real-Time PCR analysis software v1.3 (Thermo Fisher Scientific) according to the manufacturer's instructions. Some samples were also quantified with the Qubit dsDNA HS Assay Kit (Thermo Fischer Scientific) using a Qubit® 3.0 Fluorometer system following the manufacturer's protocol.

For full quantification SOPs, see Appendix A2.4 for the Quantifiler™ Human protocol, Appendix A2.5 for the Quantifiler™ Trio protocol, and Appendix A2.6 for the Qubit dsDNA HS Assay protocol.

Figure 2.5: The Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific) using the QuantStudio 5 Real-Time PCR (qPCR) system.

2.5.1 QUANTIFILER™ HUMAN DNA QUANTIFICATION KIT

Briefly, 23 µL of master mix containing 10.5 µL of Quantifiler™ human primer mix and 12.5 µL Quantifiler™ PCR reaction mix was dispensed into the wells of a MicroAmp™ optical 96-well reaction plate (Applied Biosystems). Then, 2 µL of each DNA standard was loaded in duplicate, and 2 µL of the extracted DNA samples were loaded on the plate to give a final total volume of 25 µL per reaction. Finally, the plate was sealed with an optical adhesive cover (Applied Biosystems) and placed in the QuantStudio 5 Real-Time PCR (qPCR) system for DNA quantification. The thermal cycler protocol was performed according to the manufacturer's instructions (Applied Biosystems): holding stage 1, 50 °C for 2 min and holding stage 2 at 95 °C for 10 min followed by a two-step cycle of 40 cycles; step 1 at 95 °C and step 2 at 60 °C. After completion of amplification, the DNA concentration of each sample was estimated in ng/µl.

2.5.2 QUANTIFILER™ TRIO DNA QUANTIFICATION KIT

Briefly, 18 µL of prepared master mix containing 8 µL of Quantifiler™ Trio primer mix and 10 µL Quantifiler™ PCR reaction mix was dispensed into the wells of a MicroAmp™ optical 96-well reaction plate (Applied Biosystems). Then, 2 µL of each DNA standard concentration was loaded in duplicate, and 2 µL of the extracted DNA samples were loaded on the plate to give a final total volume of 25 µL per reaction. Finally, the plate was sealed with an optical adhesive cover (Applied Biosystems) and placed in the

QuantStudio 5 Real-Time PCR (qPCR) system for DNA quantification. The thermal cycler protocol was performed according to the manufacturer's instructions (Applied Biosystems): holding stage 1, 50 °C for 2 min and holding stage 2 at 95 °C for 10 min followed by a two-step cycle of 40 cycles; step 1 at 95 °C and step 2 at 60 °C. After completion of amplification, the DNA concentration for each sample was estimated in ng/µl.

2.6 DNA AMPLIFICATION

After extraction and quantification of the samples, amplification was performed using the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific Inc., 2016) according to the manufacturer's instructions using the ABI GeneAmp® 9700 PCR System (Thermo Fisher Scientific) with a 29 or 30 cycle protocol (Table 2.1). Test samples were processed by preparing 15 µL of input volume containing the extracted DNA, diluted if required, plus 10 µL of PCR reaction mixture (7.5 µL Master Mix and 2.5 µL Primer Set) to make a total volume of 25 µL per reaction for PCR (for full amplification protocol see Appendix A2.7).

Table 2.1: GlobalFiler™ PCR Amplification Kit thermal cycling conditions (Thermo Fisher Scientific Inc., 2016).

Initial incubation step	Cycle (29 or 30 cycles)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C, 1 minute	94°C, 10 seconds	59°C, 90 seconds	60°C, 10 minutes	4°C, Up to 24 hours[1]

2.7 CAPILLARY ELECTROPHORESIS

Amplified samples were separated by size and detected using the ABI 3500 Genetic Analyser (Thermo Fisher Scientific) (Figure 2.6). The MicroAmp™ Optical 96-Well reaction plate was prepared using 1 µL of PCR amplified product, 9.6 µL of Hi-Di™ formamide, and 0.4 µL of GeneScan™ 600 LIZ® Size Standard (Thermo Fisher Scientific) for each sample. One microliter of the allelic ladder was used for every 23 samples (1 per 3 injections). The samples were denatured at 95 °C for 5 min and immediately cooled on ice for 5 min before being subjected to electrophoresis on a 50-cm capillary array

with POP-6™ polymer (Thermo Fisher Scientific) using the standard injection parameters (1.2 kV/15 s). The capillary electrophoresis products (STR data) were sized and typed using GeneMapper® ID-X Software Version 1.2 following the GlobalFiler™ PCR Amplification Kit manufacturer's validated analytical thresholds, see the GlobalFiler PCR Amplification Kit User Guide (Pub. no. 4477604E) for full details (Thermo Fisher Scientific Inc., 2016) and Appendix A2.8 for the full electrophoresis sample preparation protocol.

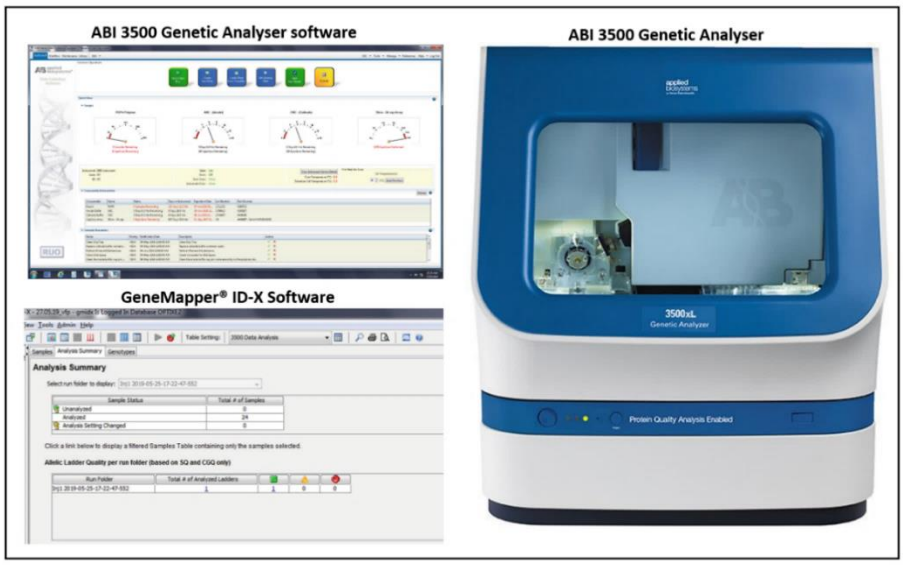


Figure 2.6: Capillary electrophoresis was performed on an ABI 3500 Genetic Analyser and the products were analysed using GeneMapper® ID-X Software.

2.8 DATA ANALYSIS

The study data were processed by Microsoft Excel (Version 1901) and R-studio (Version 0.98.1049). Microsoft Excel was used to perform simple statistical analyses and to generate tables and figures. R-studio was used for the statistical analysis of the variables using factorial analysis of variance (ANOVA). The data were organised in separate files and uploaded to R-studio. The analyses were performed manually, firstly by defining the linear model (e.g., one or two categorical explanatory variables), then by checking if the model was appropriate with the use of a QQ plot, and finally, the ANOVA results were produced and the p-values were reported. In ANOVA, the p-value derived from the F-distribution was different for every pair of degrees of freedom (df) values [F value = variance of the variables means (Mean Square Between) / mean of the within variables variances (Mean Squared Error)]. In this study, 'n' represents the total number of Touch DNA deposits or the number of samples collected.

CHAPTER THREE

3. COLLECTION TECHNIQUES

This chapter started by evaluating the shedding status of the participants involved in this study. Thereafter, examined the effect of collection techniques using a cotton swab on Touch DNA. Such techniques include the wetting and collection process, and drying or freezing of the swabs before extraction.

3.1 EVALUATING THE SHEDDING STATUS

The shedding status of the person who has handled the items can impact the amount of DNA deposited. Typically, individuals can be classified as either good or poor shedders (Lowe *et al.,* 2002; Wickenheiser, 2002), with some individuals having the ability to deposit more or less DNA than others. Nonetheless, it can be difficult to categorise the shedding status of individuals (Goray *et al.,* 2016; Kanokwongnuwut *et al.,* 2018b), as discussed in Section 1.3.1.

Therefore, the aim of this experiment was not to determine the shedding status of the participants involved in this study but rather to estimate their shedding abilities to use the participants more efficiently when conducting experiments.

3.1.1 EXPERIMENTAL SETUP

A group of five participants were asked to wash their hands with antibacterial soap (LabGUARD), refrain from any activity for 10 minutes and avoid touching other surfaces, then rub both their hands together for a few seconds before collection to help shed more biological materials. Copan cotton swabs (150C) were used to collect the samples as described in Section 2.3. Biological material from the palms of both hands and fingers was collected using one swab for each participant (Figure 3.1) over 5 days at room temperature.

The DNA was extracted from the swabs using the QIAamp® DNA Investigator Kit following the procedure detailed in Section 2.4, then quantified using the Qubit dsDNA HS Assay Kit (Thermo Fischer, UK) and a Qubit® 3.0 Fluorometer according to the manufacturer's protocols (procedure detailed in Section 2.5).

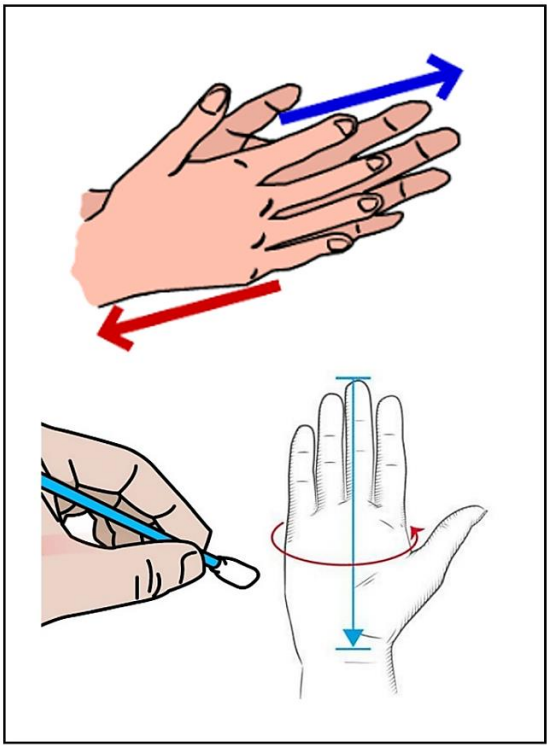


Figure 3.1: The participants' shedding abilities were evaluated by collecting DNA using cotton swabs moistened with 100 µL of molecular-grade water at room temperature over 5 days following the procedure detailed in Section 2.3. The participants first washed their hands with antibacterial soap, refrained from activity for 10 min and then rubbed both hands together for a few seconds. One swab was used to collect the biological material from the palms of both hands and fingers of each participant.

3.1.2 RESULTS

The DNA collected from the participants' hands over five days showed some variation and indicated the shedding abilities of each participant (Table 3.1). Even though the shedding status of each participant was not consistent, the mean DNA collected over five days showed that some participants typically shed more DNA than others.

Participant one had the highest shedding ability (avg. 7.20 ng/µL) and participant two had the lowest shedding ability (avg. 2.42 ng/µL), with participants three, four and five being intermediate between participants one and two. Consequently, the participants were classified based on their shedding abilities and subsequently referred to as low (avg. 0.0–4.0 ng/µL), moderate (avg. 4.0–7.0 ng/µL) and high shedders (avg. 7.0 ng/µL and above). However, it is important to note that different scenarios and conditions may yield different results when testing participants' shedding abilities.

Table 3.1: Amount of DNA (ng/µL) collected from the participants over five days (n= 25).

Days	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5
Day 1	7.78	1.78	5.15	7.04	3.74
Day 2	9.26	3.36	2.21	3.41	6.58
Day 3	4.87	2.75	4.89	5.49	4.91
Day 4	3.74	1.86	5.17	2.17	8.69
Day 5	10.34	2.34	6.21	6.51	7.46
Mean	**7.20**	**2.42**	**4.73**	**4.92**	**6.28**
SD	**2.82**	**0.66**	**1.49**	**2.07**	**1.98**

One DNA sample collected from each participant's hand was amplified and analysed following the procedures described in Section 2.6 and 2.7. All tested samples produced a full single DNA profile and were used as a reference to evaluate the Touch DNA deposited samples collected from the surfaces tested in this study.

3.2 COTTON SWAB WETTING TECHNIQUES

Successful swabbing of an item usually demands a moistened swab with the use of some pressure and the rotation of the swab head applied to the target area for DNA collection. However, this is often not the case, as a moist cotton swab may collect less than half of the available DNA leaving some biological material on the surface (Van Oorschot *et al.,* 1999). Choosing the appropriate collection technique for use with a cotton swab can improve Touch DNA recovery from the surfaces, for instance, using an appropriate amount of solution to moisten the swab or using a double swab technique (wet and dry) (Pang & Cheung 2007; Van Oorschot *et al.,* 2010). Therefore, this experiment evaluated three recovery techniques, two of which are commonly used with cotton swabs: single swab technique (half wet and half dry), double swab technique (wet and dry), and a single swab technique using a plastic spray bottle to moisten the swab.

3.2.1 EXPERIMENTAL SETUP

A participant, previously confirmed as a moderate shedder in Section 3.1, was asked to perform the deposition process described in Section 2.2 on a 5 x 7 cm area of a glass surface (smooth non-porous). The participant was requested to repeat the same process of DNA deposition 24 times (n= 24, 8 replicates per each collection technique). Before use, the surfaces were sterilised with 2% Virkon and ultraviolet radiation (UV) for 15 min. The process was conducted at room temperature. After deposition, the samples were collected immediately using a Copan cotton swab (150C) moistened with 100 µL of molecular grade water via three different techniques as follows:

a) Half of the swab head using a pipette.
b) Full swab head using a spray bottle as described in Section 2.3.
c) Full swab head using a pipette, followed by the use of dry swab after first collection (double swab technique – swabs were extracted individually then the final sample elution was combined).

Next, swabs heads were extracted immediately after collection using the QIAamp® DNA Investigator Kit following the procedures in Section 2.4. Extracted samples were quantified using the Quantifiler™ Human DNA Quantification Kit as described in Section 2.5. Some samples collected by each technique were amplified and analysed following the procedures in Section 2.6 and 2.7 to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed following the procedures in Section 2.8.

3.2.2 RESULTS

There was a considerable difference between the three collection techniques used to recover Touch DNA with a cotton swab ($p < 0.001$) (Figure 3.2) (see Appendix A3.1 for the complete results). The spray bottle technique to moisten the swab head (b) or the use of the double swab technique (wet and dry) (c) were more efficient to collect Touch DNA, with technique (b) being slightly better ($p < 0.001$). The single swab technique with the use of the pipette to moisten half of the swab head (a) resulted in some trace DNA being left on the surface ($p < 0.001$) (mean a. 0.05, b. 0.09 and c. 0.07 all in ng/µL) (Figure 3.3).

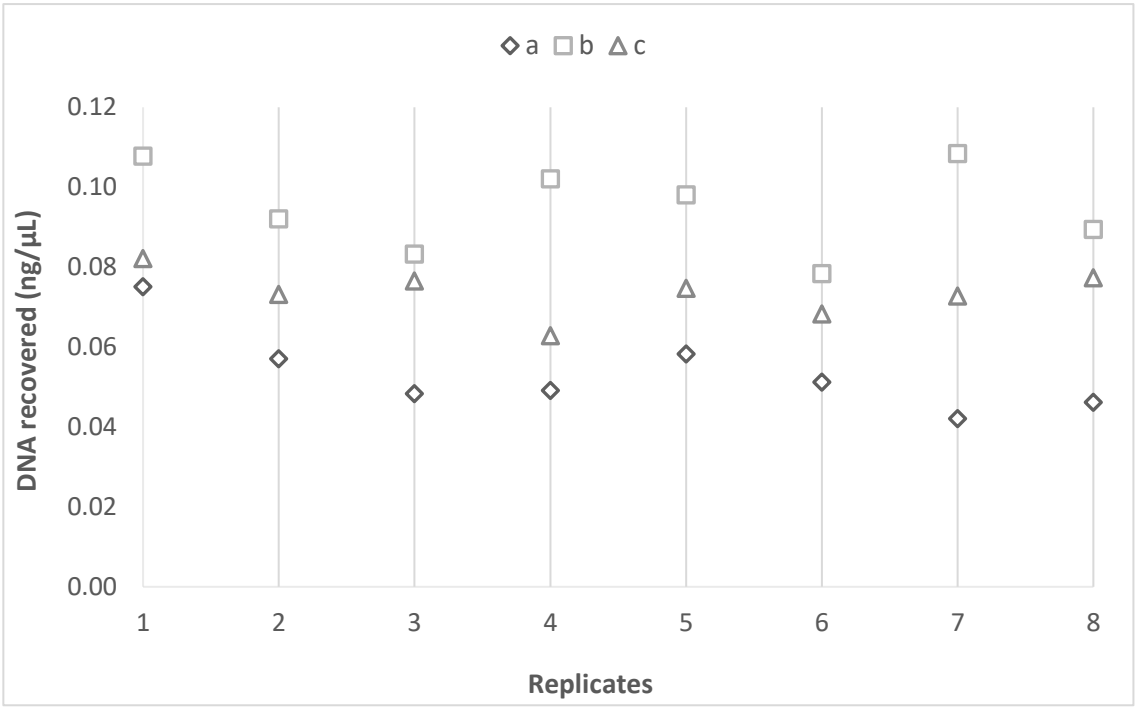


Figure 3.2: Amount of DNA recovered from eight replicates (n= 24) by each technique: **(a)** single swab, **(b)** spray bottle and **(c)** double swab.

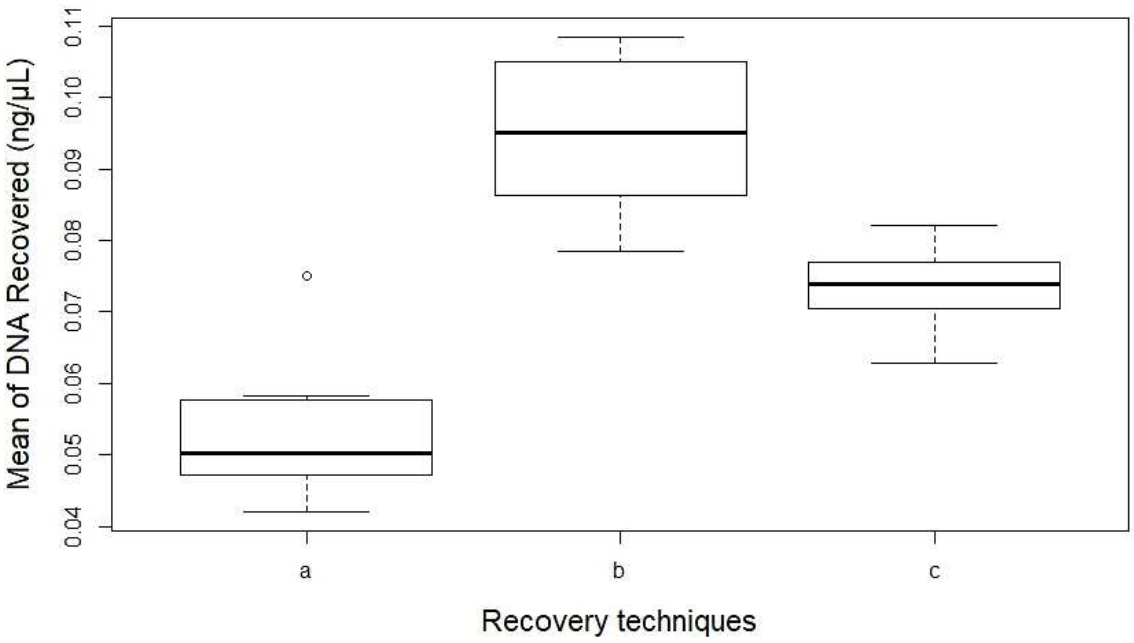


Figure 3.3: Mean DNA recovered (n= 24) by each technique: **(a)** single swab, **(b)** spray bottle and **(c)** double swab.

3.3 DRYING OR FREEZING BEFORE EXTRACTION

Biological materials are often recovered using a moistened cotton swab and after collection, some of the cotton swabs are extracted immediately, while others are stored in the freezer. Most forensic laboratories practice drying the swabs at room temperature or use swab drying cabinets before extraction or freezing and such practices can affect the amount of trace DNA collected as the cotton swabs can retain some DNA during extraction (Van Oorschot *et al.,* 2003). Some studies suggest that extracting DNA from the cotton swab or freezing it while moist can help to improve Touch DNA recovery (Van Oorschot *et al.,* 2010).

Therefore, this experiment examined the influence of immediate extraction, drying and freezing of collected Touch DNA using moistened cotton swabs.

3.3.1 EXPERIMENTAL SETUP

A participant, previously confirmed as a moderate shedder in Section 3.1, was asked to perform the deposition process described in Section 2.2 on a 5 x 7 cm area of a glass surface (smooth non-porous). The participant was requested to repeat the same process of DNA deposition 24 times (n= 24, 8 replicates per each condition). Before use, the surfaces were sterilised with 2% Virkon and UV for 15 min. The process was conducted at room temperature.

After deposition, samples were collected immediately using a Copan cotton swab (150C) moistened with molecular grade water using a spray bottle as described in Section 2.3. After collection, the swabs were treated as follows:

a) Immediate extraction following collection.
b) Frozen at -20 °C for a week while the swabs were moist.
c) Dried for 24 hours at room temperature, then frozen at -20 °C for 6 days.

The DNA was then extracted from the swab heads using the QIAamp® DNA Investigator Kit following the procedures in Section 2.4. Extracted samples were quantified using the Quantifiler™ Human DNA Quantification Kit following the procedures detailed in Section 2.5.

Some samples collected by each technique were amplified and analysed following procedures outlined in Section 2.6 and 2.7 to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed as described in Section 2.8.

3.3.2 RESULTS

There was a significant difference in the amount of DNA collected from the different cotton swab conditions before extraction ($p < 0.001$) (Figure 3.4) (see Appendix A3.2 for the complete results). More Touch DNA was extracted from the cotton swabs that had been extracted immediately after DNA collection (a) or frozen when still moist (b), with condition (a) being slightly better ($p < 0.001$). Drying the moist swab following collection and before freezing (c) led to some loss of the trace DNA collected ($p < 0.001$) (mean a. 0.09, b. 0.08, and c. 0.05 all in ng/µL) (Figure 3.5).

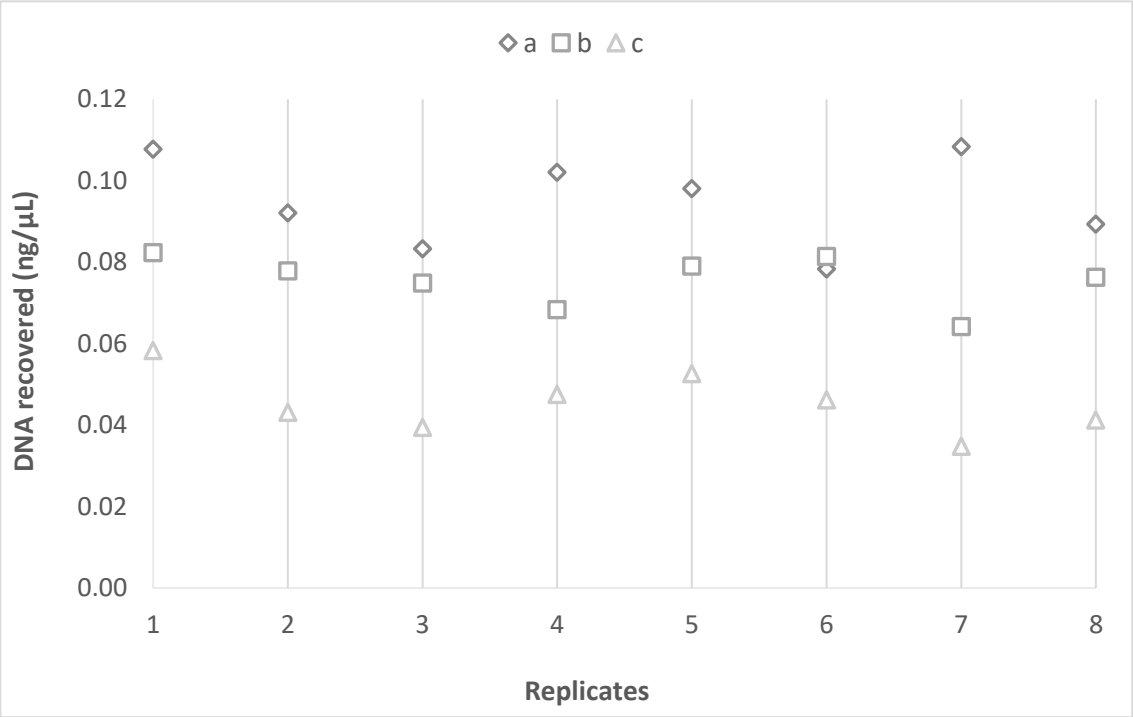


Figure 3.4: Amount of DNA recovered from eight replicates (n= 24) from each differently treated swab: **(a)** immediate extraction of swabs, **(b)** swabs were only frozen before extraction and **(c)** swabs were dried and frozen before extraction.

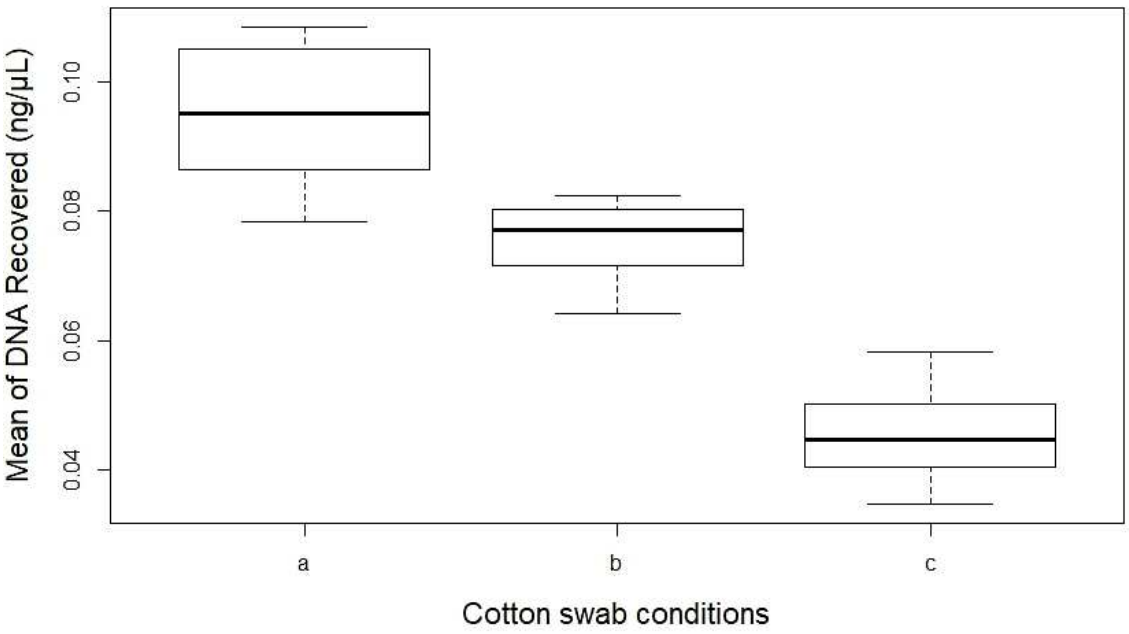


Figure 3.5: Mean DNA recovered (n= 24) from each differently treated swab: **(a)** immediate extraction of swabs, **(b)** swabs were only frozen before extraction and **(c)** swabs were dried and frozen before extraction.

3.4 CHAPTER DISCUSSION AND CONCLUSION

Individuals have different DNA shedding abilities, and thus can be classified as high, moderate or low shedders. However, the quantity may differ after each deposition, therefore, it is recommended to collect DNA over time to calculate the mean DNA shed to determine the individual's shedding ability. These variations in shedding status among individuals were also supported by some previous studies (Murray *et al.,* 2001; Lowe *et al.,* 2002; Phipps & Petricevic, 2007; Allen *et al.,* 2008; Quinones, 2011).

After deposition, samples are often collected using a cotton swab moistened with molecular grade water, a common moistening agent used by forensic laboratories to wet the swabs (Van Oorschot *et al.,* 1999). Cotton swabs are used routinely to recover trace DNA even though some DNA can be retained by the cotton swab depending on the efficiency of the extraction method used (Van Oorschot *et al.,* 2003). Therefore, using an appropriate collection technique could help improve the DNA quantity recovered from the cotton swab.

The double swab technique (wet and dry) can lead to more trace DNA being recovered from the surface than a single swab technique (half wet and half dry) (Pang & Cheung, 2007), but is dependent on the size of the area from which the sample is collected. Furthermore, the double swab technique is challenging (Van Oorschot *et al.,* 2003). For instance, if the two swabs are processed in the same extraction tube, the extraction will not be efficient as discussed previously in Section 1.3.9. Therefore, extracting the swabs individually in separate extraction tubes before combining the final sample elutes is preferred, however, this is time-consuming and costly. The use of a plastic spray bottle to moisten the swab is preferable to a pipette because it spreads the molecular-grade water evenly over the swab without soaking it (Alketbi & Goodwin, 2019d). In addition, there is less risk of contamination compared to the use of a pipette. However, the amount of water on the cotton swab might not be consistent if the spray bottle is held at different distances from the swab before spraying. Based on this study, it is recommended to hold the swab approximately 25 cm from the bottle and use 3 to 4 sprays only. Furthermore, it is important to consider the quantity of solution sprayed by plastic bottles as different spray bottles spray different quantities.

Drying the cotton swab before freezing it could be useful when long-term storage is required for some biological materials, particularly for body fluids to avoid fungal growth on swabs (Van Oorschot *et al.,* 2010). However, this is not the case for Touch DNA, if the cotton swab is allowed to dry before extraction, less DNA is retrieved than if the moist swab was used immediately (Alketbi & Goodwin, 2019d). The collected biological materials attach to the water in the outer layer of the swab, and when dried, the cells attach to the swab making DNA extraction difficult. Therefore, freezing the swab following DNA recovery while it is moist rather than drying it before extraction could result in similar quantities of DNA extracted to the immediate extraction after collection. Based on this study, it is recommended to freeze cotton swabs immediately after Touch DNA collection for better DNA recovery.

CHAPTER FOUR

4. INFLUENCE OF SURFACE, COLLECTION AND EXTRACTION

This chapter examined a range of factors that impact Touch DNA profiling including how a range of porous and non-porous surfaces can retain deposited biological materials and the efficiency of DNA collection methods from these selected surfaces. It also investigated the DNA extraction methods and their effectiveness for extracting DNA collected by different collection methods. It also examined dual recovery of DNA and fingerprints using minitapes. Finally, the performance of the two quantification methods were examined on the same DNA samples.

4.1 INFLUENCE OF SURFACE TYPE

In some cases, the amount of DNA left by touch can be sufficient in terms of quality and quantity to produce a DNA profile. However, some variables affect the success of obtaining a quality DNA profile, including surface type and the methods used for DNA collection and extraction, as discussed previously in Section 1.3.

Although previous studies have investigated the effectiveness of different methods used for DNA recovery from various biological materials such as body fluids (Plaza *et al.,* 2016**;** Verdon *et al.,* 2014a**)**, there are some deficiencies in the published information related to Touch DNA **(**Brownlow *et al.,* 2012**)**. There is a necessity to include the duration of the deposit on the surface (Tobias *et al.,* 2017), alongside recovery and extraction method efficiencies in the interpretation of trace DNA (Taylor *et al.,* 2017). Therefore, the following study aimed to investigate the effect of surface type, DNA collection methods, as well as extraction methods on Touch DNA recovery.

4.1.1 EXPERIMENTAL SETUP

Five surfaces, including stainless steel, glass, textured plastic, textured wood, and white copier paper were selected to replicate common surfaces encountered in crime scenes and provide a variety of smooth, rough, porous, and non-porous surfaces (Figure 4.1). In addition, a banana skin was also included since there is a lack of published research regarding Touch DNA collected from eaten fruits and often offenders consume food in various home-related burglary cases (based on current professional experience), making

six surface types in total. All non-porous surfaces were sterilised using 2% Virkon and UV-irradiation for 15 min, while porous surfaces were only irradiated using UV light for 25 min.

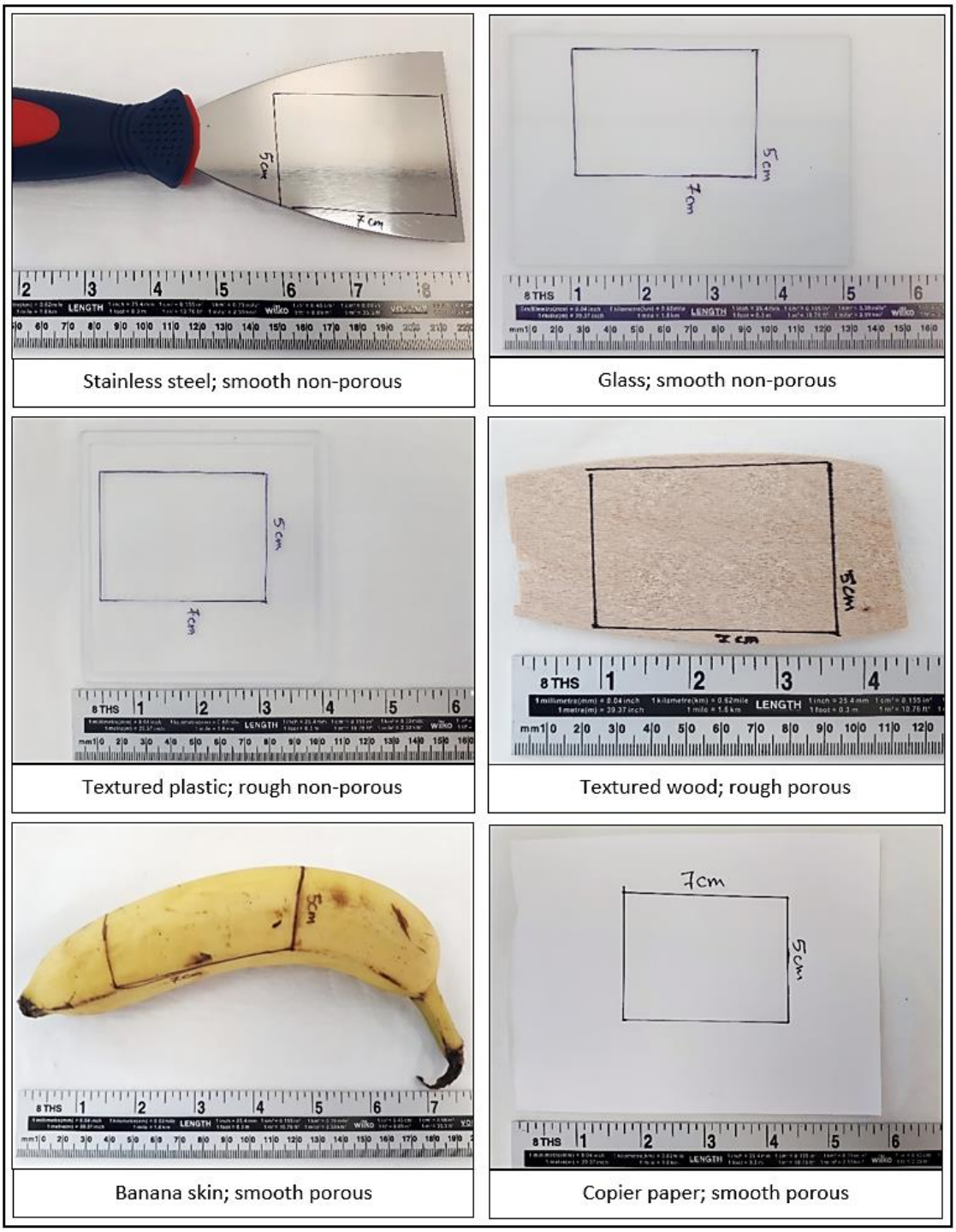


Figure 4.1: A selection of smooth, rough, porous and non-porous surfaces including stainless steel (SS), glass (G), textured plastic (TP), textured wood (TW), banana skin (BS) and copier paper (CP).

Two participants, previously evaluated to be high and low shedders in Section 3.1, were instructed to perform the deposition process described in Section 2.2 on a 5 x 7 cm area of the test surfaces (n= 72, 2 replicates per each variable; surface type, collection and extraction methods). Each participant deposited their DNA separately on the selected six surfaces to avoid any contamination. The process was conducted at room temperature.

For DNA recovery, three different methods were used: Copan cotton swab (150C) (CS), Copan nylon flocked swab (4N6FLOQSwabs®) (NS), and SceneSafe Fast™ minitape (K545) (MT) (Figure 2.2 – Section 2.3) following the same collection procedure detailed in Section 2.3.

Touch DNA was recovered within 30 minutes from the surfaces after deposition and extracted immediately with the PrepFiler Express BTA™ kit using an AutoMate Express Forensic DNA Extraction System (EXT1) and manually using the QIAamp® DNA Investigator Kit (EXT2) following the procedures in Section 2.4. However, only nylon swabs were extracted using NAOBasket™ as recommended by Copan to increase the DNA yield.

Extracted samples were quantified using the Quantifiler™ Human DNA Quantification Kit following the procedures in Section 2.5. Some samples were then amplified and analysed as described in Section 2.6 and 2.7 to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed following the procedures mentioned in Section 2.8.

4.1.2 RESULTS

The results indicated that the amount of DNA collected from the test surfaces was significantly affected by the type of surface ($p < 0.05$), as well as the extraction method ($p < 0.05$) (Figure 4.2) (see Appendix A4.1 for the complete results). However, the collection method used ($p > 0.05$) and the interaction between the type of collection and extraction ($p > 0.05$) did show some variation in the DNA quantity among the collection methods used but there was a lack of replicates.

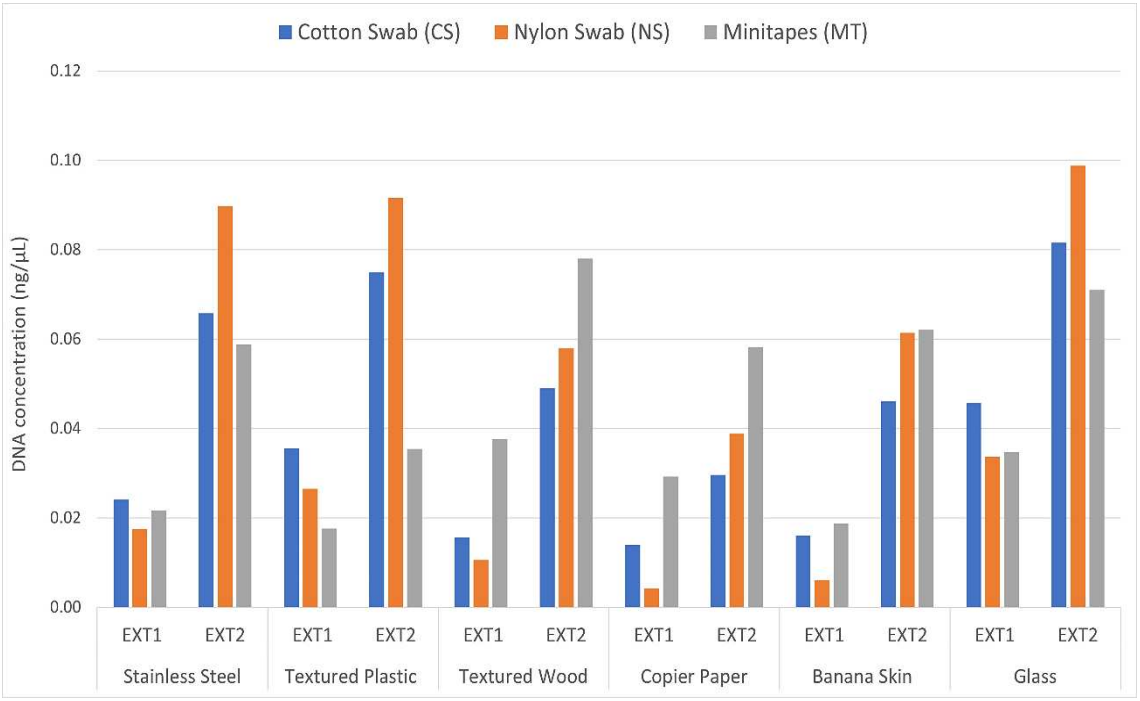


Figure 4.2: Mean amount of DNA recovered from the six surfaces (n= 72 - two replicates were used to make each bar) using three collection methods and two extraction methods, PrepFiler Express BTA™ kit (EXT1) and QIAamp® DNA Investigator Kit (EXT2). The CS performed better with EXT1 and NS performed better with EXT2 when used on non-porous surfaces. MT performed better when used on porous surfaces regardless of the extraction method used.

Despite similar techniques followed for deposition, differing amounts of DNA were recovered from the test surfaces. The largest average amount of DNA was recovered from glass (smooth and non-porous surface) with both extraction types, with the least amount of DNA recovered from copier paper (smooth and porous surface), as shown in Figure 4.3. Moreover, despite the variation in the amount of DNA collected, there was a similar trend for the amount of DNA recovered from the surfaces touched by both participants.

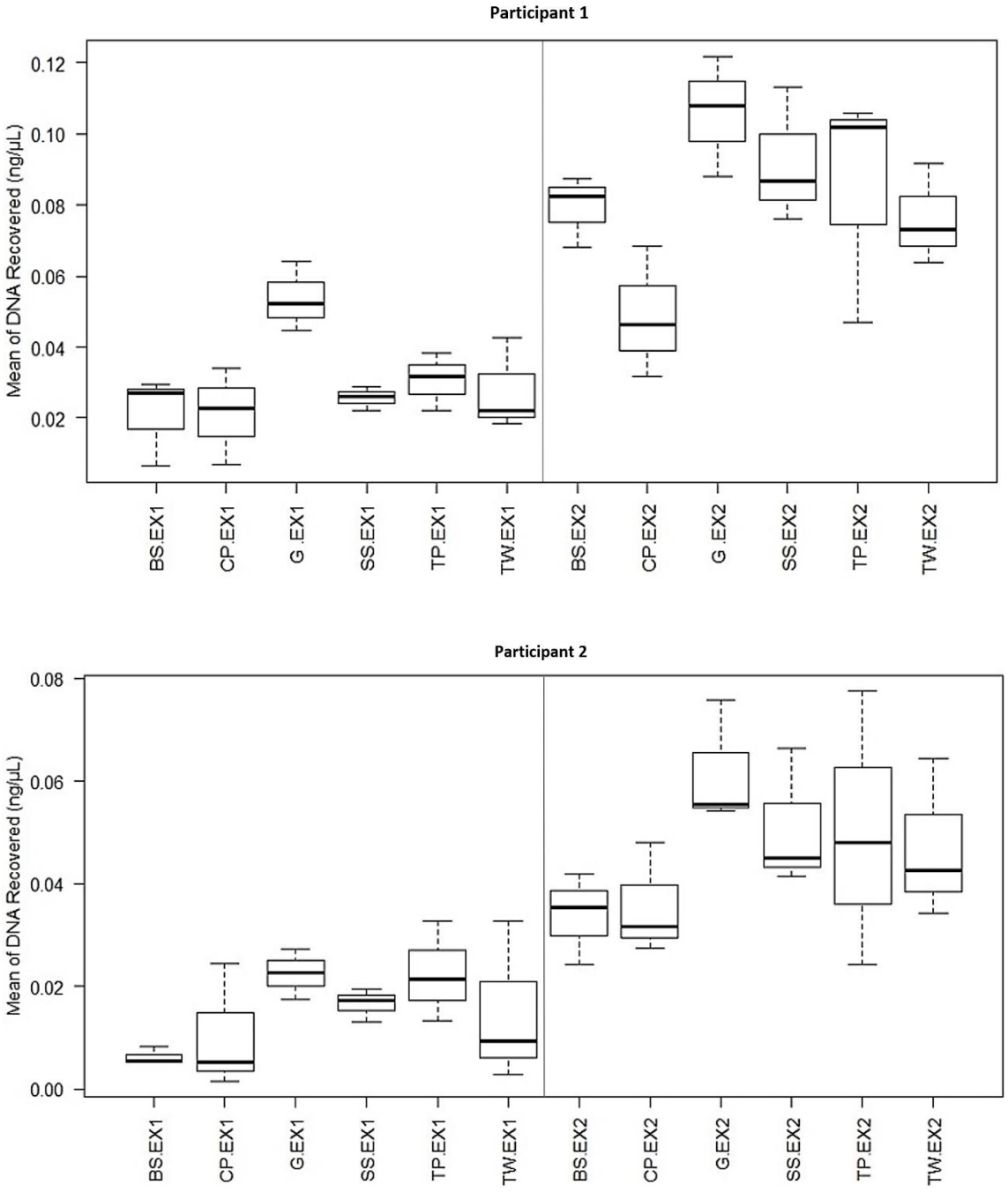


Figure 4.3: Mean amount of DNA recovered from six surfaces (n= 72) [banana skin (BS), copier paper (CP), glass (G), stainless steel (SS), textured plastic (TP) and textured wood (TW)] extracted by two extraction methods [PrepFiler Express BTA™ kit (EX1) and QIAamp® DNA Investigator Kit (EX2)].

The extraction process can contribute to a loss of the initial amount of DNA and in this experiment, there was a major difference in DNA recovery between both extraction methods, with EXT2 performing better than EXT1. This may be due to the amount of

lysis buffer solution, which was 230 µL for PrepFiler and 420 µL for QIAamp® **(**Joël *et al*., 2015**)**, which was recommended by the manufacturers.

Despite the differences in the amount of DNA, the collection methods performed differently for each surface (Figure 4.2), with CS and NS performing better with smooth and rough non-porous surfaces (SS, TP and G), whereas the MT performed better with smooth and rough porous surfaces (CP and BS). Although the CS was the best performing collection method for most of the test surfaces, the use of NS with the NAOBasket™ was best for improving DNA recovery when the sample was extracted using the QIAamp® DNA Investigator Kit. Supporting these findings, previous studies reported that minitapes were the preferred method of collection for porous surfaces, especially fabric **(**Hansson *et al.,* 2009; Verdon *et al.,* 2014b**;** Verdon *et al.,* 2015).

Moreover, the use of tape to recover DNA from smooth non-porous surfaces allows dual recovery, that is, recovery of DNA and fingerprints **(**Van Oorschot & Jones 1997; Zamir *et al.,* 2000**)**. In this study, fingerprints were visible after deposition on stainless steel and glass, and they were still visible after the use of MT to collect the DNA (fingerprints shown in Figure A4.1 - Appendix A4.2). Therefore, MT can be a useful tool for dual recovery, especially from smooth non-porous surfaces such as metal or glass because they reflect the light and fingerprints are often visible on these surfaces. However, this needs to be investigated further.

Most extracted samples had sufficient DNA for amplification, with the amount of DNA ranging from 1.5–121.7 pg/µL (Appendix A4.1). Samples that were deposited by participant one and collected from textured wood (TW) by the three collection methods while extracted by EXT1 were amplified to determine the quality of the profiles obtained from the three collection methods. Full profiles were obtained from the amplified samples (Figure 4.4). Moreover, samples that were deposited by participant two and collected from the six surfaces using a nylon swab while extracted by EXT2 were amplified to determine the quality of the profiles collected from the surfaces. Full profiles were obtained from the amplified samples (Figure 4.5).

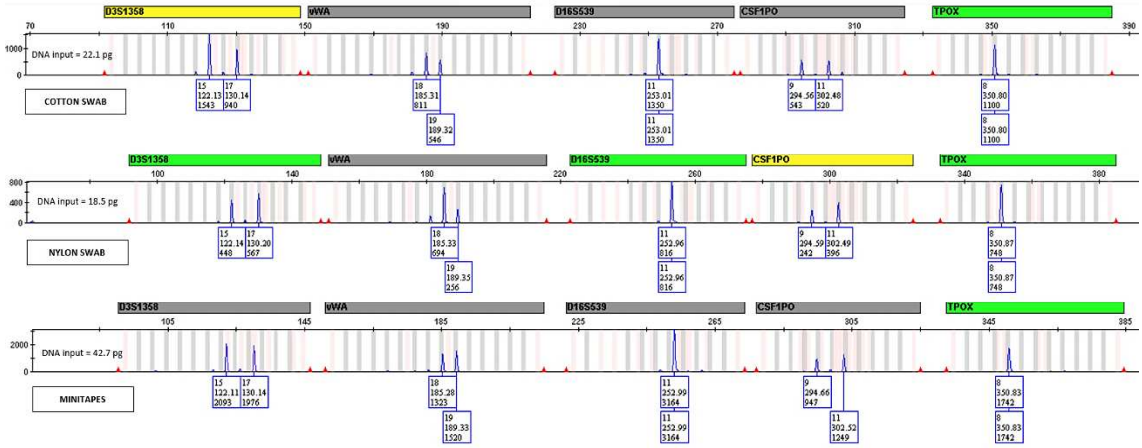


Figure 4.4: Electropherograms of the samples deposited by participant one and collected from textured wood using a cotton swab, nylon swab and minitapes and extracted using the PrepFiler Express BTA™ kit. The profiles show the difference in peak height at 5 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX). Maximum volume of DNA was added.

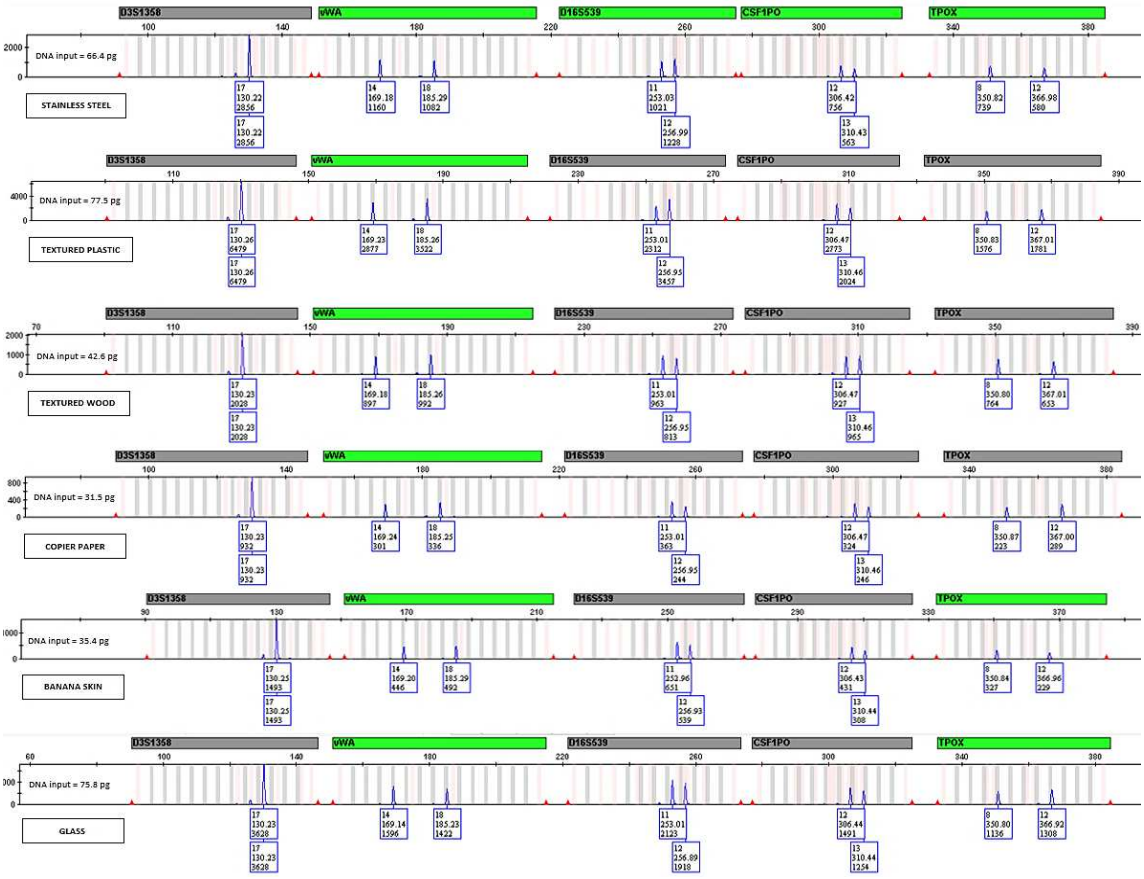


Figure 4.5: Electropherograms of the samples deposited by participant two and collected from the six surfaces by a nylon swab and extracted using the QIAamp® DNA Investigator Kit. The profiles show the difference in peak height at 5 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX). Maximum volume of DNA was added.

4.2 The PrepFiler EXPRESS BTA™ DNA EXTRACTION KIT

The extraction technique can influence the amount of recovered DNA (Ottens *et al.,* 2013: Joël *et al*., 2015). From the previous experiments (Section 4.1), more DNA was extracted by the QIAamp® DNA Investigator Kit than from the PrepFiler Express BTA™ kit, which may be influenced by the amount of the lysis buffer.

Therefore, this experiment investigated whether the amount of lysis buffer used with the cotton swab could affect the amount of DNA extracted.

4.2.1 EXPERIMENTAL SETUP

A participant, previously confirmed as a moderate shedder in Section 3.1, was asked to perform the deposition process described in Section 2.2 on a 5 x 7 cm area of a glass surface (smooth non-porous). The participant was requested to repeat the same process of DNA deposition 24 times (n= 24, 8 replicates per each extraction technique). Before use, the surfaces were sterilised with 2% Virkon and UV for 15 min. The process was conducted at room temperature.

After deposition, samples were collected immediately using a Copan cotton swab (150C) moistened with molecular grade water using a spray bottle as described in Section 2.3.

After collection, three techniques were used to extract the swabs by the PrepFiler Express BTA™ with AutoMate Express System as described in Section 2.4:

a) Full swab head with 230 µL of lysis buffer.
b) Half swab head with 230 µL of lysis buffer (Figure 4.6).
c) Full swab head with 460 µL of lysis buffer.

Extracted samples were quantified using the Quantifiler™ Human DNA Quantification Kit following the procedures detailed in Section 2.5. Some samples collected by each technique were amplified and analysed as outlined in Section 2.6 and 2.7 to evaluate the quality of the samples collected.

All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed as described in Section 2.8.

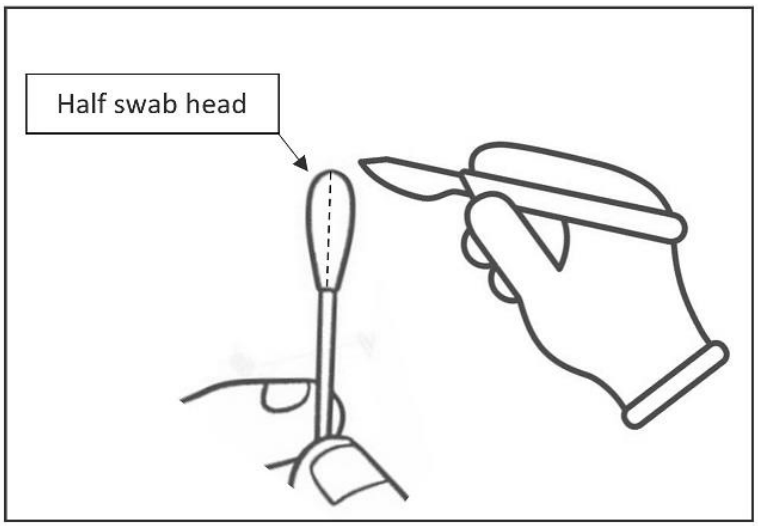


Figure 4.6: Half of the swab head was cut from the tip downward into the extraction tube using a sterilised (DNA-free) scalpel.

4.2.2 RESULTS

The PrepFiler Express BTA™ is an effective extraction kit and has been validated for many types of trace samples. However, the amount of lysis buffer used in relation to the size of the sample for extraction can influence the amount of DNA extracted. The quantity of DNA extracted from cotton swabs was affected by the amount of lysis buffer used with the PrepFiler Express BTA™ Extraction Kit ($p < 0.001$) (Figure 4.7) (see Appendix A4.3 for the complete results).

There was no significant difference in the mean amount of DNA collected when the swab head (a) or half swab head (b) were extracted with 230 µL of lysis buffer ($p > 0.05$), but there was a significant difference when the swab head was extracted with 460 µL of lysis buffer (c) ($p < 0.001$) (mean a. 0.06, b. 0.05, c. 0.08 all in ng/µL) (Figure 4.8).

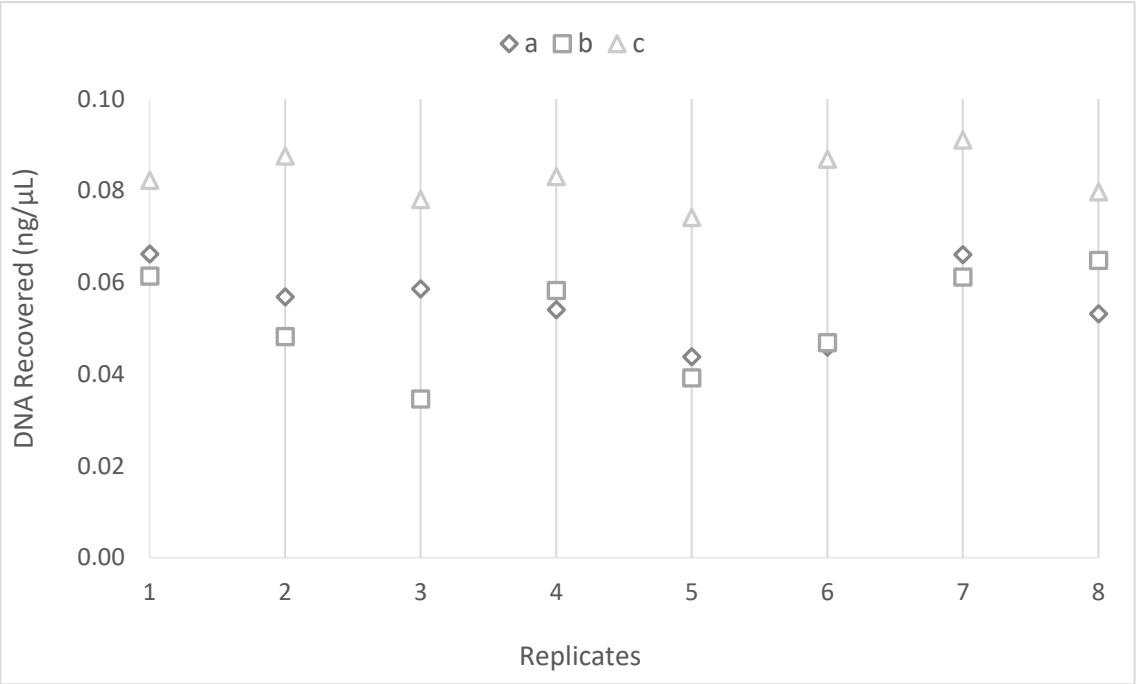


Figure 4.7: Amount of DNA recovered from eight replicates (n= 24) by each extraction technique: **(a)** full swab head with 230 µL of lysis buffer, **(b)** half swab head with 230 µL of lysis buffer and **(c)** full swab head with 460 µL of lysis buffer.

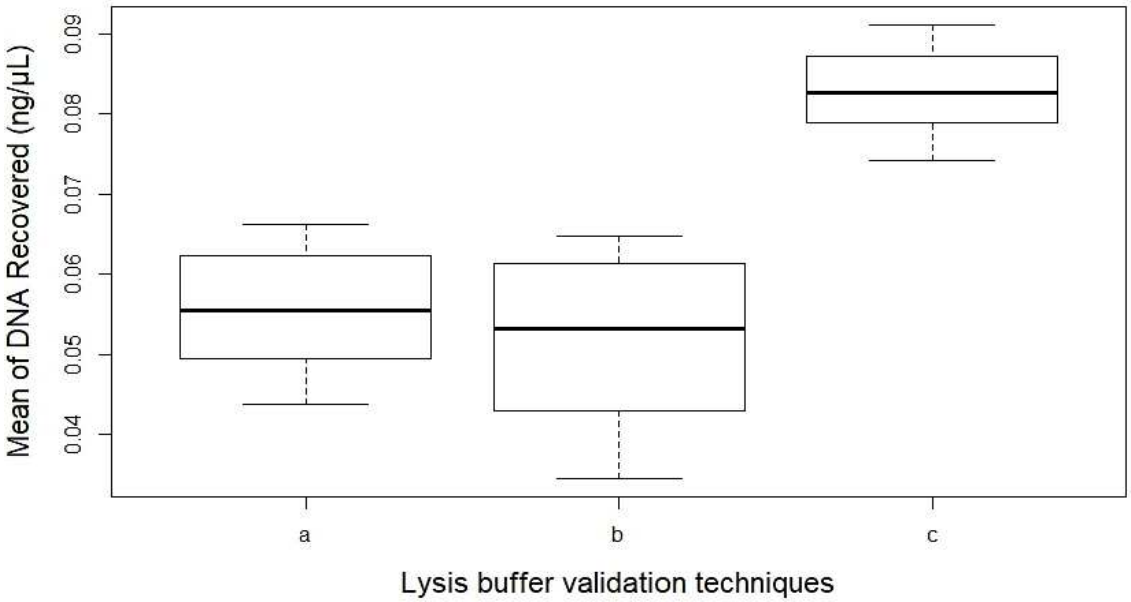


Figure 4.8: Mean amount of DNA recovered (n= 24) by each extraction technique: **(a)** full swab head with 230 µL of lysis buffer, **(b)** half swab head with 230 µL of lysis buffer and **(c)** full swab head with 460 µL of lysis buffer.

Despite the difference in the amount of DNA recovered (Figure 4.8), most quantities were good enough to produce a good quality amplification profile. The sample extracted by technique (a), and another sample by technique (c) were amplified and both produced full profiles (Figure 4.9). However, extracting crime scene samples is more challenging because of the low quantity of samples present (Raymond *et al.* 2009a).

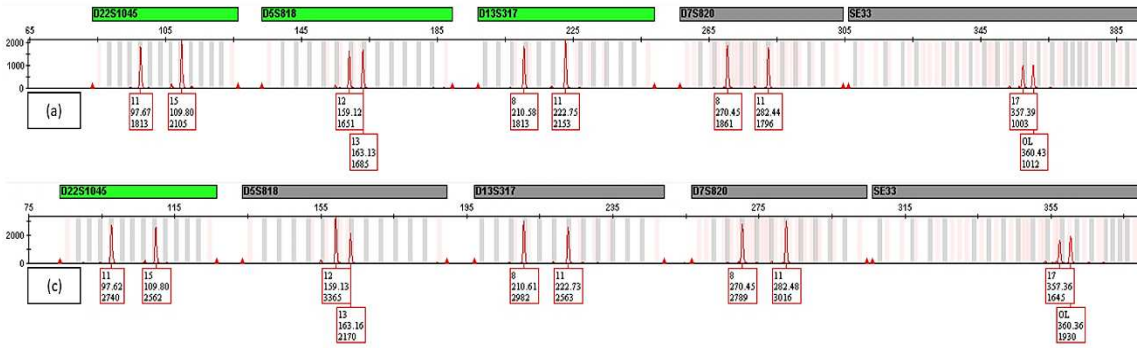


Figure 4.9: Electropherograms of the samples extracted by a **(a)** full swab head with 230 µL of lysis buffer and **(c)** full swab head with 460 µL of lysis buffer. The profiles show the difference in peak height at 5 autosomal STR loci (D22S1045, D5S818, D13S317, D7S820, SE33). Maximum volume of DNA was added.

Based on these results, it is advisable to use half of the cotton swab with the manufacturer's recommended extraction process when using the PrepFiler Express BTA™ Extraction Kit for Touch DNA collected by cotton swabs to save money. If needed, the process could be repeated with the second half of the swab and the two extracted samples combined to increase the DNA yield. Alternatively, a full swab head can be used with 460 µL of lysis buffer instead of 230 µL to maximise the extracted DNA yield from the swab.

4.3 INFLUENCE OF COLLECTION AND EXTRACTION METHODS

In Section 4.1 the collection method used for different surfaces and the interaction between the types of collection and extraction did not reach significance because of the lack of replicates. This experiment set up more replicates for glass and paper to determine if the collection method works better for a particular surface.

4.3.1 EXPERIMENTAL SETUP

Participant one from Section 4.1, previously confirmed as a high shedder in Section 3.1, was asked to perform the deposition process described in Section 2.2 on a 5 x 7 cm area of a glass (smooth non-porous) and copier paper (smooth porous) surfaces. The participant was requested to repeat the DNA deposition 18 times per surface (n= 18, 3 replicates per each variable; collection and extraction methods). Before use, the glass (G) surfaces were sterilised using 2% Virkon and UV-irradiation for 15 min, while copier paper (CP) surfaces were only irradiated using UV for 25 min. The process was conducted at room temperature.

For DNA recovery, three different methods were used: Copan cotton swab (150C) (CS), Copan nylon flocked swab (4N6FLOQSwabs®) (NS), and SceneSafe Fast™ minitape (K545) (MT) (Figure 2.2 – Section 2.3) following the same collection procedure detailed in Section 2.3.

Touch DNA was recovered within 30 minutes from the surfaces after deposition and extracted immediately with the PrepFiler Express BTA™ kit using an AutoMate Express Forensic DNA Extraction System (EXT1) and manually using the QIAamp® DNA Investigator Kit (EXT2) as described in Section 2.4. However, based on the finding from Section 4.2, for EXT1, 460 µL of lysis buffer was used instead of 230 µL with a full swab.

Extracted samples were quantified using the Quantifiler™ Human DNA Quantification Kit following the procedures in Section 2.5. Some samples were amplified and analysed as described in Section 2.6 and 2.7 to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed as described in Section 2.8.

4.3.2 RESULTS

The amount of DNA collected from glass (G) was significantly affected by collection type ($p < 0.001$), as well as the interaction between collection type and the extraction method ($p < 0.001$), but not by the extraction method alone when 460 µL of lysis buffer was used instead of 230 µL ($p > 0.05$) (Figure 4.10) (see Appendix A4.4 for the complete results).

There was a slight difference in the average DNA extracted by both extraction methods when samples were collected by CS and NS, but the highest average DNA recovered when extracted by EXT1 was with CS (mean: 0.08 ng/µL), and NS (mean: 0.09 ng/µL) for samples extracted by EXT2 (Figure 4.12). However, some of the collected DNA was not efficiently extracted by EXT2 for samples collected by MT (mean: 0.03 ng/µL) compared to samples collected by MT and extracted by EXT1 (mean: 0.06 ng/µL).

The amount of DNA collected from copier paper (CP) was also significantly affected by collection type ($p < 0.05$), as well as the interaction between collection type and the extraction method ($p < 0.05$), but not by the extraction method alone when 460 µL of lysis buffer were used with EXT1 instead of 230 µL ($p > 0.05$) (Figure 4.11) (see Appendix A4.4 for the complete results).

MT was the most suitable collection type for CP and EXT1 was the most efficient extraction method for MT (mean: 0.04 ng/µL) when compared to the EXT2 (mean: 0.02 ng/µL) (Figure 4.12). Supporting this, similar findings were found by Joël *et al.,* (2015) in their study regarding the extraction using minitapes. There was a slight difference in the average DNA extracted by both extraction methods when samples were collected by CS and NS (mean: 0.01–0.02 ng/µL).

Despite the difference in the amount of DNA recovered from both surfaces, most samples had sufficient DNA to produce a good quality profile when amplified. The sample collected from G by CS and another sample collected from CP by MT, both extracted by EXT1, were amplified and both produced full profiles (Figure 4.13).

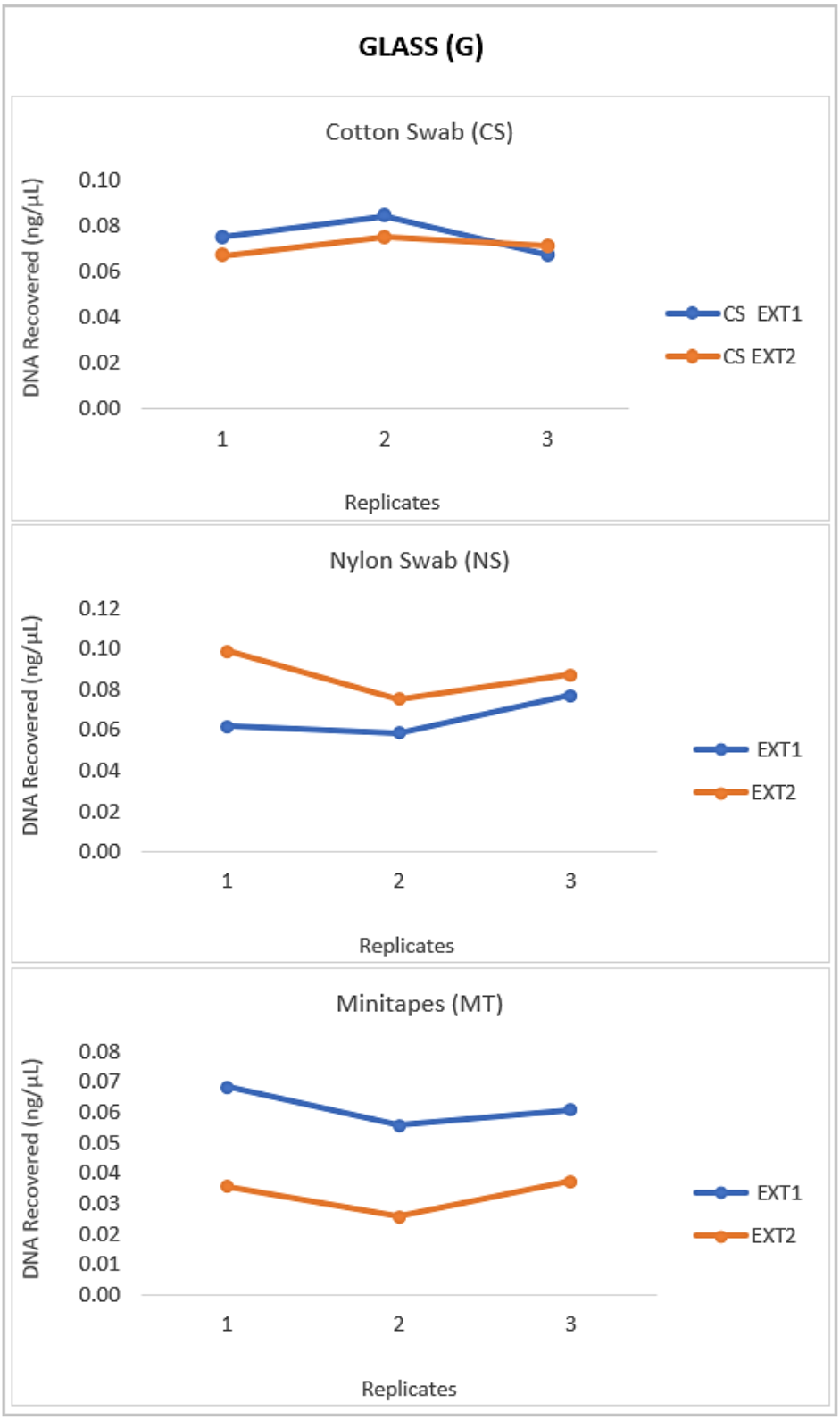


Figure 4.10: Amount of DNA recovered from glass (n= 18) using three collection methods, cotton swab (CS), nylon swab (NS) and minitapes (MT) and extracted by two extraction methods, PrepFiler Express BTA™ kit (EXT1) and QIAamp® DNA Investigator Kit (EXT2).

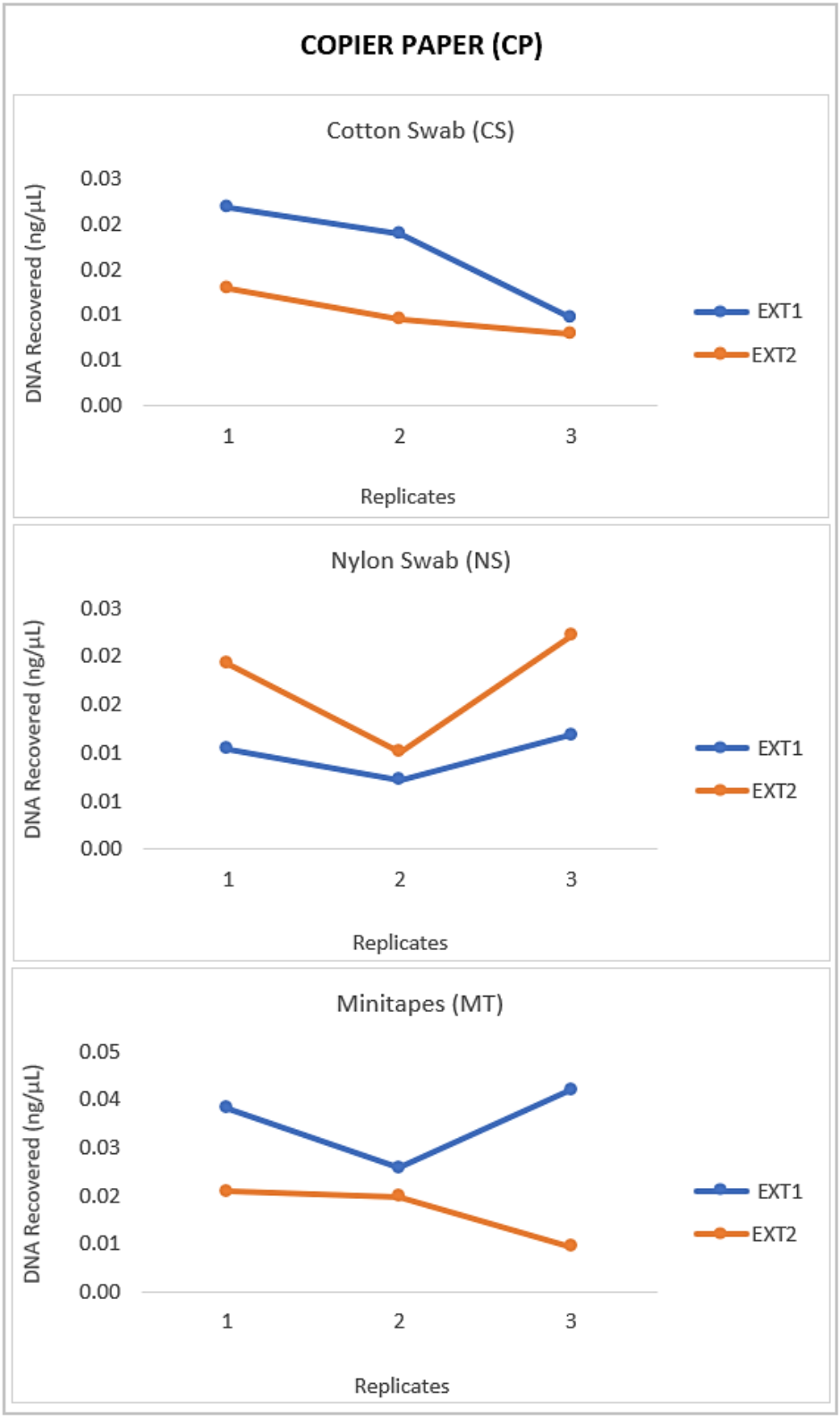


Figure 4.11: Amount of DNA recovered from copier paper (n= 18) using three collection methods, cotton swab (CS), nylon swab (NS) and minitapes (MT) and extracted by two extraction methods, PrepFiler Express BTA™ kit (EXT1) and QIAamp® DNA Investigator Kit (EXT2).

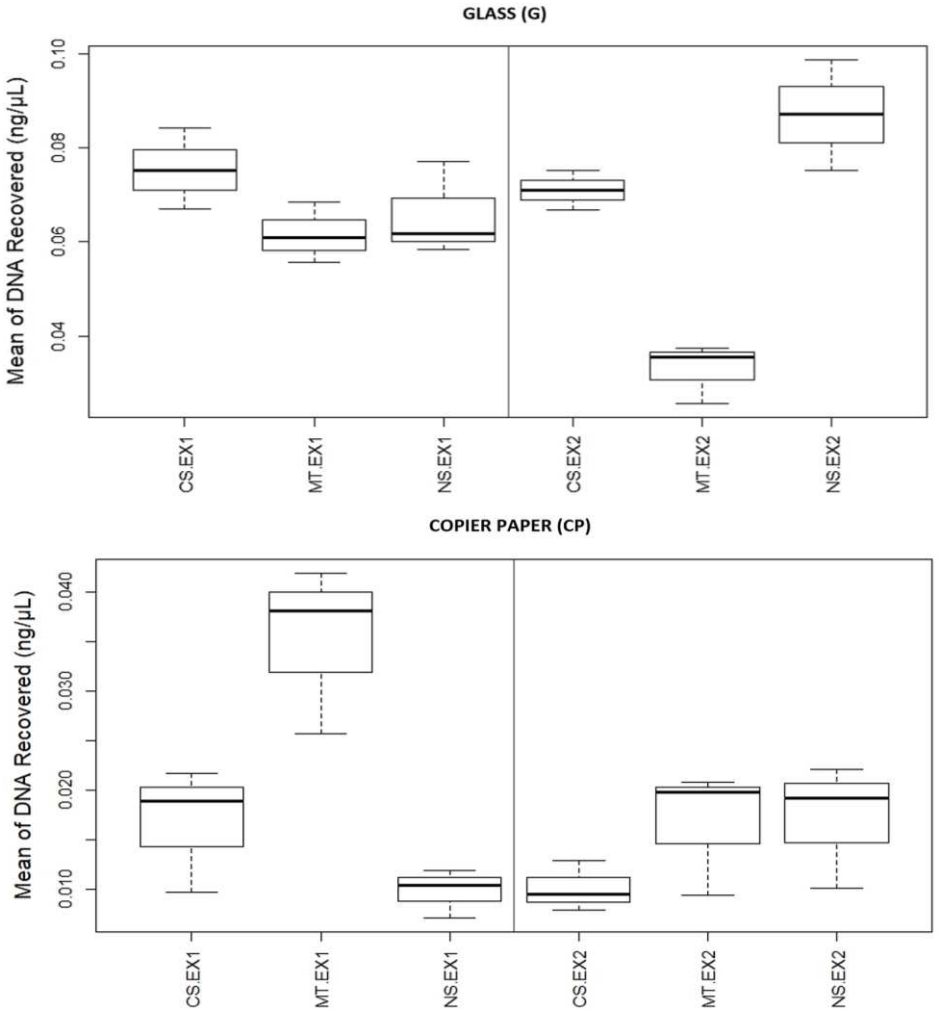


Figure 4.12: Mean of DNA recovered from glass and copier paper (n= 36) using three collection methods, cotton swab (CS), nylon swab (NS) and minitapes (MT) and extracted by two extraction methods, PrepFiler Express BTA™ kit (EX1) and QIAamp® DNA Investigator Kit (EX2).

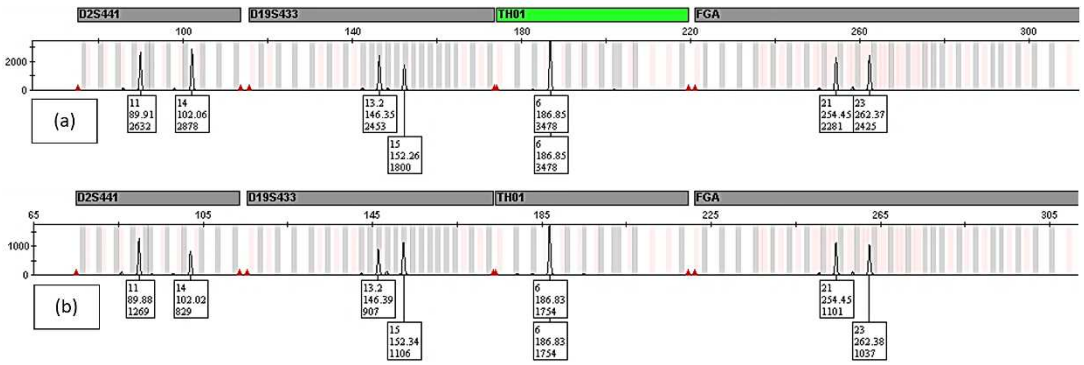


Figure 4.13: Electropherograms of the samples extracted by with PrepFiler Express BTA™ kit (EXT1) and collected by **(a)** cotton swab (CS) from glass surface, and **(b)** minitape (MT) from copier paper (CP). The profiles show the difference in peak height at 4 autosomal STR loci (D2S441, D19S433, TH01, FGA). Maximum volume of DNA was added.

4.4 EVALUATION OF 4N6FLOQSwabs

Nylon flocked swabs are designed to help increase DNA collection and improve the extraction efficiency (Hansson *et al.,* 2009; Dadhania *et al.,* 2013; Verdon *et al.,* 2014a) because cotton swabs retain some of the extracted DNA leading to a loss in the amount of DNA recovered.

From the findings of Section 4.1, the cotton swab and nylon swab performed equally well in the extraction of DNA from non-porous surfaces. Even though cotton swabs were slightly better for samples extracted by the PrepFiler Express BTA™, there were not sufficient replicates to compare the performance of the two swabs. The cotton swab was slightly more efficient with smooth non-porous surfaces such as glass and stainless steel, while the nylon swab was slightly more efficient with textured non-porous surfaces such as textured plastic.

Therefore, this experiment aimed to set up more replicates of textured plastic (rough non-porous) surfaces to compare the performance of the two swabs.

4.4.1 EXPERIMENTAL SETUP

A participant, previously confirmed as a moderate shedder in Section 3.1, was asked to perform the deposition process described in Section 2.2 on a 5 x 7 cm area of a textured plastic surface (rough non-porous). The participant was requested to repeat the same process of DNA deposition 36 times (n= 36, 12 replicates per each collection method). Before use, the surfaces were sterilised with 2% Virkon and UV for 15 min. The process was conducted at room temperature.

After deposition, samples were collected immediately using a Copan cotton swab (150C) (CS) and Copan nylon flocked swab (4N6FLOQSwabs®) (NS) (Figure 2.2 – Section 2.3) moistened with molecular grade water as follows:

a) Cotton swab with 100 µL using a spray bottle as described in Section 2.3.
b) Nylon swab with 100 µL using a spray bottle as prescribed in Section 2.3.
c) Nylon swab with 30 µL by pipette as recommended by the manufacturer.

The swabs heads were extracted immediately after collection using PrepFiler Express BTA™ with the AutoMate Express System as described in Section 2.4. However, based on the finding from Section 4.2, for EXT1, 460 µL of lysis buffer was used instead of 230 µL with a full swab.

Extracted samples were quantified using the Quantifiler™ Human DNA Quantification Kit following the procedures detailed in Section 2.5. Some samples collected by each technique were amplified and analysed as described in Section 2.6 and 2.7 to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed as described in Section 2.8.

4.4.2 RESULTS

There was a significant difference between the collection techniques used to recover Touch DNA with CS and NS from textured plastic (TP) ($p < 0.001$) (Figure 4.14) (see Appendix A4.5 for the complete results).

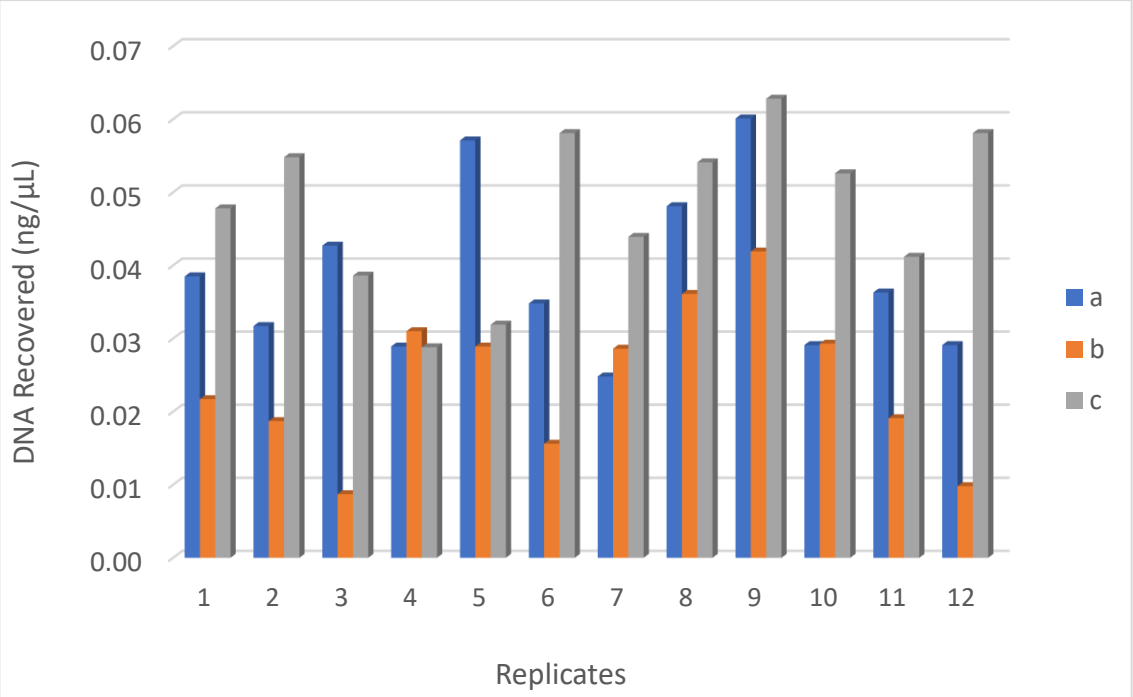


Figure 4.14: Amount of DNA recovered from twelve replicates (n= 36) by each technique: **(a)** cotton swab moistened with 100 µL, **(b)** nylon swab moistened with 100 µL and **(c)** nylon swab moistened with 30 µL.

Using NS with 30 µL of molecular grade water (c) was more efficient than CS with 100 µL of molecular grade water (a) when collecting Touch DNA from rough non-porous surfaces such as TW (mean = a. 0.04 and c. 0.05 all in ng/µL) ($p < 0.05$).

The increased wetting reagent on NS such as using 100 µL of molecular grade water resulted in reduced efficiency for collection (mean: 0.02 ng/µL) ($p < 0.001$) (Figure 4.15), as it is more sensitive to the wetting solution than CS because of the nature of the swab fabric. However, NS was more efficient for the collection of Touch DNA from textured surfaces.

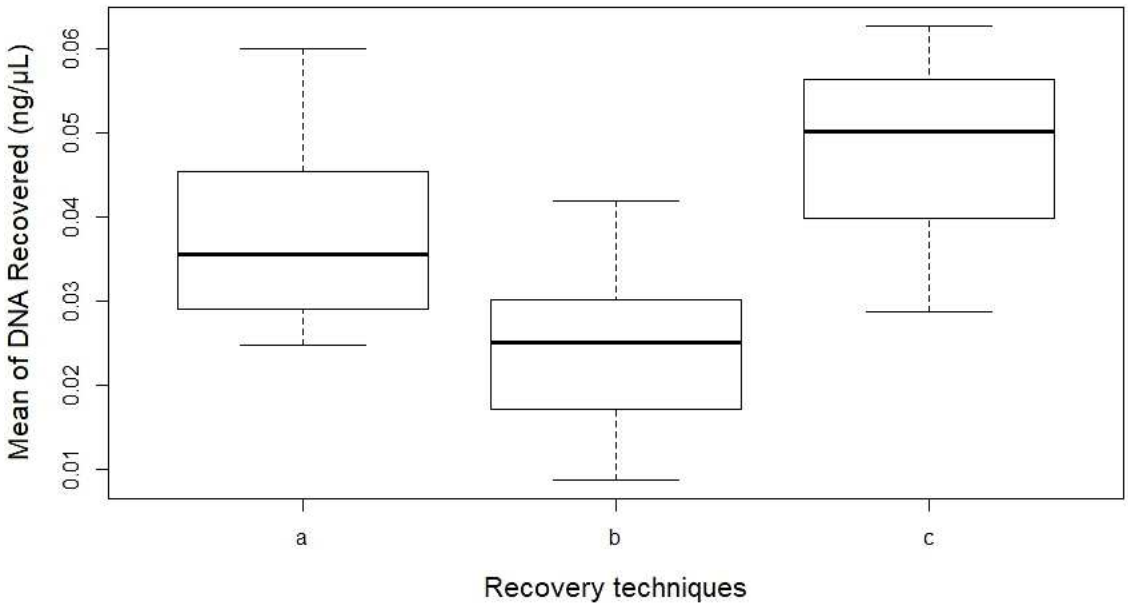


Figure 4.15: Mean of DNA recovered (n= 36) by each technique: **(a)** cotton swab moistened with 100 µL, **(b)** nylon swab moistened with 100 µL and **(c)** nylon swab moistened with 30 µL.

4.5 DUAL RECOVERY OF DNA AND FINGERPRINTS USING MINITAPES

Collecting Touch DNA and fingerprints from crime scenes is common, and often used in combination connect the suspects to the crime scene. However, it is challenging to collect both DNA and fingerprints when they are located in the same spot on a surface, i.e. where the touch has left both DNA and a fingerprint. DNA recovered from fingerprints is often found in minute quantities (Raymond *et al.,* 2009a), and the powder used to recover fingerprints can also interfere by damaging the DNA, thereby inhibiting DNA profiling. Collecting Touch DNA and fingerprints from the same deposit is called dual recovery, and it has been previously investigated by swabbing for DNA after lifting fingerprints (Alem *et al.,* 2017; Sinelnikov & Reich, 2017). In Section 4.1, there were visible fingerprints after DNA deposition on stainless steel and glass surfaces, and they were still visible after the use of minitapes to recover DNA. Therefore, this study aimed to examine the possibility of recovering Touch DNA prior to fingerprint collection using minitapes.

4.5.1 EXPERIMENTAL SETUP

The participant previously confirmed as a high shedder in Section 3.1, was asked to perform the DNA deposition process described in Section 2.2 on stainless steel (SS: 7.5 x 5 cm) and glass (G: 7.5 x 2.5 cm), smooth non-porous surfaces (n= 20, 2 replicates per each collection method). Prior to deposition, the surfaces were sterilised using 2% Virkon and UV-irradiation for 15 min. DNA samples were collected using SceneSafe FAST™ minitape (1-Tape Kit) (MT) following the same recovery procedure described in Section 2.3, and fingerprints were collected using Black Fingerprint Powder (EVIDENT) (Figure 4.15). Touch DNA was recovered with five different methods: one tape-lift (1L), three tape-lifts (3L), six tape-lifts (6L), nine tape-lifts (9L), and fifteen tape-lifts (15L). Fingerprints were collected following the different tape-lifts, to determine whether the number of lifts could damage the fingerprint. This process of deposition and dual recovery was conducted at room temperature. Samples were extracted with the PrepFiler Express BTA™ kit, using an AutoMate Express Forensic DNA Extraction System (EXT1) following the procedures described in Section 2.4. However, based on the findings from Section 4.2, 460 µL of lysis buffer was used instead of 230 µL. The samples

were quantified using the Quantifiler™ Trio DNA Quantification Kit, following the procedures from Section 2.5. All samples were then amplified and analysed as described in Sections 2.6 and 2.7, to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed following the procedures mentioned in Section 2.8. For analysis, when the total RFUs observed were reported, this was directly counted for homozygous loci and by adding the peak heights of each allele for heterozygous loci.

Figure 4.16: Tools used to recover fingerprints. Fingerprints were collected by revealing them with a dusting of EVIDENT black powder using brush, then lifted with a clear tape and deposited on white backing cards.

4.5.2 RESULTS

Touch DNA recovered from the deposited fingerprints on the smooth non-porous surfaces with MT was 100% successful, as all the collected samples produced full DNA profiles. The quantity of DNA collected was dependent on the surface area size (p <0.05), with more DNA recovered from the 7.5 x 5 cm SS than the 7.5 x 2.5 cm G surface (mean SS. 0.24 ng/µL vs. G. 0.09 ng/µL) (Figure 4.17). Likewise, the quantity of DNA recovered was influenced by the number of tape-lifts (p <0.01), with the amount of collected DNA increasing with more tape-lifts (Figure 4.18).

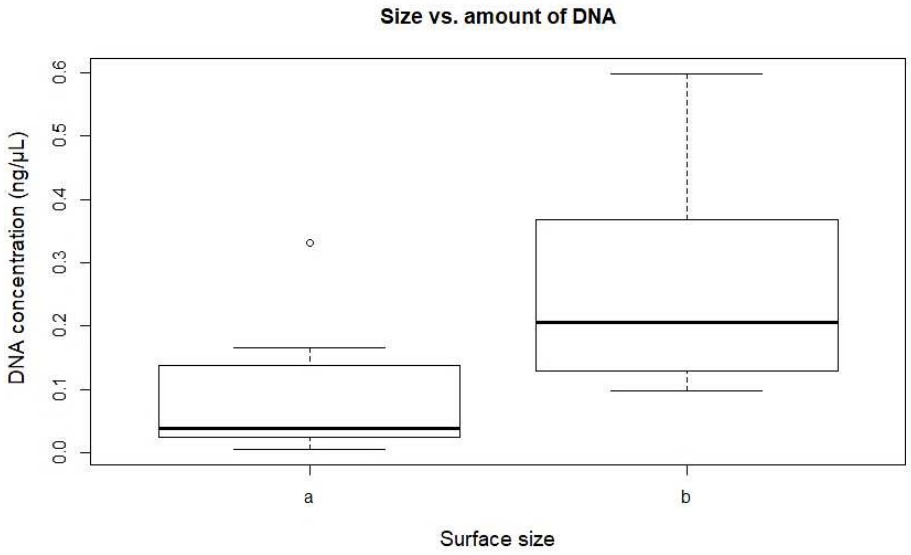


Figure 4.17: Mean of DNA recovered by minitapes (MT) number of tape-lifts from deposited fingerprints (n= 20) on **(a)** glass (G: 7.5 x 2.5 cm) and **(b)** stainless steel (SS: 7.5 x 5 cm).

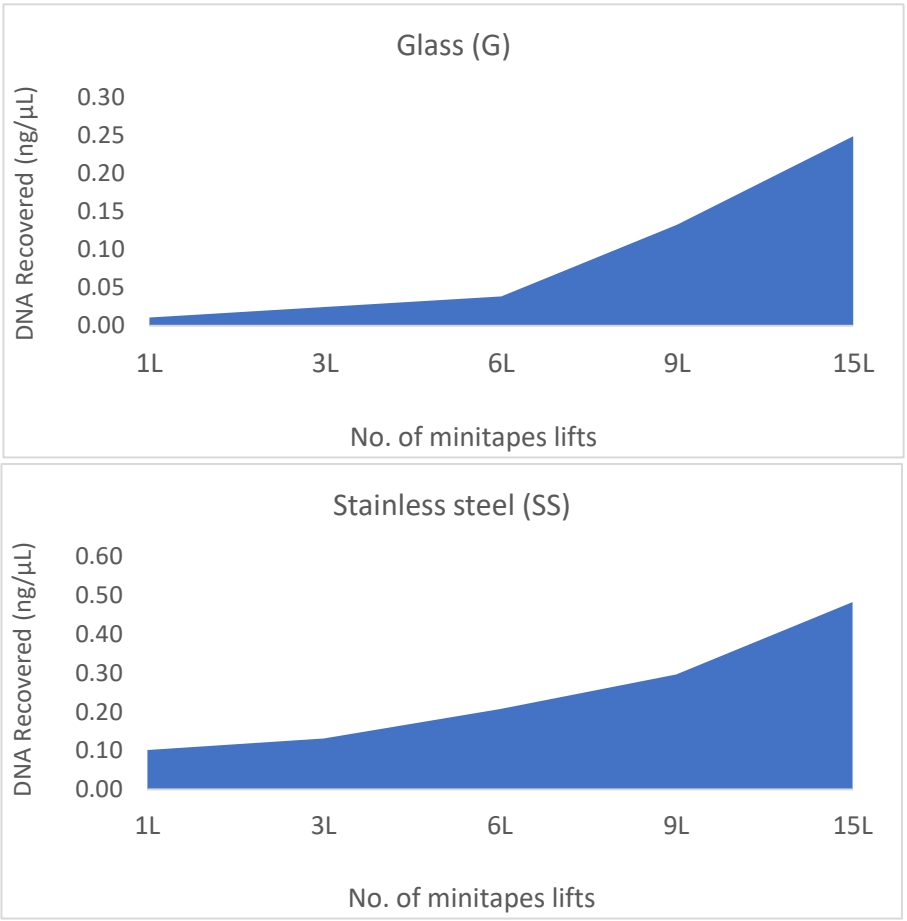


Figure 4.18: Amount of DNA recovered by minitapes (MT) is dependent on the number of tape-lifts from deposited fingerprints (n= 20) on glass (G) and stainless steel (SS).

In addition, the average signal (RFU) was impacted by the surface area size (p <0.01) and the number of tape-lifts (p <0.01), with a higher average peak height for SS than G (RFU mean SS. 4788 vs. G. 2623) (Figure 4.19). similarly, the mean of peak height increased with the number of tape-lifts (RFU means 1L. 1803, 3L. 2050, 6L. 3074, 9L. 3772, and 15L. 7828) (Figure 4.20).

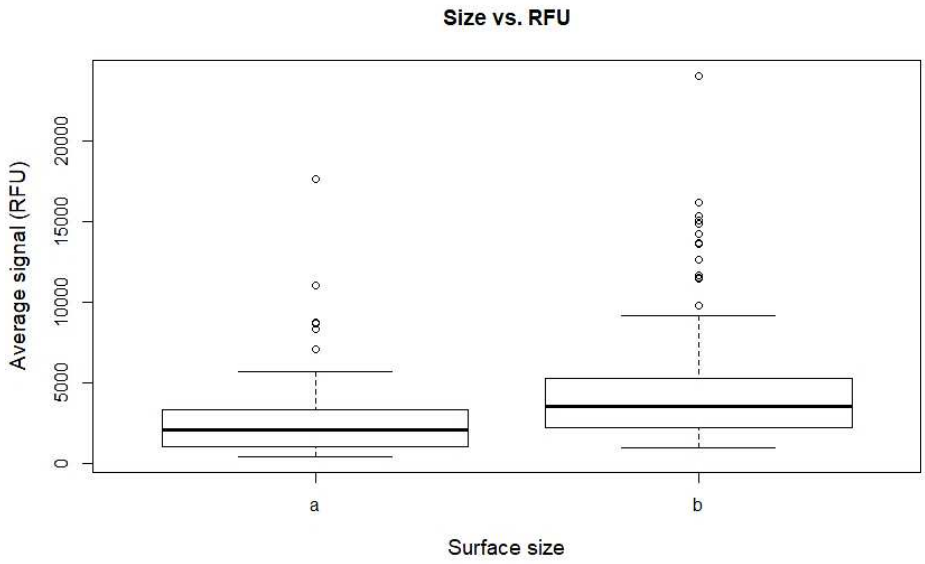


Figure 4.19: Average signal (RFU) recovered by minitapes (MT) from deposited fingerprints (n= 20) on **(a)** glass (G: 7.5 x 2.5 cm) and **(b)** stainless steel (SS: 7.5 x 5 cm).

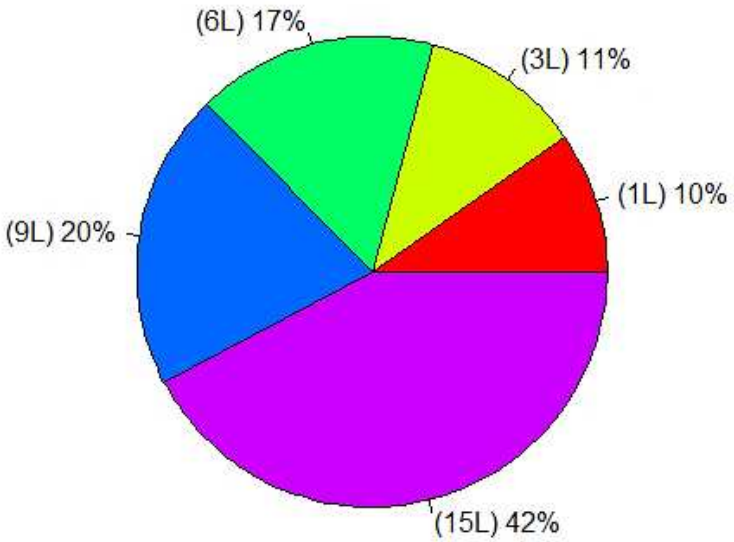


Figure 4.20: Percentage signal (RFU) recovered by minitapes (MT) by number of tape-lifts from deposited fingerprints (n= 20) on glass (G) and stainless steel (SS).

The collection of fingerprints after DNA recovery was 90% successful from the G, and 100% from the SS surfaces (Figure 4.21). Fingerprint recovery was not influenced by the recovery of DNA or the number of tape-lifts ($p > 0.05$), when samples were deposited by a participant considered to be a high shedder. Identifiable patterns and features were detected in most fingerprints, which were suitable for database comparison.

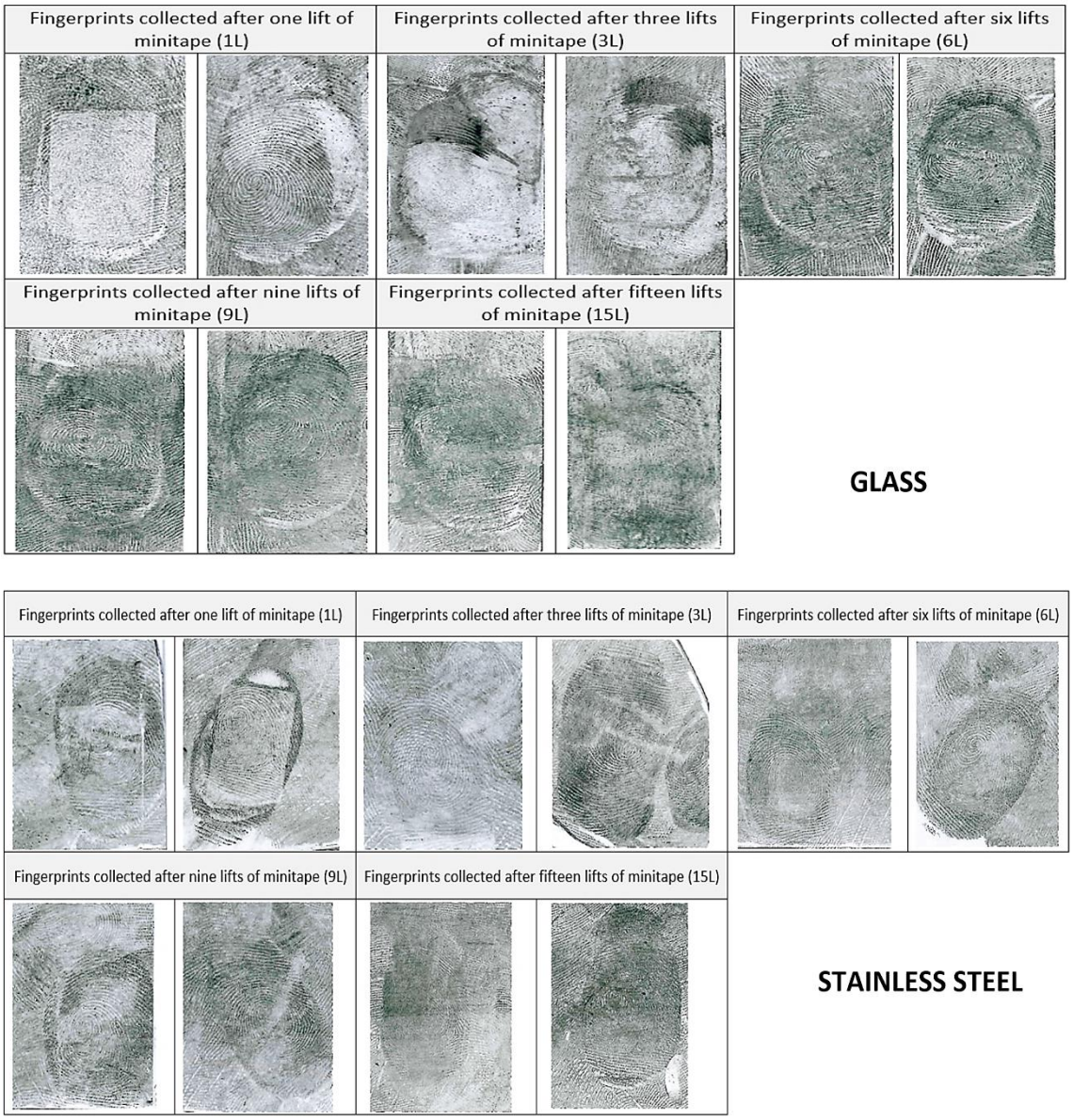


Figure 4.21: Fingerprints collected after each minitape (MT) per number of tape-lifts from deposited fingerprints (n= 20) on glass (G) and stainless steel (SS), collected by Black Fingerprint Powder (EVIDENT). Fingerprints were collected by revealing them with a dusting of black powder using a brush, then lifted with clear tape, and deposited on white backing cards.

4.6 EVALUATION OF THE QUANTIFILER™ TRIO DNA QUANTIFICATION KIT

The efficiency of DNA extraction, as well as quantification methods, can impact the amount of trace DNA collected (Ottens *et al.,* 2013). However, new quantification methods that use real-time PCR (qPCR) are more sensitive than alternative quantification methods such as NanoDrop™ or Gel electrophoresis. Quantifiler™ Human and Quantifiler™ Trio (Thermo Fisher Scientific) are Quantitative real-time PCR kits commonly used in forensic laboratories to quantify casework samples.

Therefore, this study tested the efficacy of Quantifiler™ Human and Quantifiler™ Trio quantification kits on Touch DNA.

4.6.1 EXPERIMENTAL SETUP

Previously extracted samples that were deposited by participant one from Section 4.1 were quantified using the Quantifiler™ Trio DNA Quantification Kit following the procedures outlined in Section 2.5 for comparison to the Quantifiler™ Human Quantification Kit. Some of the samples quantified by both kits were amplified and analysed as described in Section 2.6, 2.7, and Section 2.8.

4.6.2 RESULTS

There was no significant difference in the quantification methods used (Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Trio DNA Quantification Kit) to quantify the recovered Touch DNA from the variety of surfaces tested ($p > 0.05$) (Figure 4.22).

The amount of DNA recovered from the samples was similar according to both quantification methods. All the samples were similarly diluted (10 µL from the sample with 5 µL of diluent) for amplification with the GlobalFiler™ PCR Amplification Kit, apart from one sample that was collected by a cotton swab from glass (circled in Figure 4.22), in which, 9.5 µL of sample was diluted with 5.5 µL of diluent for the Quantifiler™ Human and 8.6 µL of sample was diluted with 6.4 µL of diluent for the Quantifiler™ Trio. The sample was processed for amplification in two different dilutions to check the quality of the DNA profile, with both dilutions producing a full profile. However, the peaks

produced from the dilution suggested by Quantifiler™ Human were much higher than the dilution suggested by Quantifiler™ Trio (Figure 4.23). This small change in the dilution between both quantification kits may be related to a human pipetting error or using an uncalibrated pipette.

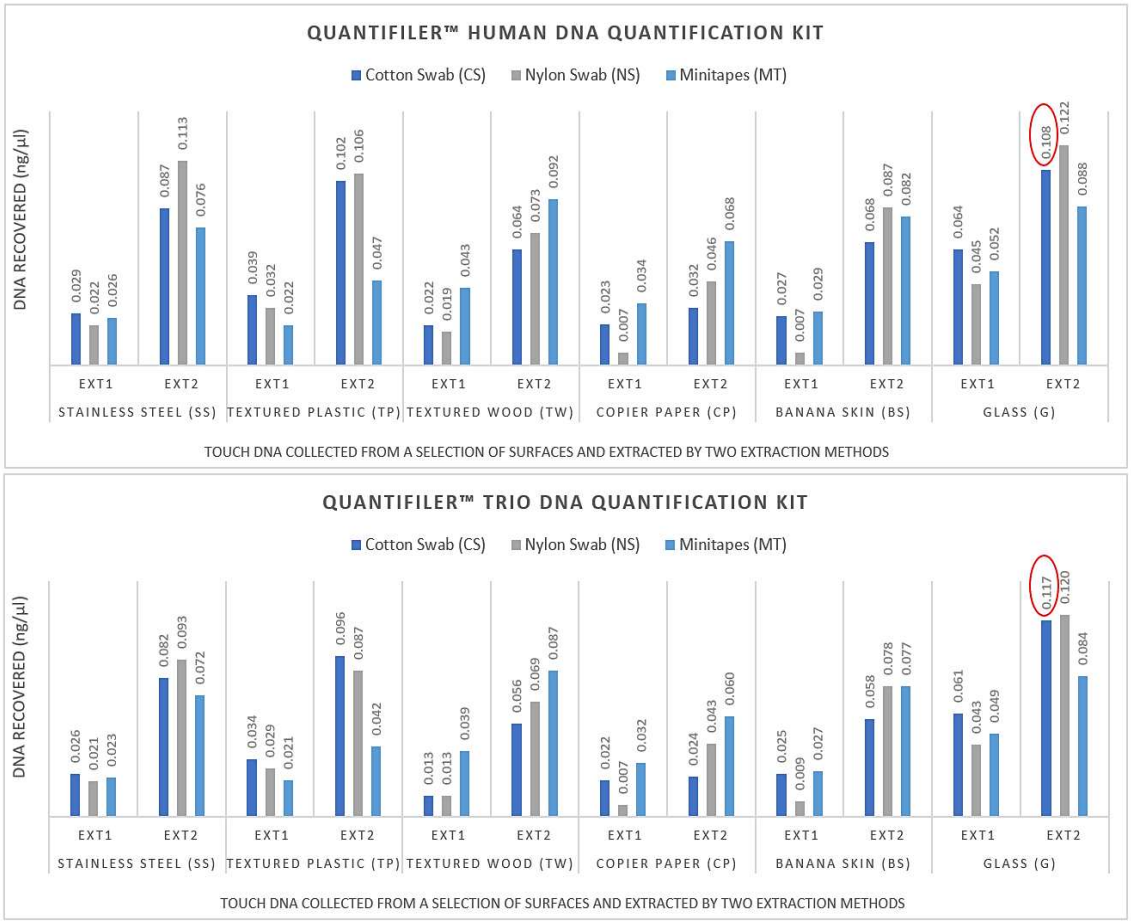


Figure 4.22: Amount of DNA recovered (n= 36) by three collection methods and two extraction methods, then quantified by the Quantifiler™ Human and Quantifiler™ trio quantification kits.

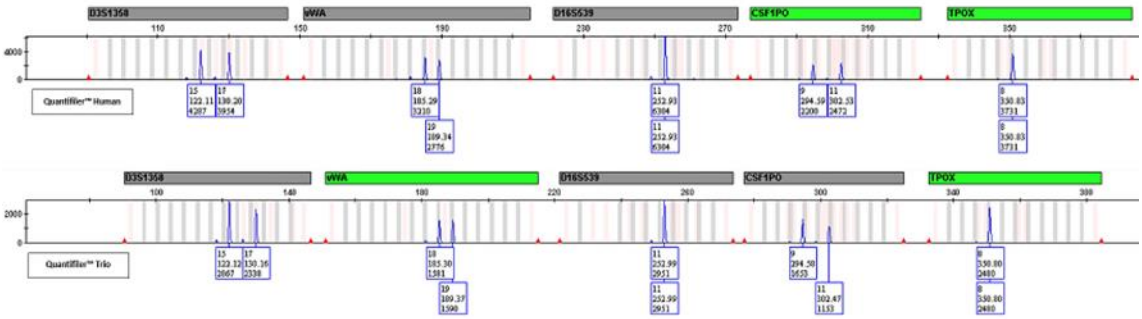


Figure 4.23: Electropherograms of a sample quantified by the Quantifiler™ Human and Quantifiler™ Trio kits. The profiles show a difference in peak height at 5 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX). Maximum volume of DNA was added.

4.7 CHAPTER DISCUSSION AND CONCLUSION

Different types of surfaces whether they are smooth, rough, porous or non-porous retain Touch DNA differently and it is important to consider the most appropriate collection method to improve DNA recovery (Alketbi & Goodwin, 2019a). Based on these experimental findings, it is recommended to use a cotton swab for smooth, non-porous surfaces such as glass and stainless steel and a nylon swab for rough, non-porous surfaces such as textured plastic, whereas the use of minitapes is better for porous surfaces such as paper or wood. More importantly, following the recommended procedures, such as using the appropriate amount of wetting solution with different swabs, can influence the amount of DNA recovered. Even though it is easier to extract DNA from the nylon swab (Verdon *et al.,* 2014a), the cotton swab has increased absorbance ability compared to the nylon swab because of the nature of the swab fabric. Therefore, it is not recommended to use more than 30-50 µL of wetting solution with the swab before collection.

The QIAamp® DNA Investigator Kit (Qiagen) and PrepFiler Express BTA™ kit (Thermo Fisher Scientific) are popular extraction methods used in forensic laboratories and have been evaluated in many studies regarding their extraction efficacy for trace samples collected by different collection methods (Brownlow *et al.,* 2012; Ip *et al.,* 2015). However, different collection methods require different extraction processes, for example, in this study more DNA was recovered when minitapes were extracted using PrepFiler Express BTA™ than when extracted by the QIAamp DNA Investigator. Nevertheless, different extraction methods, whether automated or manual, have different requirements to process the sample, with the size of the specimen in combination with the amount of lysis buffer influencing the maximum DNA yield. For example, using a half swab head with the recommended 230 µL of lysis buffer for the PrepFiler Express BTA™ extraction kit is better than using a full swab head (Alketbi & Goodwin, 2019a; Joël *et al.,* 2015).

Regarding the collection methods used in this study, the Copan nylon flocked swab (4N6FLOQSwabs®) was the easiest to use, as the handle was flexible and the swab head was easy to break into the tube by bending the swab. The Copan cotton swab (150C)

requires a scissor or scalpel to cut the swab, similarly, SceneSafe Fast™ minitape (K545) needs to be cut into small pieces by scissor for effective extraction. These requirements need to be taken into consideration when using the collection methods to avoid contamination. For example, the use of minitapes is better done in an indoor environment and placed in a tube immediately after collection to avoid any contamination, while cotton or nylon swabs can be used for outdoor collection and transferred to the lab to be processed. Moreover, the cotton swab has another advantage over the other collection methods, only half of the swab head can be used, with the other half stored for future extraction if required, while a nylon swab head must be processed entirely (because of the nature of the swab fabric). All the factors mentioned above should be considered when designing protocols for the collection of Touch DNA from various surfaces, irrespective of whether it is indoor or outdoor.

Most fingerprints can be invisible in various types of surfaces, though smooth non-porous surfaces, such as metal or glass, reflect light to some degree, rendering fingerprints often visible on these surfaces, which can enable Dual recovery (Alketbi & Alsoofi, 2022b). Dual recovery of DNA and fingerprints from Touch DNA was successful from clear fingerprints deposited by high shedders on non-porous surfaces, with the use of SceneSafe FAST™ minitape and Black Fingerprint Powder. The number of tape-lifts necessary to recover the DNA does not impact upon the quality of the fingerprint, if performed with care, while using low-medium pressure to avoid smearing the fingerprint. However, additional studies should investigate other types of shedders to confirm these results.

The process of Touch DNA deposition in this study was effective and provided consistent results, which lead to the solid evaluation of the tested variables and can be used for future studies of Touch DNA.

CHAPTER FIVE

5. INFLUENCE OF TIME AND ENVIRONMENTAL CONDITIONS

This chapter examined the impact of environmental conditions on Touch DNA collected from outdoor surfaces. First, the influence of humidity found on non-porous surfaces on recovery techniques was evaluated. Next, by simulating outdoor crime scene casework scenarios, the effect of environmental conditions (e.g., temperature, humidity) and time since deposition on Touch DNA deposited on a range of surfaces were examined. This was done by simulating conditions that are common in Dubai and London such as high temperatures with moderate humidity and low temperatures with high humidity, respectively. Finally, the impact of sand found on outdoor surfaces in countries such as the UAE was examined.

5.1 INFLUENCE OF HUMIDITY ON RECOVERY TECHNIQUES

Humid environments can lead to reduced traces of biological material in samples (Raymond *et al.,* 2008). Therefore, an investigation on swabs commonly used is needed to find the most effective way to collect Touch DNA deposited on humid surfaces and exposed to different conditions. The aim of this study was to test the use of moist/dry cotton and nylon swabs to collect DNA from humid non-porous surfaces.

5.1.1 EXPERIMENTAL SETUP

A participant, previously confirmed as a moderate shedder in Section 3.1, was asked to perform the deposition process described in Section 2.2 on a 5 x 7 cm glass surface (smooth non-porous). The participant was requested to repeat the same process of DNA deposition 32 times (n= 32, 8 replicates per each collection technique). Before use, the surfaces were sterilised with 2% Virkon and subjected to ultraviolet radiation (UV) for 15 min. The process was conducted at room temperature.

After deposition, the surfaces were stored in a fridge for 24 h before collection to expose the surfaces to low temperature and high humidity (5 °C/78%) (LT) (Figure 5.1). The temperature and humidity were recorded during the exposure period using an Oria digital thermometer hygrometer, indoor/outdoor humidity meter and temperature monitor (Calibrated against Oregon scientific THGR221) (Figure A5.1 - Appendix A5.1).

Figure 5.1: Selected surfaces stored at high temperature with moderate humidity (40 °C/50%) in the oven, and low temperature with high humidity (5 °C/78%) (LT) in the fridge. Temperature and humidity were monitored during the exposure period.

Samples were collected using a Copan cotton swab (150C) (CS) and Copan nylon flocked swab (4N6FLOQSwabs®) (NS) (Figure 2.2 – Section 2.3) moistened with molecular grade water as follows:

a) Cotton swab with 100 µL water using a spray bottle, as prescribed in Section 2.3.
b) Dry cotton swab; no water added.
c) Dry nylon swab; no water added.
d) Nylon swab with 30 µL water added using a pipette, as prescribed in Section 2.3.

Next, swabs heads were extracted immediately after collection using QIAamp® DNA Investigator Kit following the procedures outline in Section 2.4. Extracted samples were quantified using the Quantifiler™ Human DNA Quantification Kit following the procedures detailed in Section 2.5. Some samples collected by each technique were amplified and analysed following the procedures outlined in Section 2.6 and 2.7 to evaluate the quality of the samples. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed as described in Section 2.8.

5.1.2 RESULTS

There was a significant difference amongst the recovery techniques of the four methods used to recover Touch DNA with CS and NS from a glass surface exposed to high humidity and low temperature (78%, 5 °C) ($p < 0.001$) (Figure 5.2) (see Appendix A5.2 for the complete results).

The dry cotton swab (b) or dry nylon swab (c) was more efficient at collecting trace DNA from non-porous humid surfaces such as glass ($p < 0.001$), with no significant difference between the two collection techniques ($p > 0.05$). Using the moist cotton swab (a) or moist nylon swab (d) resulted in some biological materials left uncollected from the surface ($p > 0.05$) (mean a. 0.04, b. 0.08, c. 0.07, and d. 0.05 ng/µL) (Figure 5.3).

Although the moisture content in a sample can lead to increased rate of degradation (Goray *et al.,* 2010; Burrill *et al.,* 2019). It may also enhance DNA transfer when the right

collection technique is used (Alketbi & Goodwin, 2021), and depending on the periods between original deposition and recovery.

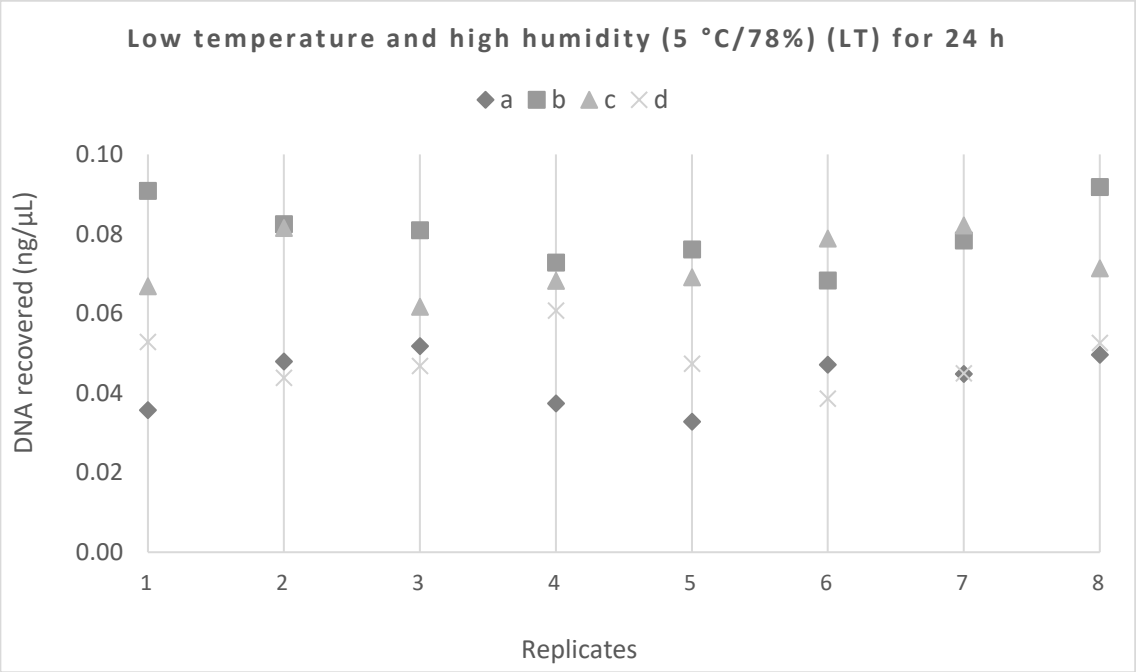


Figure 5.2: Amount of DNA recovered from eight replicates of samples collected from glass surface exposed to high humidity and low temperature (78%, 5 °C) (n= 32) by **(a)** moist cotton swab, **(b)** dry cotton swab, **(c)** dry nylon swab, and (d) moist nylon swab.

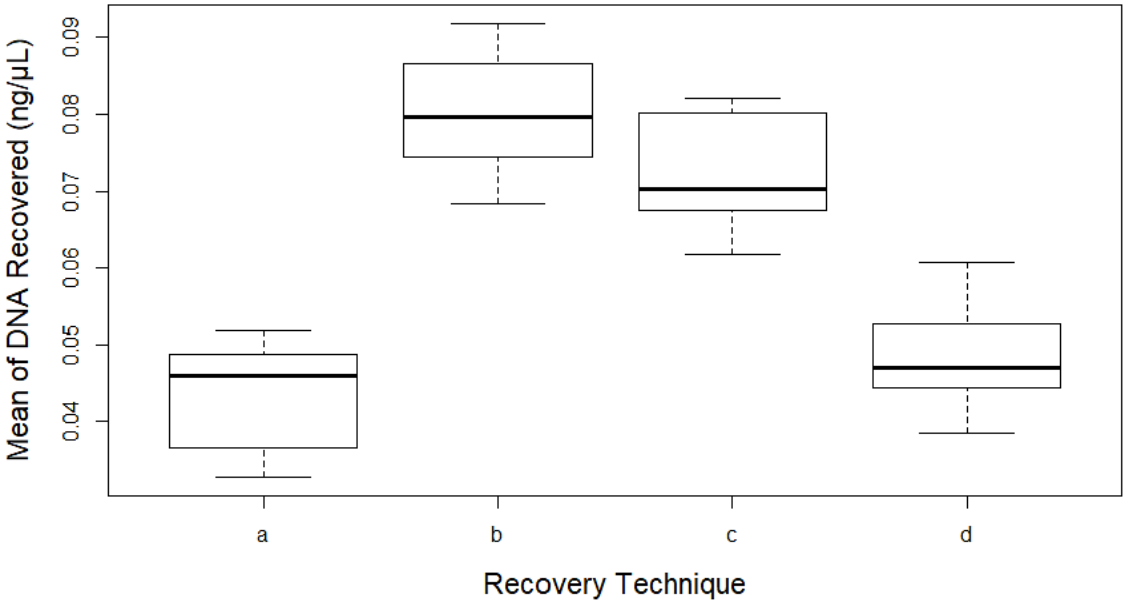


Figure 5.3: Mean of DNA recovered from eight replicates of samples collected from glass surface exposed to high humidity and low temperature (78%, 5 °C) (n= 32) by **(a)** moist cotton swab, **(b)** dry cotton swab, **(c)** dry nylon swab, and (d) moist nylon swab.

5.2 SIMULATING OUTDOOR CRIME SCENE CASEWORK SCENARIOS

In addition to the factors investigated in Section 4.1, time and environmental conditions can also affect Touch DNA. Although few studies have looked at the effects of time between deposition and recovery of Touch DNA (Li *et al.,* 2003; Raymond *et al.,* 2009a; Frégeau *et al.,* 2010), there is a lack of research concerning the influence of environmental conditions such as temperature and humidity, which can reduce traces of biological material in samples (Poinar, 2003; Lindahl, 1993; Raymond *et al.,* 2008).

Therefore, the aim of this study was not only to investigate the effect of time between deposition and recovery of Touch DNA, but also the impact of temperature and humidity on a range of porous and non-porous surfaces.

5.2.1 EXPERIMENTAL SETUP

Four surfaces were selected to replicate common items encountered in outdoor crime scenes and have a variety of smooth, rough, porous, and non-porous surfaces. These surfaces included stainless steel (SS), glass (G), textured plastic (TP), and textured wood (TW) (previously tested in Section 4.1). All surfaces were sterilised using 2% Virkon and exposed to UV-irradiation for 15 min. A participant previously confirmed as a high shedder in Section 3.1 was instructed to perform the deposition process described in Section 2.2 on a 5 x 7 cm area of the selected surfaces (n= 96, 2 replicates per each variable; surface type, conditions and time).

To assess the influence of time since deposition under different environmental conditions, the surfaces were left for four time periods (3 hours, 12 hours, 24 hours and one week) after deposition under the following conditions:

a) Room temperature with moderate humidity (20–25 °C/50%) (RT).
b) High temperatures with moderate humidity (40 °C/50%) (HT), to replicate the weather in Dubai.
c) Low temperatures with high humidity (5 °C/78%) (LT), to replicate the weather in London.

The tested surfaces were stored in a general-purpose lab oven and regular fridge for the HT and LT conditions, respectively (Figure 5.1 – Section 5.1). The temperature and humidity were recorded during the exposure period using an Oria digital thermometer hygrometer, indoor/outdoor humidity meter and temperature monitor (Calibrated against Oregon scientific THGR221) (Figure A5.1 - Appendix A5.1). As ovens are often dry and have very low humidity, plastic containers full of water were kept inside the oven and were refilled regularly to maintain a moderate humidity.

In addition to the mentioned surfaces, the same participant previously confirmed as a high shedder in Section 3.1 was instructed to perform the deposition process described in Section 2.2 on a 5 x 7 cm area of a banana skin (BS) (n= 10, 2 replicates per each time period). After the deposition, the surfaces were stored for the four periods only at RT (condition a) to evaluate whether Touch DNA can be collected from a rotten banana skin (Figure 5.4).

Figure 5.4: Banana skin surfaces (smooth porous) stored for four periods (3 h, 12 h, 24 h, and one week) at room temperature with moderate humidity (20–25 °C/50%) (RT).

Samples were collected using a Copan cotton swab (150C) moistened with molecular grade water using a spray bottle, as described in Section 2.3. Based on the findings from Section 5.1, the swabs were moistened with molecular grade water only when samples were collected at RT and HT, but not at LT because of the high humidity on the surfaces. Once the samples were collected, extraction from the full swab head was immediately carried out using PrepFiler Express BTA™ with AutoMate Express System, as described in Section 2.4. Based on the findings presented in Section 4.2, 460 µL of lysis buffer was used instead of 230 µL with the full swab.

Extracted samples were quantified using the Quantifiler™ Human DNA Quantification Kit following the procedures detailed in Section 2.5. Some samples collected from each condition were amplified and analysed (Section 2.6 and 2.7) to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed, as described in Section 2.8.

5.2.2 RESULTS

At RT , the amount of collected DNA from the selected surfaces (SS, TP, TW, and G) (n=96) was not affected over the one week period ($p > 0.05$). At HT and LT, the amount of collected DNA was significantly affected by the condition of the surfaces ($p < 0.05$), the interaction between each surface type and the conditions ($p < 0.05$), the interaction between the conditions and time ($p < 0.05$) as well as the interaction between all the variables (surface type, time, and environmental conditions) ($p < 0.05$) (Figure 5.5) (see Appendix A5.3 for the complete results).

Over the four periods of time (3 h, 12 h, 24 h, and one week), the amount of DNA recovered from each surface was stable at RT, but at HT and LT there was a slight change in the amount of DNA collected from the selected surfaces (Figure 5.6). This is more likely to be caused by the interaction between each surface type and the conditions they were exposed to.

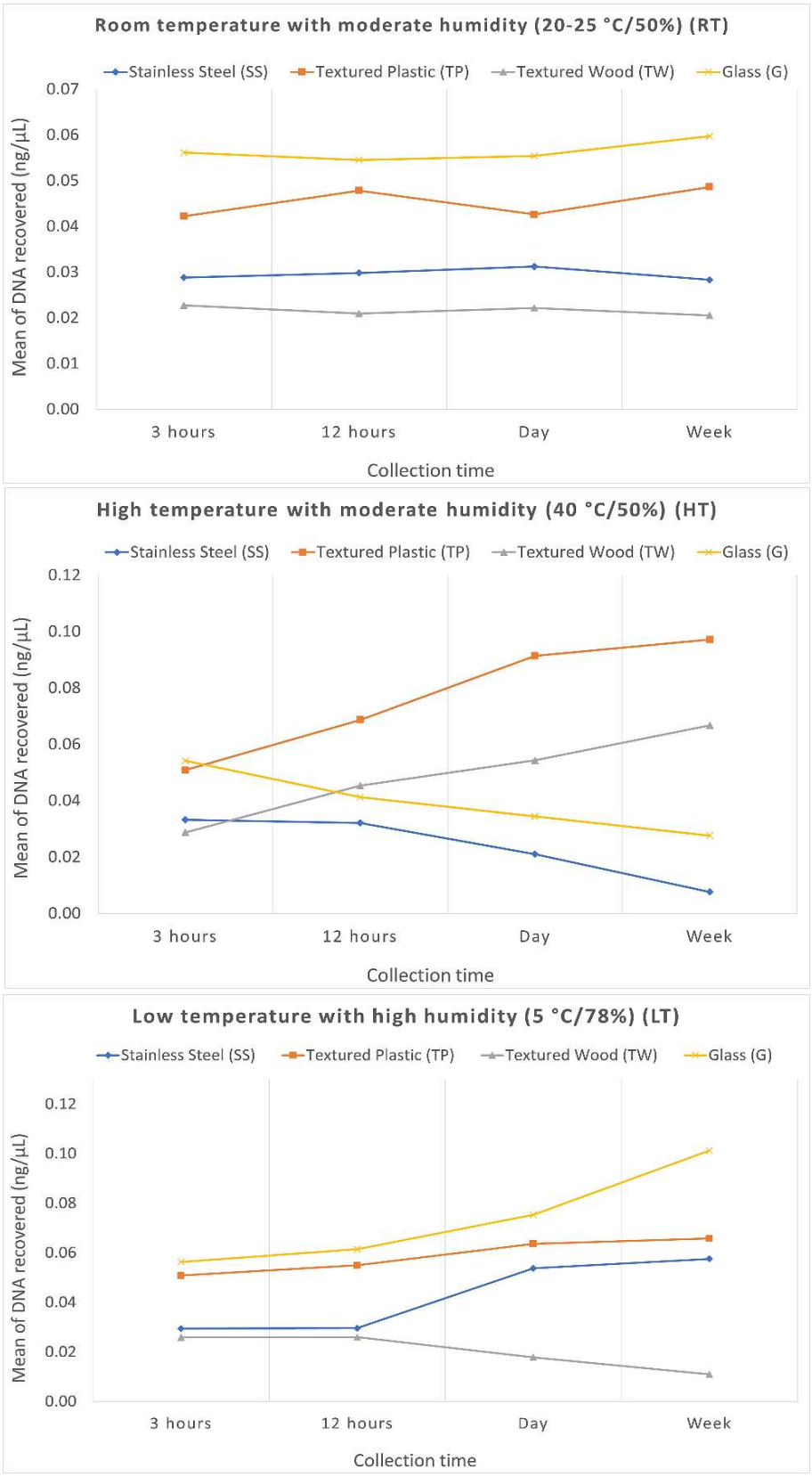


Figure 5.5: Amount of DNA collected (n= 96) from the selected surfaces (SS, TP, TW, and G) over four periods (3 h, 12 h, 24 h, and 168 h) at room temperature with moderate humidity (RT), high temperature with moderate humidity (HT), and low temperature with high humidity (LT).

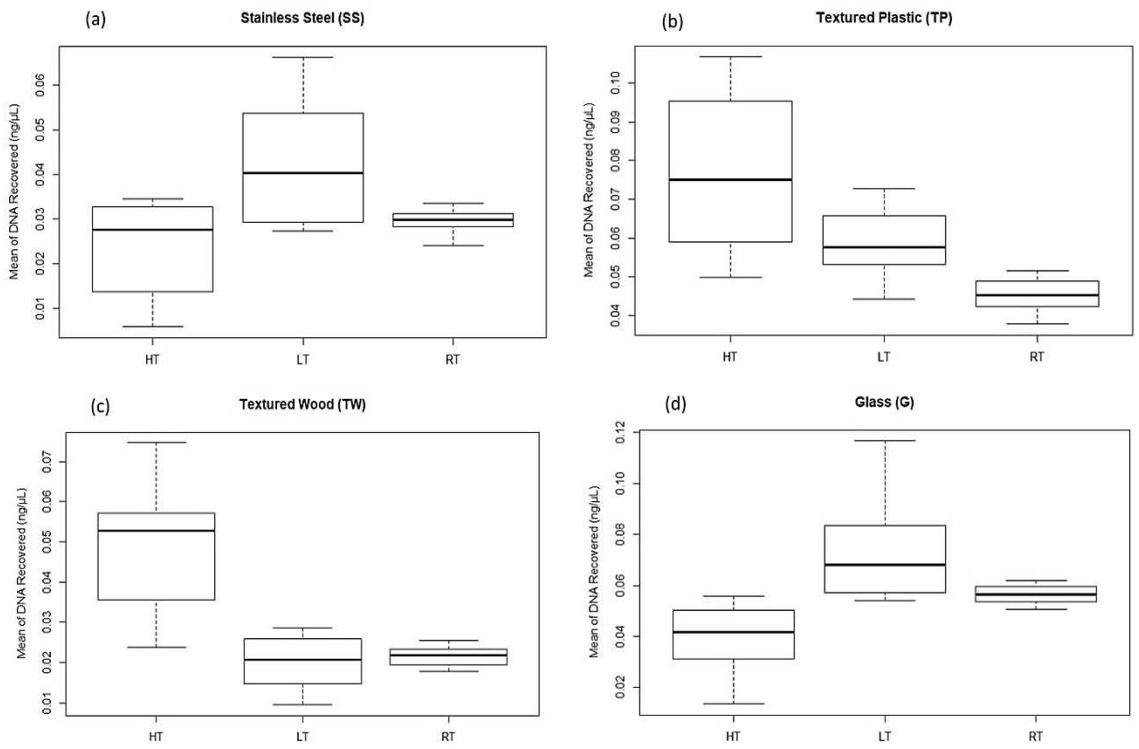


Figure 5.6: Mean of DNA recovered (n= 96) from stainless steel **(a)**, textured plastic **(b)**, textured wood **(c)** and glass **(d)** over four periods (3 h, 12 h, 24 h and 168 h) at room temperature with moderate humidity (RT), high temperature with moderate humidity (HT), and low temperature with high humidity (LT).

The results show that each surface behaved differently, with the mean of DNA collected from G and SS (smooth non-porous surfaces) decreasing over time in HT, and increasing in LT (Figure 5.6). High temperatures can increase the rate of hydrolytic cleavage, which can lead to direct damage of the DNA strands due to drying (Poinar, 2003), as most non-porous surfaces such as SS and G can absorb heat. Even though the quantity of the absorbance can be low, it is still more effective in comparison to porous surfaces. Moisture can increase the rate of sample degradation (Goray et al., 2010), but as reported in Section 5.1, moisture from humidity on the surfaces can enhance DNA transfer, similarly in this case, more DNA was recovered from G and SS (non-porous surfaces) in LT when surfaces were exposed to high humidity.

In contrast, the mean of DNA collected from TP (rough non-porous surface) increased in HT, and it was slightly stable in LT (Figure 5.6). Even though the surface is non-porous, it is rough, which can retain more DNA than smooth surfaces (Wickenheiser, 2002).

Moreover, DNA left on rough surfaces is less likely to be affected by heat, especially if the surfaces are porous.

Contrary to G and SS surfaces, the mean of DNA collected from TW (rough porous surface) increased in HT but decreased in LT (Figure 5.6). In this case, high humidity influenced the amount of DNA collected from the TW surface. It is a porous surface, therefore, the humidity was higher due to the water moving through the pores, which deteriorates the traces of DNA left on the object.

Most extracted samples when quantified had enough DNA to be amplified, but only samples collected from SS (Figure 5.7), TP (Figure 5.8), and TW (Figure 5.9) that were stored under the three conditions (RT, HT, and LT) for a week were amplified to validate the quality of the samples collected. All samples produced full profiles, even those that were as low as 9.3 pg, but there was some variation in the peak height in the sample profiles. Samples with higher DNA content produced a profile with higher peaks, which was influenced by the surface conditions.

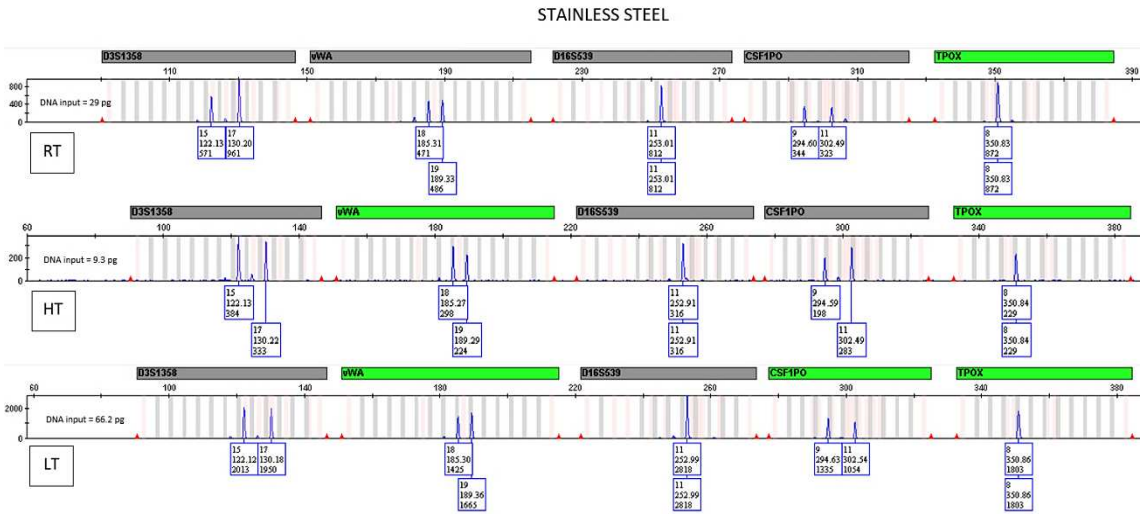


Figure 5.7: Electropherograms of samples collected from stainless steel surface stored at room temperature with moderate humidity (RT), high temperature with moderate humidity (HT), and low temperature with high humidity (LT) over a week. The profiles show the difference in peak height at 5 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX). Maximum volume of DNA was added.

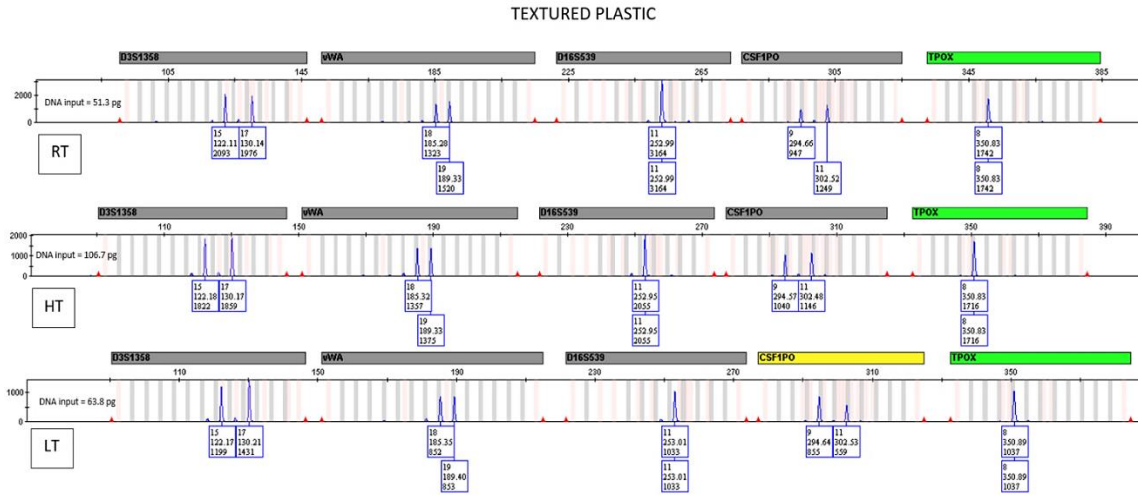


Figure 5.8: Electropherograms of samples collected from textured plastic surface stored at room temperature with moderate humidity (RT), high temperature with moderate humidity (HT), and low temperature with high humidity (LT) over a week. The profiles show the difference in peak height at 5 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX). Maximum volume of DNA was added.

Figure 5.9: Electropherograms of samples collected from textured wood surface stored at room temperature with moderate humidity (RT), high temperature with moderate humidity (HT), and low temperature with high humidity (LT) over a week. The profiles show the difference in peak height at 5 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX). Maximum volume of DNA was added.

Including the results from the previous surfaces, the amount of DNA recovered from BS was not affected during the one-week period at RT ($p > 0.05$). The amount of collected DNA was consistent (mean 0.3 ng/µL) (Figure 5.10), and a full DNA profile was generated even when the banana skin was rotten after a week (Figure 5.11).

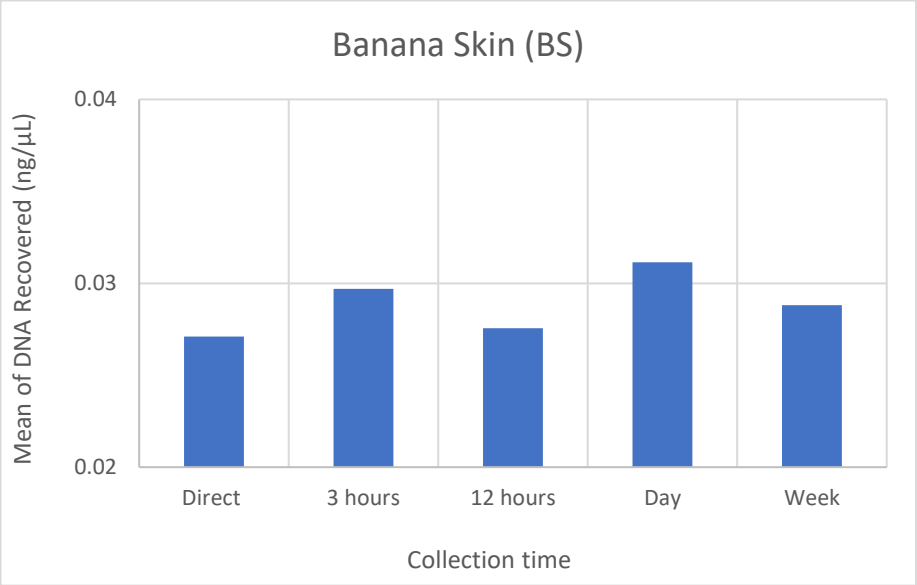


Figure 5.10: Amount of DNA collected (n= 10) from banana skin over 3 h, 12 h, 24 h and 168 h at room temperature with moderate humidity (RT).

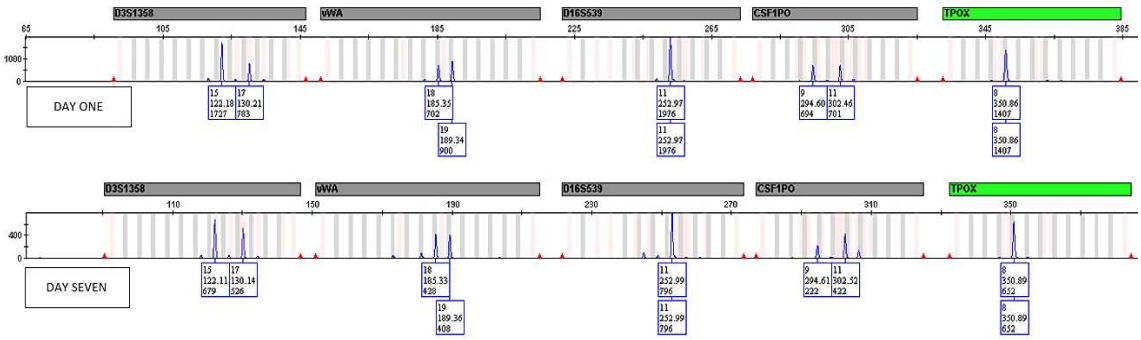


Figure 5.11: Electropherograms of samples collected from the surface of normal banana skin (day one) and rotten banana skin (day seven), stored at room temperature with moderate humidity (RT). The profiles show the difference in peak height at 5 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX). Maximum volume of DNA was added.

5.3 INFLUENCE OF SANDY SURFACES

In addition to heat and humidity (Alketbi & Goodwin, 2019b), other environmental factors that affect Touch DNA collection from outdoor items is sand or dust, particularly in dry hot climates such as Dubai where sand moves in the air all the time because of the wind. In this regard, there is a lack of research, therefore, this experiment tested how sandy surfaces can affect the recovery of Touch DNA by examining the most appropriate collection and extraction techniques.

5.3.1 EXPERIMENTAL SETUP

Four surfaces were selected to replicate common items encountered in outdoor crime scenes and have a variety of smooth, rough, porous and non-porous surfaces. These surfaces included stainless steel (SS), glass (G), textured plastic (TP), textured wood (TW) (previously tested in Section 4.1). All surfaces were sterilised using 2% Virkon and exposed to UV-irradiation for 15 min. A participant, previously confirmed as a high shedder in Section 3.1, was instructed to perform the deposition process described in Section 2.2 on a 5 x 7 cm area of the selected surfaces (n= 48, 3 replicates per each variable; collection and extraction methods).

After the DNA deposition, sand from Dubai (common sand found outdoors) was dusted on the surfaces, which were then stored in a general-purpose lab oven at high temperature with moderate humidity (40 °C, 50%) (HT) (Figure 5.1 – Section 5.1) for three hours to replicate Dubai weather during midday.

Samples were collected using a Copan cotton swab (150C) (CS), and Copan nylon flocked swab (4N6FLOQSwabs®) (NS) following the same collection procedure detailed in Section 2.3. After collection, samples were extracted immediately with the PrepFiler Express BTA™ kit using an AutoMate Express Forensic DNA Extraction System (EXT1) and manually using the QIAamp® DNA Investigator Kit (EXT2) following the procedures outlined in Section 2.4. However, based on the finding from Section 4.2, for EXT1, 460 µL of lysis buffer was used instead of 230 µL.

Extracted samples were quantified using the Quantifiler™ Human DNA Quantification Kit following the procedures outlined in Section 2.5. Some samples were then amplified and analysed, as described in Section 2.6 and 2.7 to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed following the procedures described in Section 2.8.

5.3.2 RESULTS

The amount of DNA recovered from the sandy surfaces was significantly impacted by the collection method ($p < 0.05$), extraction type ($p < 0.05$), as well as the interaction between collection and extraction types ($p < 0.05$) (Figure 5.12) (see Appendix A5.4 for the complete results).

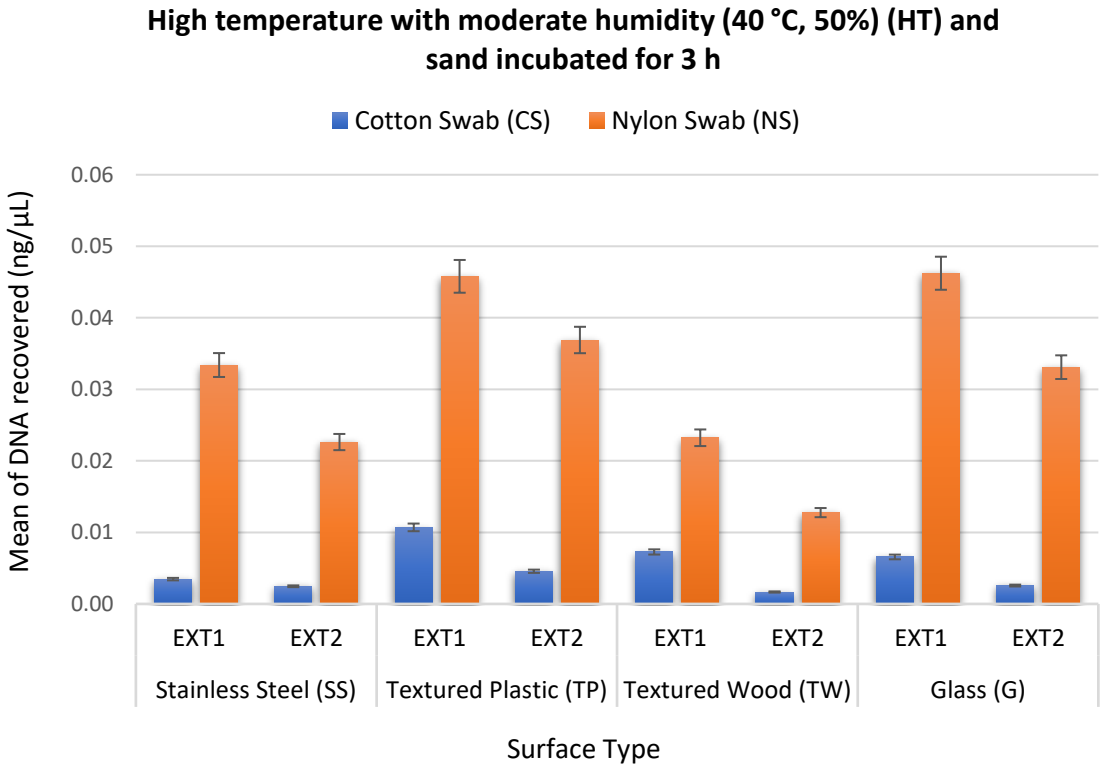


Figure 5.12: DNA concentration of samples recovered from four sandy surfaces (n= 48 - three replicates were used to make each bar) using cotton (CS) and nylon (NS) swabs, extracted using a PrepFiler Express BTA™ kit (EXT1) and QIAamp® DNA Investigator Kit (EXT2). Error bars represent 95% confidence intervals.

Collected samples yielded more DNA when extracted by EXT1 than EXT2, when the swabs used for recovery contained sand. PrepFiler Express BTA™ kit was developed to enhance the yield and purity of DNA prepared from forensic samples by removing any inhibitors present (Applied Biosystems 2010; Rubinstein, 2020), and it was effective in cleaning the collected samples from sand.

Nevertheless, NS performed best for collecting Touch DNA from sandy surfaces, when compared to CS (Figure 5.13). CS retained more sand than the NS owing to the amount of molecular grade water used to moisten the swabs (100 µL with CS and 30 µL with NS), or because of the nature of the swab fabric. It has been reported that nylon flocked swabs can enhance extraction efficiency, because the swab is designed to rapidly absorb and release biological materials more effectively when compared to traditional fibre swabs (Verdon *et al.,* 2014a; Frippiat & Noel, 2016).

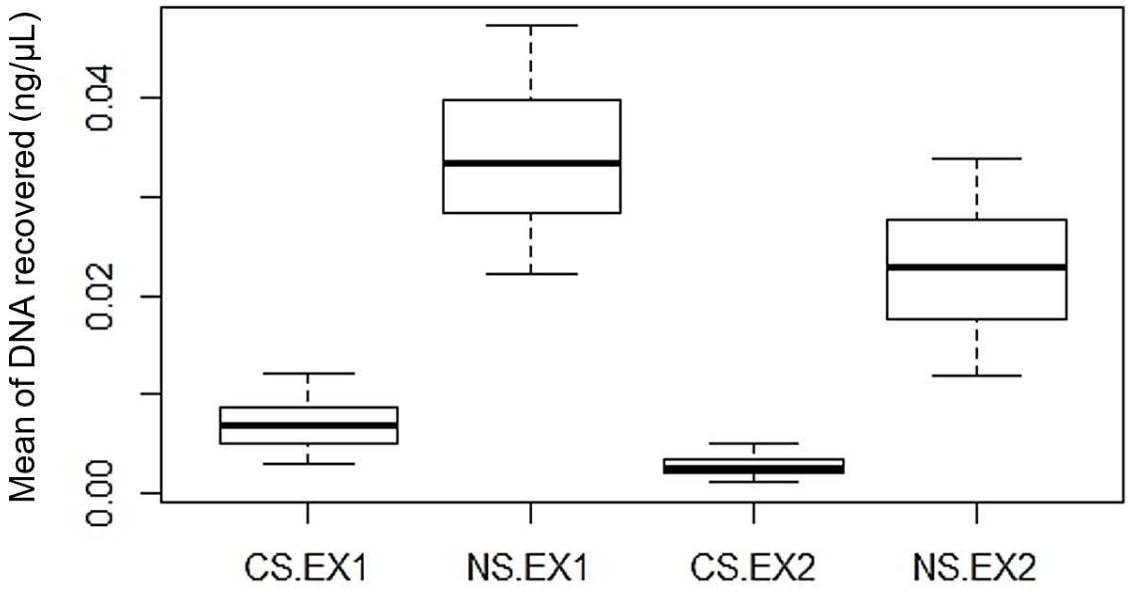


Figure 5.13: Mean of DNA recovered from the sandy surfaces (n= 48) using cotton (CS) and nylon (NS) swabs, then extracted using a PrepFiler Express BTA™ kit (EX1) and QIAamp® DNA Investigator Kit (EX2).

Samples recovered from SS were amplified to validate the quality of the samples collected. DNA recovered by NS produced full profiles, and samples collected by CS produced partial DNA profiles with few allele dropouts (Figure 5.14).

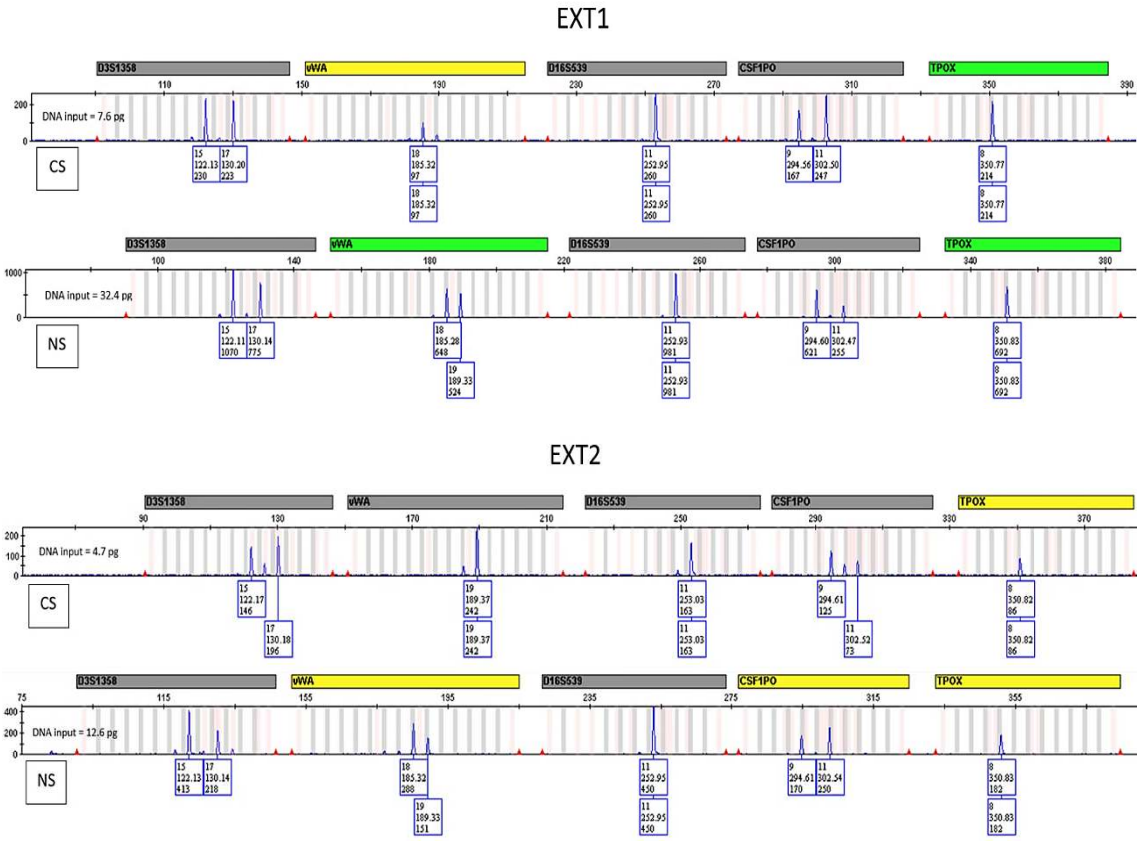


Figure 5.14: Electropherograms of samples collected from sandy stainless steel surface by cotton (CS) and nylon (NS) swabs, while extracted using a PrepFiler Express BTA™ kit (EXT1) and QIAamp® DNA Investigator Kit (EXT2). The profiles show the difference in peak height at 5 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX). Maximum volume of DNA was added.

5.4 CHAPTER DISCUSSION AND CONCLUSION

There is limited systematic investigation on the impact of time since deposition on trace DNA degradation, especially in either casework-like touch deposit samples or over a significant period (Burrill *et al.,* 2019). Usually, more DNA is retrieved from freshly touched surfaces when compared to surfaces that have been stored over a long period (Frégeau *et al.,* 2010), however, that is highly dependent on the conditions the touched object is exposed to (Raymond *et al.,* 2009a). Time itself does not impact Touch DNA on the surfaces at room temperature (Breathnach *et al.,* 2016), but the influence of other environmental conditions such as low/high temperature or low/high humidity can affect the persistence of DNA (Alketbi & Goodwin, 2019b). Longer time since deposition and recovery could lower the DNA yield, but low temperatures can help preserve the biological samples. In addition, DNA sample contamination is common when the sample has been left for a long period of time. Therefore it is important to recover biological materials as soon after deposition as possible in order to obtain higher DNA yields and to avoid cross-contamination, particularly if items containing Touch DNA are found outdoors.

Exposure to humidity can reduce traces of biological material in samples (Raymond *et al.,* 2008), however it can also improve DNA transfer and recovery (Goray *et al.,* 2010; Alketbi & Goodwin, 2021). Therefore, it is recommended to use dry cotton or nylon swabs to collect biological materials from outdoor humid non-porous surfaces such as glass for a better DNA recovery. In contrast, porous surfaces such as wood found outdoor are impacted by high humidity. Hence, trace DNA can be decreased or washed away because of the ability of porous surfaces to absorb moisture. Thus, it is a better practice to collect DNA samples as soon as possible from porous surfaces exposed to outdoor conditions with high humidity or transfer the items containing the touch deposit to a dry area for storage until collection.

Similarly, high temperatures from the sun can lead to a decrease in DNA quantities because some non-porous surfaces can interact with the sun, and get heated at different rates such as stainless steel and glass. The examination of vehicles is common at outdoor crime scenes where Touch DNA is often collected from the outer surface such as door

handles, which are often made of metal. Thus, it is recommended to move vehicles to a shaded controlled environment to help preserve the DNA.

Sand or dust on objects found outdoors can impact the recovery of Touch DNA (Alketbi & Goodwin, 2019c), which is a common in hot dry climates such as Dubai. The use of nylon flocked swabs such as Copan 4N6FLOQSwabs® in combination with the PrepFiler Express BTA™ extraction kit can help improve the yield and purity of DNA. However, it is the recommended to use of 460 µL of lysis buffer instead of 230 µL with the full swab (Joël *et al.,* 2015; Alketbi & Goodwin, 2019a).

CHAPTER SIX

6. SIMULATING SEXUAL HARASSMENT CASEWORK SCENARIOS

This chapter examined the impact of collection techniques and time on Touch DNA recovered from human skin. Three recovery techniques were tested with two collection methods to evaluate their efficacy on recovering the deposited DNA from the neck following a strangulation scenario. Time and its effects were investigated by collecting DNA deposits over a period of two days. As well as human activity such as washing the area of the skin where the DNA is deposited. Finally, the effect of collection type and time were investigated by collecting biological materials from human hands after deposition.

This chapter also looked into the recovery of Touch DNA from fabric. This was done by investigating variables previously tested such as extraction and collection types on how they influence trace DNA recovery. Fabric type and area size were examined by investigating two commonly used fabric types and by collection of DNA from two different area sizes. Finally, deposition area and time were investigated by collecting deposited DNA from t-shirts and trousers, over a 24 h period.

6.1 TOUCH DNA RECOVERY FROM HUMAN SKIN

In scenarios of violent crimes like assault, sexual offences, or even homicide, usually Touch DNA is recovered from the skin of the victim and sometimes from the offender hands if caught within a short time frame from committing the crime. However, there is limited shared data on trace DNA recovery from human skin (de Bruin *et al.,* 2011; Bowman *et al.,* 2018; Kallupurackal *et al.,* 2021), which is only collected with cotton swabs using double swabbing technique (Sweet *et al.,* 1996; Pang & Cheung, 2007). Recovering Touch DNA evidence from contacted areas, not only from frequently handled objects or worn clothes, but also from the skin surface of victims or corpses, can provide valuable information to the investigation of criminal cases. Therefore, there is an urgent need for more effective ways to recover Touch DNA from human skin.

6.1.1 INFLUENCE OF COLLECTION TECHNIQUES ON TOUCH DNA COLLECTED FROM NECK

During manual strangulation, there is an intense physical contact between the perpetrator and the victim, thus the biological materials from the hand of the offender are deposited on the neck of the victim. However, the recovery of Touch DNA from the skin of the victim can be complex because of the mixture of DNA present containing a small amount of the DNA of the offender compared to that of the victim. Touch DNA transfer on human skin has been widely examined (Wiegand & Kleiber, 1997; Rutty, 2002; Graham & Rutty, 2008; Bowman *et al.,* 2018), but only few studies have investigated the performance of different collection methods from human skin (de Bruin *et al.,* 2011; Kallupurackal *et al.,* 2021). Examining different collection techniques with commonly used methods can improve Touch DNA sampling. Therefore, the aim of this study was to evaluate three collection techniques involving cotton and nylon swabs to test their efficiency for the collection of Touch DNA from the human neck following a strangulation scenario.

6.1.1.1 EXPERIMENTAL SETUP

In this scenario, the DNA was deposited by hands to the neck, two male donor participants, previously confirmed as a high and low shedders in Section 3.1, were requested to wash their hands with antibacterial soap (LabGUARD) and abstain from any activity related to using their hands for 10 min. The neck skin of a male and female receiver participants was disinfected using alcohol wipes (70% isopropyl alcohol), then cleaned with water based moist wipes and air-dried for 10 min. Prior disinfecting the surfaces, measurements of the hands and necks from the participants were taken for accurate sampling (Figure 6.1). Next, a male donor (perpetrator) was instructed to hold the neck of the female receiver (victim) (male vs. female) as described in Figure 6.2. After deposition, the receiver neck was marked in three equal sections (11 cm x 10 cm) with a temporary marker pen for the recovery of Touch DNA via three collection techniques. Similar DNA deposition process was repeated for the next donor and receiver (male vs. male). It is important to note that the deposition and DNA recovery process was conducted at room temperature to avert any environmental factors related to low/high

temperatures that can influence the skin such as sweating. Furthermore, three collection techniques were used to collect the randomly deposited DNA form the marked three sections (11 cm x 10 cm) to avoid using the same collection technique for the same area. This was done to have a more efficient sampling average for each technique used, as DNA quantities may shed differently from the hand of the donor during the physical contact in the strangulation process.

After each deposition (n= 48, 8 replicates per each collection technique), Touch DNA was recovered immediately with a Copan cotton swab (150C) (CS) and Copan nylon flocked swab (4N6 FLOQSwabs®) (NS) (Figure 2.2 – Section 2.3) using three recovery techniques as prescribed in Figure 6.2. Following DNA recovery, the swabs were extracted immediately manually using the QIAamp® DNA Investigator Kit following the procedures in Section 2.4.

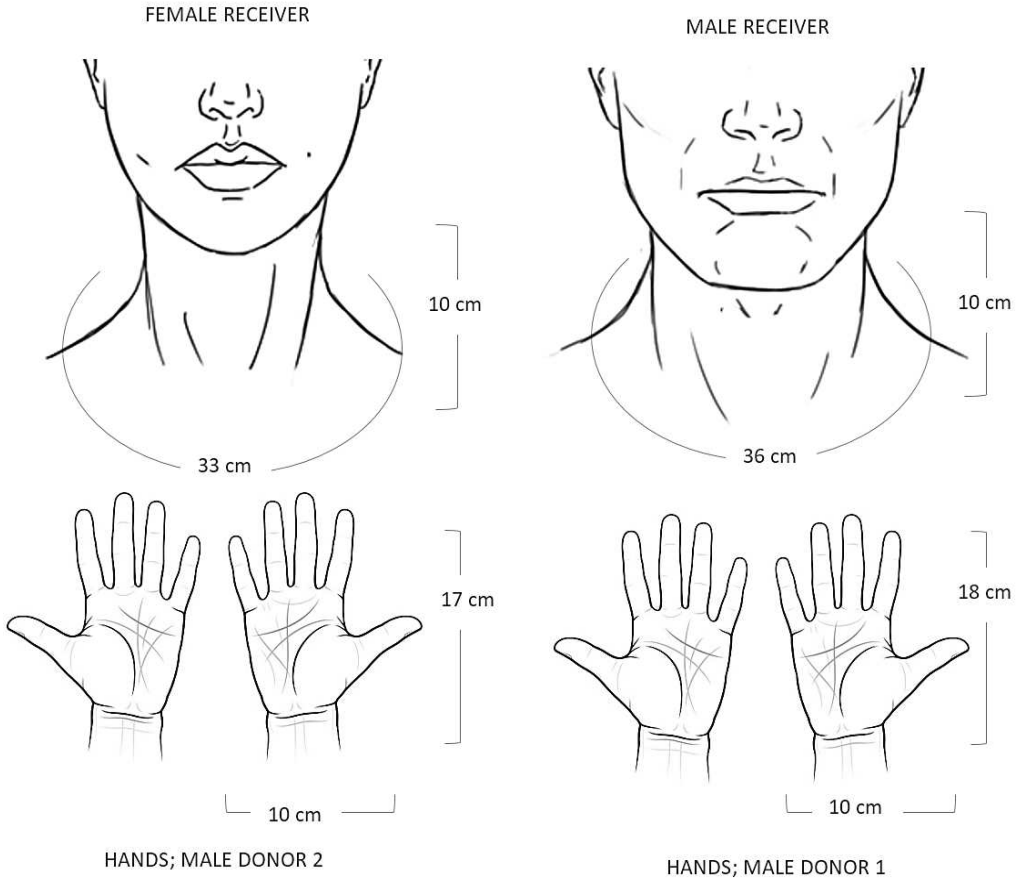


Figure 6.1: Measurements of the hands and necks of the participants involved in the strangulation scenario, male donor vs. female receiver and male donor vs. male receiver. The hands measurement of the donors is very similar, while the neck measurement of male receiver was 3 cm more in circumference than that of the female receiver.

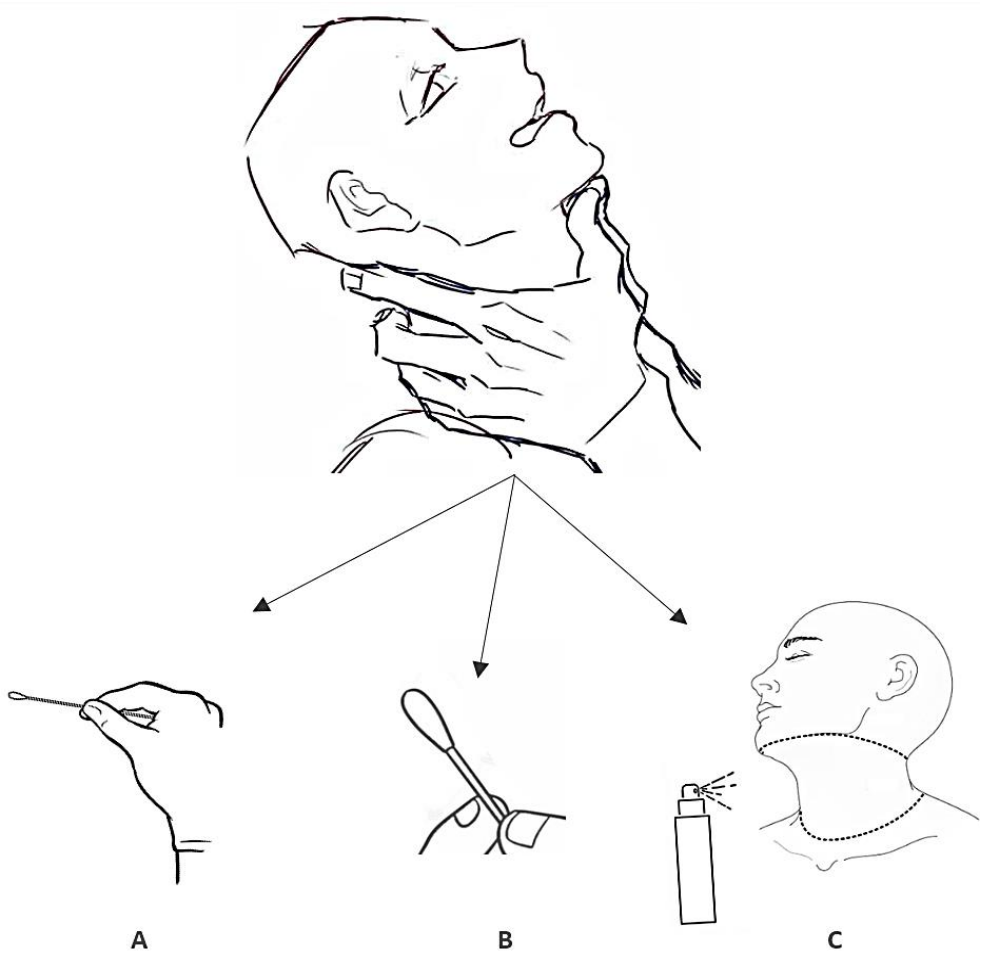


Figure 6.2: DNA deposition process was carried out to replicate a strangulation scenario, by holding the neck semi tight for 2 min while rubbing the hands over the skin (no pressure was applied to sensitive areas of the neck such as throat and areas with major blood vessels). DNA recovery was done using Copan cotton swab (150C) (CS) and Copan nylon flocked swab (4N6FLOQSwabs®) (NS), via three techniques **(a)** moistening the swab with 100 µL of molecular grade water using a spray bottle for CS and moistening the swab with 30 µL of molecular grade water using a pipette for NS as prescribed in Section 2.3 **(b)** dry swab and **(c)** moistening the neck with 100 µL of molecular grade water using a spray bottle before collection with dry swabs.

The extracted samples were quantified using the Quantifiler™ Trio DNA Quantification Kit following the procedures in Section 2.5. All the samples were then amplified and analysed as described in Section 2.6 and 2.7 to evaluate the quality of the samples collected. Control samples from the hands of the donors and the neck of the receivers were collected after each sterilisation, which produced full single DNA profiles related to the participants without any sign of mixtures or contamination. Statistical analysis was performed following the procedures mentioned in Section 2.8.

6.1.1.2 RESULTS

There was a significant difference between the three recovery techniques used to collect Touch DNA with CS ($p < 0.05$) and NS ($p < 0.05$). For the CS, moistening the neck prior collection (c) collected more DNA than moistening the swab first (a) or using a dry swab (b) ($p < 0.05$) (mean a. 0.25, b. 0.37, c. 0.59 all in ng/µL; Figure 6.3) (see Appendix A6.1 for the complete results).

By contrast, for the NS, using a dry swab (b) collected more DNA than moistening the swab first (a) or moistening the neck prior collection (c) ($p < 0.05$) (mean a. 0.62, b. 1.02, c. 0.54 all in ng/µL; Figure 6.3) (see Appendix A6.1 for the complete results), which can be related to the neck skin being naturally moist and a nylon swab usually is more sensitive to moist or humid surfaces than a cotton swab as discussed in Section 4.4 and Section 5.1. Nylon swabs recovered more DNA quantities from the neck skin than cotton swabs ($p < 0.05$), which may be explained by the nature of the nylon fibres. The NS is much rougher on the skin surface compared to the CS, therefore it may collect more biological materials from the skin of the victim (Squassina *et al.,* 2014).

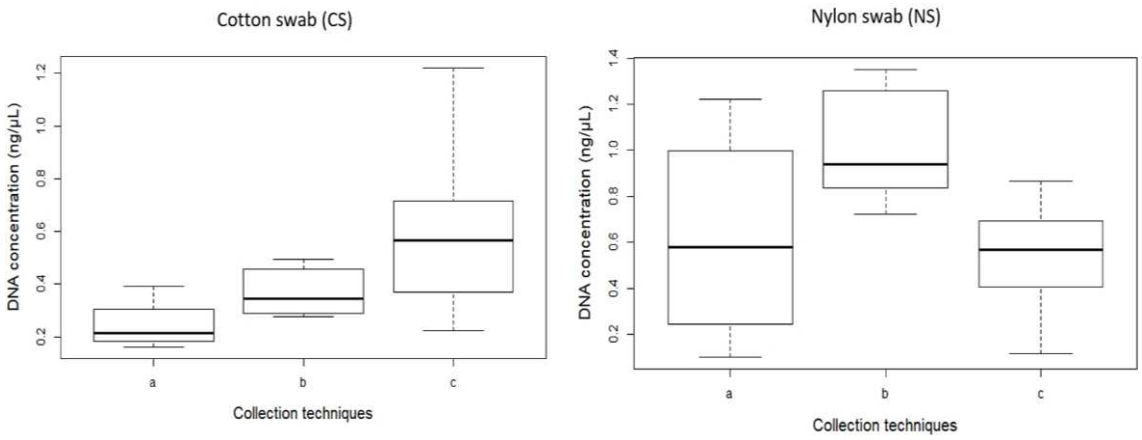


Figure 6.3: Mean of DNA recovered (n= 48) from neck skin by the three techniques using a cotton swab (CS) and nylon swab (NS): **(a)** moistening the swab with 100 µL of molecular grade water using a spray bottle for CS and moistening the swab with 30 µL of molecular grade water using a pipette for NS; **(b)** dry swab; and **(c)** moistening the neck with 100 µL of molecular grade water using a spray bottle before collection using dry swabs.

The DNA recovered from the skin of the victim after physical contact usually contains a mixture of profiles comprising alleles from the DNA of the victim and that of the perpetrator, hence the quantitates of DNA recovered does not necessarily lead to more alleles being recovered. All the collected samples generated mixed DNA profiles (see Appendix A6.2 for the complete results), but the number of alleles observed was not consistent among the three used techniques ($p < 0.05$). The average number of alleles observed was more consistent when the skin was moistened before collection (c) for both swabs (CS avg. alleles recovery a. 81%, b. 87%, and c. 94% vs. NS avg. alleles recovery a. 87%, b. 88%, and c. 96%; Figure 6.4).

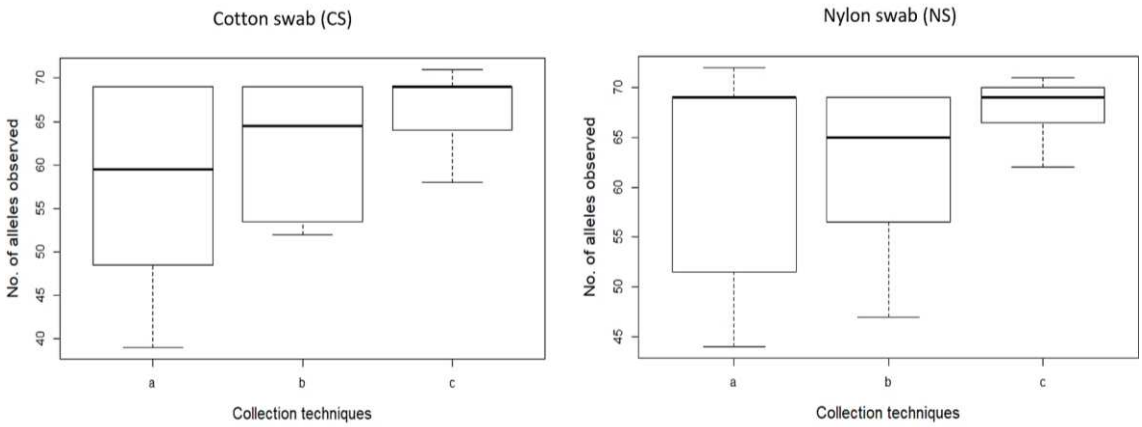


Figure 6.4: Number of alleles observed (n= 48) for each technique using a cotton swab (CS) and nylon swab (NS): **(a)** moistening the swab with 100 µL of molecular grade water using a spray bottle for CS and moistening the swab with 30 µL of molecular grade water using a pipette for NS; **(b)** dry swab; and **(c)** moistening the neck with 100 µL of molecular grade water using a spray bottle before collection using dry swabs. Homozygous loci were counted as one allele, while heterozygous loci were counted as two alleles.

Similarly, the mixture ratio among the minor (perpetrator) and major (victim) contributors in the mixture DNA profiles was easily distinguished in the profiles collected using technique (c) for both swabs ($p < 0.05$), when compared to the other two techniques (Figure 6.5). Usually, a 1:1 mixture ratio between the minor and major contributors in a DNA profile is hard to interpret, and the increase in the mixture ratio leads to a better evaluation, which helps to distinguish between the minor and major contributor (e.g. Figure 6.6).

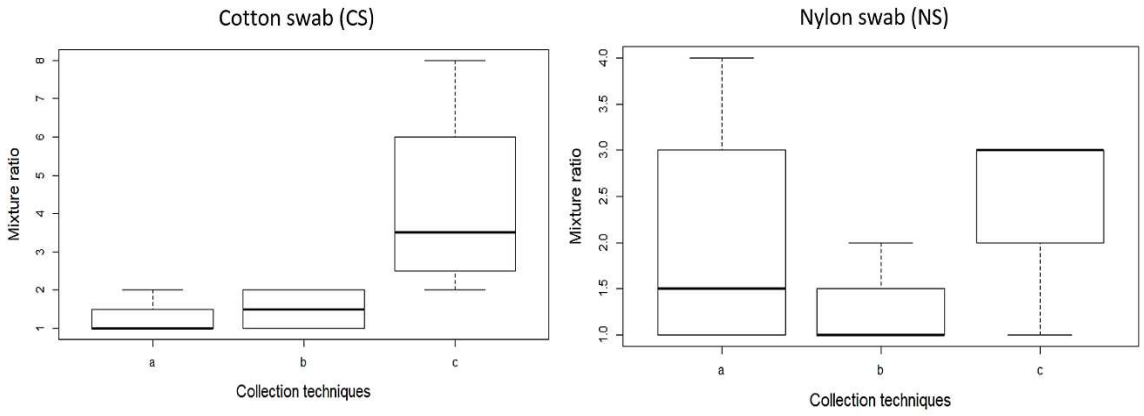


Figure 6.5: Mean of the mixture ratio between the minor and major contributors (n= 48) in the DNA profiles collected by each technique using a cotton swab (CS) and nylon swab (NS): **(a)** moistening the swab with 100 µL of molecular grade water using a spray bottle for CS and moistening the swab with 30 µL of molecular grade water using a pipette for NS; **(b)** dry swab; and **(c)** moistening the neck with 100 µL of molecular grade water using a spray bottle before collection using dry swabs.

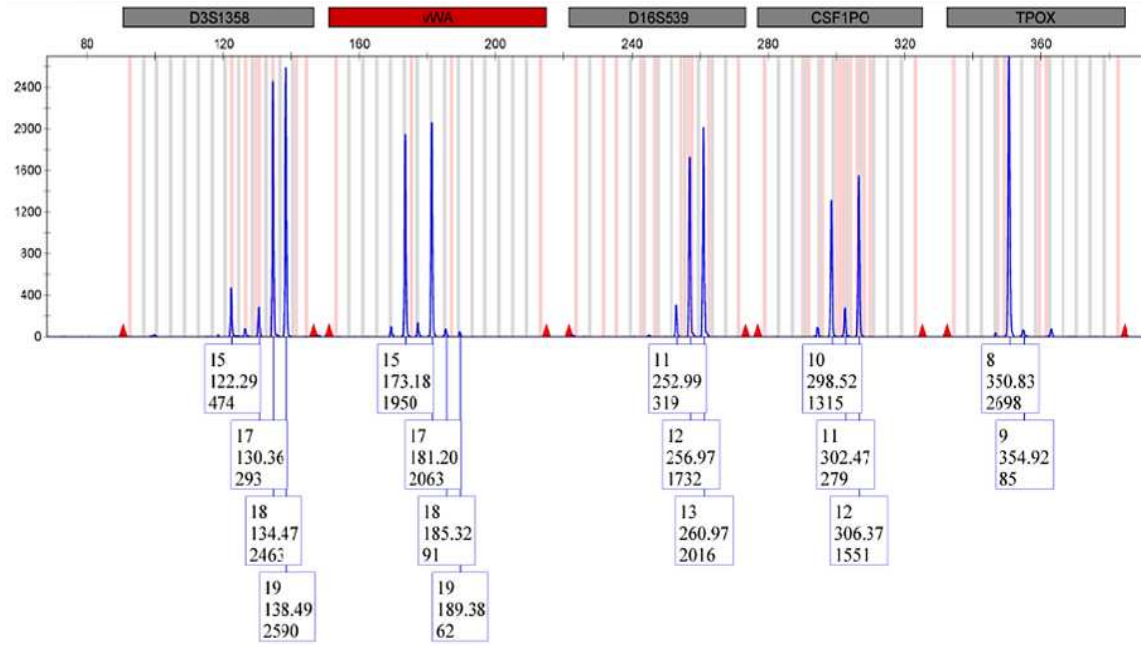


Figure 6.6: An electropherogram of the sample collected from the neck of the female (victim) by technique (c) with a cotton swab. The DNA profile shows the mixture ratio (1 in 8) of the minor (perpetrator) and major (victim) contributors, with alleles observed at five autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, and TPOX).

Furthermore, by looking the at Peak height (RFU) of the DNA profiles, it was noted that there is correlation with the number of alleles observed ($p < 0.05$). The mean of RFU increased with less alleles observed and decreased with more alleles observed (Figure 6.7). This could be explained by the difference in peak height between minor and major contributors, which lowers the mean of the RFU in the mixture DNA profile. Peak height ratio (PHR%) was balanced in all the amplified samples in the minor and major contributing alleles with an average above 70%.

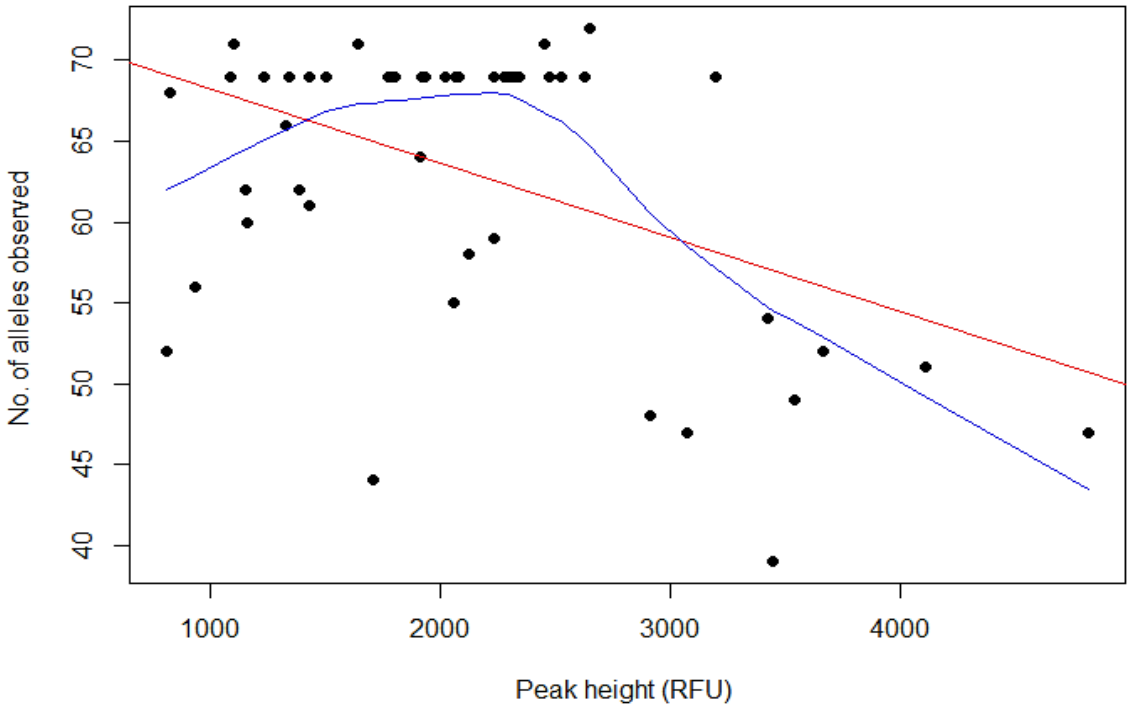


Figure 6.7: Relationship between the number of alleles observed and peak height (RFU) in all the generated mixture DNA profiles (n= 48). The average of RFU increased when there were less alleles, and decreased when there were more alleles. The red and blue lines represent the regression (y~x: line of best fit), and lowess (x,y: line of the relationship between variables) lines, respectively.

6.1.2 INFLUENCE OF TIME ON TOUCH DNA COLLECTED FROM NECK

Once DNA is deposited on human skin, the frequent question is how long it would last, but that is depended on the factors that affect its persistence. Some of these factors include time between deposition and collection, exposure to contamination, and human intervention such as washing. Previous studies observed offender alleles up to 48 h (Wiegand and Kleiber, 1997), and partial offender profiles up to 10 d after contact with the skin of the victim. However, shedding status of offender and nature of contact can also play a role on the persistence of the DNA deposited on the skin. Furthermore, these studies used profiling technologies that are less sensitive than those currently in use. Therefore, the aim of this study was not only to investigate the effect of time between deposition and recovery of Touch DNA from human skin following a strangulation scenario, but also the impact of washing on the DNA deposited.

6.1.2.1 EXPERIMENTAL SETUP

In this scenario, the DNA was deposited by hands to the neck, in which a donor participant (perpetrator) previously confirmed as a high shedder in Section 3.1 was requested to wash both hands with antibacterial soap (LabGUARD) and abstain from any activity related to using hands for 10 min. The neck skin of the receiver participant (victim) was disinfected using alcohol wipes (70% isopropyl alcohol), then cleaned with water based moist wipes and air-dried for 10 min. DNA deposition was done to replicate a strangulation scenario, by holding the neck semi tight for 2 min while rubbing the hands over the skin (Figure 6.2 – Section 6.1.1) (n= 28, 4 replicates per each time period). The deposited DNA was left on the skin of the receiver over different periods of time (1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 48 hours, and 72 hours). The receiver participant was instructed to continue his normal activity without washing the neck.

Touch DNA was recovered with a Copan cotton swab (150C) following the technique of moistening the neck with 100 µL of molecular grade water using a spray bottle technique before collecting the biological materials with dry swab (technique (c) in Figure 6.2 – Section 6.1.1). The DNA recovery was performed from the whole neck, and after each collection the same process of sterilising and DNA deposition was repeated.

Also, some samples were collected over the six hours period while the participant was instructed to shower within the three hours period after deposition.

Following DNA recovery, the swabs were extracted immediately manually using the QIAamp® DNA Investigator Kit following the procedures in Section 2.4. Extracted samples were quantified using the Quantifiler™ Trio DNA Quantification Kit following the procedures in Section 2.5.

All the samples were then amplified and analysed as described in Section 2.6 and 2.7 to evaluate the quality of the samples collected. Control samples from the hands of the donor and neck of the receiver were collected after each sterilisation process, all of which produced full single DNA profiles related to the participants without any sign of mixtures or contamination. Statistical analysis was performed following the procedures described in Section 2.8.

6.1.2.2 RESULTS

The amount of collected DNA increased over time ($p < 0.05$), which may be the result of the receiver participant (victim) not washing the neck (Figure 6.8) (see Appendix A6.3 for the complete results).

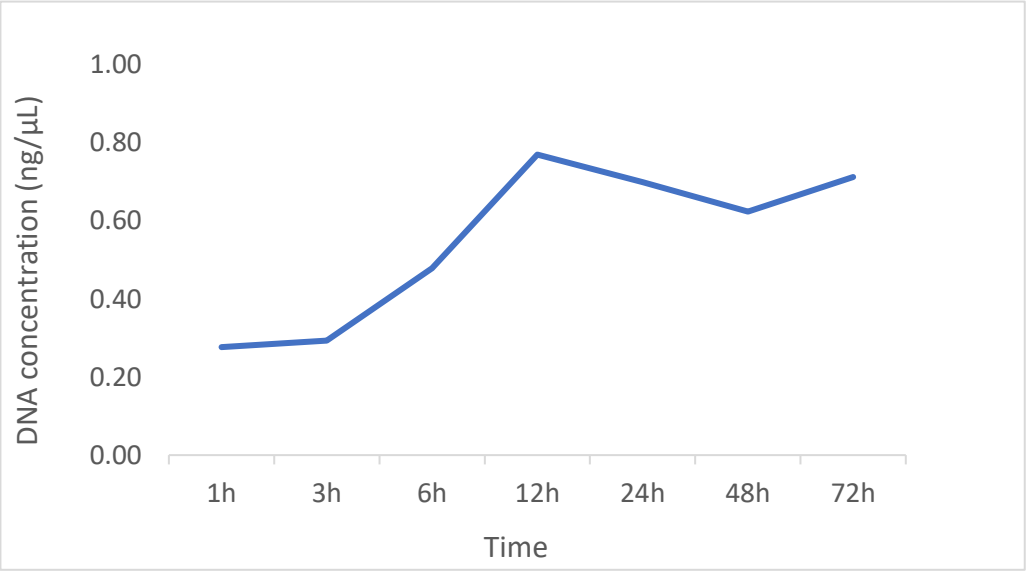


Figure 6.8: Mean DNA recovered (n= 28) from neck skin using cotton swabs over different period of time after deposition (1 h, 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h).

The number of alleles observed in the mixture DNA profiles was affected by 72 h (p < 0.05). When the receiver participant (victim) did not wash the neck, full mixture DNA profiles were obtained at 48 h, but after 72 h, no alleles remained of the donor (perpetrator). Furthermore, mixture ratio increased between the minor (perpetrator) and major (victim) contributor over time (p < 0.001) (Figure 6.9). The participant has been active during the period of the study, which might lead to donor DNA being washed away from the receiver neck through the mechanism of sweating.

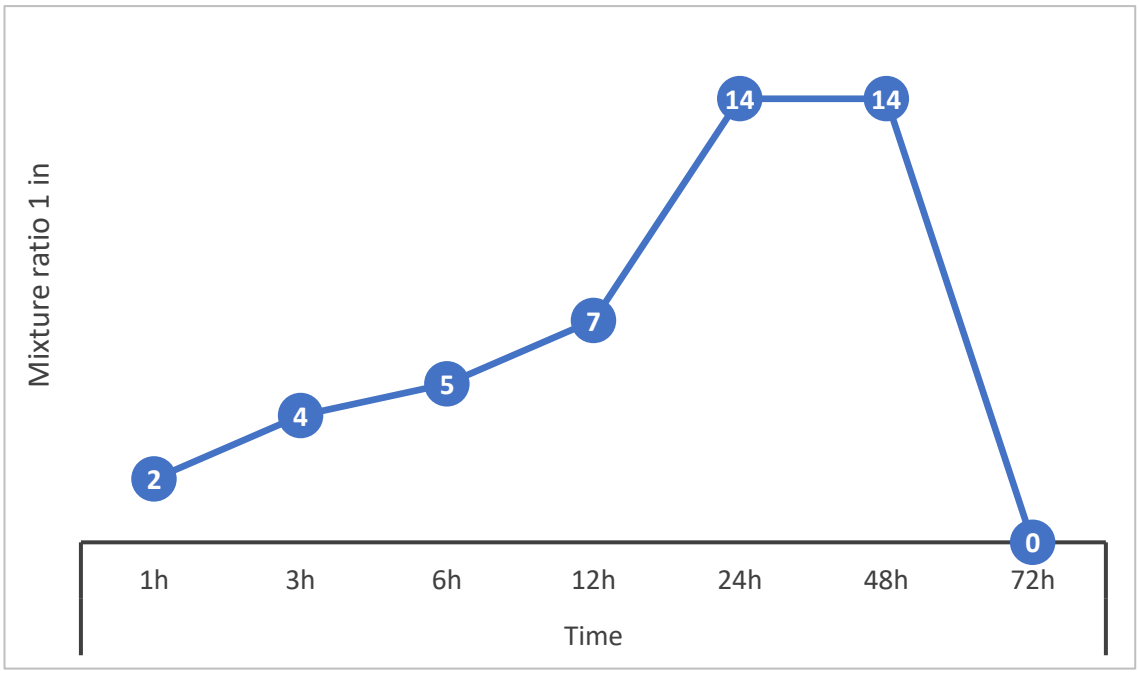


Figure 6.9: Mean of the mixture ratio between the minor and major contributors (n= 28) over different period after deposition (1 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h). The participant instructed to continue his normal activity without washing his neck.

In contrast, when the receiver participant (victim) did wash the neck within the six hours period after deposition (n= 12), there were no alleles observed of the donor's DNA (perpetrator) in the samples collected after 6 h (p < 0.05). Therefore, activities such as showering, or sweating could affect the presentence of Touch DNA deposited on the neck area.

6.1.3 INFLUENCE OF COLLECTION METHOD AND TIME ON TOUCH DNA COLLECTED FROM HANDS

After an assault, when the hands of the offender touch the skin of the victim, the DNA is not only transferred from the perpetrator to victim but vice versa (Wiegand & Kleiber, 1997; Rutty, 2002). In such cases, often forensic examiners collect DNA from the hand of the offender. However, the time between committing the crime and DNA collection can influence the quality of the DNA collected, as the biological materials of the victim deposited on the hands of the offender may deteriorate quickly. This study aimed to examine the influence of collection type and time of collection following a strangulation scenario.

6.1.3.1 EXPERIMENTAL SETUP

In this scenario, the DNA was deposited on hands from the neck following the strangulation scenario. A receiver participant (perpetrator) previously confirmed as a low shedder in Section 3.1 was requested to wash both hands with antibacterial soap (LabGUARD) and abstain from any activity related to using hands for 10 min. The neck skin of the donor participant (victim) was disinfected using alcohol wipes (70% isopropyl alcohol), then cleaned with water based moist wipes and air-dried for 10 min. DNA deposition was done to replicate a strangulation scenario, by holding the neck semi tight for 2 min while rubbing the hands over the skin (Figure 6.2 – Section 6.1.1) (n= 16, 8 replicates per each collection method).

The DNA was recovered from both hands immediately after the strangulation, and after each DNA recovery the same process of sterilising and DNA collection was repeated (hands measurement in Figure 6.1 (male vs. female) – Section 6.1.1). Touch DNA was recovered with a Copan cotton swab (150C) (CS) and Copan nylon flocked swab (4N6FLOQSwabs®) (NS) (Figure 2.2 – Section 2.3). CS was used following the technique of moistening the skin with 100 µL of molecular grade water using spray bottle technique before collecting the biological materials with dry swab (technique (c) in Figure 6.2 – Section 6.1.1). While NS was used following the same recovery procedure detailed in Section 2.3. The process was conducted at room temperature.

After the strangulation, some samples were collected at 3 h and 6 h from the participant hands, while instructed not to wash the hands and refrain from any activity related to using the hands (n= 21, 7 replicates per each time period). These samples were collected with Copan cotton swab (150C) (CS) following the same technique mentioned above. Following DNA recovery, the swabs were manually extracted immediately using the QIAamp® DNA Investigator Kit following the procedures in Section 2.4. Extracted samples were quantified using the Quantifiler™ Trio DNA Quantification Kit following the procedures in Section 2.5.

All the samples were then amplified and analysed as described in Section 2.6 and 2.7 to evaluate the quality of the samples collected. Control samples from the hands of the donor and neck of the receiver was collected after each sterilisation process, all of which produced full single DNA profiles related to the participants without any sign of mixtures or contamination. Statistical analysis was performed following the procedures mentioned in Section 2.8. For analysis, when the total RFUs observed were reported, this was directly counted for homozygous loci and by adding the peak heights of each allele for heterozygous loci. Also, when the total number of alleles observed was reported, homozygous loci were counted as one allele, while heterozygous loci were counted as two alleles.

6.1.3.2 RESULTS

There was a significant difference between the two collection methods used to recover the DNA from hands of the perpetrator ($p < 0.001$). More DNA was recovered with a CS than that with NS (Table 6.1) (see Appendix A6.4 for the complete results). Considering the average peak height (RFU) of the alleles in the mixture DNA profiles, CS samples generated higher peaks than those of NS ($p < 0.001$).

Table 6.1: Mean of the data observed from the samples recovered using cotton swab (CS) and nylon swab (NS) from the hands after the strangulation scenario (n= 16).

Method	DNA con. ng/µL	Alleles called	avg. RFU	Mixture Ratio	PHR%
CS	0.33	72	2526	1;9	75
NS	0.04	67	982	1;5	79

Similarly, more alleles were sampled with CS than NS, including full mixture DNA profile in almost all the samples collected. In this study, the participant collecting the DNA with hands is considered a low shedder, however hand skin is different from other parts of the body.

The palms of the hands and soles of the feet have more sweat glands, hence can produce more sweat secretions than any other part of the body (Baker, 2019). Nylon swabs are usually more sensitive to moist or humid surfaces than a cotton swab as discussed in Section 4.4 and Section 5.1. Thus, it is not recommended to use NS as recovery method of biological materials collected from the hands following a strangulation scenario. However, examining different techniques with NS can enhance the DNA recovery.

The data observed from the samples collected from the hands of the perpetrator was impacted by 6 h ($p < 0.05$) (Table 6.2) (see Appendix A6.4 for the complete results). The amount of DNA quantities increased slightly over time, which may be the result of not washing the hands. Up to 3 hours usable partial mixture of usable DNA profiles (Based on the Biology and DNA Section lab in the General Department of Forensic Science and Criminology) were obtained for up to 3 h, but after 6 h there was no alleles observed from the minor contributor (victim). Also, the mixture ratio increased between the minor (victim) and major (perpetrator) contributor over time ($p < 0.001$), which was similar to the data collected in Section 6.1.2.

Table 6.2: Mean of the observed data from the samples collected from hands using cotton swabs after after the strangulation scenario over the period of 6 h (n= 21).

Time	DNA con. ng/µL	Alleles called	avg. RFU	Mixture Ratio	PHR%
0 hrs	0.33	72	2526	1;9	75
3 hrs	0.37	64	2196	1;19	73
6 hrs	0.45	39	2740	-	78

6.1.4 SECTION DISCUSSION AND CONCLUSION

Collecting Touch DNA deposited on human skin is more challenging than the collection from items, because Touch DNA deposited by a human on a skin often produces a mixture DNA profile. Therefore, recovery methods can impact the quality of the DNA profile collected. Based on a study by Kallupurackal *et al.,* (2021), cotton and nylon swabs are equally effective on collecting Touch DNA from human skin, while SceneSafe Fast™ minitapes appear to be the least effective method for the scenario of Touch DNA collection from the skin of the victim. However, using the appropriate technique with cotton or nylon swabs can enhance their performance on recovering Touch DNA from human skin. Moistening the neck with 100 µL of molecular grade water using a spray bottle before sample collection with a dry cotton or nylon swab increased the alleles recovery rate in the strangulation scenario. It is worth noting that the amount of solution used can impact the amount of DNA collected, hence it is recommended not to exceed 100 µL which is the amount usually used to wet cotton swabs.

The amount of DNA collected from the skin of the receiver following a strangulation scenario increased over time. This led to the increase in the mixture ratio between the minor and major contributors in the mixed DNA profiles, until it deteriorated completely after 48 h, which was likely the result of the activity of the receiver. With the activity of washing by the receiver, there were no alleles observed from the DNA of the donor in the samples collected after 6 h of deposition. Similar results were observed from previous studies, Wiegand and Kleiber (1997) successfully recovered offender alleles in a real strangulation case where visible marks on the neck of the victim were swabbed 48 h after death. A recent study examined the stability of Touch DNA on pig skin specimens (Meixner *et al.,* 2022) and reported that a complete STR profile from Touch DNA could be recovered several days after deposition. The results from these studies were collected from dead bodies in a controlled environment. Nonetheless, this study supports the observation that the DNA of the offender can be collected from the skin of the victim several days or even more after deposition. In some cases, a partial DNA profile from an offender can be observed on the skin of a victim up to 10 days after contact (Rutty, 2002). However, washing or sweating could influence the presence of the deposited DNA. Furthermore, there is increased possibility of DNA contamination

over time in human skin through coming in contact with other individuals or used objects (Rutty, 2002).

The hands can be sweatier in some people than others. In this study, cotton swabs performed better on collecting better quality mixture DNA profiles than nylon swabs. Furthermore, the location of the deposited DNA on the body of the victim (e.g. neck, hands, and hand wrist) can impact the stability of the DNA of the offender. The biological materials of the victim collected from the hands of the donor following the strangulation deteriorated much quicker than those deposited on the neck. Hands are used in everyday activity more often than the neck, and collecting biological materials once crimes are committed is essential to be able to recover the DNA of the victim from the hands of the offender.

Finally, the technique developed in this study of moistening the skin first with a spray bottle before recovery with cotton swabs produced full mixture DNA profiles from the receiver neck skin after deposition for up to 48 h, whereas usable partial mixture DNA profiles were obtained from the donor hands after deposition for up to 3 h.

6.2 TOUCH DNA RECOVERY FROM FABRIC

In many cases such as sexual assault, homicide, and theft, the clothing of the victim is often sampled for trace DNA. Trace DNA deposits as a result of short or prolonged contact with a person, and when Touch DNA is deposited on the surface containing the background DNA of the frequent user a mixed DNA profile is often generated which can complicate the interpretation (Petricevic *et al.,* 2011). Collecting Touch DNA from fabric can be impacted by many variables such as fabric type, deposition area and size, time between deposition and collection, collection type, and DNA extraction method. While there is little shared data from casework, there is a need for an evaluation of these variables to improve Touch DNA recovery from fabric.

6.2.1 INFLUENCE OF COLLECTION AND EXTRACTION TYPES

Tape-lifting is commonly used in forensic analysis to collect biological materials from porous substrates such as fabric (Hall & Fairley, 2004; Barash *et al.,* 2010; Gunnarsson *et al.,* 2010; Keller *et al.,* 2012; Alketbi & Will, 2019a). Furthermore, cutting a piece of fabric containing the DNA is also used for direct extraction, however it can produce PCR inhibitors with the biological sample (Van Oorschot *et al.,* 2010). There has been limited direct comparison between the performance of swabbing and tape-lifting as collection methods (Verdon *et al.,* 2014a), considering that different types of surfaces require different types of collection methods in combination with the right extraction method to maximise Touch DNA recover. Therefore, this study investigated the impact of collection and extraction methods on Touch DNA collected from fabric.

6.2.1.1 EXPERIMENTAL SETUP

A fabric type composed of 65% polyester and 35% cotton was selected as it is a popular synthetic material used for garments in the fashion industry (Textile Exchange, 2017) (Figure 6.10). For easier DNA deposition and collection, the fabric was cut into 5 x 7 cm pieces. Next, two participants, previously confirmed to be high and low shedders in Section 3.1, were asked to wash their hands with antibacterial soap (LabGUARD), cease from activity related to using their hands for 10 min, then rub a fabric piece (5 x 7 cm) for 1 min between both hands (n= 24, 8 replicates per each collection method).

This process was repeated for each deposition at room temperature. The fabric surfaces were washed at 50 °C, dried, and disinfected with ultraviolet radiation (UV) for 25 min before use.

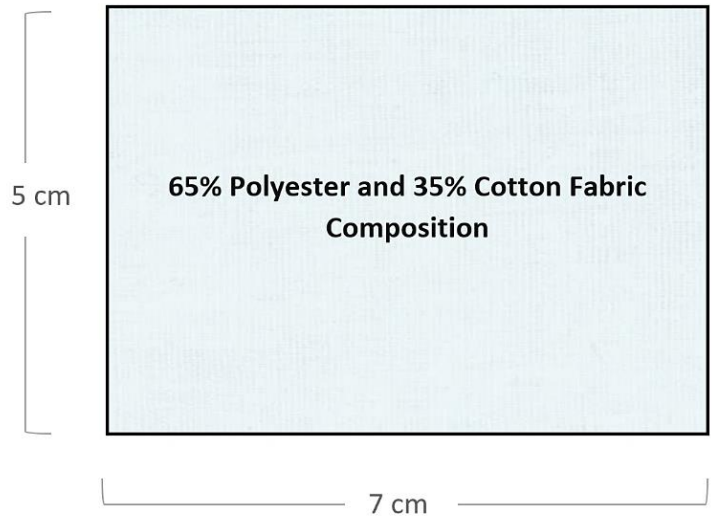


Figure 6.10: Piece of fabric (5 x 7 cm) composed of 65% polyester and 35% cotton fabric used for Touch DNA deposition.

For DNA collection, three different methods were used: Copan cotton swab (150C) (CS), Copan nylon flocked swab (4N6FLOQSwabs®) (NS), and SceneSafe Fast™ minitape (K545) (MT) (Figure 2.2 – Section 2.3) following the same recovery procedure detailed in Section 2.3. Touch DNA was recovered within 15 min from the surfaces after deposition and extracted immediately with the PrepFiler Express BTA™ kit using an AutoMate Express Forensic DNA Extraction System (EXT1) and manually using the QIAamp® DNA Investigator Kit (EXT2) following the procedures in Section 2.4. However, based on the findings detailed in Section 4.2, 460 µL of lysis buffer was used for EXT1 instead of 230 µL.

Extracted samples were quantified using the Quantifiler™ Trio DNA Quantification Kit following the procedures in Section 2.5. Some samples were then amplified and analysed as described in Section 2.6 and 2.7 to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed following the procedures mentioned in Section 2.8.

6.2.1.2 RESULTS

The amount of recovered DNA from the fabric was significantly impacted by collection type ($p < 0.05$), with MT being more effective than a cotton swab (CS) and nylon swab (NS) to recover Touch DNA from fabric samples (5 x 7 cm, 65% polyester and 35% cotton) ($p < 0.05$) (Figure 6.11 & 6.12) (see Appendix A6.5 for the complete results).

Similarly, the use of minitapes can be more efficient than swabbing on collecting Touch DNA from fabric (Hansson *et al.,* 2009; Hess & Haas, 2017; Alketbi, 2022d). Though, recovery area size needs to be put into consideration when comparing different recovery methods.

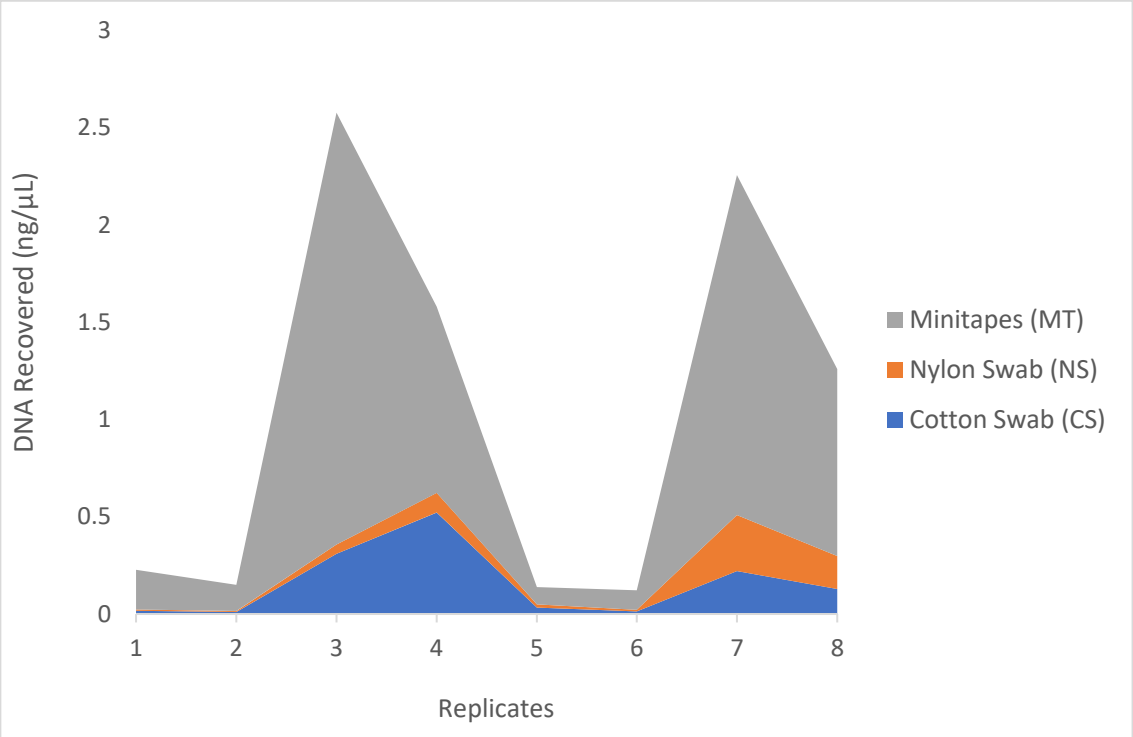


Figure 6.11: Amount of DNA recovered using cotton swabs (CS), nylon swabs (NS) and minitapes (MT) from eight replicates (n= 24) of fabric (5 x 7 cm, 65% polyester and 35% cotton).

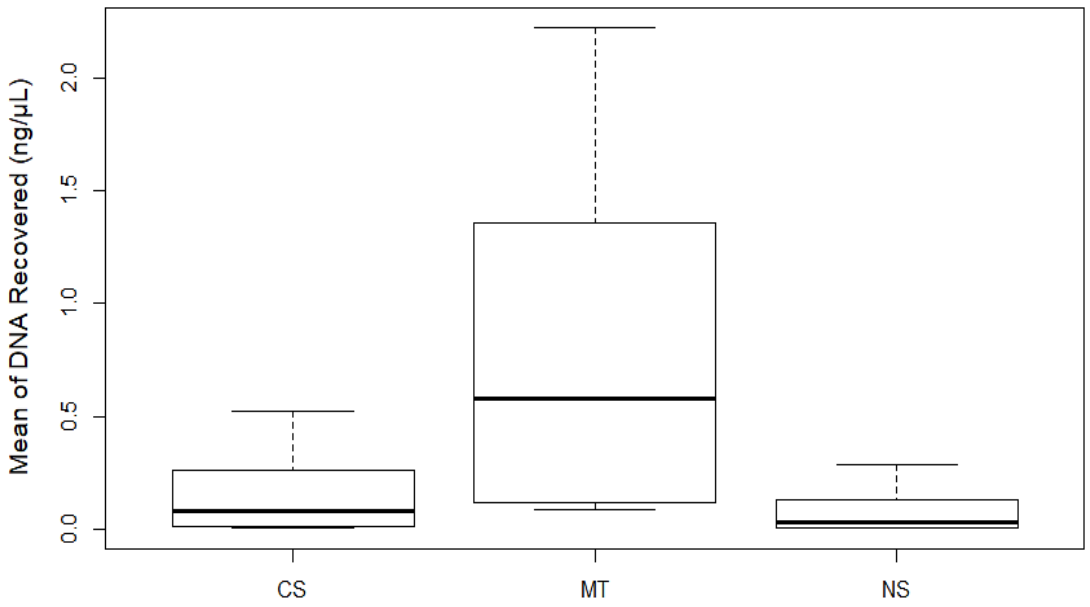


Figure 6.12: Mean of DNA recovered (n= 24) using cotton swabs (CS), nylon swabs (NS) and minitapes (MT) from fabric (5 x 7 cm, 65% polyester and 35% cotton).

The samples collected using the three methods were not affected by the extraction (p > 0.05) when 460 µL of lysis buffer was used with EXT1 instead of 230 µL. Samples extracted with EXT1 generated slightly more DNA than that with EXT2 (mean EXT1. 0.38 and EXT2. 0.31 all in ng/µL) (Figure 6.13).

Additionally, the results described in Section 4.3 indicated an interaction between collection type and extraction method, and EXT1 was more successful on extracting samples collected using MT than EXT2. Similar findings on the effectiveness of using PrepFiler Express BTA™ kit on extracting SceneSafe Fast™ minitape was also reported by Joël *et al*. (2015).

Some of the collected samples from the fabric (5 x 7 cm, 65% polyester and 35% cotton) were amplified to validate their quality. All the samples collected using MT produced full single DNA profiles, whereas only samples deposited by the high shedder and collected using NS and CS produced full single DNA profiles, while samples deposited by the low shedder produced partial DNA profiles (Figure 6.14).

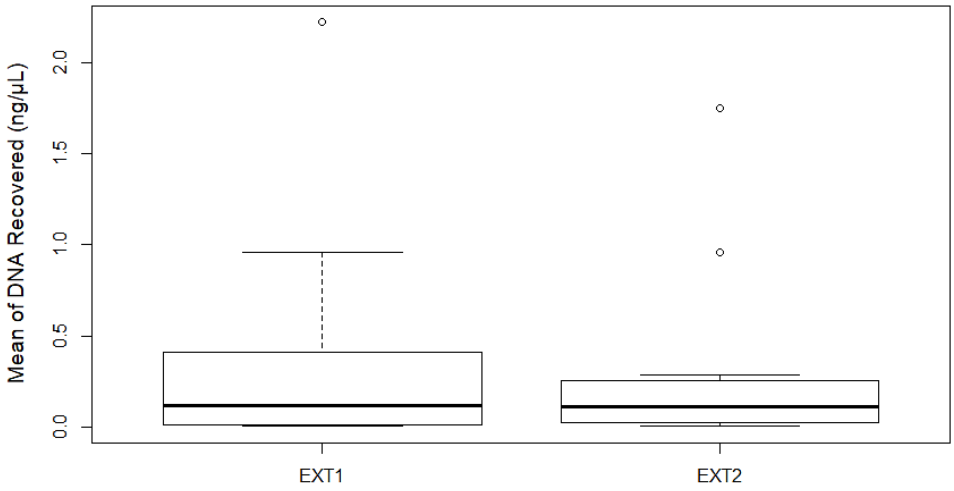


Figure 6.13: Mean of DNA extracted (n= 24) using the PrepFiler Express BTA™ kit (EXT 1) and QIAamp® DNA Investigator Kit (EXT 2) of samples collected from fabric (5 x 7 cm, 65% polyester and 35% cotton) using cotton swabs (CS), nylon swabs (NS), and minitapes (MT).

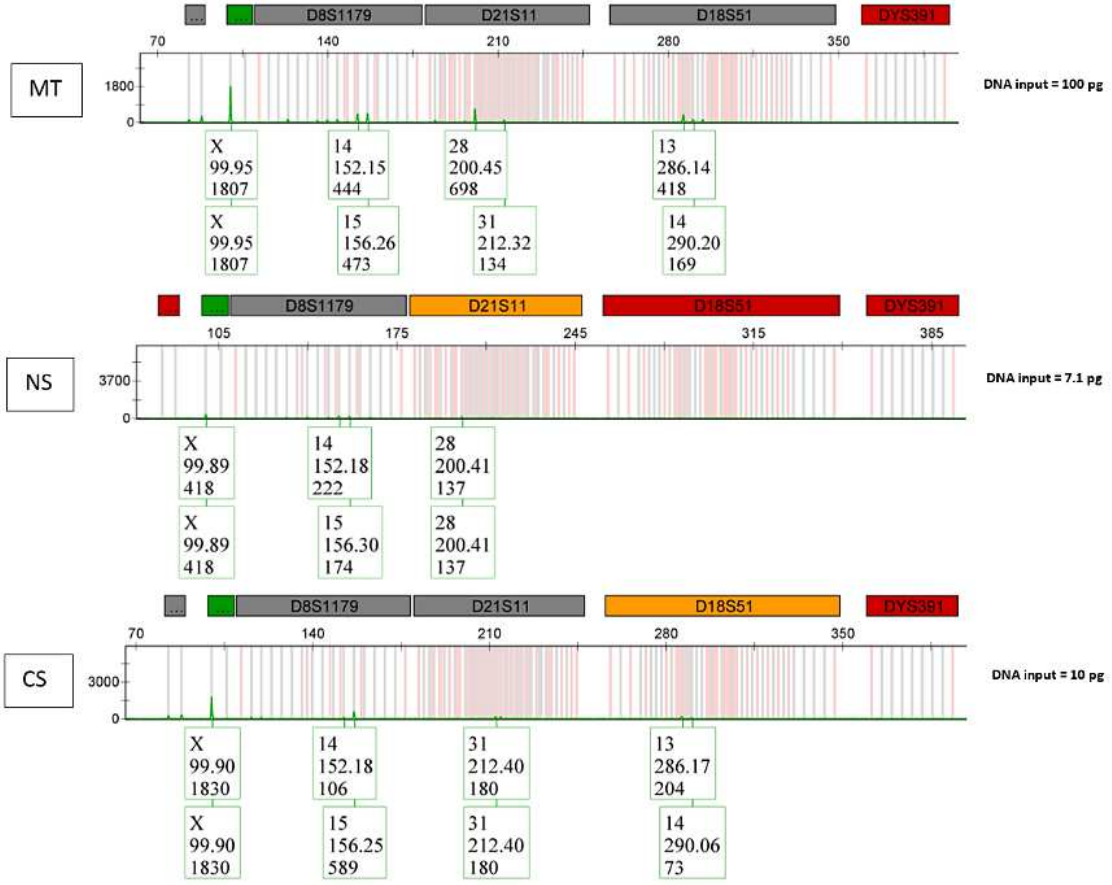


Figure 6.14: Electropherograms of samples collected from the fabric using minitape (MT), nylon swab (NS), and cotton swab (CS). The profiles show some missing alleles from DNA profiles collected using NS and CS, and some differences in peak height between the three collection types (Yindel, AMEL, D8S1179, D21S11, D18S51, DYS391). Maximum volume of DNA was added.

6.2.2 INFLUENCE OF FABRIC TYPE

Fabric differs from other types of surfaces because it is usually made of different fibres, producing different garments . Clothes are made from a wide range of different materials. Traditional materials like cotton, linen, and leather, which are sourced from plants and animals, only represent a small percentage of the clothes made today (Textile Exchange, 2017). Cotton makes up for approximately 21% of all fibre use globally, while 65% of all fibres used in the fashion industry are made mostly from synthetic materials such as polyester (Textile Exchange, 2017). However, the impact of fabric type on Touch DNA is not well understood. Therefore, this study aimed to investigate the influence of fabric type on Touch DNA.

6.2.2.1 EXPERIMENTAL SETUP

The fabrics tested were composed of 65% polyester and 35% cotton (FB1), and 100% woven cotton (FB2), the first and second most popular materials used in the fashion industry (Textile Exchange, 2017). The two fabrics were cut into 5 x 7 cm pieces for DNA deposition and collection (Figure 6.15). Next, two participants, previously confirmed to be high and low shedders in Section 3.1, were asked to wash their hands with antibacterial soap (LabGUARD), cease from activity related to using their hands for 10 min, then rub a fabric piece (5 x 7 cm) for 1 min between both hands (n= 16, 8 replicates per each fabric type). This process was repeated for each deposition at room temperature. The fabric surfaces were washed at 50 °C, dried, and disinfected before use with ultraviolet radiation (UV) for 25 min.

After deposition, samples were immediately collected using SceneSafe Fast™ minitape (K545) (MT) following the same recovery procedure detailed in Section 2.3. Samples were then extracted with the PrepFiler Express BTA™ kit using an AutoMate Express Forensic DNA Extraction System (EXT1) following the procedures detailed in Section 2.4. However, based on the finding from Section 4.2, 460 µL of lysis buffer was used instead of 230 µL.

Extracted samples were quantified using the Quantifiler™ Trio DNA Quantification Kit following the procedures in Section 2.5. Some samples were then amplified and

analysed as described in Section 2.6 and 2.7 to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed following the procedures mentioned in Section 2.8.

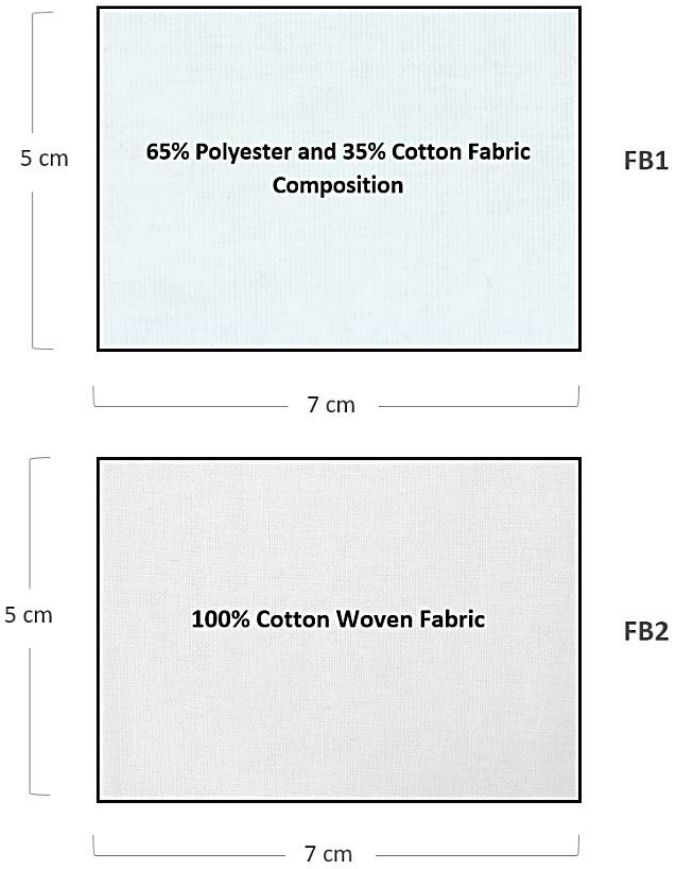


Figure 6.15: Fabric type one (FB1) made of 65% polyester and 35% cotton and fabric type two (FB2) made of 100% woven cotton.

6.2.2.2 RESULTS

The amount of Touch DNA recovered was significantly impacted by the fabric type ($p < 0.05$), with more DNA recovered from FB1 composed of 65% polyester and 35% cotton than that from FB2 composed of 100% woven cotton (mean FB1. 0.80 ng/µl vs. FB2. 0.14 ng/µL) (Figure 6.16 & 6.17) (see Appendix A6.6 for the complete results).

Some fabrics have loose fibres, which may hinder the recovery with MT and weaken the stickiness of the tape-lift, therefore swabbing may be more efficient or equally effective as minitapes (Stoop et al., 2017).

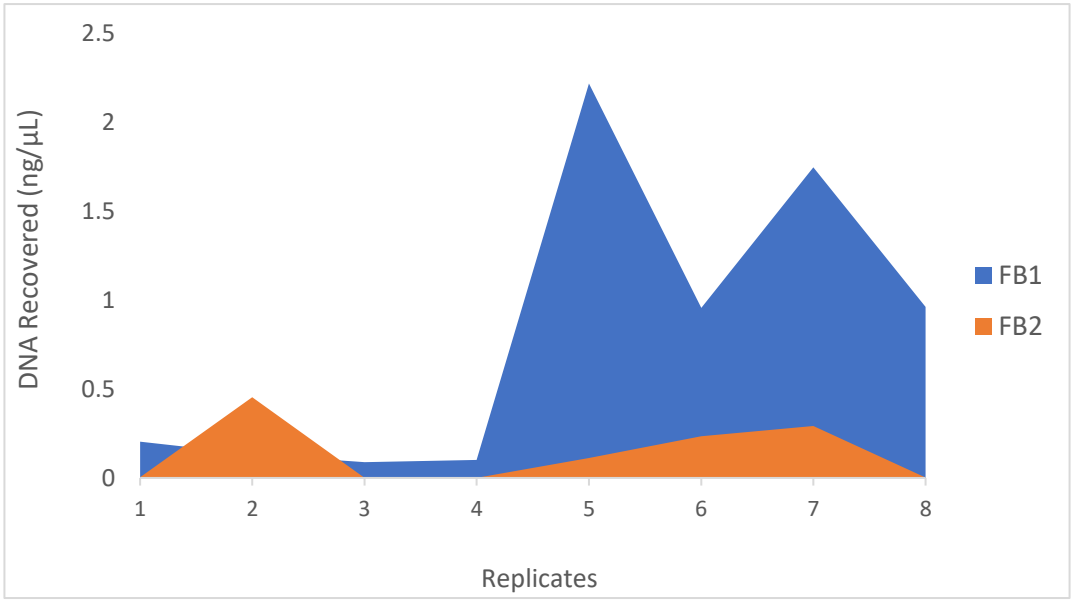


Figure 6.16: Amount of DNA recovered from eight replicates (n= 16) using minitapes (MT) from fabric type 1 (FB 1 - 65% polyester and 35% cotton) and fabric type 2 (FB 2 - 100% woven cotton).

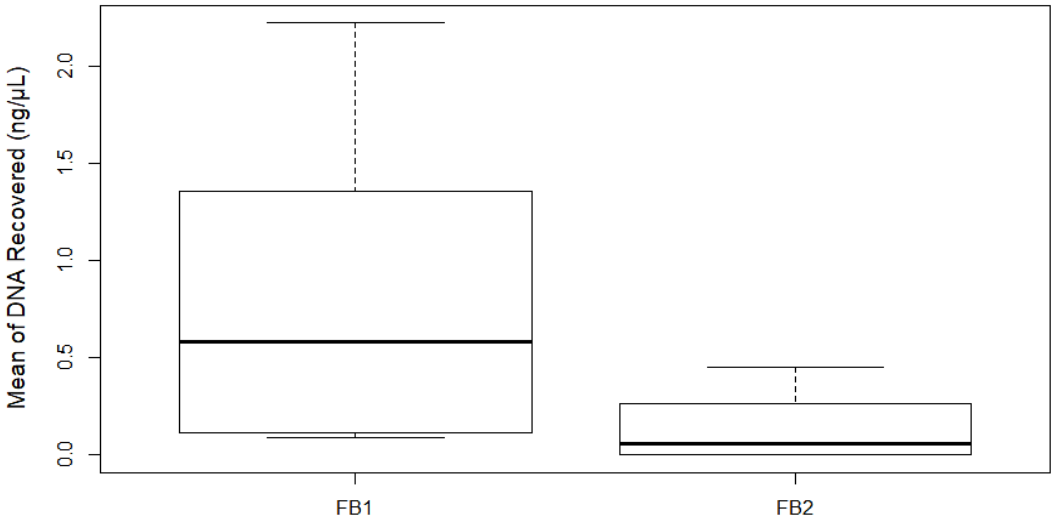


Figure 6.17: Mean of DNA recovered from (n= 16) using minitapes (MT) from fabric type 1 (FB 1 - 65% polyester and 35% cotton) and fabric type 2 (FB 2 - 100% woven cotton).

Some samples recovered from FB1 and FB2 were amplified to validate the DNA quality, with all samples producing full single DNA profiles with some variation in the peak height between the fabric types. Samples recovered from FB1 had a relatively higher peak height (RFU) compared to the profiles from FB2.

6.2.3 INFLUENCE OF AREA SIZE

Self-adhesive minitapes such as SceneSafe Fast™ minitape has been developed for forensic DNA use, with successful results from worn clothing (Gunnarsson *et al.,* 2010; Barash *et al.,* 2010; Verdon *et al.,* 2015). However, SceneSafe Fast™ minitape has limited taping resistance, which is approximately between 16 to 30 tape-lifts, thus too many applications can possibly impact collection efficiency (Verdon *et al.,* 2014b). For that reason. The efficiency of SceneSafe Fast™ minitape on collecting trace DNA from a fairly larger area of fabric was compared to swabbing, which has not been examined previously. Therefore, this study investigated the impact of area size and collection method on Touch DNA recovered from fabric.

6.2.3.1 EXPERIMENTAL SETUP

A fabric composed of 65% polyester and 35% cotton was used as it is a popular synthetic material used for garments in the fashion industry (Textile Exchange, 2017). The fabric was cut into two pieces (SZ1 = 5 x 7 cm and SZ2 = 10 x 14 cm) (Figure 6.18) for DNA deposition and collection.

Next, two participants, previously confirmed to be high and low shedders in Section 3.1, were asked to wash their hands with antibacterial soap (LabGUARD), cease from activity related to using their hands for 10 minutes, then rub a fabric piece for 1 min between both hands (n= 16, 8 replicates per each variable; area sizes and collection methods). This process was repeated for each deposition at room temperature. The fabric surfaces were washed at 50 °C, dried, and disinfected before use with ultraviolet radiation (UV) for 25 min.

After deposition, samples were collected immediately using Copan cotton swab (150C) (CS) and SceneSafe Fast™ minitape (K545) (MT) following the same recovery procedure detailed in Section 2.3. Then, extracted with the PrepFiler Express BTA™ kit using an AutoMate Express Forensic DNA Extraction System (EXT1) following the procedures detailed in Section 2.4. However, 460 µL of lysis buffer was used instead of 230 µL based on the finding from Section 4.2.

Extracted samples were quantified using the Quantifiler™ Trio DNA Quantification Kit following the procedures described in Section 2.5. Some samples were then amplified and analysed as described in Section 2.6 and 2.7 to evaluate the quality of the samples collected. All samples produced full DNA profiles without any sign of mixtures or contamination. Statistical analysis was performed following the procedures mentioned in Section 2.8.

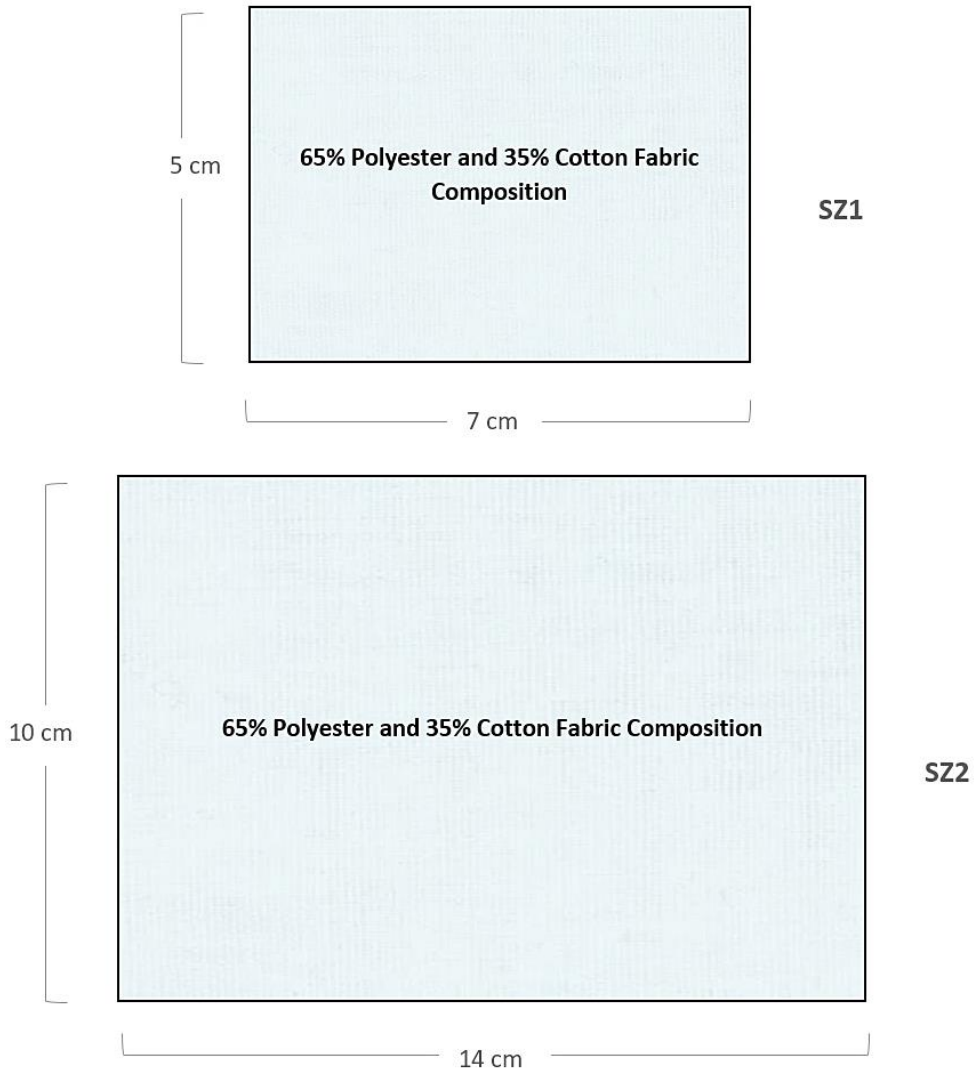


Figure 6.18: Fabric one (SZ1 - 5 x 7 cm) and fabric two (SZ2 - 10 x 14 cm) composed of 65% polyester and 35% cotton.

6.2.3.2 RESULTS

The quantity of Touch DNA collected from the fabric was significantly impacted by the interaction between fabric size and collection method ($p < 0.05$), with more DNA recovered from SZ1 using MT than CS ($p < 0.05$) (mean MT. 0.88 ng/µL vs. CS. 0.03 ng/µL). In contrast, with SZ2, more DNA was recovered using CS than that with MT ($p < 0.05$) (mean MT. 0.08 ng/µL vs. CS. 0.35 ng/µL) (Figures 6.19 & 6.20) (see Appendix A6.7 for the complete results).

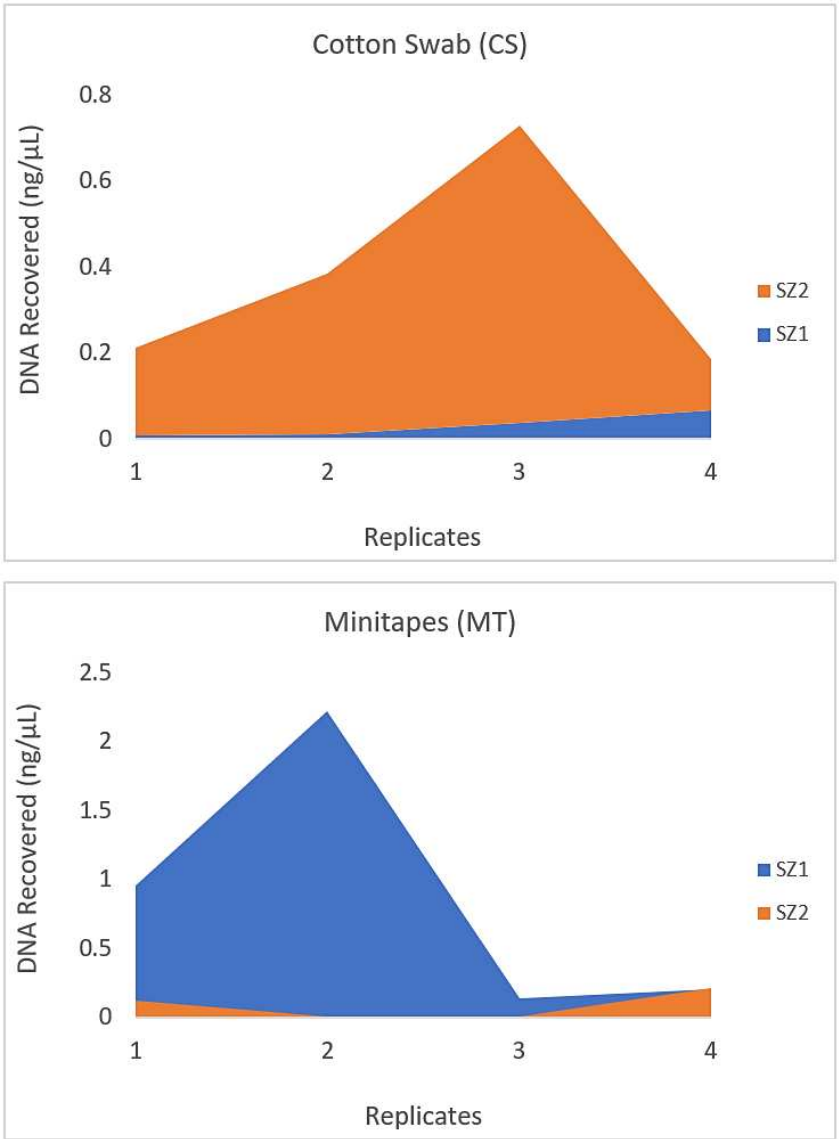


Figure 6.19: Amount of DNA collected from four replicates (n= 16) using minitapes (MT) and cotton swab (CS) from fabric one (SZ1 – 5 x 7 cm) and fabric two (SZ2 – 10 x 14 cm).

SceneSafe Fast™ minitape is mostly effective for collecting trace DNA from a small surface area, because it has limited number of tape-lifts, which can influence sampling from different area sizes (Verdon *et al.,* 2014b). Therefore, two or more minitapes are recommended to be used for areas that are larger than 5 x 7 cm, depending on the size of the area. However, using cotton swabs can also be effective on collecting trace DNA from fabric. In this study the amount of DNA increased when CS was used to recover DNA from SZ2 compared to SZ1, and the other way for MT (Figure 6.20). That indicates that cotton swabs have the potential to collect more DNA spread over larger areas, while minitapes are more efficient for collecting DNA concentrated in small areas.

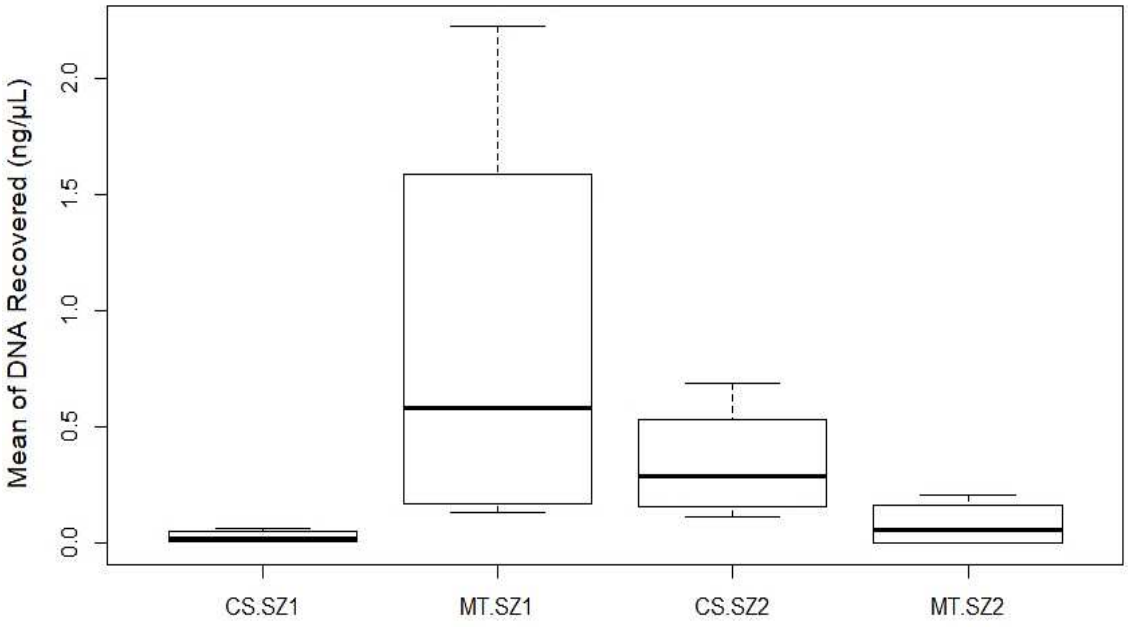


Figure 6.20: Mean of DNA collected (n= 16) using minitapes (MT) and cotton swab (CS) from fabric size one (SZ1 – 5 x 7 cm) and fabric size two (SZ2 – 10 x 14 cm).

Some samples recovered from SZ1 and SZ2 were amplified to validate the DNA quality, with all samples producing full single DNA profiles but with some variation in the peak height (RFU) between the samples collected using each collection method for each fabric size. Samples recovered by the MT from SZ1 had a relatively higher mean RFU than CS and it was the other way for samples collected from SZ2.

6.2.4 INFLUENCE OF DEPOSITION AREA AND TIME

The DNA of the wearer is deposited mainly on the interior side of the clothing through rubbing against the skin, while a minimal amount of DNA can transfer to the external surface through casual touching. Consequently, the pre-deposited biological material internally may not physically mix with an external deposit, possibly allowing a separate recovery of the deposits (Verdon *et al.,* 2015). However, wearing garments for longer period of time can cause a lot of friction between the surface of the fabric and the skin, as well sweating from the skin, which is absorbed by the fabric. All these factors could affect the persistence of any Touch DNA deposited on the outer layer of the garment. Therefore, this study investigated the impact of deposition area and time on Touch DNA collected from fabric following a sexual harassment scenario.

6.2.4.1 EXPERIMENTAL SETUP

A female t-shirt and a long pants made of 65% polyester and 35% cotton were chosen as it is a popular synthetic material used in the fashion industry (Textile Exchange, 2017). A male participant (perpetrator) previously identified as high shedder in Section 3.1, were requested to wash his hands with antibacterial soap (LabGUARD), cease from activity related to using the hands for 10 min, then charge both hands with eccrine sweat by touching his forehead to load them with enough DNA to help improve the quality and quantity of the DNA deposit. The participant was then asked to rub both hands separately on a highlighted 5 x 7 cm area for 1 min on the chest of the t-shirt (A) and the buttocks of the pants (B) to replicate a sexual harassment scenario (Figure 6.21) (n= 30, 3 replicates per each time period).

Prior to the DNA deposition, the used clothes were washed at 50 °C, dried and disinfected with ultraviolet radiation (UV) for 25 min. After the deposition, the female participant (victim) was requested to wear the clothes during the experimental duration and carry on with the normal daily activity without touching the highlighted area, avoid other people come in contact with the highlighted area, clean the clothes, or do any physical activity to avoid excessive sweating. This process was replicated for each deposition, and the DNA samples were collected after five periods of 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours.

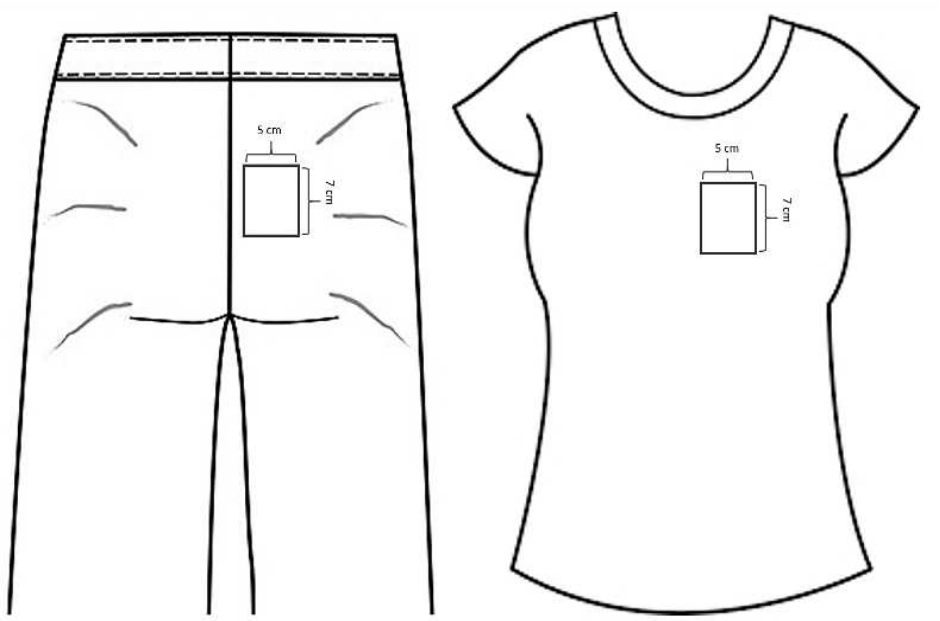


Figure 6.21: Deposition area A (5 x 7 cm) on the chest area of the t-shirt and deposition area B (5 x 7 cm) on the buttocks area of the long pants.

Samples were collected using SceneSafe Fast™ minitape (K545) (MT) following the same recovery procedure detailed in Section 2.3. The sample was then extracted with the PrepFiler Express BTA™ kit using an AutoMate Express Forensic DNA Extraction System (EXT1) following the procedures detailed in Section 2.4. However, based on the findings from Section 4.2, 460 µL of lysis buffer was used instead of 230 µL. Extracted samples were quantified using the Quantifiler™ Trio DNA Quantification Kit following the procedures in Section 2.5. All the samples were then amplified and analysed as detailed in Section 2.6 and 2.7 to evaluate the quality of the samples collected. Statistical analysis was performed following the procedures described in Section 2.8. For analysis, when the total number of alleles observed was reported, homozygous loci were counted as one allele, while heterozygous loci were counted as two alleles.

6.2.4.2 RESULTS

The quantity of the collected Touch DNA from the garments was significantly affected by the deposition area ($p < 0.05$), time ($p < 0.05$), as well the interaction between the deposition area and time ($p < 0.05$) (Figures 6.22 & 6.23) (see Appendix A6.8 for the complete results).

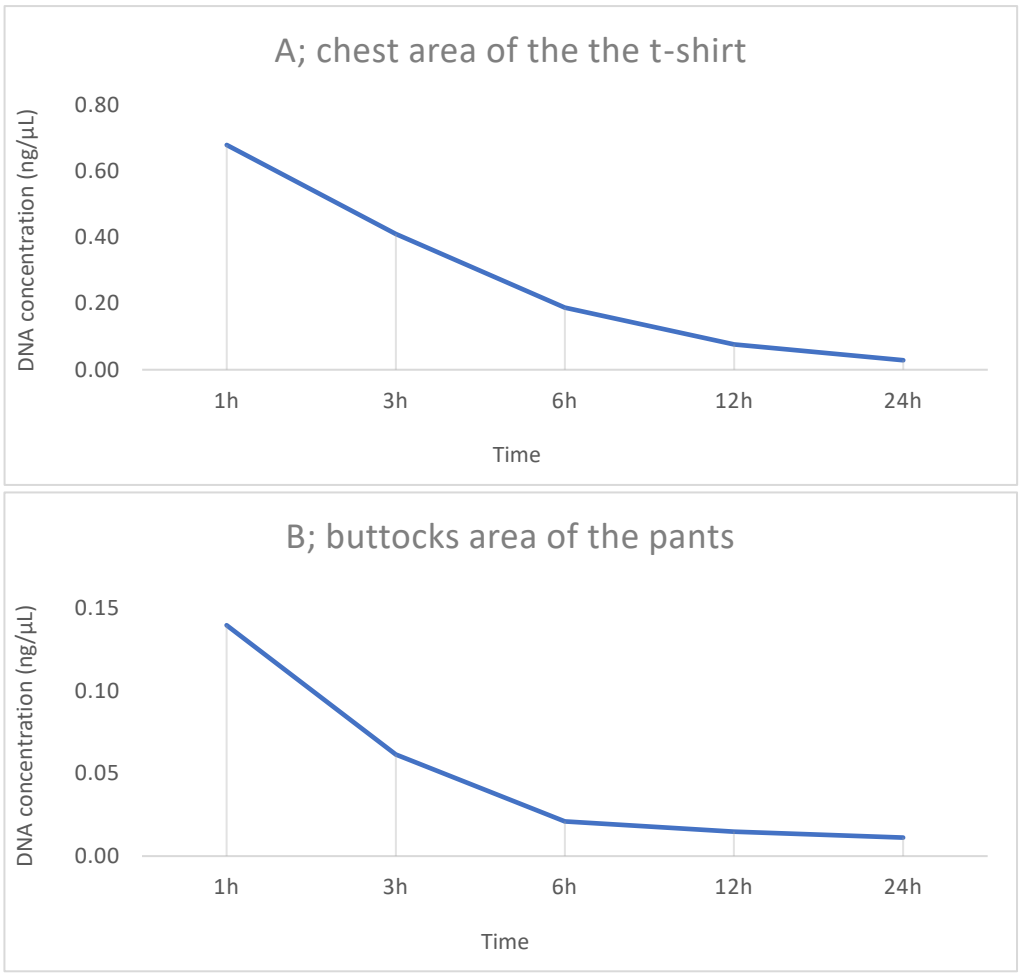


Figure 6.22: Amount of DNA recovered (n= 30) from the chest area of the t-shirt (A – 5 x 7 cm) and the buttocks area of the pants (B – 5 x 7 cm) at 1 h, 3 h, 6 h, 12 h, and 24 h.

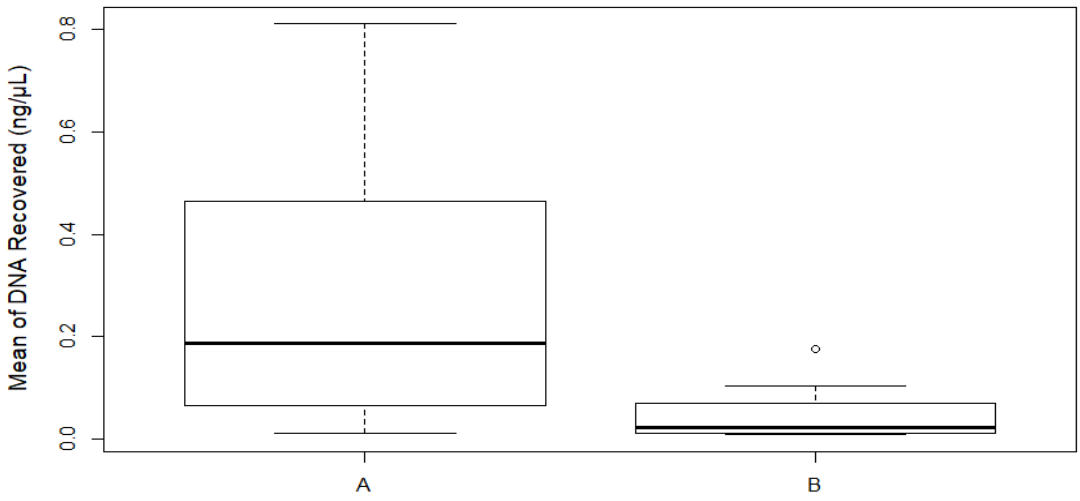


Figure 6.23: Mean of DNA collected (n= 30) from the chest area of the t-shirt (A – 5 x 7 cm) and the buttocks area of the pants (B – 5 x 7 cm) at 1 h, 3 h, 6 h, 12 h, and 24 h.

More DNA was collected from the chest area of the t-shirt (A) than that from the buttocks area of the pants (B) over the 24 h period (1 h, 3 h, 6 h, 12 h, 24 h) (mean Area A. 0.28 and Area B. 0.05 all in ng/µL). Furthermore, DNA quantities decreased over time, however, the number of alleles observed in the mixture DNA profiles was not affected by time for the samples collected from area A. Whereas full mixture DNA profiles were obtained from the samples collected from area B, however, no alleles were observed from the minor contributor (perpetrator) after 6 h (Figure 6.24).

Touch DNA deposited on the long pants decreased more rapidly than the DNA deposited on the t-shirt. In addition, location of the deposit in the garment, whether it was front/back or up/down can impact the persistence of the Touch DNA deposited. Additionally, DNA transfer between the wearer of the garment and background DNA deposited on the garment is mostly dependent on the fabric used (e.g. tightness of the weave and fibre type) and activity of the wearer (Mulligan *et al.,* 2011; Verdon *et al.,* 2013; Alketbi & Goodwin, 2022e).

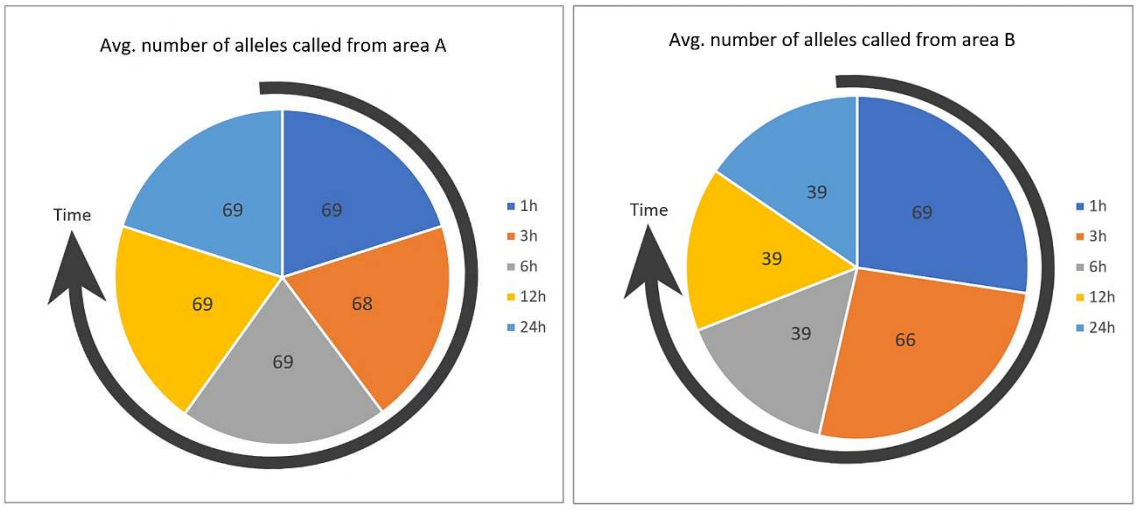


Figure 6.24: Mean number of alleles observed (n= 30) in the samples collected from the chest area of the t-shirt (A – 5 x 7 cm) and the buttocks area of the pants (B – 5 x 7 cm) at 1 h, 3 h, 6 h, 12 h, and 24 h.

6.2.5 SECTION DISCUSSION AND CONCLUSION

Collection of Trace DNA from clothes can be influenced by the collection method, and use of tape such as SceneSafe Fast™ minitape, being more effective for recovering DNA from porous surfaces like fabric (Alketbi, 2022d). However, too many applications could possibly impact the collection competence of the minitape. Using the tape until it is no longer sticky requires a subjective evaluation.

SceneSafe Fast™ minitape is mostly effective for up to 16 tape-lifts and although it can allow more tape-lifts, no extra information will be collected from the sample (Verdon *et al.,* 2014b). Moreover, there was no big difference in average of DNA extracted when samples collected from fabric using minitape and cotton swabs, and extracted using PrepFiler Express BTA™ kit and QIAamp® DNA Investigator Kit, while using 460 µL of lysis buffer was used instead of 230 µL with PrepFiler BTA™. However, PrepFiler BTA™ was more effective in extracting samples collected using minitapes than that with QIAamp® DNA Investigator Kit.

Recovery area size in combination with collection type can impact Touch DNA recovery (Alketbi & Goodwin, 2022c). SceneSafe Fast™ minitapes has limited taping durability, thus based on the findings of this study, using minitape over small areas in the garment such as an area size of 5 x 7 cm is more effective. In addition, cotton swabs can be equally effective for sampling larger areas of fabric when compared to using only minitape over an 10 x 14 cm area.

Additionally, Fabric type can influence Touch DNA recovery (Alketbi & Goodwin, 2022c), considering that more trace DNA was recovered from fabric composed of a high percentage of polyester than that from fabric composed of 100% woven cotton with SceneSafe Fast™ minitapes. Nonetheless, other fabrics with loose fibres such as flannelette can make minitapes less sticky much faster than other types of fabric (Stoop *et al.,* 2017). Therefore, considering using cotton swabs may be more efficient.

Finally, the area of deposition, time, and other factors such as physical activity can affect the amount of Touch DNA collected from garments (Alketbi & Goodwin, 2022e). The buttocks area of the pants compared to the chest area of the t-shirt is more susceptible

to friction from daily activity like repeatedly sitting on different surfaces which reduces the amount of deposited DNA available. Therefore, it is recommended to recover trace DNA from the victim's clothes as soon as the crime is committed to avoid losing valuable information related to the offenders. However, this can be challenging when people do not report sexual harassment straight away, consequently, more awareness is needed to educate victims.

CHAPTER SEVEN

7. DIRECT PCR AMPLIFICATION OF TOUCH DNA

This chapter evaluated direct PCR amplification and how it could impact Touch DNA profiling. This was done by examining the effect of collection methods on DNA recovered from a range of porous and non-porous surfaces. Extraction of samples versus direct amplification were also investigated by collecting DNA from different deposition area sizes. An innovative solution used to retrieve samples using cotton swab in combination with microFLOQ™ Direct swab for both extraction and direct amplification was examined. Finally, microFLOQ™ Direct swab was evaluated by recovering DNA from office items to replicate casework samples.

7.1 INFLUENCE OF COLLECTION TYPE

Direct amplification of casework samples can help in maximising DNA recovery and accelerate the DNA profiling process. It has proven to be successful for body fluid samples, such as blood and saliva (Park *et al.,* 2008; Barbaro *et al.,* 2008; Wang *et al.,* 2015), when processing reference samples. However, it can be much more challenging from samples containing low amounts of DNA, such as trace samples (Van Oorschot*et al.,* 2010). Developing new protocols to recover and process Touch DNA samples can better direct amplification; therefore, a set of collection methods were examined for their performance in collecting Touch DNA for direct amplification using a GlobalFiler™ PCR amplification Kit.

7.1.1 EXPERIMENTAL SETUP

Two participants, previously confirmed to be high and low shedders in Section 3.1., were instructed to perform the deposition process described in Section 2.2 on a 2.5 x 3.5 cm area of the test surfaces (n= 72, 12 replicates per each collection method). The surfaces were selected to replicate common items encountered in crime scenes, and have a variety of smooth, rough, porous, and non-porous surfaces. These surfaces included stainless steel (SS), glass (G), textured plastic (TP), textured wood (TW), copier paper (CP) (previously tested in Section 4.1), and fabric (FB- 65% polyester and 35% cotton) (previously tested in Section 6.2).

Each participant deposited their DNA separately on the selected six surfaces to avoid any contamination. Prior to deposition, all non-porous surfaces were sterilised using 2% Virkon and UV-irradiation for 15 min, while porous surfaces were only irradiated using UV light for 25 min. This process was conducted at room temperature.

Deposited DNA was collected immediately, using six collection types as displayed in Figure 7.1. The MicroFLOQ™ Direct swab (MF), mini cotton swab (MCS), and mini plastic swab (MPS) were moistened with 1 µL of molecular grade water using a pipette. Copan cotton swab (150C) (CS), Copan nylon flocked swab (4N6FLOQSwabs®) (NS), and SceneSafe Fast™ minitape (K545) (MT) were used following the same collection procedure detailed in Section 2.3.

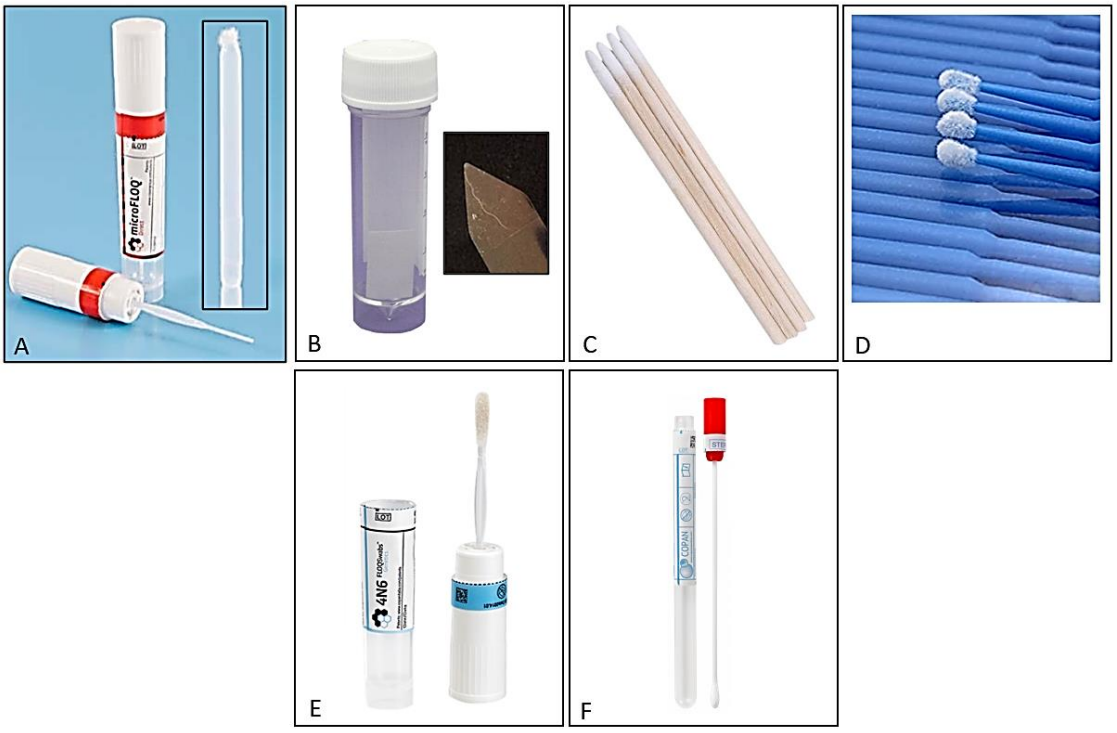


Figure 7.1: Six collection methods used for direct amplification: **(A)** microFLOQ™ Direct swab (MF) (Copan), **(B)** SceneSafe Fast™ Minitape (MT) (K545), **(C)** Mini Cotton Swab (MCS) (Fenshine), **(D)** Mini Plastic Swab (MPS) (G2Plus), **(E)** Copan Nylon flocked Swab (4N6FLOQSwabs®) (NS) and **(F)** Copan Cotton Swab (CS) (150C).

After collection, the samples were directly transferred to 0.2-ml PCR tubes, for amplification using the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific). The MF swab head was broken directly into the tube by bending the swab, an area of

approximately 2 mm^2 from the tip of the other swabs (MCS, MPS, NS, and CS) was cut into the tube by sterilised scissors, and the tip of the MT (triangular shape of approximately 2 mm^2) was cut into the tube using sterile scissors. Tubes were prepared by adding PCR master mix (10 µL), while the total reaction volume was made up with TE buffer (15 µL). All the samples were then amplified and analysed as described in Sections 2.6 and 2.7, to evaluate the quality of the samples collected. There was no sign of mixtures or contamination. Statistical analysis was performed following the procedures mentioned in Section 2.8. For analysis, when the total number of alleles observed was reported, homozygous loci were counted as one allele, while heterozygous loci were counted as two alleles.

7.1.2 RESULTS

The number of alleles observed in the DNA profiles was impacted by collection type ($p < 0.001$), as well as surface type ($p < 0.05$) (Figures 7.2 & 7.3) (see Appendix A7.1 for the complete results).

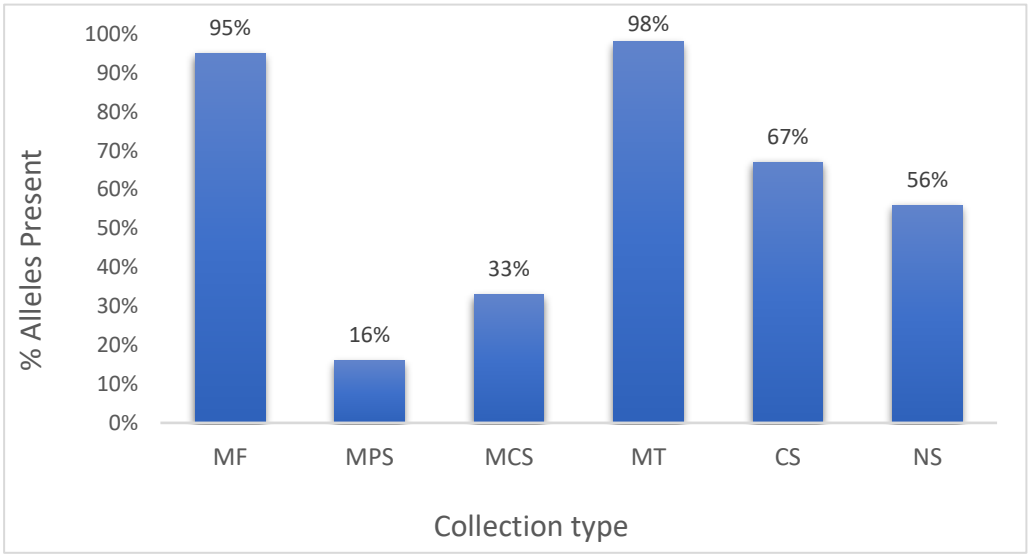


Figure 7.2: Percentage of alleles recovered from Touch DNA profiles (n= 72) collected from various porous and non-porous surfaces by six collection methods, and generated by direct amplification with the GlobalFiler™ kit. *MF*: microFLOQ™ Direct swab, *MPS*: Mini Plastic Swab, *MCS*: Mini Cotton Swab, *MT*: Minitape, *CS*: Cotton Swab, and *NS*: Nylon flocked Swab.

More alleles were recovered from non-porous surfaces than porous surfaces ($p < 0.05$), when samples were processed for direct PCR (allele recovery for SS. 77%, G. 72%, TP. 65%, TW. 56%, CP. 49%, and FB. 51%). However, samples were only collected by swabbing and tape-lifting, thus cutting a small piece from non-porous surfaces, such as fabric, for direct amplification can improve the recovery from such a surface (Linacre *et al.,* 2010). MF and MT recovered a higher percentage of alleles and were more effective for direct DNA amplification than MPS and MCS ($p < 0.05$). In addition, CS and NS recovered a reasonable percentage of alleles, but the results were not consistent when compared to MF and MT.

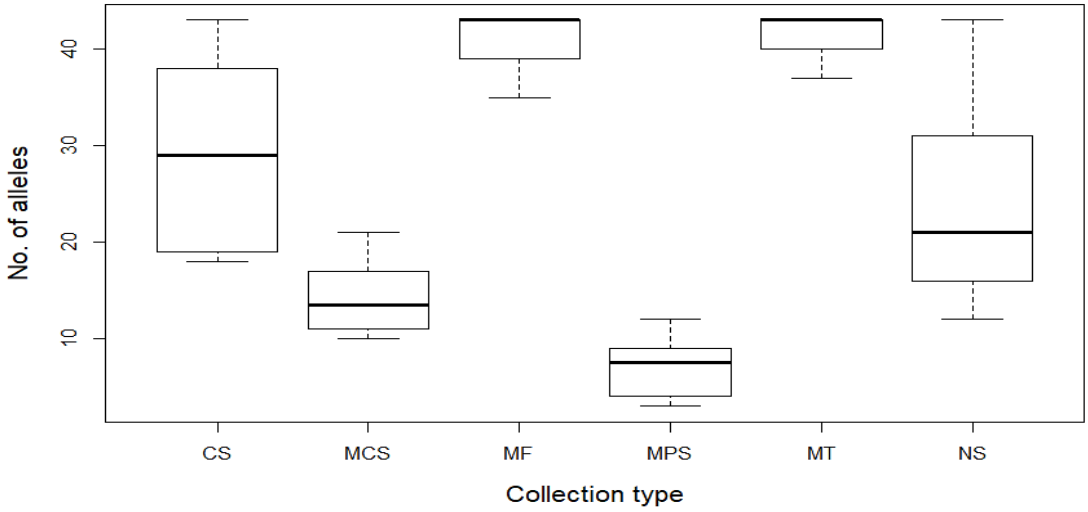


Figure 7.3: Number of alleles recovered from Touch DNA profiles (n= 72) collected from various of porous and non-porous surfaces by six collection methods, and generated by direct amplification with the GlobalFiler™ kit. MF: microFLOQ™ Direct swab, MPS: Mini Plastic Swab, MCS: Mini Cotton Swab, MT: Minitape, CS: Cotton Swab, and NS: Nylon flocked Swab. Mean alleles recovered: MF= 41, MPS= 7, MCS= 14, MT= 42, CS= 29, and NS= 24.

Samples were collected from a selection of surfaces to test the sensitivity of the collection method used for direct amplification. As it was previously observed in section 4.1 that different surface types could affect the recovery of Touch DNA. MF and MT produced full and partial DNA profiles with balanced loci; however, the other recovery methods only produced partial DNA profiles, or no data (Figure 7.4). In addition, direct amplification produced some artifacts in 40% of the collected samples, which were mostly split and shoulder peaks (Figure 7.5). Similar artifacts associated with direct PCR have been reported previously (Verheij *et al.,* 2012; Ambers *et al.,* 2018).

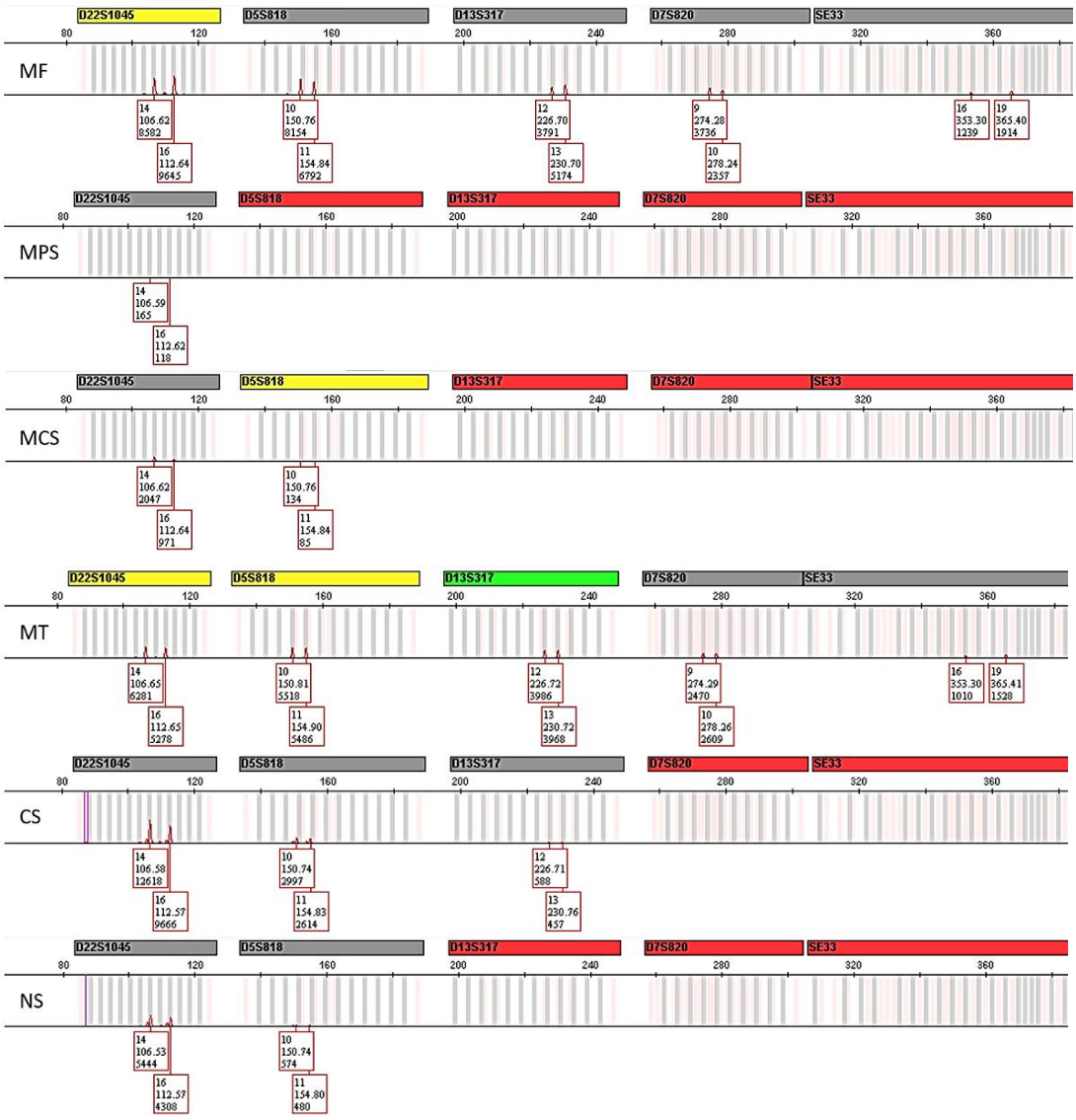


Figure 7.4: Comparison of electropherograms generated by direct amplification with the GlobalFiler™ kit between the six collection methods at five loci (D22S1045, D5S818, D13S317, D7S820, and SE33). Maximum volume of DNA was added. MF: microFLOQ™ swabs, MPS: Mini Plastic Swabs, MCS: Mini Cotton Swabs, MT: Minitapes, CS: Cotton Swabs, NS: Nylon flocked Swabs.

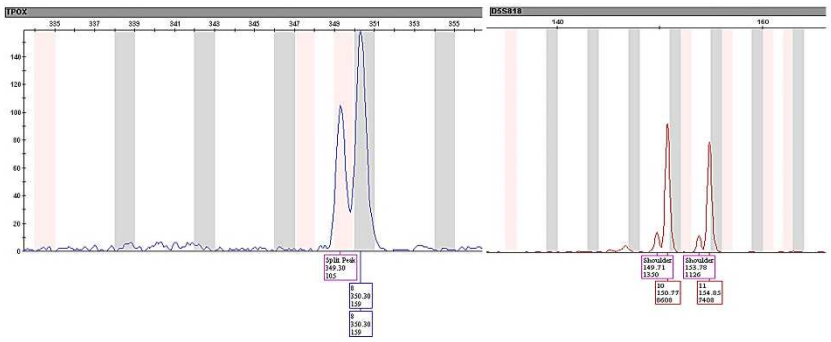


Figure 7.5: Split and shoulder peaks observed in most of the DNA profiles generated by the direct amplification of samples collected by the six different methods.

7.2 DIRECT AMPLIFICATION VERSUS EXTRACTION

Body fluid samples often yield relatively large volumes of DNA upon extraction, while trace samples generate far less DNA. During extraction, column-based purification methods can result in loss of DNA quantities, therefore impacting successful DNA profiling of degraded or low copy number samples (Barbaro *et al.,* 2004; Mumy *&* Findlay, 2004; Dabney *et al.,* 2013; Garvin *&* Fritsch, 2013; Noren *et al.,* 2013; Kemp *et al.,* 2014). In addition, this can be time-consuming, labour intensive, and increase the risk of cross-contamination. Considering that many standard amplification systems demand more than 0.1 ng to consistently produce full DNA profiles (Green *et al.,* 2012; Ensenberger *et al.,* 2016; Thermo Fisher Scientific, 2016; Kraemer *et al.,* 2017), the collected samples would initially require containing roughly 250 cells (approximately 1.45 ng) to retain enough quantity of DNA for amplification after extraction, whereas a direct PCR process only requires around 17 cells (Ottens *et al.,* 2013). Thus, this study investigated the efficiency of sample extraction versus direct amplification from Touch DNA collection from different deposition area sizes.

7.2.1 EXPERIMENTAL SETUP

Two participants, previously confirmed to be high and low shedders in Section 3.1., were instructed to perform the deposition process described in Section 2.2 on two different surface area sizes (a= 2.5 x 3.5 cm, and b= 5 x 7 cm) (n= 72, 36 replicates per each variable; direct amplification and extraction). DNA was deposited on the surfaces tested previously in Section 7.1, and each participant deposited their DNA separately, to avoid any contamination. Prior deposition, all non-porous surfaces were sterilised using 2% Virkon and UV-irradiation for 15 min, while porous surfaces were only irradiated using UV light for 25 min. This process was conducted at room temperature.

Deposited DNA was collected immediately using Copan nylon flocked swab (4N6FLOQSwabs®) (NS), and SceneSafe Fast™ minitape (K545) (MT) for extraction, while using MicroFLOQ™ Direct swab (MF), and also MT for direct amplification. The recovery process followed the same collection procedure detailed in Section 2.3. DNA was extracted from samples with the PrepFiler Express BTA™ kit using an AutoMate Express

Forensic DNA Extraction System (EXT1), following the procedures detailed in Section 2.4. However, 460 µL of lysis buffer was used instead of 230 µL, based on the finding from Section 4.2. Touch DNA was quantified using the Quantifiler™ Trio DNA Quantification Kit, following the procedures described in Section 2.5. For direct amplification, samples were transferred directly to PCR tubes (0.2 ml). The MF swab head was broken directly into the tube by bending the swab, and the tip of the MT (triangle shape of approximately 2 mm^2) was cut into the tube with sterilised scissors. Direct PCR tubes were prepared by adding PCR master mix (10 µL), while the reaction volume was made up with TE buffer (15 µL).

All the samples that were processed with extraction and direct amplification were then amplified and analysed as described in Section 2.6 and 2.7, to evaluate the quality of the samples collected. There was no sign of mixtures or contamination. Statistical analysis was performed following the procedures mentioned in Section 2.8. For analysis, when the total RFUs observed were reported, this was directly counted for homozygous loci and by adding the peak heights of each allele for heterozygous loci.

7.2.2 RESULTS

There was a noticeable difference on the average signal (RFU) between direct amplification (MT and MF) and extraction when samples were collected from the small surfaces area (2.5 x 3.5 cm) ($p < 0.05$). MF and MT with direct amplification generated higher peak heights when compared to extracted samples (RFU mean: MT. 5779, MF. 7354, and EXT. 3703) (Figures 7.6 & 7.7) (see Appendix A7.2 for the complete results).

However, samples were collected from a small area of the surfaces where the deposited cellular materials might have been concentrated, which means using MF and MT to collect Touch DNA from a small area is more effective than processing samples from extraction. The use of direct detection enabled DNA profiling from sample collection to final result in less than 2 hours, but similar artifacts (split and shoulder peaks) reported in Section 7.1 were observed. Nevertheless, true alleles in the DNA profiles were easy to distinguish, as they originated from a single source.

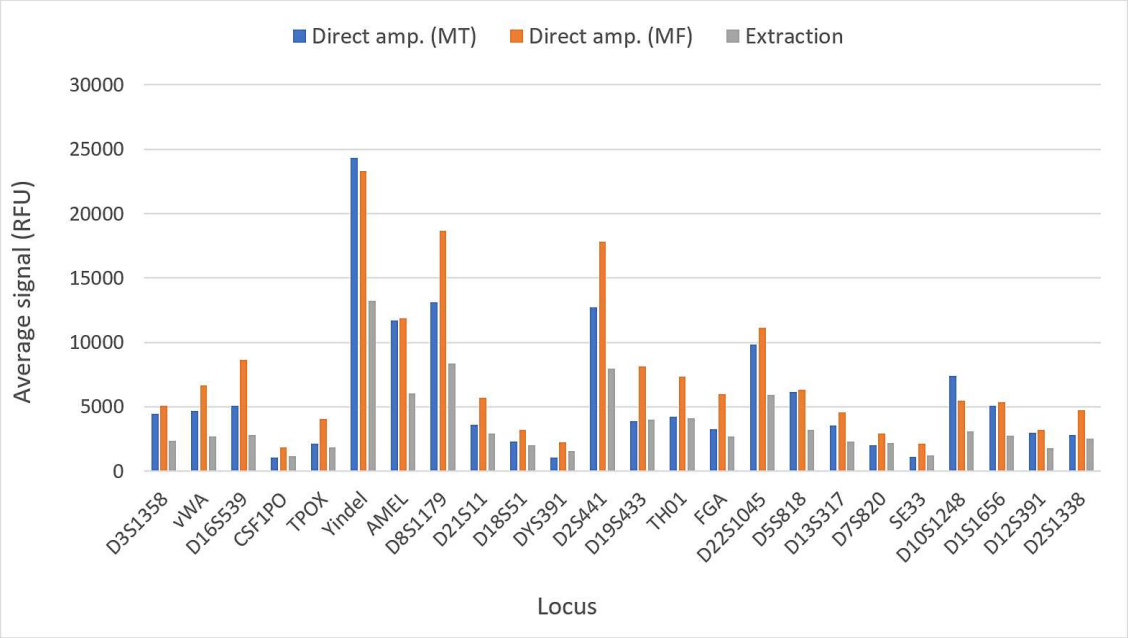


Figure 7.6: Average signal (RFU) per locus of samples (n= 72) collected from a small surface area (2.5 x 3.5 cm) and processed with direct amplification using MicroFLOQ swabs (MF) and Minitapes (MT) with GlobalFiler™ kit, versus samples processed with extraction using PrepFiler Express BTA™ kit and amplified with the GlobalFiler™ kit.

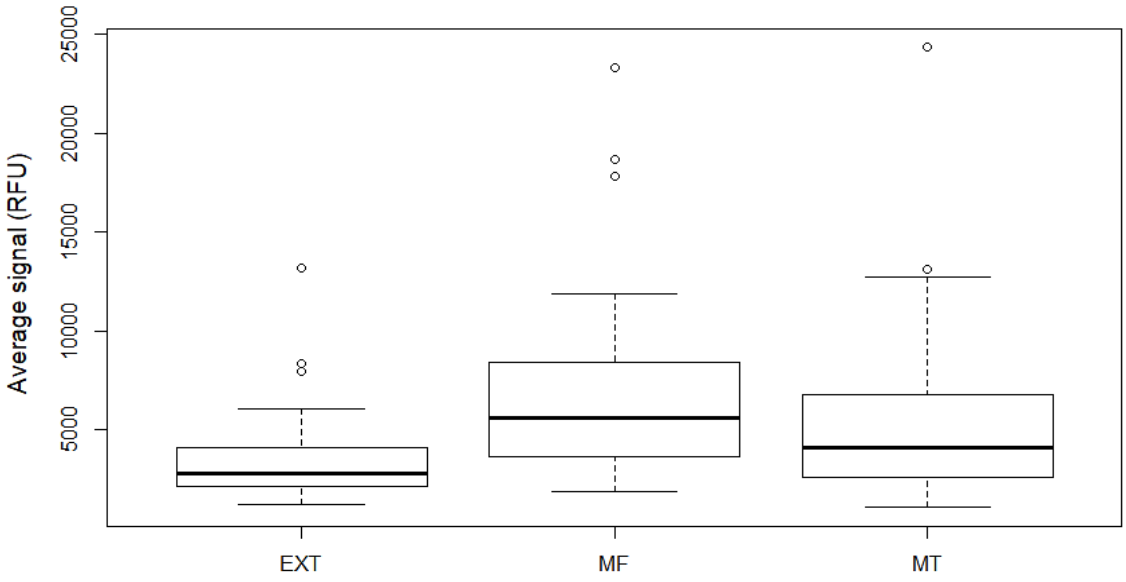


Figure 7.7: Mean of average signal (RFU) per locus of samples (n= 72) collected from a small surface area (2.5 x 3.5 cm) and processed with direct amplification using MicroFLOQ swabs (MF) and Minitapes (MT) with GlobalFiler™ kit, versus samples processed with extraction using PrepFiler Express BTA™ kit and amplified with the GlobalFiler™ kit.

In contrast, when samples were collected from a larger surface area (5 x 7 cm), there was a notable difference on the average signal (RFU) between direct amplification (MT and MF) and extraction ($p < 0.001$). Extracted samples generated higher peak heights when compared to microFLOQ™ swab (MF) and Minitape (MT) with direct amplification (RFU mean: MT. 6201, MF. 7105, and EXT. 13859) (Figures 7.8 & 7.9) (see Appendix A7.3 for the complete results).

Even though DNA extraction from samples prior to amplification increased the sample processing time for DNA profiling, DNA extraction has a slight advantage over direct amplification when more cellular materials are collected from the surface. In addition, MF collects only minute amounts of trace DNA from the surface, because of its small swab tip design, which leads to some DNA being left uncollected from the surface. Therefore, a combination of using MF for direct amplification and collecting the rest of the sample from the surface using another swab as a backup would be beneficial, to maximise the retrieval of DNA from the surface.

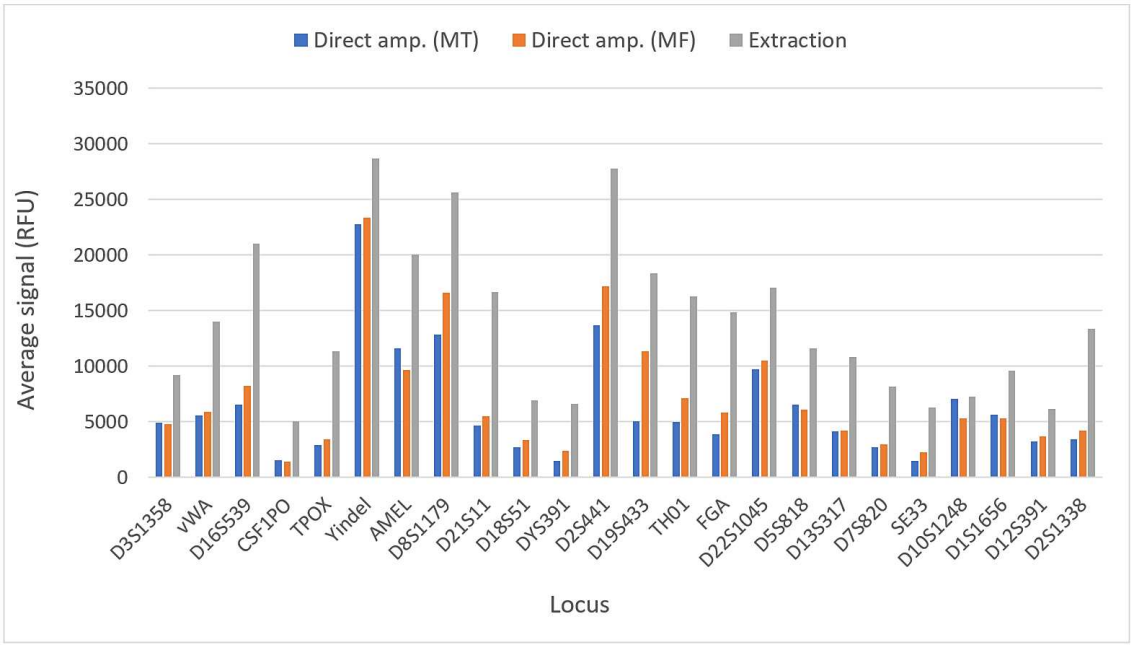


Figure 7.8: Average signal (RFU) per locus of samples (n= 72) collected from a large surface area (5 x 7 cm) and processed with direct amplification using MicroFLOQ swabs (MF) and Minitapes (MT) with GlobalFiler™ kit, versus samples processed with extraction using PrepFiler Express BTA™ kit and amplified with the GlobalFiler™ kit.

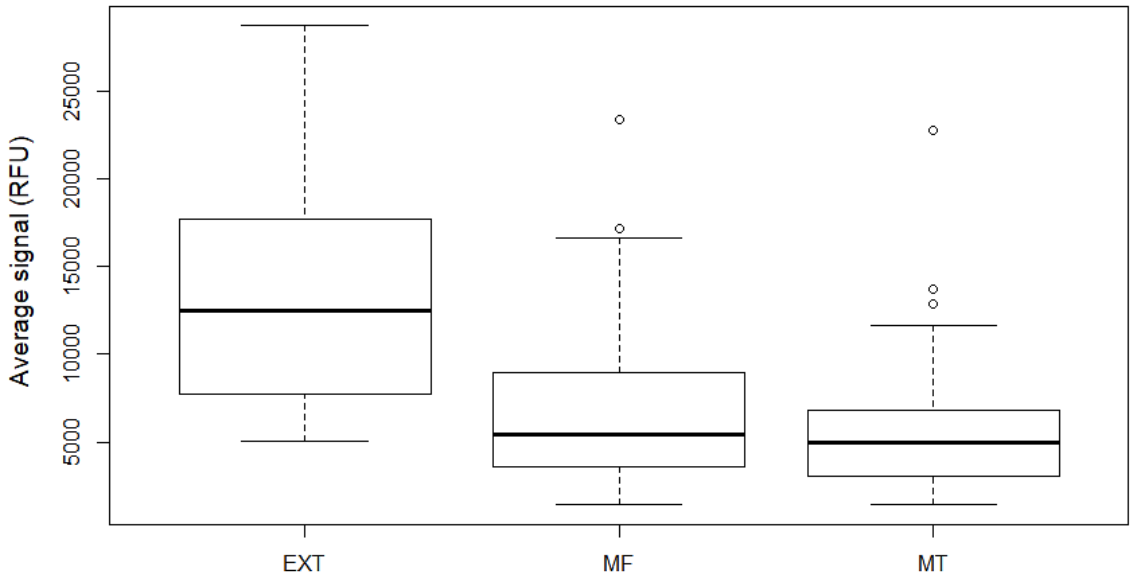


Figure 7.9: Mean of average signal (RFU) per locus of samples (n= 72) collected from a large surface area (5 x 7 cm) processed with direct amplification using MicroFLOQ swabs (MF) and Minitapes (MT) with GlobalFiler™ kit, versus samples processed with extraction using PrepFiler Express BTA™ kit and amplified with the GlobalFiler™ kit.

7.3 INNOVATIVE SOLUTION

Direct PCR has the prospect of becoming a useful application for the analysis of Touch DNA evidence samples, which often face the limits of detection when using standard methodologies. However, there is limited shared knowledge regarding collecting techniques of trace DNA for direct PCR from casework samples (Cavanaugh *et al.,* 2018). Cutting a small piece of the fabric to process for direct amplification has proven to be an effective procedure, though it can be hindered by some PCR inhibitors (Linacre *et al.,* 2010). In addition, collecting trace DNA from fabric for direct PCR is more challenging for casework samples, as Touch DNA is invisible when compared to stains of blood (Verheij *et al.,* 2012), since the small piece of fabric (approximately 2 mm^2) processed for direct PCR might not contain DNA. The finding of Section 7.2 proposed that a combination between direct PCR and extraction systems to address casework samples can maximise Touch DNA recovery from the surface and be cost-effective. Therefore, an innovative solution was explored to recover Touch DNA using a cotton swab in combination with a microFLOQ® swab for direct PCR, to allow the preservation of the collected samples for re-analysis or additional testing.

7.3.1 EXPERIMENTAL SETUP

Two participants, previously evaluated to be high and low shedders in Section 3.1., were asked to perform the deposition process described in Section 2.2 on a 5 x 7 cm area of the test surfaces (n= 48, 24 replicates per each variable; direct amplification and extraction). Biological materials were deposited on the surfaces tested previously in Section 7.1, while each participant deposited their DNA separately to avoid any contamination. Prior to deposition, all non-porous surfaces were sterilised using 2% Virkon and UV-irradiation for 15 min, while porous surfaces were only irradiated using UV light for 25 min. This process was conducted at room temperature.

Deposited DNA was collected immediately using a moistened Copan cotton swab (150C) (CS) following the same collection procedure detailed in Section 2.3. Then, MicroFLOQ™ Direct swab (MF) and SceneSafe Fast™ minitape (K545) (MT) were used to collect the biological materials from the cotton swab (innovative solution), following the same collection procedure detailed in Section 2.3.

Samples collected by MF and MT were processed for direct amplification, and transferred directly to PCR tubes (0.2 ml). The MF swab head was broken directly into the tube by bending the swab, and the tip of the MT (triangle of approximately 2 mm^2) were cut into the tube by sterilised scissors. Direct PCR tubes were prepared by adding PCR master mix (10 µL), while the reaction volume was made up with TE buffer (15 µL).

Samples collected by CS were extracted with the PrepFiler Express BTA™ kit using an AutoMate Express Forensic DNA Extraction System (EXT1), following the procedures detailed in Section 2.4. However, 460 µL of lysis buffer was used instead of 230 µL based on the finding from Section 4.2. Then, quantified using the Quantifiler™ Trio DNA Quantification Kit, following the procedures described in Section 2.5. All the samples that were processed with extraction and direct amplification were then amplified and analysed as described in Sections 2.6 and 2.7, to evaluate the quality of the samples collected. There was no sign of mixtures or contamination. Statistical analysis was performed following the procedures mentioned in Section 2.8. For analysis, when the total number of alleles observed was reported, homozygous loci were counted as one allele, while heterozygous loci were counted as two alleles.

7.3.2 RESULTS

Touch DNA was first collected by CS to concentrate the biological materials in a small area, to be then collected with MF and MT (innovative solution). All extracted samples collected by CS produced full single DNA profiles (100% allele recovery), however allele recovery by direct amplification ranged from 84–88% (figure 7.10) (see Appendix A7.4 for the complete results). When comparing the number of alleles obtained from direct amplification with MF and MT to the amount of DNA collected by cotton swabs (CS), it was noticeable that the number of alleles collected by direct amplification was impacted by the amount of DNA collected by CS ($p < 0.001$). The number of alleles observed increased when there was more DNA collected by the cotton swabs (> 0.03 ng/µL) (Figure 7.11). Therefore, the amount of DNA deposited on the surface can influence the success of direct amplification. However, the innovative solution has proven to be effective when there is 30 pg or more of DNA available at the tested surface.

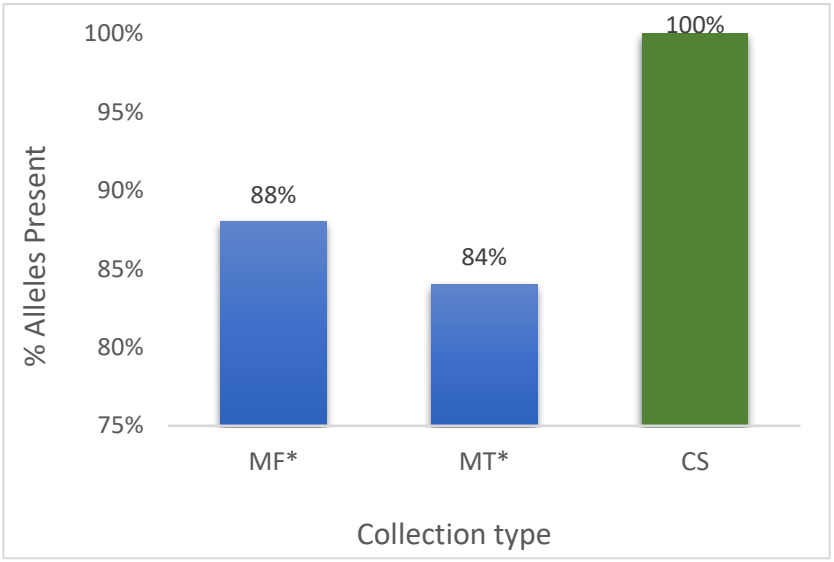


Figure 7.10: Percentage of alleles recovered from DNA profiles collected by the Innovative solution (MF* and MT*) and amplified directly with GlobalFiler™ PCR Kit (n= 24), versus samples collected by cotton swabs (CS) and extracted using PrepFiler Express BTA™ kit then amplified with the GlobalFiler™ kit (n= 24). *MF*:* collected biological materials from cotton swabs using microFLOQ™ Direct swab, *MT*:* collected biological materials from cotton swabs using SceneSafe Fast™ minitape (K545).

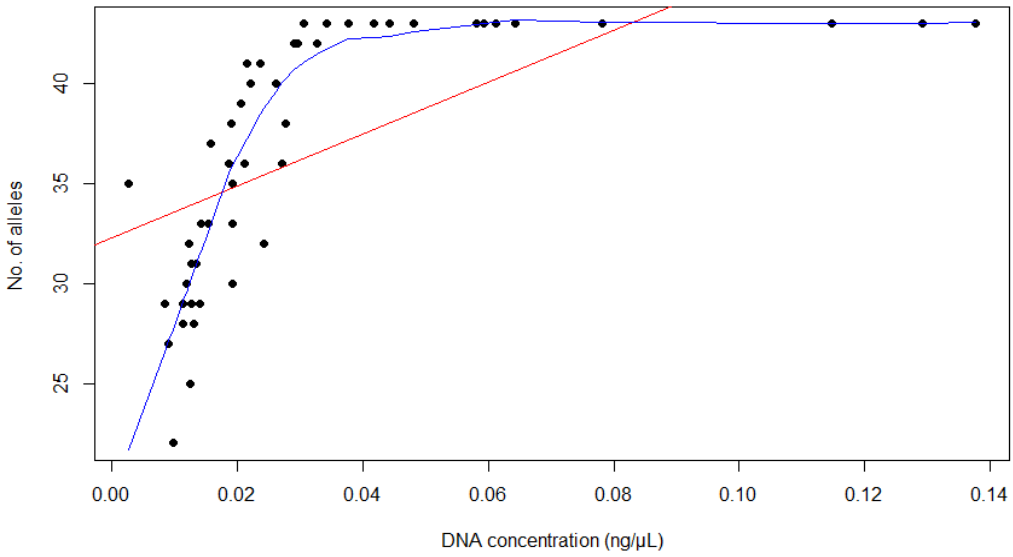


Figure 7.11: The relationship between the number of alleles observed in the DNA profiles (n= 24) collected by the Innovative solution (MF and MT) and amplified directly with GlobalFiler™ PCR Kit, versus the amount of DNA collected by cotton swabs (CS) (n= 24). The number of alleles observed increased when there was more DNA collected by the cotton swabs (> 0.03 ng/µL). The red and blue lines represent the regression (y~x: line of best fit), and lowess (x,y: line of the relationship between variables) lines, respectively.

7.4 EVALUATION OF MicroFLOQ™ swab

Due to the ingrained variability of Touch DNA samples, DNA profiling success cannot be ensured from any type of item. However, some collection methods have more advantages than others for effective direct amplification. When compared to foam and cotton swabs, nylon flocked swabs were found to produce the highest profile peak following direct PCR (Templeton *et al.,* 2013). The composition of nylon swabs enables increased efficiency of sample recovery and biological material release during extraction when compared to traditional swabs (Benshop *et al.,* 2010; Dadhania *et al.,* 2013). MicroFLOQ® Direct nylon swab is a new swab co-developed by the French Gendarmerie Forensic Research Institute, IRCGN™ and Copan, for direct PCR after evidence recovery. The micro-fibres of microFLOQ® Direct swab are designed in the same fashion as 4N6 FLOQSwabs®, to eliminate the need for DNA extraction and quantification, and produce DNA profiles within 2 hours (IRCGN™ & Copan, 2022). These have proven successful for saliva and blood (Ambers *et al.,* 2018), but for trace DNA more validation is required. Therefore, microFLOQ™ Direct swab was evaluated, by recovering Touch DNA from office items to replicate casework samples.

7.4.1 EXPERIMENTAL SETUP

MicroFLOQ Direct swab (MF) were used to recover trace DNA following the same collection procedure detailed in Section 2.3. Biological materials were recovered from a diverse set of surfaces that can be found in an office space, to examine the efficiency of MF. The surfaces were chosen randomly to simulate casework workflow, and the period of DNA deposition on these surfaces was unknown. The surfaces included three computer mouses, three computer keyboards, two door handles, two window handles, two pens, an old handprint on a window, a leather wallet, and a cell phone (Figure 7.12).

After collection, samples were processed for direct amplification by transferring them directly to PCR tubes (0.2 ml). The MF swab head was broken directly into the tube by bending the swab and the tip of the MT (triangle shape approximately 2 mm^2) was cut into the tube with sterile scissors. Direct PCR tubes were prepared by adding PCR master mix (10 µL), while the reaction volume was made up with TE buffer (15 µL).

Samples then were amplified and analysed as described in Sections 2.6 and 2.7, to evaluate the quality of the samples collected. Statistical analysis was performed following the procedures mentioned in Section 2.8.

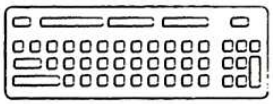
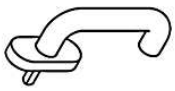
	Items	quantity
	Computer mouse (CM)	3
	Computer keyboard (CK)	3
	Door handle (DH)	2
	Window handle (WH)	2
	Pen (PN)	2
	Handprint on a window (HP)	1
	Leather wallet (LW)	1
	Cell phone (CP)	1

Figure 7.12: Office items used for Touch DNA collection with MicroFLOQ Direct swab (MF) to be processed for direct amplification with GlobalFiler™ PCR Kit.

7.4.2 RESULTS

The results of using MF with GlobalFiler™ amplification Kit for direct PCR, to recover Touch DNA from random items in an office area produced a 73% success rate within three hours from sample collection.

From a total of 15 collected samples, 11 STR profiles were good enough to use for database search (Based on the Biology and DNA Section lab in the General Department of Forensic Science and Criminology), with samples producing full single, partial single, full mixture, and partial mixture DNA profiles (Figures 7.13 & 7.14). However, it was unknown weather some of these items were used or cleaned, which might have impacted the success of DNA profiling.

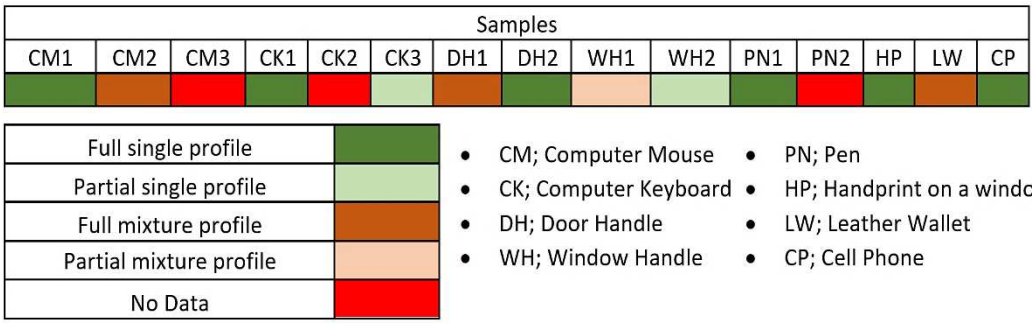


Figure 7.13: Trace DNA profiles (n= 15) recovered from office items with MicroFLOQ Direct swab (MF) and directly amplified using GlobalFiler™ PCR Kit.

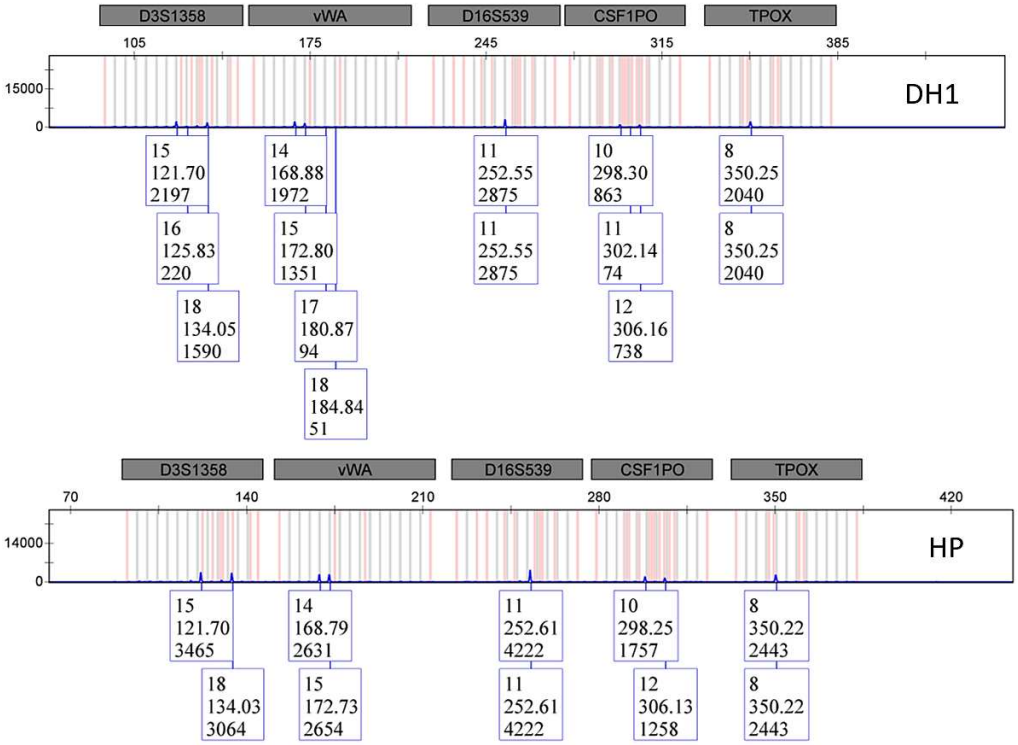


Figure 7.14: Electropherograms of samples collected from a door handle (DH1) and from a visible handprint on the window (HP). The profiles show the difference in peak height at five autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, and TPOX). Maximum volume of DNA was added.

7.5 CHAPTER DISCUSSION AND CONCLUSION

Direct amplification is a sample processing procedure, in which an evidence swab or a small piece of substrate (approximately 2 mm^2) is added directly to a PCR reaction, without prior extraction or quantification. This has the potential to ameliorate the generation of genotyping data from such samples. The success of the direct amplification of trace DNA is subjected to the amount of biological material available on the surface; however, it can be improved by an effective recovery method.

By comparing six collection methods in the study of Touch DNA collected from a selection of surfaces, it was observed that surface type influenced recovery method. However, MicroFLOQ™ Direct swab and SceneSafe Fast™ minitape (K545) were the most reliable to collect and process Touch DNA samples for direct PCR with the GlobalFiler™ PCR Amplification Kit. MicroFLOQ™ Direct swab has a more appropriate design, which enables easy recovery and sample processing, when compared to the minitape, which demands more handling.

By comparing direct amplification to extraction, direct PCR produced the highest DNA yield, as evaluated by the relative fluorescence units (RFU) of peak heights. Previous studies came to the same conclusion when investigating methods for collection and direct amplification of Touch DNA against extraction (Templeton *et al.,* 2013; Liu, 2015). However, these results were based on samples collected from fingerprints.

This study reported that extraction has a slight advantage over direct amplification, when samples were collected from larger surface area (5 x 7 cm). Since there was more biological material to be retrieved from the surface, MicroFLOQ™ Direct swab, which has a small swab tip design, is not efficient for covering more surface area. In addition, common inhibitors found in samples are often removed during extraction, such as indigo carmine dye in denim fabrics, which can impact direct amplification (Yoshii *et al.,* 1993). However, this was not an issue in the directly amplified samples in this study.

Furthermore, this study proposed an innovative solution: a combination of using cotton swab and MicroFLOQ™ Direct swab to maximise DNA recovery from the tested surface (Alketbi, 2022a). The use of a cotton swab to collect the biological material, then

swabbing the cotton swab with a microFLOQ® direct swab to recover the DNA was a successful method for direct PCR. Swabbing with cotton swab covers more surface area, which allow more recovery of DNA. This process concentrates the biological material in a small surface area for a better sample collection with the microFLOQ® Direct swab, and allows re-analysis or additional testing if needed, which is a limitation of using direct PCR alone.

The method of direct amplification should be evaluated for casework samples that contain low amounts of DNA, to better understand how to use it more effectively. MicroFLOQ™ Direct swab has the potential to improve Touch DNA profiling, as it is time- and cost-effective. However, there are some observed effects with direct PCR, such as increased stutter ratios, elevated baseline, heterozygous allele imbalance, and split and shoulder peaks (Gouveia *et al.,* 2015), which can make data interpretation of a mixture of DNA profiles much more challenging.

CHAPTER EIGHT

8. TRACE DNA CASEWORK AT DUBAI POLICE FORENSIC DEPARTMENT

This chapter gave a brief overview of the city of Dubai, its police force history and the establishment of the General Department of Forensic Science and Criminology and more importantly, the Department of Specialised Evidence and the role of the divisions within the Biology and DNA Section. In addition, this chapter covered the number of cases received and the total number of samples processed by the Biology and DNA Section in the last three years, particularly trace DNA success rates relating to casework samples. Finally, recommended procedures to improve Touch DNA recovery were applied to forensic casework received in the Biology and DNA Section, and the results were reported in this study.

8.1 DUBAI AND ITS POLICE FORENSIC FORCE

Dubai is one of the seven cities often referred to as Emirates that make up the United Arab Emirates (UAE) (Figure 8.1). Abu Dhabi (AD) is the nation's capital city, while Dubai is the most populous city, with a population of around 3.4 million (Dubai Statistics Centre, 2015). In the early 18th century, Dubai was thought to have been founded as a fishing village (Nonneman, 1999) but nowadays, it is an international hub hosting 195 nationalities living and working in the city (The United Arab Emirates Government Portal, 2022).

Dubai Police Force was instituted in 1956 to protect the people of the city and to serve justice. It is thought to be the most progressive of all Arab police forces for introducing new advanced law enforcement techniques in the region, which include electronic fingerprinting and forensic DNA testing (Al Theeb, 2006).

Besides the eleven police stations covering the city, the Dubai Police Force has many departments that include the General Department of Operations, General Department of Artificial Intelligence, General Department of Criminal Investigation, and General Department of Forensic Science and Criminology, which all work together to fight crime and establish a sense of security (Dubai Police, 2022).

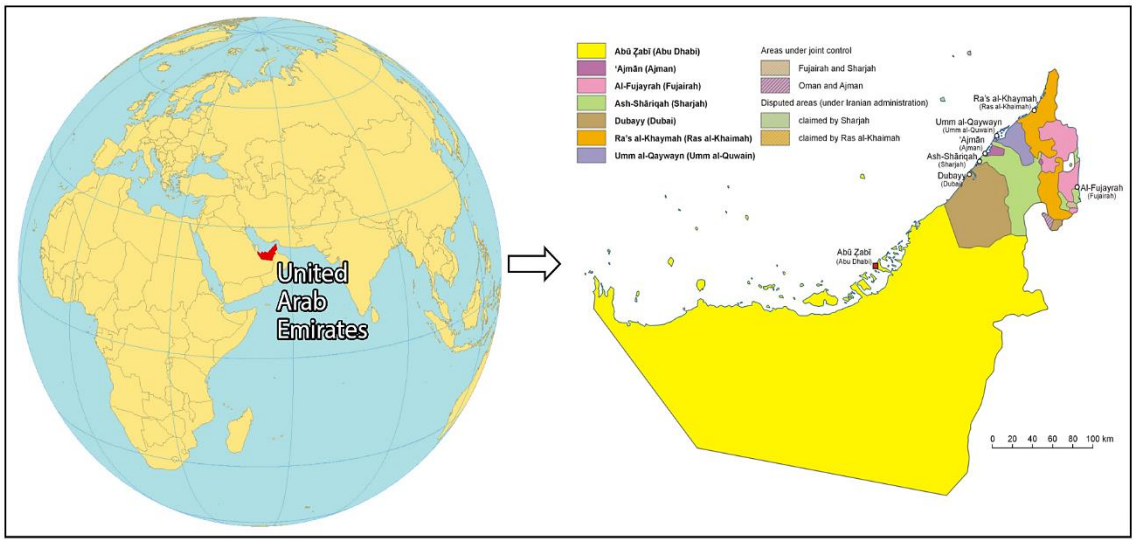


Figure 8.1: Map of the United Arab Emirates (UAE) and its seven cities; Abu Dhabi, Dubai, Sharjah, Ajman, Ras Al Khaimah, Fujairah and Umm al-Quwain (adapted from Map produced by Maps.com, 1999).

The first Forensic Lab was established in 1981 under the General Department of Criminal Investigation. In 2016, the Dubai Police founded one of the largest forensic labs in the Middle East and instituted the General Department of Forensic Science and Criminology fully occupied by highly proficient staff including forensic scientists, experts, technicians and medical examiners who were estimated to work on more than forty thousand forensic cases per year (Dubai Police, 2022).

Furthermore, the Forensic Department has many international accreditations including ISO 17025 and ASCLD, and in May of 2022, it launched the first regional forensic science platform which focuses on collaborative research and training (Fouda, 2022; International Centre for Forensic Sciences, 2022). The General Department of Forensic Science and Criminology has eight major departments including the Fingerprint Department, Electronic Evidence Department, Forensic Medicine Department, Department of Specialised Evidence, and others.

The Biology and DNA Section is one of the largest in the Department of Specialised Evidence (Figure 8.2) and consists of the Biology division, DNA division, and Reference samples division. The Biology division is responsible for the examination of exhibits, presumptive/confirmatory tests and sample collection, while the DNA division is responsible for the DNA profiling process starting from DNA extraction to data analysis

and DNA database comparison. The Reference samples division deals with all the suspects or individuals in question with samples starting from direct amplification to data analysis and DNA database comparison.

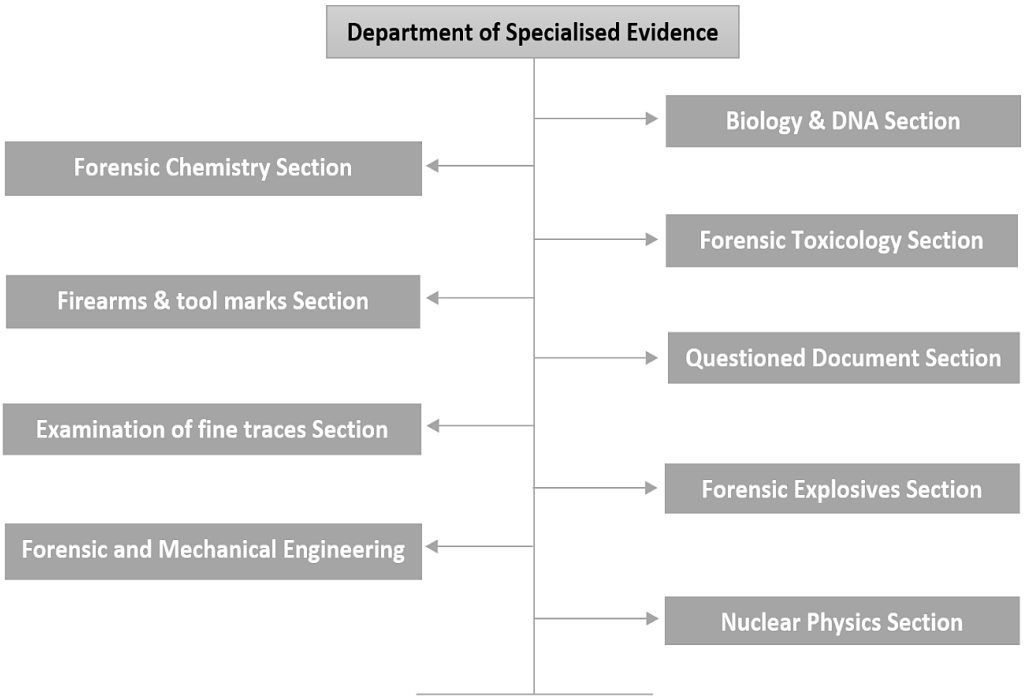


Figure 8.2: Sections of the Department of Specialised Evidence in the General Department of Forensic Science and Criminology of Dubai Police Force.

8.2 NUMBER OF CASES AND TRACE DNA SUCCESS RATES

During the last six years, the Biology and DNA Section of the General Department of Forensic Science and Criminology of Dubai Police Force received an average of two thousand cases per calendar year ranging from homicide, suicide, sexual assault, missing persons, paternity, burglary, etc.

A total of 6277 cases were received between 2019 to 2021 (Figure 8.3), with each crime case comprising one or multiple exhibits. In addition, 14552 samples were collected from the examined items, of which only 8696 samples were processed for DNA profiling (Figure 8.4). These samples produced 7103 positive DNA results with a success rate of 82%. Positive DNA results in the Biology and DNA Section refer to a DNA profile containing homozygous or heterozygous, or even a mixture of alleles in at least nine loci of a sample amplified with the GlobalFiler™ PCR Amplification Kit.

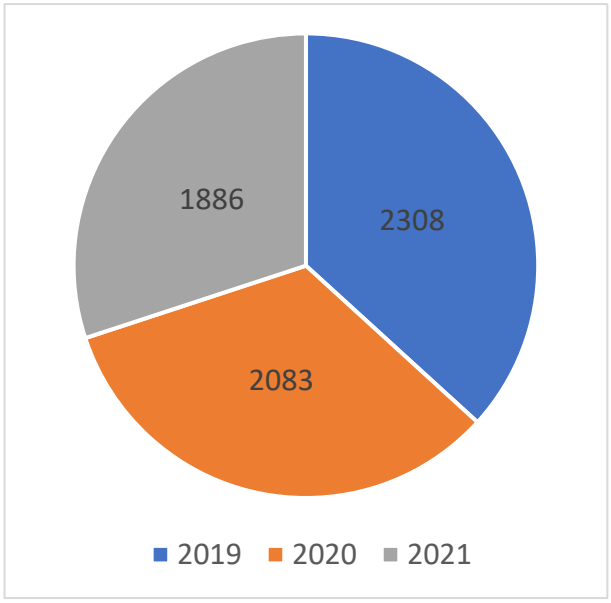


Figure 8.3: Number of cases (n= 6277) received between 2019 to 2021 in the Biology and DNA section in the General Department of Forensic Science and Criminology of Dubai Police Force.

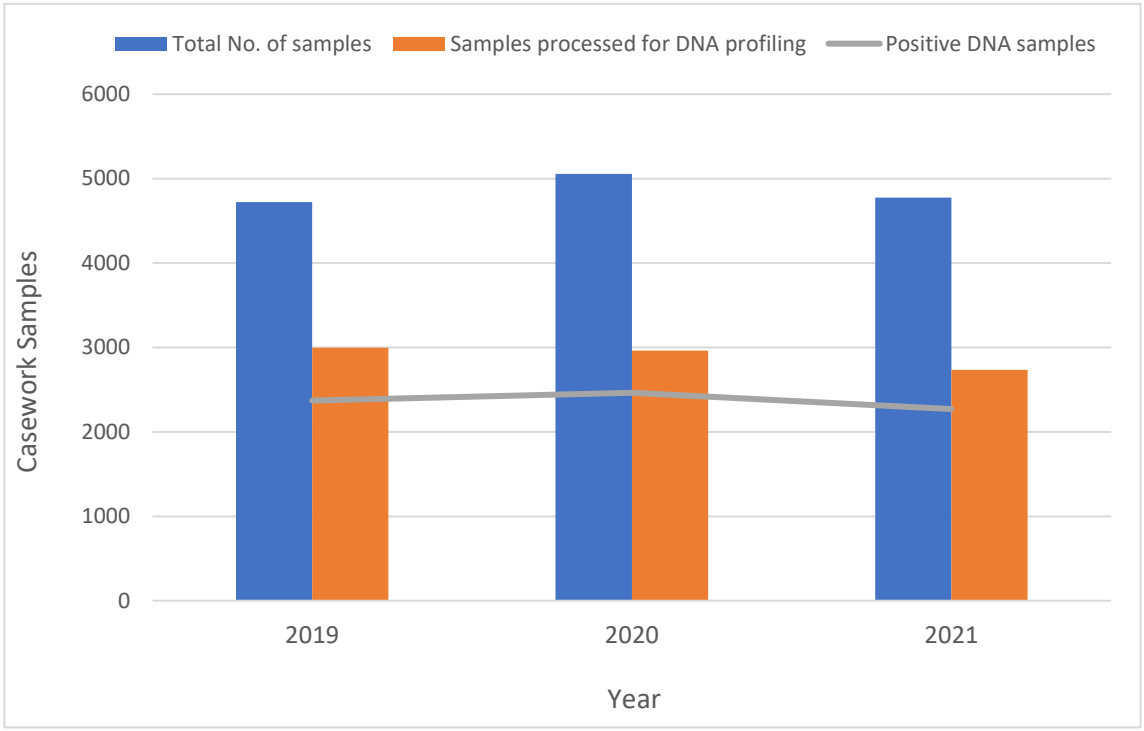


Figure 8.4: Total number of samples (n= 14552, avg. 4851), samples processed for DNA profiling (n= 8696, avg. 2899) and number of positive DNA samples (n= 7103, avg. 2368) between 2019 to 2021 in the Biology and DNA section in the General Department of Forensic Science and Criminology of Dubai Police Force.

From the total number of samples processed for DNA profiling between 2019 to 2021, 5488 (63%) were trace samples recovered from various touched or used items. The samples were sorted into six different categories (Figure 8.5), tools (e.g. screwdrivers and anything used as a weapon such as knives, axes, machetes, bats etc.), stolen items (e.g. mobile phones, wallets, handbags, etc.), wearable items (clothes, shoes/sandals, jewellery, glasses, etc.), packaging (e.g. plastic or any type of bags or containers used to hold drugs), vehicles (e.g. cars, motorcycles, trucks, buses, scooters etc.), and any other touched items.

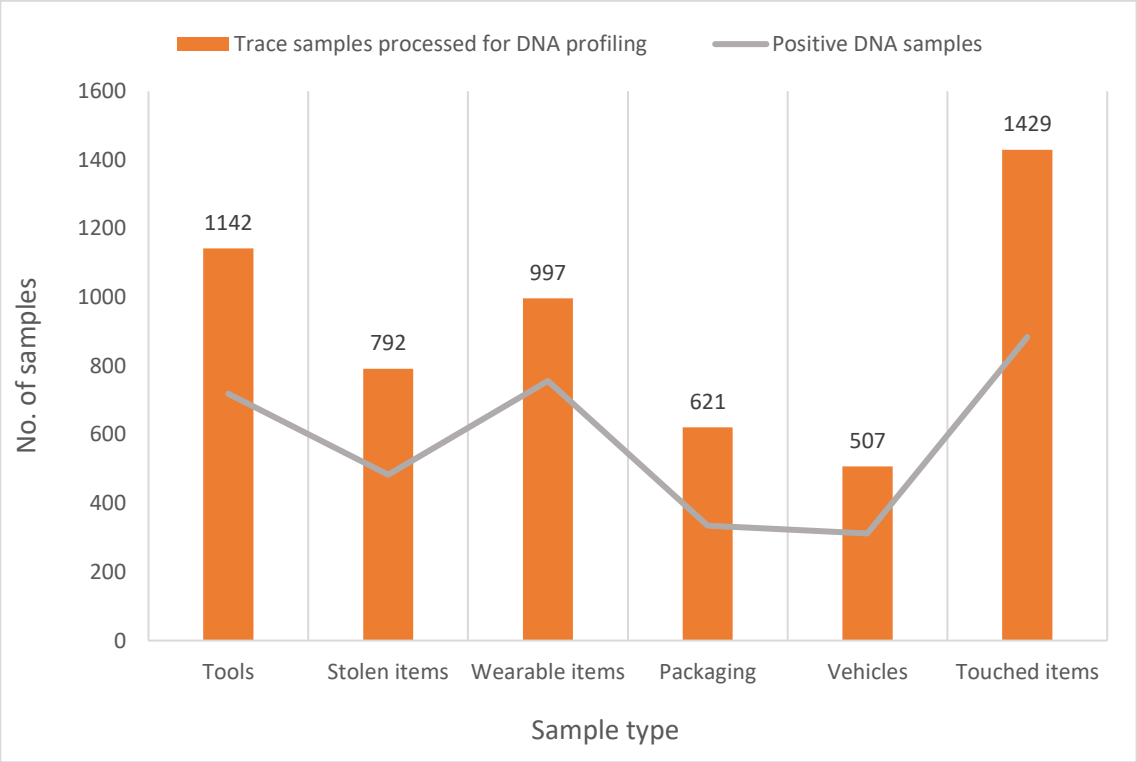


Figure 8.5: Total number of trace samples (n= 5488) processed for DNA profiling between 2019 to 2021 sorted into six different categories: tools, stolen items, wearable items, packaging, vehicles and touched items.

The success rate of trace samples was 64%, with only 3489 producing positive DNA results. Regarding each category, it was noted that samples collected from wearable items achieved the highest success rate (76%), and samples collected from packaging had the lowest success rate (54%), while the rest of the samples from the other categories had an average of 62% success rate (Figure 8.6). Wearable items such as clothes usually produce positive DNA results when compared to other touched items as

discussed in Section 6.2, and that can be related to the DNA of the wearer often present in the DNA profile produced. In contrast, packaging items used to hold illegal substances such as drugs are often small and when found go through different check-up points, which could impact the amount of biological material deposited on those items.

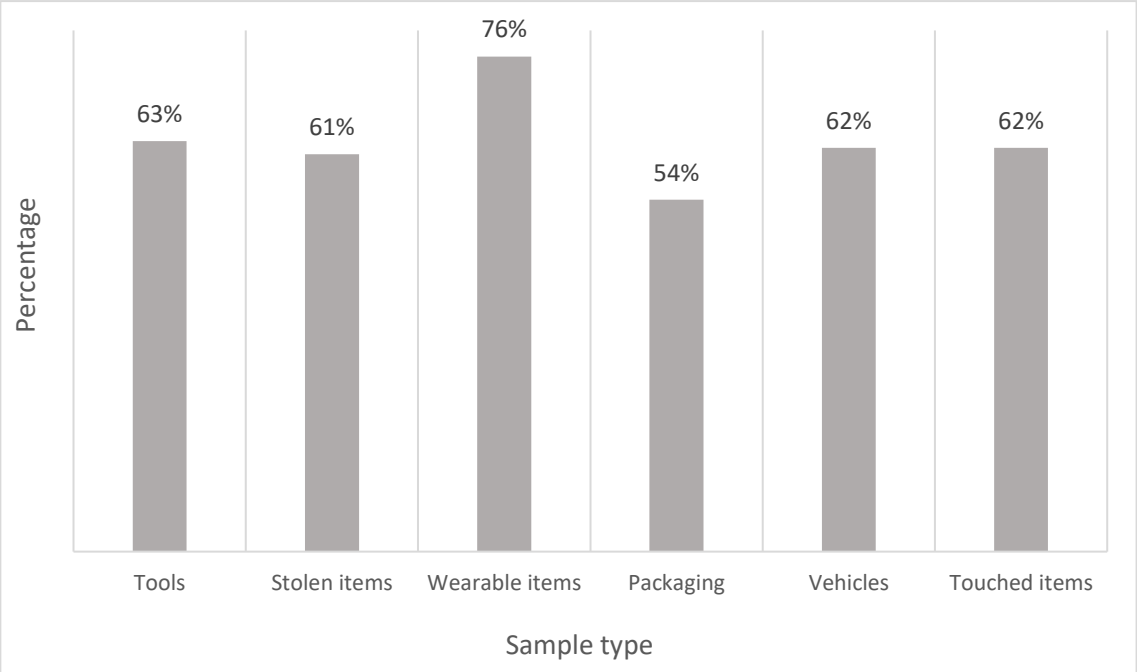


Figure 8.6: Success rate of the positive DNA trace samples (n= 3489) that was processed for DNA profiling between 2019 to 2021 sorted into six different categories; tools, stolen items, wearable items, packaging, vehicles and touched items.

All samples were collected using cotton swabs moistened with 100 µL of molecular grade water using a plastic spray bottle (Figure 2.3 – Section 2.3) and then extracted with the PrepFiler Express kit (Thermo Fisher Scientific) using the liquid handling and automation Tecan robot according to the manufacturer's recommendations. Extracted samples were quantified using Qiagen Investigator Quantiplex Pro Quantification Kit and the QuantStudio 5 Real-Time PCR (qPCR) system with HID Real-Time PCR analysis software (Thermo Fisher Scientific) according to the manufacturer's instructions. Then, 15 µL of the extract (maximum volume of DNA was added, diluted if required) was amplified in 25 µL and 29 cycles using the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific) on an ABI 3500xl Genetic Analyser.

8.3 IMPLEMENTATION OF THE RECOMMENDED PROCEDURES TO IMPROVE TOUCH DNA RECOVERY FROM FORENSIC CASEWORK

Surfaces retain biological materials deposited by touching differently and the results presented in previous chapters suggested that different methods or techniques to collect and process Touch DNA are beneficial for Touch DNA profiling. Therefore, this fieldwork aimed to apply different collection methods and techniques developed in this study to recover Touch DNA from casework samples in the Biology and DNA section of the General Department of Forensic Science and Criminology and compare the results to the samples recovered by the lab.

8.3.1 METHODOLOGY

Casework items were examined by the lab assistants and experts that were assigned these cases at the biology laboratory. Samples were first recovered by the lab tech as a priority to solve the crime. However, for the purpose of this study, samples were collected simultaneously from the same item by the lab assistants/experts to have a compatible process, which allows for better comparison of the results. The lab trace samples were collected by cotton swab as it is the method used to recover all sample types, then were dried in the fume hood until processed for DNA profiling following the procedures described in Section 8.2.

For this study, trace DNA recovery was recovered using a Copan cotton swab (150C) (CS), Copan nylon flocked swab (4N6FLOQSwabs®) (NS), and SceneSafe Fast™ minitape (1-Tape Kit) (MT) for extraction, and microFLOQ™ Direct swab (MF) (Copan) for direct amplification (Figure 2.2 – Section 2.3). Each collection method was used to recover trace DNA from different surfaces as detailed in Table 8.1, following the same collection procedure detailed in Section 2.3 and summarised in Table 8.2. After sample collection, the samples were stored in a freezer until extraction which took place in the same week of sample recovery. Samples were extracted with PrepFiler Express BTA™ Kit using an AutoMate Express Forensic DNA Extraction System as described in Section 2.4. However, based on the finding from Section 4.2, 460 µL of lysis buffer was used instead of 230 µL with a full swab or full minitape.

Table 8.1: Description of each collection method used to collect trace samples from casework items.

Collection method	Used for
Cotton swabs (CS)	Smooth non-porous surfaces.
Nylon swabs (NS)	Textured non-porous surfaces and for all type of non-porous sandy surfaces.
Minitapes (MT)	Fabric items and for all type of porous surfaces.
MicroFLOQ™ swabs (MF)	All type of surfaces for direct amplification and for dual recovery from cotton swabs (CS+MF) to create an innovative solution to save money and time.

Table 8.2: Summary of the methodology used for each collection method.

Collection method	Used for
Cotton swabs (CS)	A volume of 100 µL of molecular grade water was applied to moisten the swab using a plastic spray bottle (approx. 3-4 sprays). Then the swab was cut by scissors directly into an extraction tube and stored in the freezer while moist until extraction.
Nylon swabs (NS)	A volume of 30 µL of molecular grade water was applied to moisten the swab using a plastic spray bottle (approx. 1-2 sprays). Then swab was self-broken directly into an extraction tubes and stored in freezer until extraction.
Minitapes (MT)	No water was added to the MT, but to increase the amount of Touch DNA collected, each minitape was applied 16 times to the area. The MT was cut into small pieces by scissors into an extraction tube and stored in a freezer until extraction.
MicroFLOQ™ swabs (MF)	A volume of 1 µL of molecular grade water was added to the swabs prior to collection as recommended by the manufacturer. Then, the swabs were self-broken directly into a PCR tube (0.2

	ml) and amplified directly by the GlobalFiler™ PCR Amplification Kit. The volume of sample solution required by the kit manufacturer was replaced with TE, PCR master mix was added directly to the tube and immediately amplified.

Extracted samples were quantified using the Quantifiler™ Trio DNA Quantification Kit following the procedures in Section 2.5. Amplification of all samples was performed with the GlobalFiler™ PCR amplification Kit as described in Section 2.6 on an ABI GeneAmp® 9700 PCR System (Life Technologies) for 29 cycles, according to the manufacturer's recommended conditions. Amplified products were size-separated and detected on an ABI 3500xl Genetic Analyser as described in Section 2.7. Electrophoresis was performed on a 36-cm capillary array with POP-4™ polymer (Life Technologies) using standard injection parameters (1.2 kV, 24 s). STR data were sized and typed with GeneMapper® ID-X Software Version 1.2 (Life Technologies) using the manufacturer-validated analytical thresholds. Statistical analysis was performed as described in Section 2.8. For analysis, when the total RFUs observed were reported, this was directly counted for homozygous loci and by adding the peak heights of each allele for heterozygous loci.

8.3.2 RESULTS OF THE CASEWORK SAMPLES

Over the three months from September to December of 2021, 256 trace samples were collected using various collection methods as discussed below. Results from each category were compared directly to lab trace samples which were collected solely with cotton swabs.

8.3.2.1 COTTON SWAB (CS) SAMPLES

The amount of trace DNA collected from smooth non-porous surfaces of casework items (n=52) was affected by the collection technique used with the cotton swab (CS) ($p < 0.05$) (see Appendix A8.1 for the results). More DNA was collected with the technique developed in this study when swabs were frozen (a), while moist prior to extraction than when the swabs were dried before extraction (b; the lab technique) (mean a. 0.18 and b. 0.07 all in ng/µL) (Figure 8.7). Similarly, the average signal (RFU) was also affected by the collection technique ($p < 0.001$), with the samples collected by technique (a)

generating profiles with higher peak heights than samples collected by technique (b) (RFU mean a. 7044, and b. 971) (Figure 8.8).

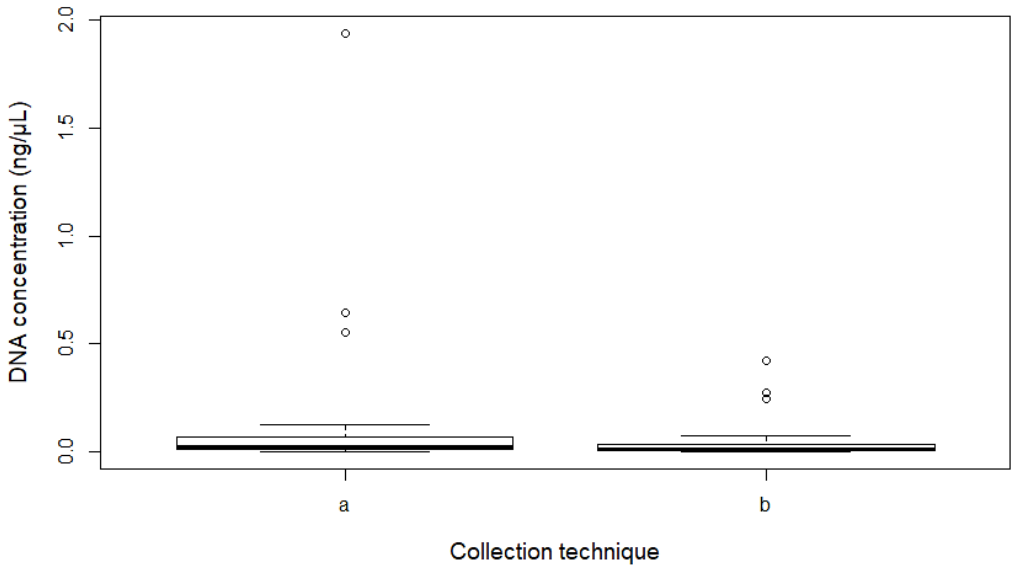


Figure 8.7: Mean DNA recovered from casework items (smooth non-porous surfaces) (n= 52) collected by two collection technique with cotton swab (CS). CS **(a)** swabs were frozen while moist before extraction and CS **(b)** swabs were dried prior to extraction.

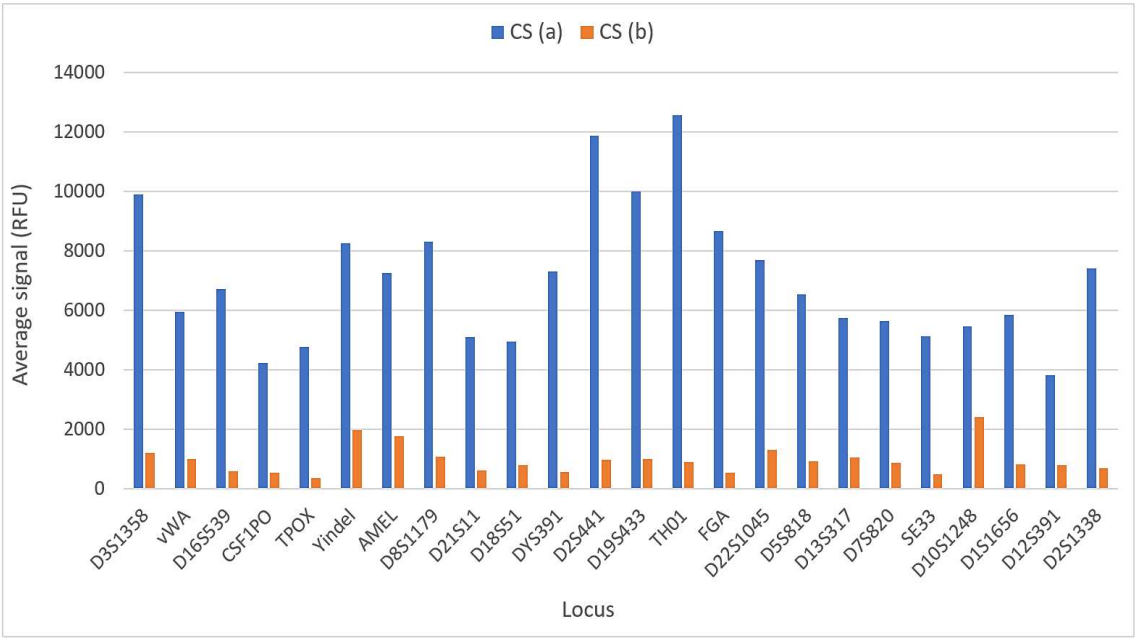


Figure 8.8: Average signal (RFU) per locus of full single (FS) DNA profiles in trace samples collected from casework items (smooth non-porous surfaces) (n= 52) by two collection techniques with cotton swabs (CS). CS **(a)** swabs were frozen while moist before extraction and CS **(b)** swabs were dried prior to extraction.

In addition, CS (a) recovered more usable DNA profiles from the collected trace samples than CS (b) (Pct. of positive DNA profile recovery CS (a). 96% and CS (b). 73%), and the CS (a) recovered more alleles than CS (b) (avg. of alleles recovered in mixture profiles CS (a). 82 and CS (b). 59). See Figure 8.9 for the DNA profiles recovered by each collection technique.

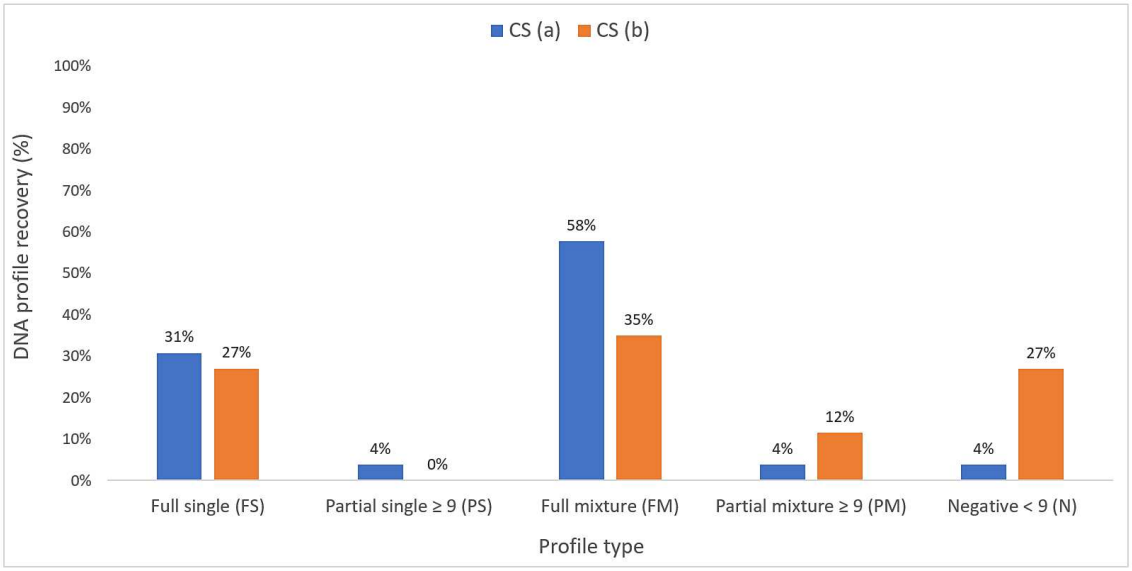


Figure 8.9: DNA profiles recovered from casework items (smooth non-porous surfaces) (n= 52) collected by two collection techniques with cotton swabs (CS). CS (a) swabs were frozen while moist prior extraction and CS (b) swabs were dried prior extraction. The DNA profiles recovered were classified into five categories: (FS) Full single, (FM) Full mixture, (PS & PM) Partial single and Partial mixture that contained alleles in nine loci or more, (N) negative refers to DNA profiles containing alleles on less than nine loci or no data.

8.3.2.2 NYLON SWAB (NS) SAMPLES

The average DNA quantity collected from textured non-porous surfaces was less than that collected from smooth non-porous surfaces. Furthermore, when analysing nylon and cotton swab performance, it was observed that the amount of trace DNA collected from textured non-porous surfaces of casework items (n=52) was affected by collection type ($p < 0.05$) (see Appendix A8.2 for the sample results). More DNA was collected with nylon swabs (NS) than with cotton swabs (CS) (mean NS. 0.040 and CS. 0.023 all in ng/µL) (Figure 8.10). Likewise, the average signal (RFU) was also affected by the collection type

used (p < 0.001). The samples collected by NS generated profiles with higher peak heights than samples collected by CS (RFU mean NS. 1951 and CS. 777) (Figure 8.11).

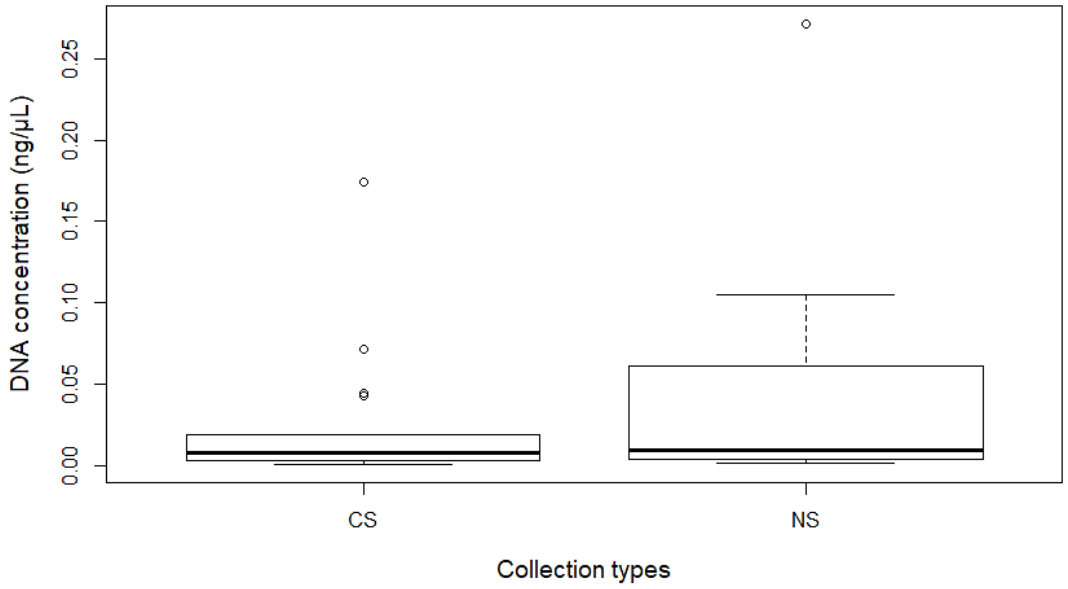


Figure 8.10: Mean DNA recovered from casework items (textured non-porous surfaces) (n= 52) collected by a nylon swab (NS) and cotton swab (CS).

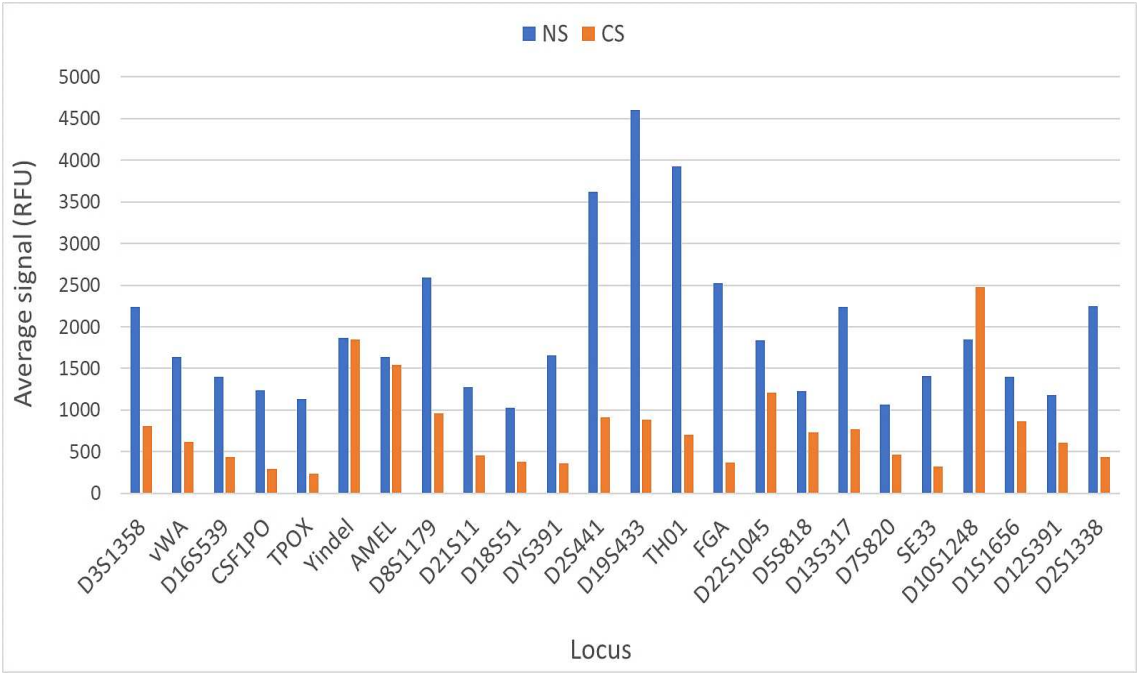


Figure 8.11: Average signal (RFU) per locus of full single (FS) DNA profiles of trace samples collected from casework items (textured non-porous surfaces) (n= 52) collected by nylon swab (NS) and cotton swab (CS).

Moreover, NS recovered more usable DNA profiles from the collected trace samples than CS (Pct. of positive DNA profile recovery NS. 89% and CS. 77%), and NS recovered more alleles than CS (avg. of alleles recovered in mixture profiles NS. 80 and CS. 62). See Figure 8.12 for the DNA profiles recovered by each collection method.

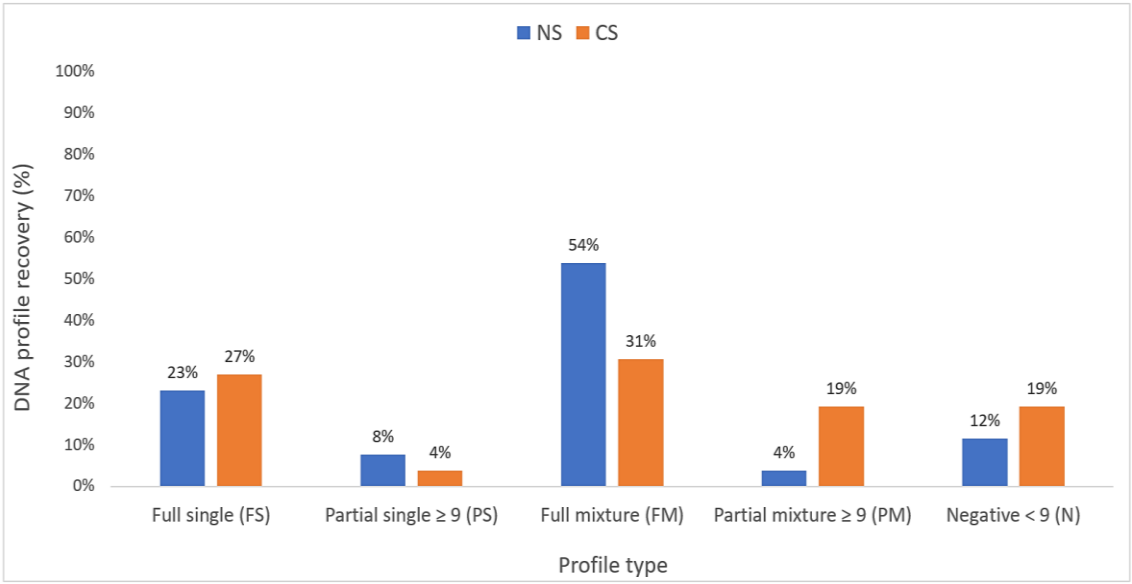


Figure 8.12: DNA profiles recovered from casework items (textured non-porous surfaces) (n= 52) collected by nylon swab (NS) and cotton swab (CS). The DNA profiles recovered were classified into five categories: (FS) Full single, (FM) Full mixture, (PS & PM) Partial single and Partial mixture that contained alleles in nine loci or more, (N) negative refers to DNA profiles containing alleles on less than nine loci or no data.

8.3.2.3 MINITAPES (MT) SAMPLES

The average DNA quantity collected from fabric items and porous surfaces was more than that collected from smooth non-porous surfaces and textured non-porous surfaces. Furthermore, when examining minitape and cotton swab performance, it was observed that the amount of trace DNA collected from fabric items and porous surfaces of casework items (n=52) was affected by collection type ($p < 0.05$) (see Appendix A8.3 for the sample results). The mean DNA recovered with minitapes (MT) was more than double the mean amount of DNA recovered with cotton swabs (CS) (mean MT. 0.38 and CS. 0.15 all in ng/µL) (Figure 8.13).

Also, the average signal (RFU) was affected by the collection type used ($p < 0.001$). The samples collected by MT generated profiles with much higher peak heights than samples collected by CS (RFU mean MT. 7010 and CS. 886) (Figure 8.14).

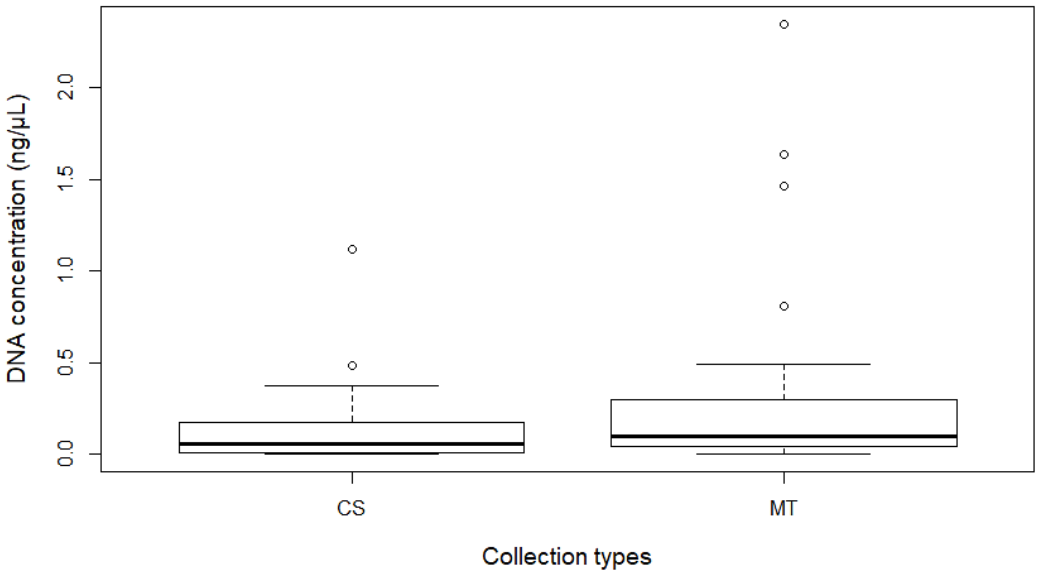


Figure 8.13: Mean DNA recovered from casework items (fabric and porous surfaces) (n= 52) collected by minitapes (MT) and cotton swab (CS).

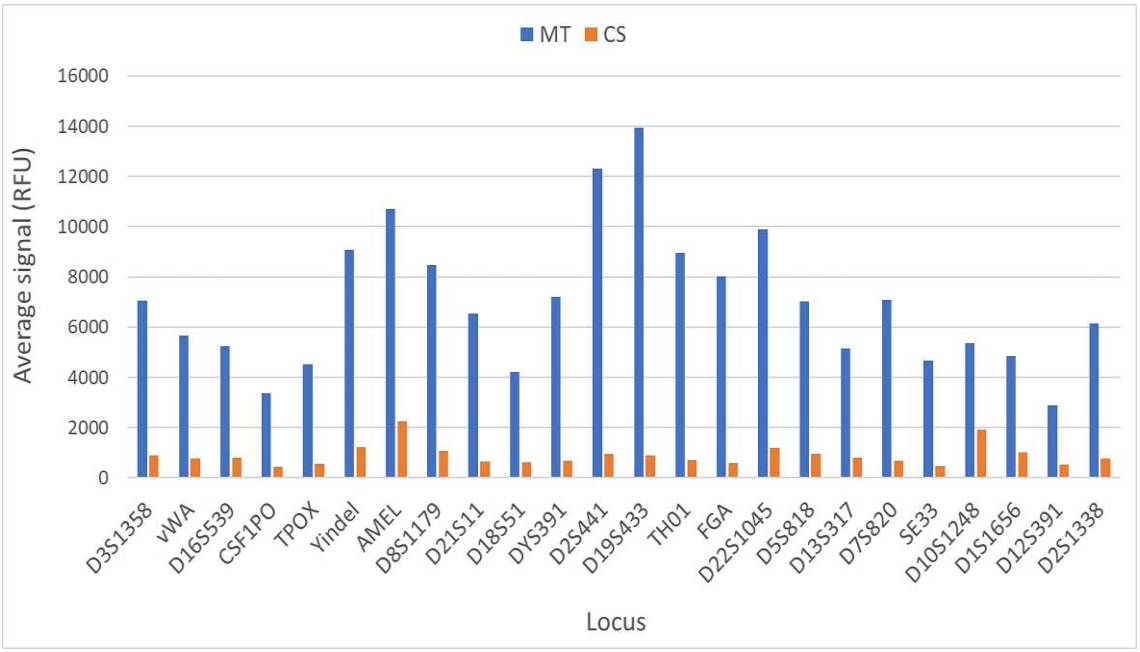


Figure 8.14: Average signal (RFU) per locus of full single (FS) DNA profiles of trace samples collected from casework items (fabric and porous surfaces) (n= 52) collected by minitapes (MT) and cotton swab (CS).

Furthermore, MT recovered more usable DNA profiles from the collected trace samples than CS (Pct. of positive DNA profile recovery NS. 96% and CS. 81%), and more alleles than CS (avg. of alleles recovered in mixture profiles NS. 93 and CS. 72). See Figure 8.15 for the DNA profiles recovered by each collection method.

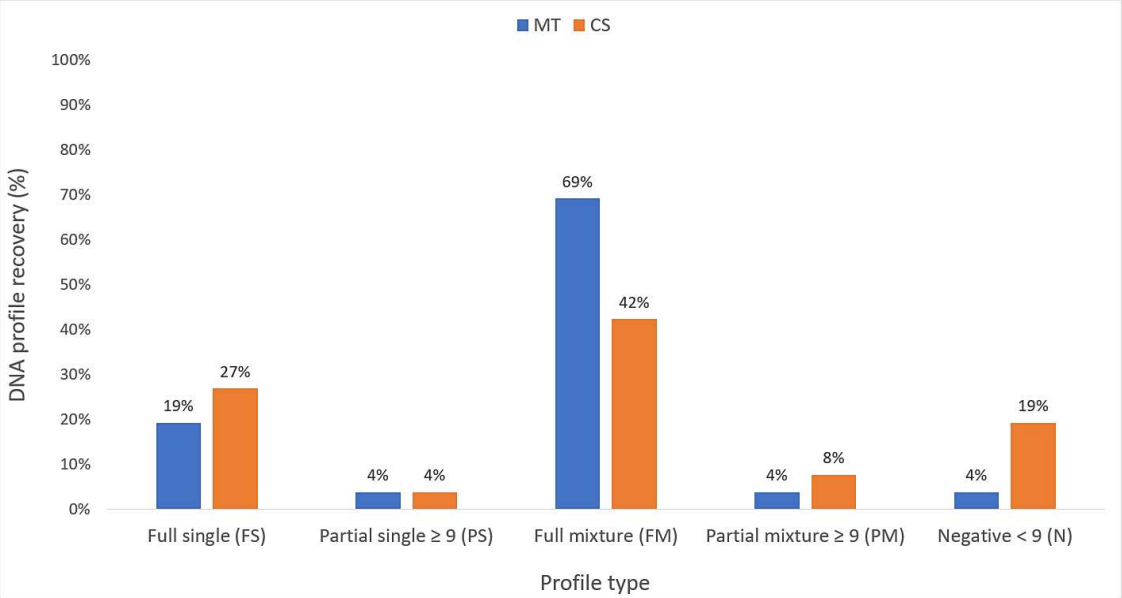


Figure 8.15: DNA profiles recovered from casework items (fabric and porous surfaces) (n= 52) collected by minitapes (MT) and cotton swab (CS). The DNA profiles recovered were classified into five categories: (FS) Full single, (FM) Full mixture, (PS & PM) Partial single and Partial mixture that contained alleles in nine loci or more, (N) negative refers to DNA profiles containing alleles on less than nine loci or no data.

8.3.2.4 DIRECT AMPLIFICATION SAMPLES

There was a considerable difference in the average signal (RFU) between the trace samples collected from casework items (n=100) that were directly amplified (a) and extracted (b) ($p < 0.001$) (Figure 8.16) (see Appendix A8.4 for the sample results).

Using a microFLOQ® Direct swab (MF) to collect trace DNA from the surface or using a microFLOQ® Direct swab to collect the collected sample from cotton swab (CS+MF; innovative solution) for direct amplification (a) generated profiles with higher peak heights than samples collected by cotton swab (CS) for extraction (b) before amplification (RFU mean MF (a). 7793, CS+MF (a). 6100 and CS (b). 805).

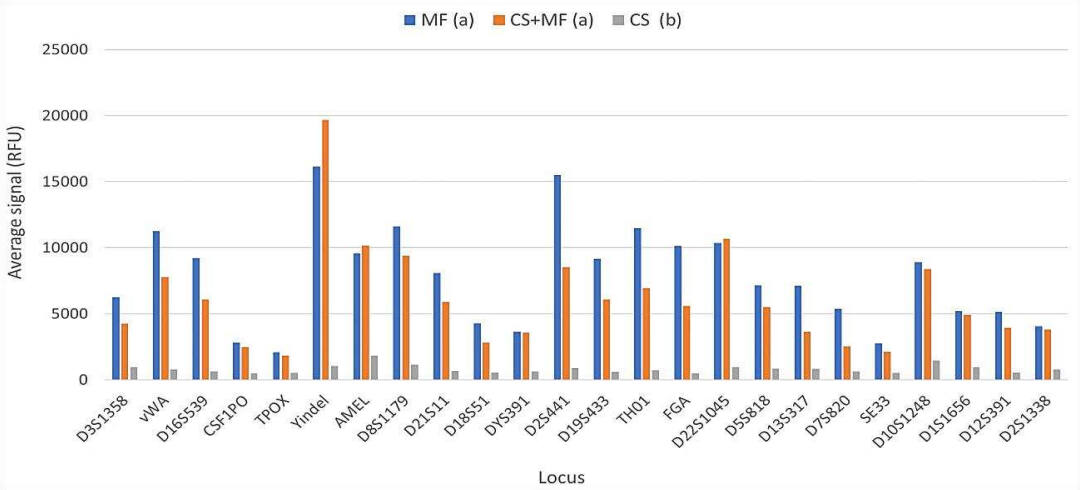


Figure 8.16: Average signal (RFU) per locus of full single (FS) DNA profiles of trace samples collected from casework items (n= 100) by **(a)** directly amplified microFLOQ® Direct swab (MF) and microFLOQ® Direct swab collected from cotton swabs (CS+MF), **(b)** extracted cotton swabs (CS).

Trace samples collected by CS and extracted (b) before amplification had a 75% positive DNA profile recovery rate. Even though direct PCR generated DNA profiles in less than three hours, there were fewer full single/partial DNA profiles, and most of the DNA profiles contained some artifacts such as increased stutter ratios and split and shoulder peaks. However, direct amplification produced 70% usable DNA results with CS+MF, which was much better than using MF alone which produced 55% usable DNA results (Figure 8.17).

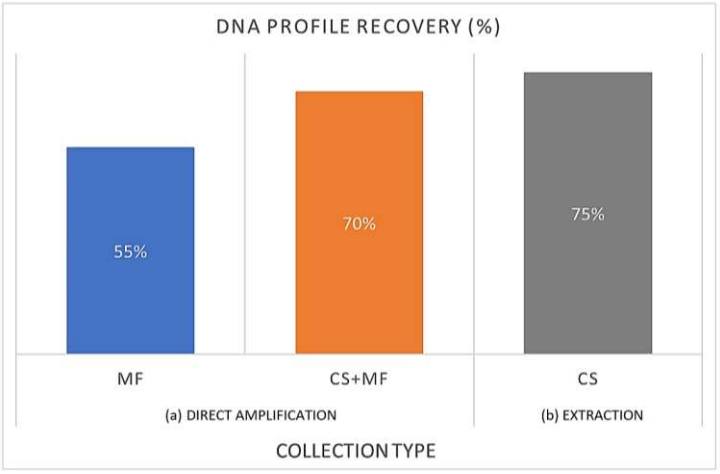


Figure 8.17: Percentage positive DNA recovery rate of trace samples collected from casework items (n= 100) collected by **(a)** directly amplified microFLOQ® Direct swab (MF) and microFLOQ® Direct swab collected from cotton swabs (CS+MF), **(b)** extracted cotton swabs (CS). Positive DNA results refers to DNA profiles with alleles in nine loci or more.

8.4 CHAPTER DISCUSSION AND CONCLUSION

There were difficulties encountered through the data collation from casework trace samples in the Biology and DNA Section of the General Department of Forensic Science and Criminology of Dubai Police Force, which could not be done automatically by computer systems. The sample type collected was not stored in the system, therefore each case file had to be manually searched to sort the trace samples into six different categories. This was time-consuming but of benefit to the data regarding policy direction, method application as well training needs. To improve trace DNA recovery, more work needs to be done to gather and analyse data from casework which will eventually help to find the limitations that impact Touch DNA recovery. This data should also be shared to enhance the general knowledge regarding trace DNA profiling.

Trace DNA collected using cotton swabs by the lab tech in Dubai Police was extracted using PrepFiler Express kit, and trace DNA collected using the three collection methods (cotton swab, nylon swab and minitapes) was extracted using PrepFiler Express BTA™ kits and 460 µL of lysis buffer instead of 230 µL with a full swab or full minitape. Nevertheless, the PrepFiler Express and PrepFiler Express BTA™ kits are effective in extracting trace DNA because they have been designed to improve the yield and overall purity of DNA isolated from both routine and challenging forensic samples (Thermo Fisher Scientific Inc., 2018a), therefore both are efficient in extracting Touch DNA collected by cotton swabs. However, the PrepFiler Express BTA™ was designed specifically for challenging and adhesive-based samples such as tape lifts. Consequently, trace DNA collected by minitapes are better extracted by the PrepFiler Express BTA™ than the PrepFiler Express (Joël *et al*., 2015). Using multiple collection methods developed in this study for different surfaces improved the trace DNA profile recovery rate by 17% in the samples collected from casework over the three months. Textured non-porous surfaces trace samples generated the least amount of DNA, and fabric and porous surfaces trace samples generated the most DNA. Using cotton swabs to collect the biological material from smooth non-porous surfaces is ideal, however, the technique used can impact the quantity and quality of DNA. Immediate freezing of cotton swabs after collection while the swabs were moist improved the trace DNA profile recovery rate by 23% compared to swabs that were dried prior to extraction.

Using only cotton swabs to collect Touch DNA from various surfaces can influence the quality and quantity of DNA recovered, therefore another collection type can be useful. Collecting trace DNA from textured/rough non-porous surfaces is more challenging than other surfaces, but using nylon swabs instead of cotton swabs improved the trace DNA profile recovery rate by 12%. Similarly, when using minitapes on fabric and non-porous surfaces, the trace DNA profile recovery rate improved by 15%. However, minitape requires more handling compared to swabs, which might make trace samples prone to contamination.

Direct amplification can be beneficial especially when there are only minute quantities of biological material, as some loss may occur during the extraction process. However, the recovery method for direct amplification is complex. Firstly, trace samples are not visible on most surfaces (fingerprints are visible on some surfaces that reflect light such as glass and stainless steel), and secondly, collection methods developed for direct amplification such as the microFLOQ® Direct swab have a tiny tip (limited by the PCR tube size which is 0.2-ml) which can only cover a small surface area, resulting in some biological material left on the surface uncollected. From casework trace samples, 45% collected by a MicroFLOQ® Direct swab produced unusable DNA profiles when compared to the same trace samples collected by a cotton swab which produce only 25% unusable DNA profiles. This study proposed an innovative solution to improve trace DNA recovery for direct PCR which is a combination of the cotton swab and MicroFLOQ™ Direct swab to maximise DNA recovery. This involved collecting the sample first using the cotton swab, then swabbing the cotton swab with a microFLOQ® direct swab to process the samples for direct amplification, while storing the cotton swab in the freezer for reanalysis if needed. It can be beneficial in urgent cases when quick results are needed because it can produce a DNA profile in less than two hours. For the casework trace samples, this innovative solution produced 70% usable DNA results when compared to the same trace samples collected by MicroFLOQ™ Direct swab alone which produced only 55% usable DNA results. However, the interpretation of the DNA profile produced by direct amplification can be challenging, especially in mixed DNA profiles because of the high frequency of artefacts present, such as a heterozygous allele imbalance which needs to be evaluated.

CHAPTER NINE

9. GENERAL DISCUSSION, FURTHER WORK AND RECOMMENDATIONS

This chapter discussed and analysed the study findings and limitations, suggested potential areas for further work and provided recommendations based on the obtained results to propose protocols for developing methodologies to improve Touch DNA profiling.

9.1 GENERAL DISCUSSION

The overall study aims were to examine the factors affecting the recovery of Touch or trace DNA to propose novel methodologies and techniques to improve Touch DNA analysis in forensic casework. This was achieved by evaluating collection and extraction methodologies/techniques for trace DNA profiling based on their common use in published research in the field of Touch DNA analysis, and their popularity among forensic laboratories. The factors impacting Touch DNA recovery, such as surface type, environmental factors, type of sampling method, etc., were discussed in length in Section 1.3 to assess their performance. However, since forensic genetics technology is constantly evolving as discussed in Section 1.1.4, continuous evaluation of the latest methodologies/techniques can also contribute to the improvement of trace DNA profiling.

Even though DNA deposits are not consistent, the duration over which the deposit on the surface and the area over which the touch occurs is important, as it helps in the evaluation of the effectiveness of sampling Touch DNA (Tobias *et al.,* 2017; Verdon *et al.,* 2014b). The deposition process was standardised in each experiment and as described in Section 2.2 in most experiments by regulating the process, duration of the deposition and the surface area. Moreover, due to the different shedding abilities of individuals which can impact the amount of DNA deposited (Allen *et al.,* 2008; Quinones, 2011; Goray *et al.,* 2016), a limited number of participants with more deposit replicates were used to minimise variation in the DNA concentration which might affect the data analysis.

Since Touch DNA is generally deposited in minute amounts, DNA contamination by direct/indirect transfer can complicate the data analysis in Touch DNA profiling (Van Oorschot *et al.,* 2019). However, this issue was addressed while performing experiments by following strict anti-contamination procedures as described in Section 2.1. Furthermore, the participants were instructed to wash their hands with antibacterial/antimicrobial soap for 45 seconds before DNA deposition. Also, blank samples were taken from materials/surfaces after sterilisation and negative controls for the DNA profiling process during collection, extraction, quantification and amplification to monitor background contamination; all of which were confirmed as DNA-free. One DNA sample was collected from each participant's hand for use as a reference to evaluate the Touch DNA deposits collected from the test surfaces.

Despite the variety of sampling methods to collect and process DNA, cotton swabs are used routinely to recover trace DNA in forensic casework (Raymond *et al.* 2009a; Castella & Mangin, 2008). However, DNA collection can be inefficient or DNA retained by the cotton swab depending on the collection technique (Alketbi & Goodwin, 2019d) and the efficiency of the extraction method used (Van Oorschot *et al*., 2003). Nonetheless, using an appropriate amount of solution to moisten the swab or using a double swab technique (wet and dry) can improve Touch DNA recovery with a cotton swab. The first aim of the project was to determine the most suitable collection process to retrieve trace DNA using cotton swabs e.g., wetting techniques, and drying or freezing before extraction. The results demonstrated that the spray bottle technique to moisten a single swab head, or the use of the double swab technique (wet and dry) were more efficient for collecting Touch DNA than the single swab moistened with a pipette. In addition, more trace DNA was obtained from the cotton swabs that had been extracted immediately after DNA collection or frozen when still moist compared to swabs that were allowed to dry before freezing.

Touch DNA can be recovered successfully from a vast range of surfaces, however, the type of surface, such as smooth or rough, porous or non-porous, can impact the amount of DNA collected (Goray *et al.,* 2010; Daly *et al.,* 2012; Alketbi & Goodwin, 2019a). There

is a lack of research in that regard, and it is necessary to determine the effect of the duration of the surface deposition (Tobias *et al.,* 2017), as well as the recovery and extraction efficiencies (Taylor *et al.,* 2017). The second research aim was to investigate whether Touch DNA can be impacted by surface type, collection and extraction methods when deposited on a range of surfaces commonly encountered at crime scenes. The analysis revealed that the amount of DNA collected from the test surfaces was significantly influenced by the surface type as well as the extraction method. Furthermore, the amount of DNA collected from glass and copier paper was notably affected by collection type but not by the extraction method when a larger volume (460 µL vs. 230 µL) of lysis buffer was used.

Manual extraction methods such as the QIAamp® DNA Investigator Kit (QIAGEN) or automated methods such as AutoMate Express™ Forensic DNA Extraction System with PrepFiler Express or PrepFiler Express BTA™ kits (Thermo Fisher Scientific) are preferred in forensic DNA laboratories (Tasker *et al.,* 2019) but can influence the amount of recovered DNA (Ottens *et al.,* 2013; Joël *et al*., 2015). Both the QIAamp® DNA Investigator Kit and PrepFiler Express BTA™ kits were effective for the extraction of Touch DNA samples in this study. However, the amount of lysis buffer recommended by the manufacturer for the PrepFiler Express BTA™ kit (230 µL) was insufficient to extract all the recovered trace DNA when a full swab head was used for extraction, so the amount of lysis buffer was increased to 460 µL, which may be costly as additional lysis buffer would be needed to be purchased separately. Furthermore, the amount of extracted DNA was impacted by the interaction between the collection type and the extraction method, with cotton and nylon swabs being equally efficient for both extraction methods, whereas minitapes were more efficient when the DNA was extracted by PrepFiler Express BTA™.

Copan cotton swab (150C) (Verdon *et al.,* 2014a), Copan nylon flocked swab (4N6FLOQSwabs®) (Brownlow *et al.,* 2012), and SceneSafe FAST™ minitape (K545) (Hansson *et al.,* 2009) were evaluated for their ability to collect trace DNA, showing that different collection methods and techniques can enhance DNA recovery. Furthermore, techniques may vary between cotton and nylon swabs, such as the amount of wetting reagent used on the swab before collection which can impact DNA recovery. Cotton

swabs absorb more wetting solution compared to nylon swabs because of the nature of the swab fabric, but it can be more challenging to extract the collected Touch DNA from the cotton swab than from the nylon swab (Van Oorschot *et al.,* 2003; Alketbi & Goodwin, 2019d). The cotton and nylon swabs performed better on non-porous surfaces, with the nylon swab being slightly better for collecting Touch DNA from rough, non-porous surfaces. Minitapes performed better on porous surfaces and enabled the dual recovery of DNA and fingerprints from touch deposits on smooth non-porous surfaces, such as metal or glass (Alketbi & Alsoofi, 2022b). In summary, each collection method has advantages and disadvantages, for example, half or full cotton swab heads can be used while a nylon swab head must be processed entirely because of the nature of the swab fabric, whereas minitapes require more handling compared to swabs which can increase the contamination risk.

The efficiency of quantification and amplification methods can influence the amount of Touch DNA collected (Ottens *et al.,* 2013), however, quantification methods that use real-time PCR (qPCR) are more effective for trace samples than alternative quantification methods such as the NanoDrop™ or gel electrophoresis. The use of Quantifiler™ Human and Quantifiler™ Trio Quantification Kits (Thermo Fisher Scientific) at different stages of the project showed that there was no significant difference in the quantification methods used. Furthermore, the Applied Biosystems™ GlobalFiler™ PCR Amplification kit to amplify trace samples provided consistent results and full DNA profiles from low DNA inputs.

Time in combination with environmental factors is crucial when collecting trace DNA from crime scenes. It has been reported that a long time between the original deposition and recovery may reduce the DNA quantities recovered but mostly in combination with the environmental conditions (Raymond *et al.,* 2009b). The third study aim was to investigate whether Touch DNA can be influenced by environmental conditions (e.g., temperature, humidity and sand) and time when deposited on a range of common surfaces encountered at crime scenes. The results showed that the amount of collected DNA was significantly affected by the conditions that the surfaces were exposed to, as well as the interaction between the conditions and time. However, at room temperature (20–25°C) the amount of DNA was not impacted, therefore, the persistence of deposited

trace DNA on the surfaces at outdoor crime scenes in countries that have low/high humidity and temperature variables may be directly affected (Alketbi & Goodwin, 2019b).

Furthermore, sand or dust on items retrieved from outdoors can impact Touch DNA recovery (Alketbi & Goodwin, 2019c), as is common in hot dry climates such as Dubai. Copan 4N6FLOQSwabs® in combination with the PrepFiler Express BTA™ extraction kit improved the yield and purity of DNA compared to samples collected by cotton swab and samples extracted using the QIAamp® DNA Investigator Kit. Exposure to humidity could reduce the amount of sample biological material (Raymond *et al.,* 2008) but improve DNA transfer and recovery (Goray *et al.,* 2010; Alketbi & Goodwin, 2021e). Dry swabs were more effective than moistened swabs for recovering Touch DNA deposited on glass surfaces (smooth non-porous) and exposed to low temperature and high humidity (5°C/78%).

The fourth aim of the project was to examine the effect of different collection methods or techniques on trace DNA deposited on human skin and fabric and investigate the influence of time after deposition to enhance Touch DNA recovery in sexual harassment casework scenarios. In scenarios of violent crimes such as assault, sexual offences, or even homicide, generally trace DNA is recovered from the skin of the victim and sometimes, from the offender's hands if caught within a short time of committing the crime. However, there is a lack of published data regarding trace DNA recovery from human skin (de Bruin *et al.,* 2011; Bowman *et al.,* 2018; Kallupurackal *et al.,* 2021), which is only collected with cotton swabs using a double swabbing technique (wet and dry) (Sweet *et al.,* 1996; Pang & Cheung, 2007). It was observed that moistening the neck with 100 µL of molecular grade water using a spray bottle before sample collection with a dry cotton or nylon swab increased the alleles recovery rate in the strangulation scenario compared to using moistened and dry swabs. Furthermore, DNA quantities recovered from a strangled victim's skin increased over time, which led to an increase in the mixture ratio of the minor (perpetrator) and major (victim) contributors, with no perpetrator alleles observed after 48 h. Furthermore, cotton swabs performed better than nylon swabs when collecting trace DNA from the perpetrator's hand in the strangulation scenario. However, the victim's biological materials recovered from the

perpetrator's hands following the strangulation decreased more rapidly than those deposited on the neck (within 6 hours) even though the participants were instructed to do their daily activity without washing during the testing period. The location of the deposited DNA on the body of the victim (e.g. neck, hands, and hand wrist) could influence the stability of the DNA of the offender, and also hands are used more often than the neck in everyday activities.

In scenarios of sexual offences, trace DNA is often collected from the victim's clothes but may produce mixed DNA profiles which can complicate the interpretation (Petricevic *et al.,* 2011). In addition, recovering Touch DNA from fabric can be impacted by many variables such as the collection type, fabric type, deposition area and size, as well as the time between deposition and collection (Alketbi & Goodwin, 2022c; Alketbi, 2022d; Alketbi & Goodwin, 2022e). Since there is a lack of published casework data, there is a need to evaluate these variables to improve trace DNA recovery from fabric. This study indicated that minitapes were more effective than cotton and nylon swabs for recovering Touch DNA from fabric when samples were collected from a 5 x 7 cm area. Similar studies by Hansson *et al.* (2009) and Hess and Haas (2017) reported that minitapes are more efficient than swabbing trace DNA from fabric. However, the cotton swab was equally effective for samples collected from a 10 x 14 cm area, indicating that cotton swabs can recover more DNA spread over larger areas, while minitapes are more efficient for collecting DNA concentrated in small areas, as they have limited number of tape lifts which can influence sampling from different sized areas (Verdon *et al.,* 2014b). Furthermore, the amount of Touch DNA collected was significantly impacted by the fabric type, with more trace DNA collected from a fabric composed of a high percentage of polyester than 100% woven cotton with minitapes. Loose fibres present in some fabric types may influence the recovery with minitapes by weakening the stickiness of the tape-lift.

The quantity and quality of the collected Touch DNA from garments were significantly affected by the deposition area and time, as well the interaction between the deposition area and time. More DNA was recovered from the chest area of a t-shirt than from the buttocks area of pants over 24 h. Additionally, the number of alleles observed in the mixture DNA profiles was not affected by time for the samples collected from the chest

area of the t-shirt, whereas full mixture DNA profiles were obtained from the samples collected from the buttocks area of the pants. However, no alleles were observed from the minor contributor after 6 h. These results could be explained by the increased friction on the buttocks area of the pants from daily activities like frequent sitting on different surfaces which often leads to a reduced amount of deposited DNA available.

Previous studies demonstrated that direct amplification of trace DNA from swabs or small items and even fabrics can produce profiles as good as or better than traditional methods (Linacre *et al.,* 2010; Templeton *et al.,* 2015; Swaran & Welch*,* 2012; Ambers *et al.,* 2018; Cavanaugh & Bathrick*,* 2018; Martin *et al.,* 2018). However, there are no consensus standardised protocols to collect and process casework Touch DNA for direct amplification. Therefore, the fifth research aim was to examine the influence of direct PCR amplification on the type of collection method used, as well as the amount of Touch DNA collected. Minitapes and MicroFLOQ™ Direct swabs recovered a higher percentage of alleles and were more effective for direct DNA amplification than other collection methods. Moreover, more alleles were recovered from non-porous surfaces than porous surfaces for samples processed for direct PCR. However, cutting a small piece of fabric has been reported to improve trace DNA recovery from such a surface (Linacre *et al.,* 2010).

Next, direct amplification was compared to extraction, direct PCR produced the highest DNA yield, as evaluated by the relative fluorescence of the peak heights. Some previous studies came to the same finding when investigating methods for collection and direct amplification of Touch DNA against extraction (Templeton *et al.,* 2013; Liu, 2015). However, the present study also showed that extraction has a slight advantage over direct PCR for samples collected from a larger surface area (5 x 7 cm). Since there was more biological material to be retrieved from the surface, the MicroFLOQ™ Direct swab, which has a small swab tip design, is not effective for covering the larger surface area. Consequently, it is proposed to use a combination of a cotton swab and MicroFLOQ™ Direct swab to maximise DNA recovery from the tested surface (innovative solution) (Alketbi, 2022a). This was proven to be 84–88% successful for direct amplification, however, the number of alleles observed increased when there was more DNA collected by the cotton swabs. The process of direct amplification for trace DNA should be

evaluated for casework samples to better understand how to use it more effectively. Artifacts are often associated with direct PCR, such as increased stutter ratios, and split and shoulder peaks observed in this study and have been reported previously (Verheij *et al.,* 2012; Gouveia *et al.,* 2015; Ambers *et al.,* 2018). The presence of such artifacts in mixed DNA profiles can make data interpretation more challenging for trace DNA analysis.

There are many available methodologies for collecting trace DNA, with most forensic laboratories developing their protocols (Hansson *et al.,* 2009; Van Oorschot *et al.,* 2010; Verdon *et al.,* 2014b; Plaza *et al.,* 2016; Verdon *et al.,* 2015) but there is limited published data regarding trace DNA recovery rates from various objects which impact the development of trace DNA profiling (Dziak *et al.,* 2018; Van Oorschot *et al*., 2019). Bond and Hammond, (2010), Mapes *et al.* (2015), and Baechler (2015) have mentioned the importance of sharing the success of data collection and comparisons of trace DNA, so the last project aim was to first share the success rate of trace samples collected from DNA casework at the Biology and DNA Section of the Dubai Police General Department of Forensic Science and Criminology in the last three years. Secondly, to implement the study findings on forensic casework and report the data at the Biology and DNA Section of the Dubai Police General Department of Forensic Science and Criminology to establish new collection methodologies and techniques to process Touch/trace samples. In total, 14,552 samples were collected from examined items of 6277 cases between 2019 to 2021, of which only 8696 samples were processed for DNA profiling. The 5488 (63%) trace samples recovered from various touched or used items were sorted into six categories based on the item type. The success rate of all the trace samples was 64%, with only 3489 producing positive DNA results. It is also important to note that one collection method was used to recover all the trace DNA samples, that is, the cotton swab moistened with approximately 100 µL of molecular grade water using a plastic spray bottle. In addition, there were difficulties encountered in the data collation of casework trace samples, as the sample type was not stored in the system, therefore each case file had to be manually searched to sort and classify the trace samples, hence, there is a small margin of human error expected.

Implementing multiple collection methods developed in this study to collect samples from different surfaces improved the trace DNA profile recovery rate by 17% in the samples collected from casework over the three months. Trace DNA was collected using a Copan cotton swab (150C) for smooth non-porous surfaces, a Copan nylon flocked swab (4N6FLOQSwabs®) for textured non-porous surfaces and all types of non-porous sandy surfaces, and SceneSafe FAST™ minitape (1-Tape Kit) for fabric items and all type of porous surfaces. Immediate freezing of cotton swabs following DNA collection while the swabs were moist enhanced the trace DNA profile recovery rate by 23% compared to swabs that were dried prior to extraction. Nylon swabs improved the trace DNA profile recovery rate by 12% compared to cotton swabs and minitapes improved the recovery rate by 15%. Finally, Copan microFLOQ™ Direct swab was used to process trace DNA samples collected from all surfaces for direct amplification and dual recovery from cotton swabs (innovative solution). In the casework trace samples, this innovative solution generated 70% usable DNA results compared to the same trace samples collected by MicroFLOQ™ Direct swab alone which generated only 55% usable DNA results. However, the interpretation of the DNA profile produced by direct amplification was challenging in mixed DNA profiles when there were no reference samples available because of the high frequency of artifacts present, such as a heterozygous allele imbalance.

9.2 FURTHER WORK AND LIMITATIONS

This project focused on investigating the factors affecting Touch DNA, therefore many experiments were conducted and small sample sizes were used to reduce costs but at least three replicates were used for each variable for valid statistical results. Furthermore, there are no published guidelines regarding the required sample size for trace DNA analysis, thus future work with larger samples should be performed to confirm the study findings.

Understanding an individual's shedding abilities is key to improving Touch or trace DNA recovery. However, it is complicated because there are many variables involved such as a person's activity or habits, nature of the physical contact etc. (Lowe *et al.,* 2002; Raymond *et al.,* 2004; Phipps & Petricevic, 2007; Allen *et al.,* 2008; Cowell, 2011), and it is not yet clear how best to classify an individual into a shedder class. As the shedder ability of an individual may be a relevant factor when interpreting trace DNA profiles in activity level assessments, a large-scale systemic study on shedder status is required.

In addition, it is still unclear what exactly is being "shed" to deposit DNA and from where the detectable DNA originates (Burrill *et al*., 2019). Based on an individual's behaviour and habits, it could come from other DNA sources present on the hand and contribute to what is deposited. Therefore, classification of the deposited biological material on surfaces would allow researchers to understand the shedding differences between people, foretell DNA deposition levels and improve DNA recovery from these sample types.

A cotton swab using the double swab technique (wet and dry) can lead to more trace DNA recovered from the surface than a single swab technique (half wet and half dry) (Pang & Cheung, 2007), but is dependent on the sample area size. For example, if DNA is collected from a small surface area, a single moistened swab may be efficient or equally effective as using the double swab technique (wet and dry), however, future investigation of the area size for single or double swabs is required.

Molecular grade water is a common moistening agent used by forensic laboratories to wet the swabs (Van Oorschot *et al.,* 1999) before Touch DNA collection, however, if the

cotton swab is allowed to dry before extraction, less DNA is often retrieved than if the moist swab was used immediately (Alketbi & Goodwin, 2019d). Therefore, investigating different wetting solutions to moisten cotton swabs may improve trace DNA recovery and allow better long-term storage solutions.

Long-term storage and its impact on trace DNA have not been investigated in depth as there are many collection methods used to collect DNA such as nylon swabs and minitapes. The influence of storage in different conditions when using these collection techniques is still unknown and understanding the best practices for storing items or garments before examination or even long-term after examination are required to improve trace DNA recovery.

Even though minitapes are the preferred choice for collecting trace DNA from fabric, the loose fibres may hinder the recovery with tape lifting and weaken the stickiness of the tape-lift (Stoop *et al.,* 2017), so future studies investigating swabbing or other collection techniques from such fabric would be beneficial.

In this study, dual recovery of DNA and fingerprints from Touch DNA was successful from clear fingerprints deposited by high shedders on non-porous surfaces using SceneSafe FAST™ minitape and Black Fingerprint Powder. However, additional studies should investigate other types of shedders and surfaces to confirm these results and advance dual recovery with minitapes.

Finally, studying the benefit of using different recent forensic genetic technologies such as next-generation sequencing (NGS) or Y-STR multiplex kits for trace DNA samples in some casework scenarios can be useful. For example, more information can be gathered using Y-STR multiplex kits in scenarios of violent crimes like assault or sexual offences when the perpetrator is known to be male, and when the recovered trace STR DNA profile did not provide sufficient information.

9.3 RECOMMENDATIONS

The following recommendations have been conceived based on the thesis results to evaluate the factors impacting Touch DNA recovery. These recommendations have been mentioned in the relevant chapters and are summarised below:

- Individuals have different DNA shedding abilities (Murray *et al.,* 2001; Lowe *et al.,* 2002; Phipps & Petricevic, 2007; Allen *et al.,* 2008; Quinones, 2011), which are often affected by a person's behaviour and activity. The quantity may differ in each deposition, therefore, to use participants more efficiently when conducting experiments, it is recommended to collect DNA from individuals' hands or their deposits over a period of time to determine the individual's shedding ability.
- The use of a plastic spray bottle to moisten the swab is preferable to a pipette or using drop bottles because it spreads the molecular grade water evenly over the swab without soaking it (Alketbi & Goodwin, 2019d). In addition, there is less risk of contamination compared to the use of a pipette. However, it is important to consider the quantity of solution sprayed by plastic bottles as different spray bottles spray different quantities. Therefore, it is recommended to measure the amount of solution by spraying it into a 1.5 ml Microcentrifuge tube first to estimate the volume before using it on swabs to determine how many sprays are required. It is recommended not to exceed 200 µL for a cotton swab and 50 µL for a nylon swab as it is more sensitive to water because of the nature of the swab fabric. In this study, the cotton swab was held approximately 25 cm from the bottle and sprayed 3-4 times while rotating.
- Freezing the swab following DNA recovery while it is moist rather than drying it before extraction could result in similar quantities of DNA extracted compared to the immediate extraction after collection (Alketbi & Goodwin, 2019d). Therefore, it is recommended to freeze cotton swabs immediately after Touch DNA collection for better DNA recovery.
- The sample size in combination with the amount of lysis buffer can influence the maximum DNA yield during extraction (Alketbi & Goodwin, 2019a; Joël *et al.,*

2015). Thus, it is advisable to use 460 µL of lysis buffer to extract the full swab head when using the PrepFiler Express BTA™ Extraction. Alternatively, use the manufacturer's recommended volume of 230 µL with half of the cotton swab head, repeating the process with the second half of the swab head and combining the samples if required to increase the DNA yield.

- Different surfaces retain trace DNA deposits differently and it is important to consider the most appropriate method to improve DNA collection (Alketbi & Goodwin, 2019a). It is recommended to use a cotton swab for smooth, non-porous surfaces such as glass and stainless steel and a nylon swab for rough, non-porous surfaces such as textured plastic, whereas the use of minitapes is better for porous surfaces such as fabric. However, the amount of trace DNA collected by SceneSafe FAST™ minitapes can be impacted by area size because of the limited number of tape lifts, therefore two or more minitapes are recommended to be used for areas larger than 5 x 7 cm.
- In cases where Touch DNA is to be collected from visible fingerprints or fingerprints on smooth non-porous surfaces, it is recommended to use SceneSafe FAST™ minitape (Alketbi & Alsoofi, 2022b). The DNA should be collected first by applying low-medium pressure while tape lifting to avoid smearing the fingerprint, then reveal the fingerprints with a dusting of EVIDENT black powder using a brush, then lift with a clear tape and deposit on white backing cards.
- The Copan nylon flocked swab (4N6FLOQSwabs®) was the simplest to use as the swab head was easily broken into the tube by bending the swab. The Copan cotton swab (150C) demanded scissors or a scalpel to cut the swab, similarly, SceneSafe FAST™ minitape (K545) needs to be cut into small pieces for effective extraction. These requirements must be considered when using the collection methods to avoid contamination. For instance, the use of minitapes is best done in an indoor environment because it requires more handling, while cotton or nylon swabs can be used for outdoor collection and transferred to the lab to be processed. In addition, the cotton swab has another advantage over the other collection methods, only half of the swab head can be used with the other half stored for future extraction if required, while a nylon swab head must be

processed entirely because of the nature of the swab fabric. All these points should be considered when designing protocols for trace DNA recovery from various surfaces, irrespective of whether it is indoor or outdoor.

- Environmental conditions in combination with time can influence Touch DNA deposits on surfaces (Alketbi & Goodwin, 2019b; Alketbi & Goodwin, 2019c; Alketbi & Goodwin, 2021). Therefore, it is recommended to use dry cotton or nylon swabs to recover biological materials from outdoor humid non-porous surfaces such as glass for better DNA recovery. In addition, it is better to collect samples from surfaces as soon as possible after deposition to obtain higher DNA yields and to avoid cross-contamination, particularly if items containing Touch DNA are found outdoors. Moreover, non-porous surfaces such as stainless steel and glass heat at different rates in the sun. The examination of vehicles such as those involved in a hit and run often requires the collection of trace DNA from outer surfaces such as door handles, which may be made of metal. Hence, it is recommended to move vehicles to a shaded controlled environment to help preserve the DNA if not examined within a short time frame from incidents. Furthermore, sand or dust on objects from outdoors impacts Touch DNA recovery, so the use of nylon flocked swabs such as Copan 4N6FLOQSwabs® in combination with the PrepFiler Express BTA™ extraction kit is recommended to help improve the DNA yield and purity.
- There is limited published information regarding the collection of Touch DNA deposited on human skin in cases of sexual offences, however, using the appropriate technique with cotton or nylon swabs can enhance the recovery of Touch DNA from human skin. It is recommended to moisten the neck with 100 µL of molecular grade water using a spray bottle before sample collection with a dry cotton or nylon swab, as it has proven to increase the alleles recovery rate in strangulation scenarios. In addition, it is better to recover DNA from the victim's skin as soon as the crime is committed, as activities such as washing or sweating could influence the presence of the perpetrator's DNA deposited on the victim's skin over time.
- The palms of the hands and soles of the feet have more sweat glands and hence can produce more sweat secretions than any other part of the body (Baker,

2019). Nylon swabs are usually more sensitive to moist or humid surfaces, thus cotton swabs are recommended to improve allele recovery from the perpetrator's hands.

- Collecting trace DNA from clothes in sexual offences is equally important as collecting trace DNA from human skin, however, the deposition area, time, and other factors such as physical activity can affect the amount of Touch DNA collected from garments (Alketbi & Goodwin, 2022e). Accordingly, it is recommended to collect trace DNA from the victim's clothes as soon as the crime is committed to avoiding losing valuable information related to the offenders. However, this can be difficult, especially when people do not report sexual offences straight away, consequently, more awareness is needed to educate victims and police officers.
- Even though direct amplification has proven to be successful for other types of DNA evidence, it is still questionable for trace DNA but may be beneficial in some cases where urgent information is needed. Therefore, this project proposed an innovative solution which is a combination of using a cotton swab and a MicroFLOQ™ Direct swab to maximise DNA recovery from the tested surface (Alketbi, 2022a). The cotton swab is first used to collect the biological material and then swabbed with a MicroFLOQ® Direct swab for processing for direct PCR. This technique concentrates the biological material in a small surface area for a better sample collection with the MicroFLOQ® Direct swab, allowing re-analysis or additional testing if needed, which is a limitation of using direct PCR alone.
- Finally, it is recommended that forensic DNA labs implement systems to help organise trace samples collected from casework into different categories based on the type of items or surfaces they were collected from. This will help to identify any weakness associated with collection methods or techniques which may impact the trace DNA success rate, and sharing such data will contribute to advancing trace DNA profiling.

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APPENDICES

A1. ETHICAL APPROVAL LETTER

17 August 2018

Will Goodwin/ Salem Khalifa Alketbi
School of Forensic and Applied Sciences
University of Central Lancashire

Dear Will and Salem

Re: STEMH Ethics Committee Application
Unique Reference Number: **STEMH 912**

The STEMH ethics committee has granted approval of your proposal application 'Transfer and Persistency of Touch DNA'. Approval is granted up to the end of project date*.

It is your responsibility to ensure that

- the project is carried out in line with the information provided in the forms you have submitted
- you regularly re-consider the ethical issues that may be raised in generating and analysing your data
- any proposed amendments/changes to the project are raised with, and approved, by Committee
- you notify EthicsInfo@uclan.ac.uk if the end date changes or the project does not start
- serious adverse events that occur from the project are reported to Committee
- a closure report is submitted to complete the ethics governance procedures (Existing paperwork can be used for this purposes e.g. funder's end of grant report; abstract for student award or NRES final report. If none of these are available use e-Ethics Closure Report Proforma).

Yours sincerely

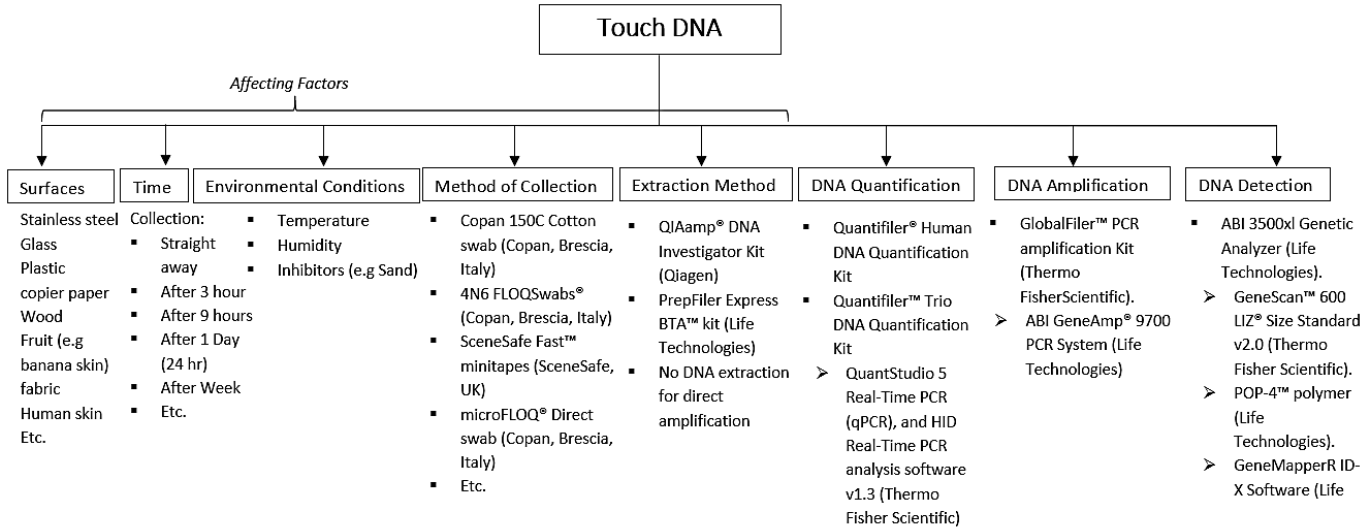
Emma Bray
Deputy Vice Chair
STEMH Ethics Committee

* for research degree students this will be the final lapse date

NB - Ethical approval is contingent on any health and safety checklists having been completed, and necessary approvals as a result of gained.

A2. MATERIALS AND METHODS

A2.1 RESEARCH OBJECTIVES AND METHODOLOGIES



Objective one: To examine the effect of the collection process using cotton swabs (e.g., wetting techniques, and drying or freezing prior to extraction) on Touch DNA.

Objective two: To examine the effect of surface type, collection and extraction methods on Touch DNA deposited on a range of surfaces.

Objective three: Simulate outdoor crime scene casework scenarios to examine the effect of environmental conditions (e.g., temperature, humidity and sand) and time on Touch DNA deposited on a range of surfaces.

Objective four: Simulate sexual harassment casework scenarios to examine the effect of different collection methods or techniques on Touch DNA deposited on human skin and fabric, and investigate the influence of time after deposition.

Objective five: To examine the effect of direct PCR amplification on the type of collection method used and the amount of Touch DNA collected.

Objective six: To implement the finding from previous objectives on forensic casework and report the data at the Biology and DNA Section of the Dubai Police General Department of Forensic Science and Criminology.

A2.2 QIAamp DNA INVESTIGATOR EXTRACTION KIT PROTOCOL

QIAamp MinElute columns should be stored at 2–8°C upon arrival if used for more than 4 weeks and are stable under these conditions for at least one year after delivery. Buffer AW1 and Buffer AW2 are supplied as concentrates. Add 25 ml ethanol (96–100%) to the bottle containing 19 ml Buffer AW1 concentrate, and add 30 ml ethanol (96–100%) to the bottle containing 13 ml Buffer AW2 concentrate. Before starting the procedure, check whether precipitate has formed in Buffer ATL and Buffer AL, if necessary, dissolve by heating to 70°C with gentle agitation. Buffers can be stored at room temperature (15–25°C) for up to 1 year.

Adding carrier RNA to Buffer AL is recommended for purification of DNA from very small amounts of sample (forensic samples), Add 310 µl Buffer ATE to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times. Add 1 µl dissolved carrier RNA to 400 µl Buffer AL to be used for each sample. Calculate the volume of Buffer AL and dissolved carrier RNA needed per batch of, to allow for pipetting errors, always prepare enough buffer for processing two extra samples time is not advisable. After preparing gently mix Buffer AL and dissolved carrier RNA by inverting the tube 10 times (do not vortex), (consumed within 48 h).

MEDIA AND SUPPLIES

QIAamp DNA Inves. Kit components

- QIAamp MinElute™ columns (50)
- Collection Tubes 2 ml (200)
- Buffer ATL (50 ml)
- Buffer AL (33 ml)
- Buffer AW1 (19 ml)
- Buffer AW2 (13 ml)
- Buffer ATE (200 ml)
- Carrier RNA (310 µg)
- Proteinase K (PK) (1.25 ml)

- (96-100%) Ethanol
- 1.5 mL Microcentrifuge
- Micropipetter
- Sterile pipet tips
- Scissors and forceps
- 70% ethanol or alcohol wipes
- Sterile water
- NAOBaskets

EQUIPMENT

- Incubator/Shaker
- Centrifuge
- Vortex mixer

Sample Preparation

- Any utensils used to cut or manipulate the swabs or other types of evidence must be cleaned between uses with alcohol wipe.
- Cuttings of swab tips or evidence substrate should be placed in a clean NAOBasket, Lyse and Spin basket with tube if recovery of lysate is a priority. Alternatively, a clean microcentrifuge tube may be used.
- Cuttings from biological stains/substrate (clothing, carpet, etc.) should be approx. 5mm x 5mm in size. Some specimens may require cuttings of differing sizes depending on the type, condition and concentration of the biological material. Larger sized cuttings may be cut into smaller pieces before placing them into the tube.

PROCEDURE

Cell Lysis

1. Add **400 µL of Buffer ATL** and **20 µL of PK** to the sample, close the lid, and mix by pulse-vortexing for 10 s.
2. Place the tube in a thermomixer and incubate at **56°C** with shaking at **900 rpm** for at least 1-3 h.
3. Briefly centrifuge (1 min) the samples to remove condensation from inside the lid.
4. Add **400 µL Buffer AL** (with **1 µl Dissolve carrier RNA**) close the lid, and mix by pulse-vortexing for 15 s.
5. Place the tube in a thermomixer and incubate at 70°C with shaking at 900 rpm for 10 min.
6. Briefly centrifuge (1 min) the samples to remove condensation from inside the lid (The solid substrate can at this point be placed in a DNA IQ™ Spin Basket. Centrifuge for 2 minutes at full speed, then discard the basket and substrate).
7. Add **200 µl ethanol (96–100%)** close the lid, and mix by pulse-vortexing for 15 s.
8. Centrifuge the tubes for 2 minutes at full speed.

Bind DNA

9. Transfer **entire supernatant** from the lysate tube to a QIAamp MinElute™ column placed in collection tube without wetting the rim of the column, close the lid, and centrifuge at 6000 x g (8000 rpm) for 1 min.
10. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

Wash DNA

11. Carefully open the QIAamp MinElute column and add **500 µl Buffer AW1** without wetting the rim. Close the lid and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
12. Carefully open the QIAamp MinElute column and add **700 µl Buffer AW2** without wetting the rim. Close the lid and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
    (Take care when removing the QIAamp MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp MinElute column)
13. Carefully open the QIAamp MinElute column and add **700 µl of ethanol** (96–100%) without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
14. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min to dry the membrane completely.
15. Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column, and incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.

Elute Purified DNA

16. Apply 20–100 µl **Buffer ATE** or distilled water to the centre of the membrane, Incubate at room temperature for 5 minutes, then Centrifuge at full speed (14,000 rpm) for 1 minute *(Repeat this step if you want to increase yield)*.
    (Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Elution with small volumes increases the final DNA concentration in the eluate significantly, but reduces the overall DNA yield. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.
17. Discard the QIAamp MinElute™ column and close the lid of the tube.

A2.3 PrepFiler EXPRESS BTA EXTRACTION KIT PROTOCOL

The AutoMate Express system enables to preform extraction up to 13 sample and it takes approximately 30 min to finish the extraction. A pre-programmed protocol card controls the purification parameters such as buffer volumes, mixing steps, and incubation time. Machine workflow: Mixes the sample lysate with magnetic particles and other reagents for subsequent DNA binding to magnetic particles in tips; Separates the DNA-bound magnetic particles from the lysate using magnetic separation; thoroughly washes the magnetic particles with wash buffers to remove PCR inhibitors; Dries the magnetic particles to remove ethanol. Elutes concentrated purified DNA in elution buffer. The kit components may be stored at 18 to 25°C.

MEDIA AND SUPPLIES

PrepFiler® BTA. Kit components

- PrepFiler® BTA Lysis Buffer (13 ml)
- PrepFiler® Express Cartridges (52)
- PrepFiler® Sample Tubes (52)
- PrepFiler® Elution Tubes (52)
- PrepFiler® LySep Columns (52)
- Tips and Tip Holders (52)
- Bone and Tooth Lysate Tubes (52)
- Bone and Tooth Lysate Tube Caps (52)
- Proteinase K (400 µL)

- (96-100%) Ethanol
- 1.5 mL Microcentrifuge
- Micropipetter
- Sterile pipet tips
- Scissors and forceps
- 70% ethanol or alcohol wipes
- Sterile water

EQUIPMENT

- Incubator/Shaker
- Centrifuge
- Vortex mixer

Sample Preparation; If the PrepFiler® Lysis Buffer contains precipitate, heat the buffer solution to 37°C for 15 minutes, then vortex the bottle for 5 seconds.

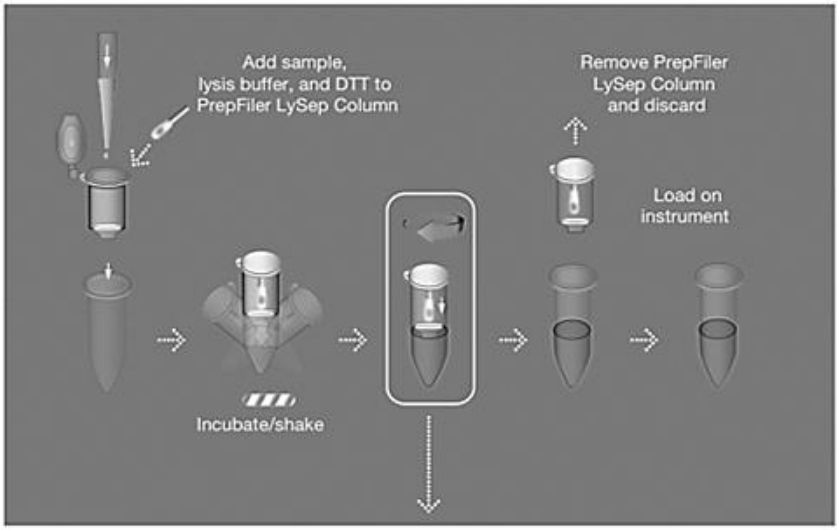
1. Bring the thermal shaker to 56°C.
2. Place each sample in PrepFiler® LySep Columns inserted in PrepFiler® Sample Tubes.
3. Prepare a fresh lysis buffer solution immediately before each experiment:
   a. Calculate the volumes of components that are needed for the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

Lysis buffer solution component	Volume	
	One reaction	96 reactions†
PrepFiler® BTA Lysis Buffer	220 µL	22 mL
1.0 M DTT	3 µL	300 µL
Proteinase K	7 µL	700 µL

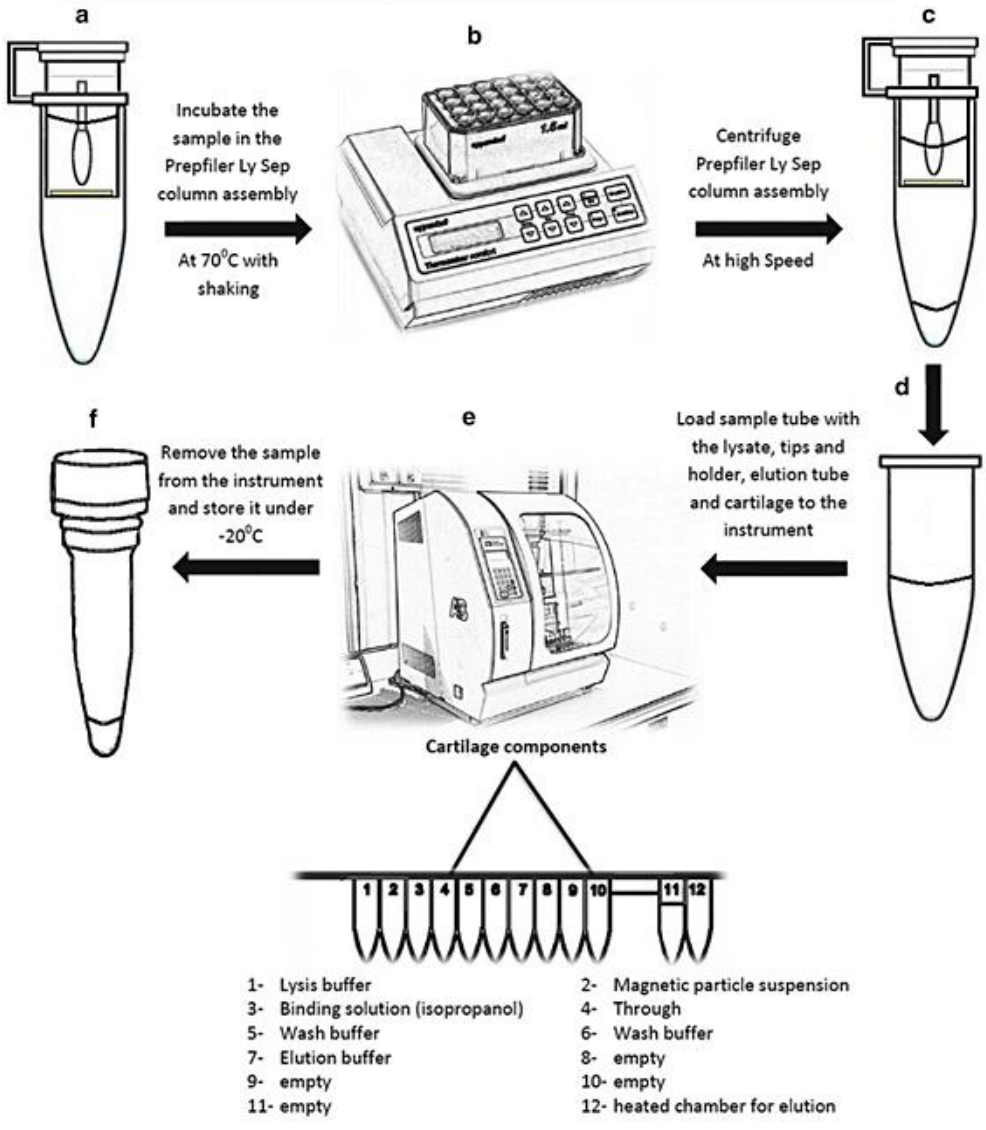
† Recommended volume; Includes approximately 4% excess volume to compensate for pipetting losses.

   b. Combine the required volumes of components, then gently mix.

4. Add 230 µL of the lysis buffer solution to each sample, Make sure the tubes are well sealed, then place the tubes in a thermal shaker and incubate at 56°C and 750 rpm for 40 minutes.
5. Centrifuge the column/tube assembly for 2 min and 90 s respectively at 10,000 × g (full speed), if the volume after the centrifugation is less than 300 µl. If the lysate solution is less than 150 µl, bring the solution up to 150 µl by adding PrepFiler lysis buffer. After that, Load AutoMate Express sample tubes, elution tubes, tips and holders, and cartridge into the instrument according to manufacturer's instruction



High-speed centrifugation enables simple and efficient separation of lysate from substrate



PROCEDURE

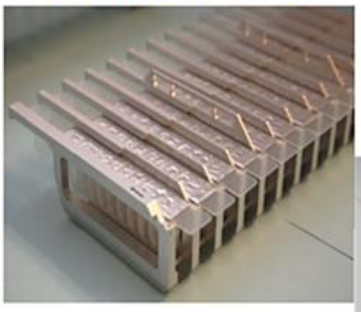
Setup AutoMate *Express*™ Instrument

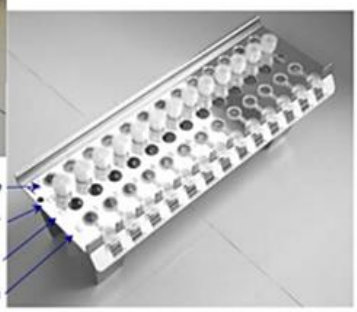
- Ensure Protocol Card is properly inserted

  - Do Not Turn on Instrument without card inserted!
  - Do Not Remove IC Card if the instrument is on!
- Power on AutoMate *Express*™ Instrument

AutoMate *Express*™ Instrument Run

- 1. Load cartridge rack with appropriate number of reagent cartridges
- 2. Load tip and tube rack
  - Sample lysate
  - Tips and tip holders
  - Elution tube
- 3. Select appropriate PrepFiler *Express*™ Protocol
- 4. Start run





A2.4 QUANTIFILER™ HUMAN DNA QUANTIFICATION KIT PROTOCOL

Real-time polymerase chain (PCR) reaction is a laboratory technique based on the polymerase chain reaction, used to amplify and simultaneously quantify a targeted DNA molecule. For one or more specific sequences in a DNA sample, Real Time-PCR enables both detection and quantification. Real-time PCR has the widest dynamic range of any DNA detection technology allowing for more accurate quantification of low level samples. Pre-formulated Internal PCR Control (IPC) included in each reaction allows for quick monitoring of amplification success and identifies samples that may contain inhibitors.

MEDIA AND SUPPLIES

Kit components:

- Quantifiler® PCR Reaction Mix (2°- 8° C).
- Quantifiler® Human DNA Standard (−15 to −25 °C)
- Quantifiler® Human Primer Mix (−15 to −25 °C).

- TE buffer
- MicroAmp Optical 96-well reaction plate - Applied Biosystems
- Optical Adhesive Covers – Applied Biosystems
- Black PCR support base (96 well)
- Eppendorf tubes
- Pipettes
- Sterile pipet tips

EQUIPMENT

- 7500 Real time PCR System
- Or QuantStudio 5
- Tabletop Centrifuge
- Vortex mixer

Preparing the DNA Quantification Standards

1. Label eight microcentrifuge tubes:

   Std. 1, Std. 2, Std. 3.. to Std 8 with the date.

   Vortex the Quantifiler HUMAN DNA Standard for 3 to 5 sec.
2. In tube labeled Std. 1, dispense 30 ul TE buffer.
3. To all tubes labeled Std. 2 to Std. 8, add 20 ul TE buffer.
4. Add 10 ul of standard into tube labeled Std. 1. mix well.
5. Add 10 ul from Std. 1 into tube labeled Std. 2. Mix well.
6. Add 10 ul from Std. 2 into tube labeled Std. 3. Mix well.
7. Add 10 ul from Std. 3 into tube labeled Std. 4. Mix well.
8. Add 10 ul from Std. 4 into tube labeled Std. 5. Mix well.
9. Add 10 ul from Std. 5 into tube labeled Std. 6. Mix well.
10. Add 10 ul from Std. 6 into tube labeled Std. 7. Mix well.
11. Add 10 ul from Std. 7 into tube labeled Std. 8. Mix well.

Standard	Concentration (ng/µL)	Example Amounts	Minimum Amounts	Dilution Factor
Std. 1	50.000	50 µL [200 ng/µL stock] + 150 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [200 ng/µL stock] + 30 µL $T_{10}E_{0.1}$ buffer	4X
Std. 2	16.700	50 µL [Std. 1] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 1] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 3	5.560	50 µL [Std. 2] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 2] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 4	1.850	50 µL [Std. 3] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 3] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 5	0.620	50 µL [Std. 4] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 4] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 6	0.210	50 µL [Std. 5] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 5] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 7	0.068	50 µL [Std. 6] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 6] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 8	0.023	50 µL [Std. 7] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 7] + 20 µL $T_{10}E_{0.1}$ buffer	3X

NOTE: The diluted standards have been shown to be stable for over 30 days when stored at 2° to 8°C without glycogen.

Preparing the Reactions

1. Calculate the volume of each component needed to prepare the reactions, using the table below.

Component	Volume Per Reaction (µL)
Quantifiler Human Primer Mix	10.5
Quantifiler PCR Reaction Mix	12.5

Note: Include additional reactions in the above calculations to provide excess volume for the loss that occurs during reagent transfers.

2. Prepare the reagents:
   - Thaw the primer mix completely, then vortex for 3 to 5 seconds.
   - Swirl the Quantifiler PCR Reaction Mix gently before using. Do not vortex it.

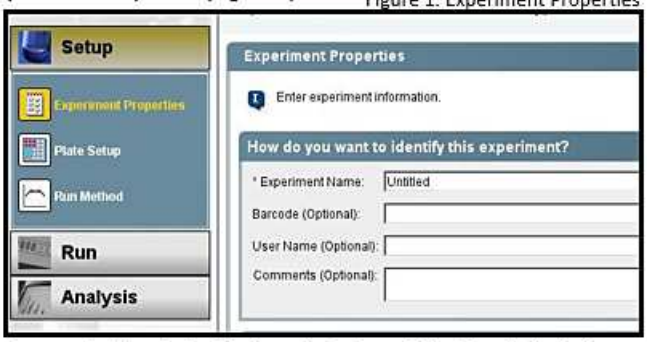
3. Pipette the required volumes of components into an appropriately sized tube. Vortex the PCR master mix 3-5 seconds, then centrifuge briefly.
4. Dispense 23 µL of the PCR mix into each reaction well.
5. Add 2 µL of sample, standard, or control to the appropriate wells.
6. Seal the reaction plate with the Optical Adhesive Cover.
7. Centrifuge the plate at 1200 rpm for about 1 min in a tabletop centrifuge with plate holders to remove any bubbles and proceed the run.

NOTE: It is recommended running duplicates of the eight DNA quantification standards for each assay and on each reaction plate.

Running the Reactions

1. Turn on the computer and then turn on the instrument
2. Position the plate in the instrument thermal block so that well A1 is in the upper-left corner
3. On the desktop, double-click HID Real-Time PCR Analysis Software v1.1.
4. In the User Name field, select your user name from the drop-down list. Click OK to open the Home screen.
5. From the icon list of HID experiments select **Quantifiler® Human**.
6. In the Experiment Menu, select Setup Experiment Properties (Figure 1).

Figure 1. Experiment Properties



7. In the "How do you want to identify this experiment?" section, enter in the Experiment Name field the *name of the plate*. In the toolbar, click the down arrow next to Save, then in the drop-down list, select **Save as** – to save the plate layout as a *.eds file.
8. In the Experiment Menu, select **Plate Setup.**

Note: Targets and an NTC (Negative Control) sample are automatically listed and named. Standards dilutions 1 to 8 are listed by default for each Quantifiler® Kit.

9. In the Define Samples area of the Define Targets and Samples tab, specify sample names.

- To define a new sample: Click **Add New Sample**. A new line appears in the Sample Name field. To save the name of the sample for future experiments, click **Save Sample.**

10. Select the **Assign Targets and Samples** tab.
11. Select the **View Plate Layout** tab in the pane on the right of the screen.

- Specify the information to display in the wells:

- Click **Show in Wells** to open the drop-down list. Items that are marked with a check (√) are selected for display.
- Click an item to select or deselect it for display.

Figure 2. Start Run button

Assign standards, NTCs, and unknown samples to well(s). In the Assign Sample(s) to the Selected Wells section to the left of the plate layout, select the check box in the Assign column corresponding to the unknown, standard, or NTC sample in the well(s).

12. In the Experiment Menu, select **Run**, select any screen, then click **Start Run** at the top left corner (Figure 2).

Data Analysis

1. Press the **green triangle icon** to automatically analyse your run. All analysed data is viewed under the **Results** tab.
2. In the **Results** tab, select the **Standard Curve** tab.
   - Examine the standard curve to see if R2 ≥ 0.98. If it is ≤ 0.98, then the run should be repeated.
3. Slopes in the range of -2.9 to -3.3 are generally considered acceptable for real-time PCR.
4. Cts < 29 are strong positive reactions indicative of abundant target nucleic acid in the sample.
5. Cts of 30-37 are positive reactions indicative of moderate amounts of target nucleic acid.
6. Cts of 38-40 are weak reactions indicative of minimal amounts of target nucleic acid which could represent an environmental contamination.

Result

Figure 3. Add a kit to the experiment

1. Open the experiment of interest.
2. In the Experiment Menu, select **STR Kit Setup.**
3. In the STR Kit Setup area, click **Add Kit to Experiment** to open the Kit Dilutions Library (Figure 3).
4. Select the kit to use in the experiment.
5. In the Experiment Menu, select any analysis screen, then select the View Well Table tab.
6. Select the check box corresponding to the unknown sample to use and the STR kit with which to use the sample.
   Note: The software automatically assigns the same kit for replicates.
7. Select the **Dilution Setup** tab to view the dilution scheme and the STR kit(s) that you selected for each sample (Figures 4 and 5).
8. Export data. In the toolbar, click (Export) to open the Export Data screen, then select the Export Properties tab (Figure 6).
9. Select the type of data to export: STR Dilution Setup – Sample dilution worksheet to prepare samples for amplification.
10. Customize the data
11. Select the **Customize Export** tab (Figure 7).

Figure 4. Dilution Setup tab (right)

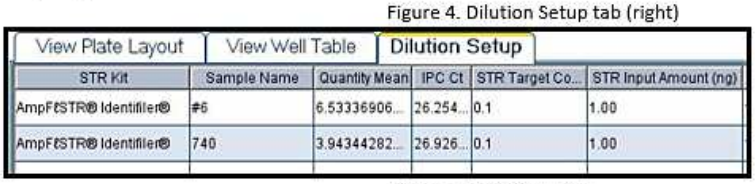


Figure 5. Dilution Setup

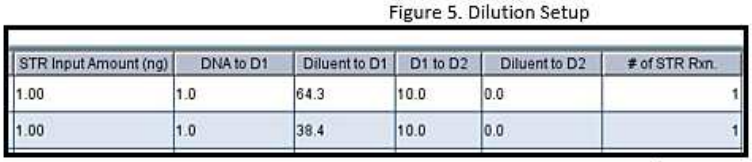
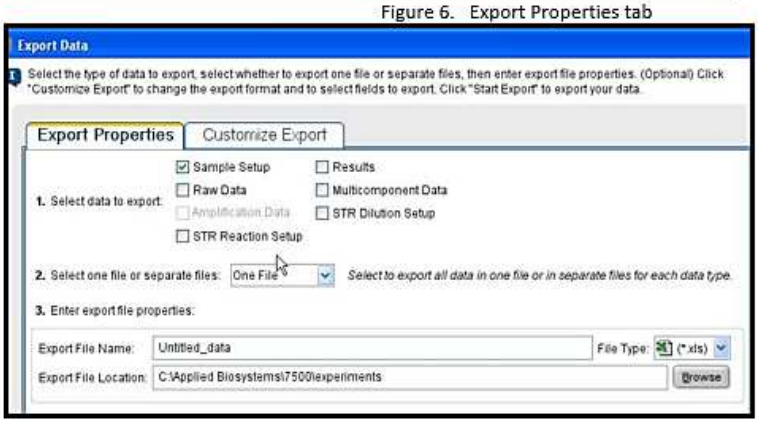


Figure 6. Export Properties tab



12. Select the information that you want to export.
13. Click Start Export to export the data to the file(s) that you selected.
14. **Print a report.** In the toolbar, click **Print Report** to display the Print Report screen (Figure 8).

Figure 7. Customize Export tab

Result Analysis

1. If the results from Quantifiler kit reactions indicate that insufficient DNA (<0.05) is present to perform a Short Tandem Repeat (STR) assay, amplify using the maximum volume of extract allowed for the amplification kit (10µl).
2. Overblown samples (<10ng/ul) can be diluted appropriately and amplified.
3. The "Ct" column is a flag for whether inhibition of a sample may be observed via the IPC, as determined by whether the cycle at which the IPC crossed the threshold was within the expected result. The IPC should cross the threshold at a similar cycle number in each sample with a degree of tolerance between samples. If a given sample's IPC CT is "Undetermined"(Figure 9), this may be an indication of inhibition and samples must be diluted. If sample's CT is "Undetermined" this means no DNA exists and sample may not be processed further (Figure 10).
4. Proceed to Amplification (PCR).

Figure 8. Print Report screen

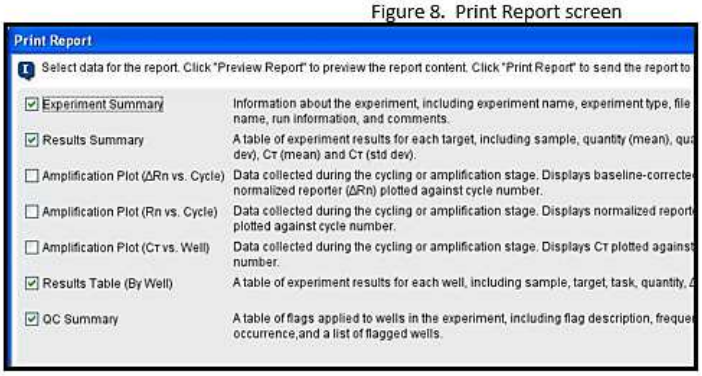


Figure 9. Analysis screen (IPC UND)

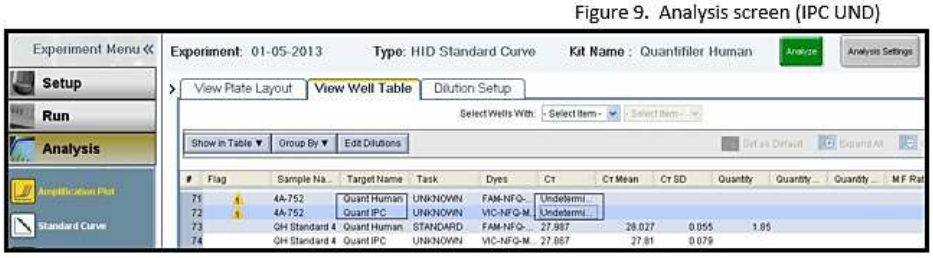


Figure 10. Analysis screen (Sample UND)

A2.5 QUANTIFILER™ TRIO DNA QUANTIFICATION PROTOCOL

The Quantifier Trio DNA Quantification Kit is a robust kit that enables forensic laboratories to simultaneously obtain a quantitative and qualitative assessment of total human and human male DNA in a single, highly sensitive real-time PCR reaction. The kit includes enhanced quantity and quality indicators that help deliver accurate, sensitive, and specific results that work together with the HID Real Time PCR Analysis Software v1.2 to enable informative and integrated workflow decisions.

Table 3 Quantifiler™ Trio DNA Quantification Kit (Cat. No. 4482910)

Reagent	Contents	Quantity	Storage†
Quantifiler™ THP PCR Reaction Mix	dNTPs, buffer, enzyme, Mustang Purple™ Passive Reference Standard, and stabilizers	4 tubes, 1 mL/tube	–25°C to –15°C upon receipt 2°C to 8°C after initial use Store protected from light
Quantifiler™ Trio Primer Mix	Target-specific primers, ABY™, JUN™, VIC™, and FAM™ dye-labeled probes, and Internal PCR Control (IPC) template	4 tubes, 0.8 mL/ tube	–25°C to –15°C upon receipt 2°C to 8°C after initial use Store protected from light
Quantifiler™ THP DNA Dilution Buffer	Genomic DNA Standard dilution buffer	2 tubes, 1.8 mL/ tube	–25°C to –15°C upon receipt 2°C to 8°C after initial use
Quantifiler™ THP DNA Standard	Genomic DNA Standard formulated at 100 ng/µL to generate standard curves	1 tube, 0.12 mL	–25°C to –15°C upon receipt 2°C to 8°C after initial use

† See reagent labels for expiration dates

Additional storage guideline for primer mix and PCR reaction mix: Keep Primer Mix and PCR Reaction Mix protected from direct exposure to light. Excessive exposure to light may affect the fluorescent probes and/or the passive reference dye.

MEDIA AND SUPPLIE

- MicroAmp Optical 96-well reaction plate - Applied Biosystems
- Optical Adhesive Covers – Applied Biosystems
- Black PCR support base (96 well)
- Eppendorf tubes
- Pipettes
- Sterile pipet tips

EQUIPMENT

- 7500 Real time PCR System
- Or QuantStudio 5
- Tabletop Centrifuge
- Vortex mixer

CALCULATING THE STANDARDS DILUTION

Standard	Concentration (ng/µL)	Example volumes	Dilution factor
Std. 1	50.000	10 µL [100 ng/µL stock] + 10 µL Quantifiler™ THP DNA dilution buffer	2X
Std. 2	5.000	10 µL [Std. 1] + 90 µL Quantifiler™ THP DNA dilution buffer	10X
Std. 3	0.500	10 µL [Std. 2] + 90 µL Quantifiler™ THP DNA dilution buffer	10X
Std. 4	0.050	10 µL [Std. 3] + 90 µL Quantifiler™ THP DNA dilution buffer	10X
Std. 5	0.005	10 µL [Std. 4] + 90 µL Quantifiler™ THP DNA dilution buffer	10X

Note: To ensure manual pipetting accuracy, pipet a minimum volume of 10 µL.

PREPARE THE DNA QUANTIFICATION STANDARDS

When using Quantifiler® THP DNA Dilution Buffer, you can store the prepared DNA quantification standards in low-binding tubes for up to **2 weeks at 2 to 8°C.** To prepare the DNA quantification standards dilution series:

1. Label five microcentrifuge tubes: Std. 1, Std. 2, Std. 3, and so on.
2. Dispense the required amount of diluent (Quantifiler® THP DNA Dilution Buffer) to each tube.
3. Prepare Std. 1:
    a. Vortex the Quantifiler® THP DNA Standard 3 to 5 seconds.
    b. Using a new pipette tip, add **10 ul** of Quantifiler® THP DNA Standard **in** tube containing **10 ul** of Quantifiler® THP DNA Dilution Buffer for your dilution series, then mix the dilution thoroughly.
4. Prepare Std. 2 through 5: Using a new pipette tip, add **90 ul** of Quantifiler® THP DNA Dilution Buffer to Std. 2 through 5, then:

- Add 10 ul from Std. 1 into tube labeled Std. 2. Mix well.
- Add 10 ul from Std. 2 into tube labeled Std. 3. Mix well.
- Add 10 ul from Std. 3 into tube labeled Std. 4. Mix well.
- Add 10 ul from Std. 4 into tube labeled Std. 5. Mix well

Preparing the Reactions

1. Calculate the volume of each component needed to prepare the reactions, using the table below.

Component	Volume Per Reaction (µL)
Primer Mix	8
PCR Reaction Mix	10

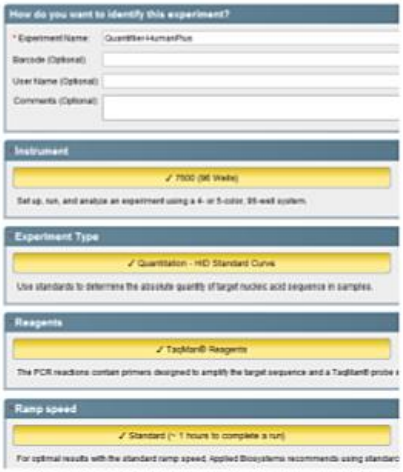
Note: Include additional reactions in the above calculations to provide excess volume for the loss that occurs during reagent transfers.

2. Prepare the reagents:
    - Thaw the primer mix completely, then vortex for 3 to 5 seconds.
    - Gently vortex the Reaction Mix before using.
3. Pipette the required volumes of components into an appropriately sized tube. Vortex the PCR master mix 3-5 seconds, then centrifuge briefly.
4. Dispense 18 µL of the PCR mix into each reaction well or tube.
5. Add 2 µL of sample, standard, or control to the appropriate wells. **Note:** *We recommend running duplicates of each sample of the DNA quantification standards for each reaction plate.*
6. Seal the reaction plate with the Optical Adhesive Cover.
7. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders to remove any bubbles. **Note:** *If a tabletop centrifuge with 96-well plate adapters is not available, visually inspect the plate for bubbles, and lightly tap the plate to remove bubbles in wells.*

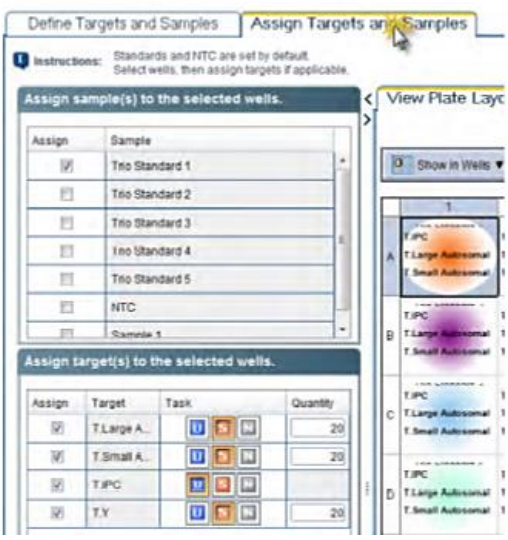
Running the Reactions

1. Turn on the computer and then turn on the instrument
2. Position the plate in the instrument thermal block so that well A1 is in the upper-left corner
3. On the desktop, double-click HID Real-Time PCR Analysis Software.
4. In the Home screen, click the icon for your application.

5. In the Experiment Properties screen, enter a name for the experiment. All other settings on this screen are automatically set for your application or are optional.



6. In the left navigational panel, click Setup -> Plate Setup. Targets are automatically specified for your application.
7. Define samples: Click **Add New Sample**, then type the name for the sample. Repeat for remaining samples.
8. Click **Assign Targets and Samples**. Targets are automatically assigned, and the standard quantities are automatically specified.
9. Assign the samples to the plate wells:
   a. To select well(s):
   • Single well—Click the well
   • Row of wells—Click a letter on the side of the layout
   • Column of wells—Click a number at the top of a column
   • More than one well, row, or column—Drag the pointer over the wells, letters, or columns to select.
   b. In the Assign sample(s) to wells section to the left of the plate layout, locate the desired sample and select the checkbox in the Assign column next to the sample name. The target for each sample is set by default.
   c. Repeat steps a and b for the remaining samples.



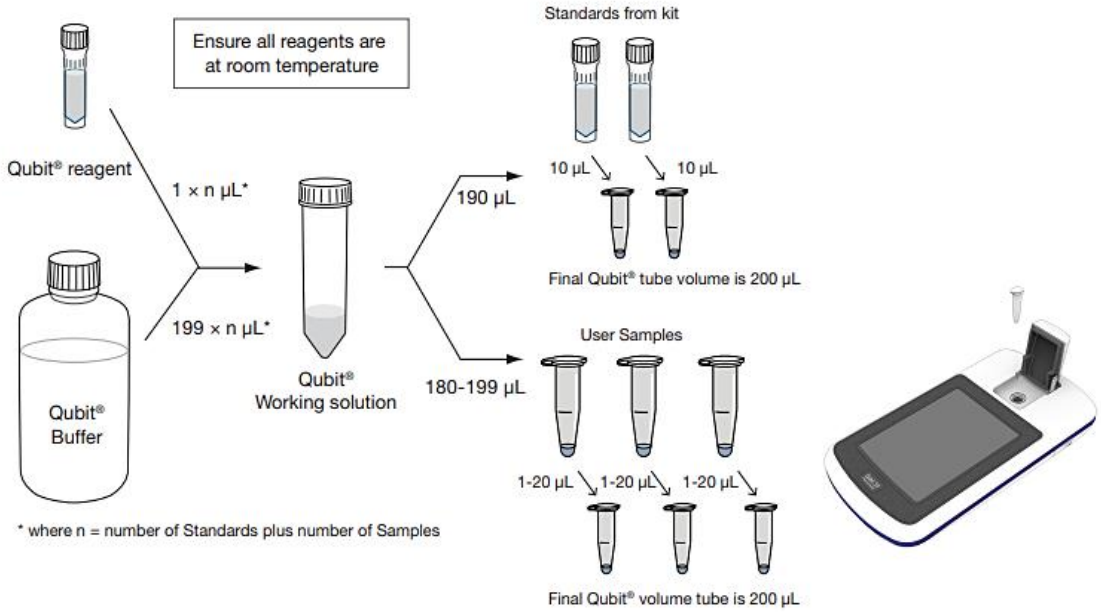
10. To change the well a sample is assigned to, click the well, deselect the sample in the Assign Samples section, click the new well, then select the sample in the Assign Samples section.
11. In the left navigational panel, click Setup - > Run Method to view the parameters. The parameters are automatically specified.

A2.6 QUBIT dsDNA HS ASSAY PROTOCOL

1. Set up two Assay Tubes for the standards (three for the protein assay) and one Assay Tube for each user sample.
2. Prepare the Qubit® Working Solution by diluting the Qubit® reagent 1:200 in Qubit® buffer. Prepare 200 µL of Working Solution for each standard and sample.
3. Prepare the Assay Tubes* according to the table below.
4. Vortex all tubes for 2–3 seconds.
5. Incubate the tubes for 2 minutes at room temperature (15 minutes for the Qubit® protein assay).
6. Insert the tubes in the Qubit® Fluorometer and take readings. For detailed instructions, refer to the Qubit® Fluorometer manual.

	Standard Assay Tubes	User Sample Assay Tubes
Volume of Working Solution (from step 2) to add	190 µL	180–199 µL
Volume of Standard (from kit) to add	10 µL	—
Volume of User Sample to add	—	1–20 µL
Total Volume in each Assay Tube	200 µL	200 µL

* Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit® assay tubes (set of 500, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830).



A2.7 GlobalFiler™ PCR AMPLIFICATION KIT PROTOCOL

The Applied Biosystems™ GlobalFiler™ PCR Amplification Kit is a 6-dye, short tandem repeat (STR) multiplex assay for the amplification of human genomic DNA. The kit amplifies: - 21 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338) - 1 Y-STR (DYS391) - 1 insertion/deletion polymorphic marker on the Y chromosome (Y indel) - Amelogenin (sex determining marker). The kit is validated for use with 1.0 ng DNA (15 µL input volume) for 29 cycles and 500 pg DNA (15 µL input volume) for 30 cycles for increased sensitivity with low-concentration samples.

Important guidelines: *store kit at –25°C to –15°C on receipt. Thaw reagents completely before first use. Store components at 2–8°C after first use to avoid freeze/thaw cycles. Keep reagents away from any source of contaminating DNA, especially from previously amplified PCR products and the allelic ladder. The primer set and allelic ladder contain primers that are labeled with light-sensitive dyes, protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use.*

KIT CONTENTS

- GlobalFiler™ Master Mix (1 × 1.5 mL)
- GlobalFiler™ Primer Set (1 × 0.5 mL)
- GlobalFiler™ Allelic Ladder (1 × 0.065 mL)
- DNA Control 007 (1 × 0.3 mL)

EQUIPMENT

- Pipettes and Pipettes Tips
- Microcentrifuge
- 1.5 or 2.0 -mL tubes
- 0.2 ml PCR Tubes
- Thermal cycler PCR
- Genetic analyser (3500)
- GeneMapper™ ID-X Software

REQUIRED MATERIALS

- GeneScan™ 600 LIZ™ Size Standard v2.0, 2
- Low-TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0)
- Hi-Di™ Formamide

PREPARE THE AMPLIFICATION KIT REACTIONS

1. Vortex the Master Mix and Primer Set for 3 seconds. Before opening the tubes or bottles, remove droplets from the caps by centrifuging the tubes briefly or tapping the bottles on the bench.
2. Pipette the required volumes of components into an appropriately sized clear (non-coloured) 1.5 or 2.0 -mL tube.
3. Vortex the reaction mixture for 3 seconds, then centrifuge briefly.
4. Dispense 10 µL of reaction mixture into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate or each MicroAmp™ tube.

Reaction component	Volume per reaction
Master Mix	7.5 µL
Primer Set	2.5 µL

Note: Include volume for additional reactions to provide excess volume for the loss that occurs during reagent transfers.

5. Adjust the sample input amount and volume as needed: If total sample input amount is >1.0 ng for 29 cycles or >500 pg for 30 cycles, dilute with low-TE buffer to achieve a 15-µL input volume... or If total sample input volume is <15 µL, bring to volume with low-TE buffer to achieve a 15-µL input volume.
6. Then Prepare samples as shown in the following table, then add them to the appropriate well or tube (final reaction volume is 25 µL).
7. Seal the MicroAmp™ Optical 96-Well Reaction Plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film, or cap the tubes. then centrifuge at 3,000 rpm for about 20 seconds.

Sample	29-cycle protocol	30-cycle protocol
Negative control	15 µL of low-TE buffer	15 µL of low-TE buffer
Test sample	15 µL of DNA	15 µL of DNA
Positive control	Combine, then add to the reaction well or tube: • 10 µL control DNA (0.1 ng/µL) • 5 µL of low-TE buffer	Combine, then add to the reaction well or tube: • 5 µL control DNA (0.1 ng/µL) • 10 µL of low-TE buffer

PERFORM PCR

IMPORTANT! This kit is validated for use with the validated thermal cyclers listed in "Instrument and software compatibility" on page 15.

1. Program the thermal cycling conditions.

   IMPORTANT! If you using are the GeneAmp™ PCR System 9700, select the Max ramping mode. If you are using the Veriti™ Thermal Cycler, select the 100% ramping rate. *Do not* use 9600 emulation mode.

Initial incubation step	Cycle (29 or 30 cycles)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C, 1 minute	94°C, 10 seconds	59°C, 90 seconds	60°C, 10 minutes	4°C, Up to 24 hours[1]

[1] The infinity (∞) setting allows an unlimited hold time.

2. Load the plate into the thermal cycler, close the heated cover, then start the run.

   IMPORTANT! If you are using adhesive clear film instead of caps to seal the plate wells, be sure to place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. The Veriti™ Thermal Cycler does not require a compression pad.

3. When the run is complete, store the amplified DNA.

If you are storing the DNA...	Then place at...
<2 weeks	2°C to 8°C
>2 weeks	–25°C to –15°C

IMPORTANT! Protect the amplified DNA from light.

A2.8 ELECTROPHORESIS SAMPLE PREPARATION PROTOCOL

PREPARE SAMPLES FOR ELECTROPHORESIS; Prepare the samples for electrophoresis immediately before loading.

1. Pipet the required volumes of components into an appropriately sized polypropylene tube:
2. Vortex the tube, then briefly centrifuge.
3. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, add: 10 µL of the formamide/size standard mixture + 1 µL of PCR product or Allelic Ladder. **Note:** *For blank wells, add 10 µL of Hi-Di™ Formamide.*

Reagent	Volume per reaction
GeneScan™ 600 LIZ™ Size Standard v2.0	0.4 µL
Hi-Di™ Formamide	9.6 µL

Note: Include volume for additional samples to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

4. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
5. To perform denaturation, heat the reaction plate in a thermal cycler at 95°C for 3 minutes. Then Immediately place the plate on ice for 3 minutes. Or set denaturation programme in PCR: Choose 'Denaturation', which is programmed with the following temperatures: 95°C for 3 min → 4°C for 3 min → 4°C ∞
6. Place the sample tray on the autosampler, then start the electrophoresis run.

OPERATING PROCEDURE

1. Switch on the 3500 Genetic Analyzer
2. When the instrument light turns green, switch on the computer.
3. When the Server monitor becomes active, a green tick mark will appear on the lower-right corner of the monitor.
4. Double-click the 3500-software icon.
5. Click 'Start Pre-heat' (optional).
6. Click 'Create new plate'. Enter 'Plate name' and select 'HID' for 'Plate Type'.
7. Click 'Assign Plate Contents'.
8. Type the sample number into the appropriate wells.
9. Click 'Customize Sample Info' arrow on the lower-right corner of the screen. A window will appear where you can assign 'Sample Type' for Allelic Ladder, Positive Control, Negative Control, Sample and HiDi-Formamide.
10. Under 'Assays', click 'Add from Library'. Select appropriate assay, e.g. IFP_POP4_5SecInj or IFP_POP4_15SecInj. Click on 'Add to Plate' and 'Close'.
11. Under 'File Name Conventions', click 'Add from Library'. Select then Click on 'Add to Plate' and 'Close'.
12. Under 'Results Group', click 'Add from Library'. Select appropriate results group, then Click on 'Add to Plate' and 'Close'.
13. Highlight all samples and tick all the boxes under, 'Assays', 'File Name Conventions' and 'Results Group'.
14. Press the instrument tray to bring the Autosampler to the front and open instrument.
15. Place the plate into the 3500 Genetic Analyzer Autosampler. The plate only fits into the instrument in one direction.
16. Close door and wait for the green light from the instrument.
17. Click 'Link Plate for run', Click 'OK' when the window '*The plate has been modified. Do you want to save this and run*'
18. Click 'OK' and 'Start Run'.

ANALYZE AND EDIT SAMPLE FILES WITH GENEMAPPER™ ID-X SOFTWARE

1. In the Project window, select **Edit ▸ Add Samples to Project**, then navigate to the disk or directory that contains the sample files.

2. Apply analysis settings to the samples in the project.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	Select **GlobalFiler Analysis Method** (or the name of the analysis method you created).
Panel	Select **GlobalFiler_Panel**.
Size Standard	Use a size range of 60–460 bp for Local Southern size-calling method or a size range of 80–460 bp for 3rd Order Least Squares size-calling method.[1]

[1] The GlobalFiler™ kit was originally validated with the GeneScan™ 600 LIZ™ Size Standard v2.0 . If you use a different size standard, perform the appropriate internal validation studies to support the use of this size standard with the GlobalFiler™ kit.

3. Click **Analyze**, enter a name for the project (in the Save Project dialog box), then click **OK** to start analysis.
   - The status bar displays the progress of analysis as a completion bar extending to the right with the percentage indicated.
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
   - The Analysis Summary tab is displayed and the Genotypes tab is available when the analysis is complete.

Allelic ladder requirements for electrophoresis

To accurately genotype samples, you must run an allelic ladder with the samples.

Instrument	Number of allelic ladders to run	One injection equals	Number of samples per allelic ladder(s)
3500	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
3500*xl*	1 per injection	24 samples	23 samples + 1 allelic ladder
3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3130*xl*	1 per injection	16 samples	15 samples + 1 allelic ladder

IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between runs. Follow the guidelines in the preceding table, which should account for normal variation in run speed. Perform internal validation studies to verify the required allelic ladder injection frequency, to ensure accurate genotyping of all samples in your laboratory environment.

Electrophoresis instrument requirements

For more information, see the "Related documentation" section in the user guide.

Table 1 3500 Series instrument requirements

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Plate templates, assays, run modules, and conditions (installed with the HID Updater)
3500 3500xL	Windows™ Vista	3500 Data Collection Software v1	HID Updater 3500 DC v2 (Cat. No. 4480670)	Plate templates: 6dye_36_POP4 (and _xl) Assays: GF+Norm_POP4 (and _xl) and GF_POP4 (and _xl), which contain instrument protocol HID36_POP4 (and_xl)_J6_NT3200 with the following conditions: • Run module: HID36_POP4 • Injection conditions: 1.2 kV/15 sec (24 sec for xl) • Run conditions: 13 kV/1550 sec • Dye Set J6
3500 3500xL	Windows™ 7	3500 Data Collection Software v2	HID Updater 3500 DC v2 (Cat. No. 4480670)	Same as 3500 Data Collection Software v1 listed above
3500 3500xL	Windows™ 7	3500 Data Collection Software v3	None	Same as 3500 Data Collection Software v1 listed above

A3. COLLECTION TECHNIQUES

A3.1 QUANTIFICATION OF COTTON SWAB WETTING TECHNIQUES

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluent to D2
GlobalFiler™	177; EXP1- Pi	0.0751	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	178; EXP1- Pi	0.0571	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	179; EXP1- Pi	0.0483	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	180; EXP1- Pi	0.0491	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	181; EXP1- Pi	0.0583	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	182; EXP1- Pi	0.0512	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	183; EXP1- Pi	0.0421	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	184; EXP1- Pi	0.0462	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	185; EXP1- Sp	0.1078	0.11	9.0	6.0	0.0	0.0
GlobalFiler™	186; EXP1- Sp	0.0921	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	187; EXP1- Sp	0.0833	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	188; EXP1- Sp	0.1021	0.10	10.0	5.0	0.0	0.0
GlobalFiler™	189; EXP1- Sp	0.0981	0.10	10.0	5.0	0.0	0.0
GlobalFiler™	190; EXP1- Sp	0.0784	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	191; EXP1- Sp	0.1084	0.11	9.0	6.0	0.0	0.0
GlobalFiler™	192; EXP1- Sp	0.0894	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	193; EXP1- Ds	0.0821	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	194; EXP1- Ds	0.0732	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	195; EXP1- Ds	0.0765	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	196; EXP1- Ds	0.0628	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	197; EXP1- Ds	0.0747	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	198; EXP1- Ds	0.0683	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	199; EXP1- Ds	0.0728	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	200; EXP1- Ds	0.0773	0.08	10.0	5.0	0.0	0.0

***EXP1**; Experiment 1 ***Pi**; Pipette ***Sp**; Spray bottle ***Ds**; Double

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

A3.2 QUANTIFICATION OF DRYING OR FREEZING PRIOR TO EXTRACTION

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluen t to D2
GlobalFiler™	185; EXP1- IM	0.1078	0.11	9.0	6.0	0.0	0.0
GlobalFiler™	186; EXP1- IM	0.0921	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	187; EXP1- IM	0.0833	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	188; EXP1- IM	0.1021	0.10	10.0	5.0	0.0	0.0
GlobalFiler™	189; EXP1- IM	0.0981	0.10	10.0	5.0	0.0	0.0
GlobalFiler™	190; EXP1- IM	0.0784	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	191; EXP1- IM	0.1084	0.11	9.0	6.0	0.0	0.0
GlobalFiler™	192; EXP1- IM	0.0894	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	201; EXP2- F	0.0824	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	202; EXP2- F	0.0779	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	203; EXP2- F	0.0749	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	204; EXP2- F	0.0684	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	205; EXP2- F	0.0791	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	206; EXP2- F	0.0814	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	207; EXP2- F	0.0642	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	208; EXP2- F	0.0763	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	209; EXP2- D	0.0583	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	210; EXP2- D	0.0432	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	211; EXP2- D	0.0395	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	212; EXP2- D	0.0476	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	213; EXP2- D	0.0527	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	214; EXP2- D	0.0462	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	215; EXP2- D	0.0348	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	216; EXP2- D	0.0413	0.04	10.0	5.0	0.0	0.0

***EXP2**; Experiment 2 ***IM**; Immediate extraction ***F**; Swabs were frozen *__D;__ Swabs were dried

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

A4. INFLUENCE OF SURFACE TYPE, COLLECTION AND EXTRACTION

A4.1 QUANTIFICATION OF THE INFLUENCE OF SURFACE TYPE

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluen t to D2
GlobalFiler™	1; SS-CS-P1-EX1	0.0288	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	2; TP-CS-P1-EX1	0.0385	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	3; TW-CS-P1-EX1	0.0221	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	4; CP-CS-P1-EX1	0.0227	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	5; BS-CS-P1-EX1	0.0271	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	6; G-CS-P1-EX1	0.0642	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	7; SS-NS-P1-EX1	0.0221	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	8; TP-NS-P1-EX1	0.0317	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	9; TW-NS-P1-EX1	0.0185	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	10; CP-NS-P1-EX1	0.0069	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	11; BS-NS-P1-EX1	0.0067	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	12; G-NS-P1-EX1	0.0447	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	13; SS-MT-P1-EX1	0.0262	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	14; TP-MT-P1-EX1	0.022	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	15; TW-MT-P1-EX1	0.0427	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	16; CP-MT-P1-EX1	0.0341	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	17; BS-MT-P1-EX1	0.0294	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	18; G-MT-P1-EX1	0.0521	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	19; SS-CS-P2-EX1	0.0194	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	20; TP-CS-P2-EX1	0.0327	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	21; TW-CS-P2-EX1	0.0092	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	22; CP-CS-P2-EX1	0.0052	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	23; BS-CS-P2-EX1	0.0051	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	24; G-CS-P2-EX1	0.0272	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	25; SS-NS-P2-EX1	0.013	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	26; TP-NS-P2-EX1	0.0213	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	27; TW-NS-P2-EX1	0.0027	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	28; CP-NS-P2-EX1	0.0015	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	29; BS-NS-PEX1	0.0054	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	30; G-NS-P2-EX1	0.0227	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	31; SS-MT-P2-EX1	0.0172	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	32; TP-MT-P2-EX1	0.0132	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	33; TW-MT-P2-EX1	0.0326	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	34; CP-MT-P2-EX1	0.0243	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	35; BS-MT-P2-EX1	0.0081	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	36; G-MT-P2-EX1	0.0174	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	CT10; EX1 CONTROL	N/A	N/A	N/A	N/A	N/A	N/A
GlobalFiler™	P0; REF. SAMPLE	12.7224	12.72	1.0	9.0	1.0	18.0

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

- CT; Control Sample
- P; Participant
- EX1; PrepFiler BTA
- Ex2; QIAamp Inve.
- P; Participant

- SS; Stainless Steel
- TP; Textured Plastic
- TW; Textured Wood
- CP; Copier Paper
- BS; Banana Skin
- G; Glass

- CS; Cotton Swab
- NS; Nylon Swab
- MT; minitabes

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluen t to D2
GlobalFiler™	37; SS-CS-P1-EX2	0.0867	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	38; TP-CS-P1-EX2	0.1018	0.10	10.0	5.0	0.0	0.0
GlobalFiler™	39; TW-CS-P1-EX2	0.0638	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	40; CP-CS-P1- EX2	0.0318	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	41; BS-CS-P1-EX2	0.0681	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	42; G-CS-P1-EX2	0.1078	0.11	9.5	5.5	0.0	0.0
GlobalFiler™	43; SS-NS-P1-EX2	0.1132	0.11	9.0	6.0	0.0	0.0
GlobalFiler™	44; TP-NS-P1-EX2	0.1057	0.11	9.5	5.5	0.0	0.0
GlobalFiler™	45; TW-NS-P1-EX2	0.0732	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	46; CP-NS-P1-EX2	0.0462	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	47; BS-NS-P1-EX2	0.0874	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	48; G-NS-P1-EX2	0.1217	0.12	9.0	6.0	0.0	0.0
GlobalFiler™	49; SS-MT-P1-EX2	0.0762	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	50; TP-MT-P1-EX2	0.0468	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	51; TW-MT-P1-EX2	0.0917	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	52; CP-MT-P1-EX2	0.0684	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	53; BS-MT-P1-EX2	0.0824	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	54; G-MT-P1-EX2	0.0879	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	55; SS-CS-P2-EX2	0.0449	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	56; TP-CS-P2-EX2	0.048	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	57; TW-CS-P2-EX2	0.0342	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	58; CP-CS-P2-EX2	0.0274	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	59; BS-CS-P2-EX2	0.0241	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	60; G-CS-P2-EX2	0.0554	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	61; SS-NS-P2-EX2	0.0664	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	62; TP-NS-P2-EX2	0.0775	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	63; TW-NS-P2-EX2	0.0426	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	64; CP-NS-P2-EX2	0.0315	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	65; BS-NS-P2-EX2	0.0354	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	66; G-NS-P2-EX2	0.0758	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	67; SS-MT-P2-EX2	0.0414	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	68; TP-MT-P2-EX2	0.0241	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	69; TW-MT-P2-EX2	0.0643	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	70; CP-MT-P2-EX2	0.0481	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	71; BS-MT-P2-EX2	0.0418	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	72; G-MT-P2-EX2	0.0541	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	CT11; EX1 CONTROL	N/A	N/A	N/A	N/A	N/A	N/A

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

- CT; Control Sample
- P; Participant
- EX1; PrepFiler BTA
- Ex2; QIAamp Inve.
- P; Participant
- SS; Stainless Steel
- TP; Textured Plastic
- TW; Textured Wood
- CP; Copier Paper
- BS; Banana Skin
- G; Glass
- CS; Cotton Swab
- NS; Nylon Swab
- MT; minitabes

A4.2 VISIBLE FINGERPRINTS ON THE SURFACE

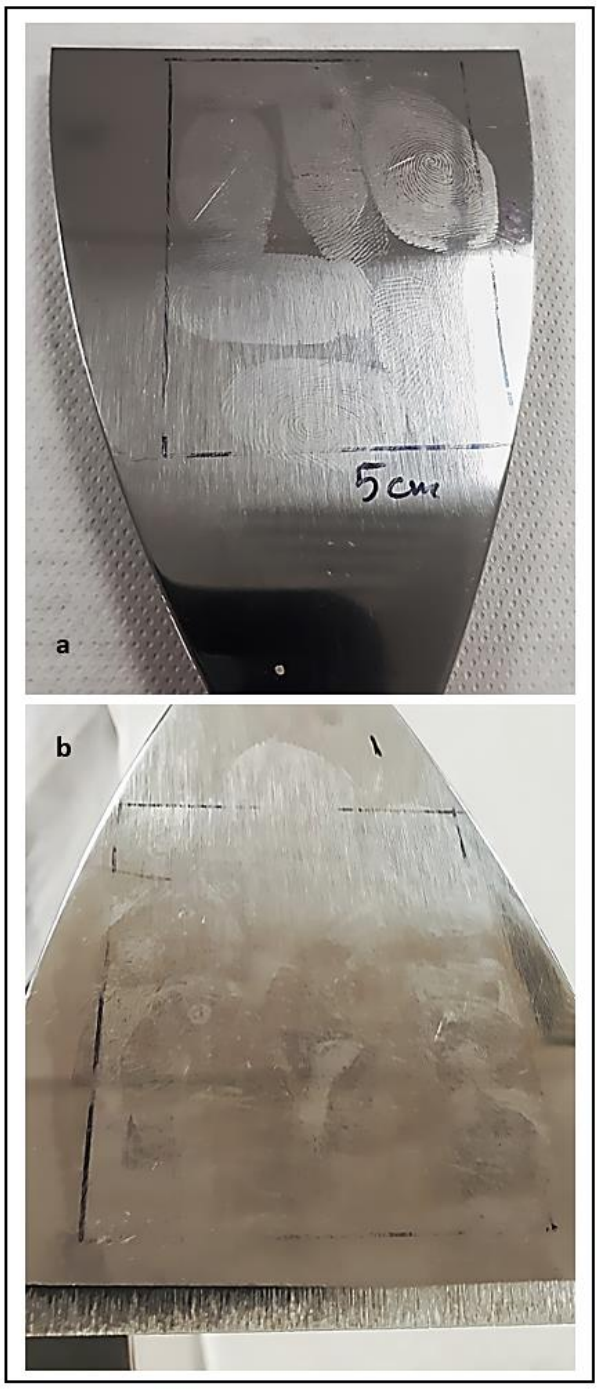


Figure A4.1: Visible fingerprints on stainless steel **(a)** after DNA deposition and **(b)** after DNA collection with SceneSafe Fast™ minitape.

A4.3 QUANTIFICATION OF PrepFiler EXPRESS BTA™ DNA EXTRACTION KIT

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluen t to D2
GlobalFiler™	217; EXP3-A	0.0662	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	218; EXP3-A	0.0569	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	219; EXP3-A	0.0586	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	220; EXP3-A	0.0541	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	221; EXP3-A	0.0438	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	222; EXP3-A	0.0459	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	223; EXP3-A	0.0661	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	224; EXP3-A	0.0532	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	225; EXP3-B	0.0614	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	226; EXP3-B	0.0482	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	227; EXP3-B	0.0346	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	228; EXP3-B	0.0583	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	229; EXP3-B	0.0392	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	230; EXP3-B	0.0469	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	231; EXP3-B	0.0612	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	232; EXP3-B	0.0648	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	233; EXP3-C	0.0823	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	234; EXP3-C	0.0876	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	235; EXP3-C	0.0781	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	236; EXP3-C	0.0831	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	237; EXP3-C	0.0742	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	238; EXP3-C	0.0869	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	239; EXP3-C	0.0911	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	240; EXP3-C	0.0798	0.08	10.0	5.0	0.0	0.0

***EXP3**; Experiment 3

***B**; Half swab head with 230µL of lysis buffer

***A;** Full swab head with 230µL of lysis buffer

***C**; Full swab head with 460µL of lysis buffer

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

A4.4 QUANTIFICATION OF THE INFLUENCE OF COLLECTION AND EXTRACTION METHODS

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluen t to D2
GlobalFiler™	241; G-CS-EX1	0.0751	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	242; G-NS-EX1	0.0617	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	243; G-MT-EX1	0.0685	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	244; G-CS-EX2	0.0668	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	245; G-NS-EX2	0.0987	0.10	10.0	5.0	0.0	0.0
GlobalFiler™	246; G-MT-EX2	0.0356	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	247; G-CS-EX1	0.0842	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	248; G-NS-EX1	0.0585	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	249; G-MT-EX1	0.0556	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	250; G-CS-EX2	0.0752	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	251; G-NS-EX2	0.0751	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	252; G-MT-EX2	0.0257	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	253; G-CS-EX1	0.0671	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	254; G-NS-EX1	0.0771	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	255; G-MT-EX1	0.0609	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	256; G-CS-EX2	0.0711	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	257; G-NS-EX2	0.0872	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	258; G-MT-EX2	0.0374	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	259; CP-CS-EX1	0.0217	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	260; CP -NS-EX1	0.0104	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	261; CP -MT-EX1	0.0381	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	262; CP -CS-EX2	0.0129	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	263; CP -NS-EX2	0.0192	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	264; CP -MT-EX2	0.0208	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	265; CP-CS-EX1	0.0189	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	266; CP -NS-EX1	0.0071	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	267; CP -MT-EX1	0.0257	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	268; CP -CS-EX2	0.0095	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	269; CP -NS-EX2	0.0101	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	270; CP -MT-EX2	0.0198	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	271; CP-CS-EX1	0.0097	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	272; CP -NS-EX1	0.0119	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	273; CP -MT-EX1	0.0419	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	274; CP -CS-EX2	0.0079	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	275; CP -NS-EX2	0.0221	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	276; CP -MT-EX2	0.0094	0.01	10.0	5.0	0.0	0.0

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

- EX1; PrepFiler BTA
- Ex2; QIAamp Inve.
- G; Glass
- CP; Copier Paper
- CS; Cotton Swab
- NS; Nylon Swab
- MT; minitabes

A4.5 QUANTIFICATION OF 4N6FLOQSwabs

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluen t to D2
GlobalFiler™	309; TP-CS	0.0385	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	310; TP-CS	0.0317	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	311; TP-CS	0.0427	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	312; TP-CS	0.0289	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	313; TP-CS	0.0571	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	314; TP-CS	0.0348	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	315; TP-CS	0.0248	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	316; TP-CS	0.0481	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	317; TP-CS	0.0601	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	318; TP-CS	0.0291	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	319; TP-CS	0.0363	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	320; TP-CS	0.0291	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	321; TP-NS - 100	0.0217	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	322; TP-NS - 100	0.0187	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	323; TP-NS - 100	0.0087	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	324; TP-NS - 100	0.0310	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	325; TP-NS - 100	0.0289	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	326; TP-NS - 100	0.0156	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	327; TP-NS - 100	0.0286	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	328; TP-NS - 100	0.0361	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	329; TP-NS - 100	0.0419	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	330; TP-NS - 100	0.0293	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	331; TP-NS - 100	0.0191	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	332; TP-NS - 100	0.0098	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	333; TP-NS - 30	0.0478	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	334; TP-NS - 30	0.0548	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	335; TP-NS - 30	0.0386	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	336; TP-NS - 30	0.0288	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	337; TP-NS - 30	0.0319	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	338; TP-NS - 30	0.0581	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	339; TP-NS - 30	0.0439	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	340; TP-NS - 30	0.0541	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	341; TP-NS - 30	0.0628	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	342; TP-NS - 30	0.0526	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	343; TP-NS - 30	0.0386	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	344; TP-NS - 30	0.0581	0.06	10.0	5.0	0.0	0.0

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

- TP; Textured plastic
- CS; Cotton Swab with 100 µl of distilled water
- NS-100; Nylon Swab with 100 µl of distilled water.
- NS-30; Nylon Swab with 30 µl of distilled water

A5. INFLUENCE OF TIME AND ENVIRONMENTAL CONDITIONS

A5.1 DIGITAL THERMOMETER HYGROMETER

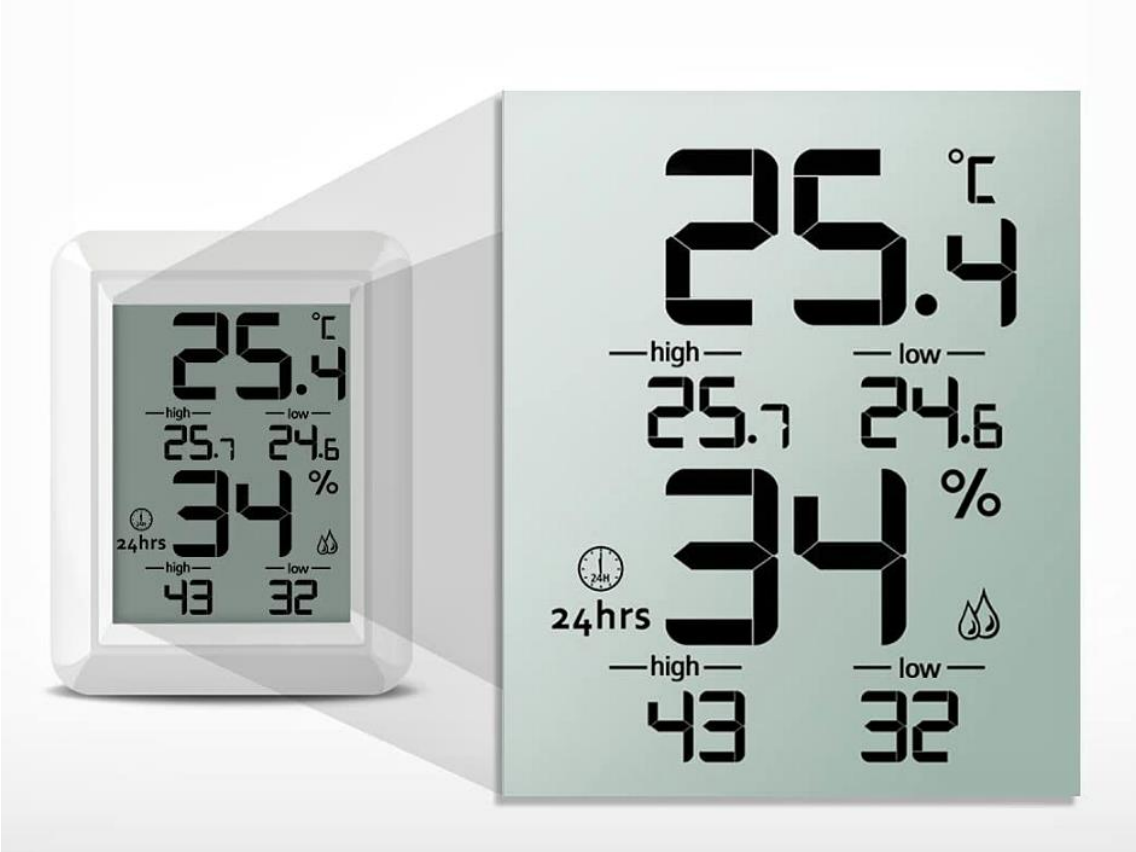


Figure A5.1: Oria digital thermometer hygrometer (L. 7.5 x W. 8.5 x H. 1.9 cm), for indoor/outdoor humidity meter and temperature monitor with wireless sensor. Calibrated against Oregon scientific THGR221.

A5.2 QUANTIFICATION OF THE INFLUENCE OF HUMIDITY ON RECOVERY TECHNIQUES

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluent to D2
GlobalFiler™	277; EXP4-CS-A	0.0357	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	278; EXP4-CS-A	0.0479	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	279; EXP4-CS-A	0.0518	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	280; EXP4-CS-A	0.0374	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	281; EXP4-CS-A	0.0328	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	282; EXP4-CS-A	0.0471	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	283; EXP4-CS-A	0.0448	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	284; EXP4-CS-A	0.0496	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	285; EXP4-CS-B	0.0908	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	286; EXP4-CS-B	0.0824	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	287; EXP4-CS-B	0.0809	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	288; EXP4-CS-B	0.0728	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	289; EXP4-CS-B	0.0761	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	290; EXP4-CS-B	0.0683	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	291; EXP4-CS-B	0.0783	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	292; EXP4-CS-B	0.0918	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	293; EXP4-NS-A	0.0668	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	294; EXP4-NS-A	0.0815	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	295; EXP4-NS-A	0.0617	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	296; EXP4-NS-A	0.0682	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	297; EXP4-NS-A	0.0691	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	298; EXP4-NS-A	0.0788	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	299; EXP4-NS-A	0.0821	0.08	10.0	5.0	0.0	0.0
GlobalFiler™	300; EXP4-NS-A	0.0713	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	301; EXP4-NS-B	0.0528	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	302; EXP4-NS-B	0.0438	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	303; EXP4-NS-B	0.0468	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	304; EXP4-NS-B	0.0607	0.06	10.0	5.0	0.0	0.0
GlobalFiler™	305; EXP4-NS-B	0.0473	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	306; EXP4-NS-B	0.0386	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	307; EXP4-NS-B	0.0449	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	308; EXP4-NS-B	0.0526	0.05	10.0	5.0	0.0	0.0

***EXP4;** Experiment 4 ***CS-A;** moist cotton swab ***CS-B;** dry cotton swab ***NS-A;** dry Nylon swab ***NS-B;** moist Nylon swab

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

A5.3 QUANTIFICATION OF SIMULATING OUTDOOR CRIME SCENE CASEWORK SCENARIOS

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1
GlobalFiler™	73; SS-CS-P1-3H-EX1-RT	0.0335	0.03	10.0	5.0
GlobalFiler™	74; TP-CS-P1-3H-EX1-RT	0.0466	0.05	10.0	5.0
GlobalFiler™	75; TW-CS-P1-3H-EX1-RT	0.0255	0.03	10.0	5.0
GlobalFiler™	76; CP-CS-P1-3H-EX1-RT	0.0268	0.03	10.0	5.0
GlobalFiler™	77; BS-CS-P1-3H-EX1-RT	0.0285	0.03	10.0	5.0
GlobalFiler™	78; G-CS-P1-3H-EX1-RT	0.0505	0.05	10.0	5.0
GlobalFiler™	79; SS-CS-P1-3H-EX1-HT	0.0345	0.03	10.0	5.0
GlobalFiler™	80; TP-CS-P1-3H-EX1-HT	0.0499	0.05	10.0	5.0
GlobalFiler™	81; TW-CS-P1-3H-EX1-HT	0.0334	0.03	10.0	5.0
GlobalFiler™	82; G-CS-P1-3H-EX1-HT	0.0523	0.05	10.0	5.0
GlobalFiler™	83; SS-CS-P1-3H-EX1-LT	0.0303	0.03	10.0	5.0
GlobalFiler™	84; TP-CS-P1-3H-EX1-LT	0.0573	0.06	10.0	5.0
GlobalFiler™	85; TW-CS-P1-3H-EX1-LT	0.0275	0.03	10.0	5.0
GlobalFiler™	86; G-CS-P1-3H-EX1-LT	0.0541	0.05	10.0	5.0
GlobalFiler™	87; SS-CS-P1-12H-EX1-RT	0.0301	0.03	10.0	5.0
GlobalFiler™	88; TP-CS-P1-12H-EX1-RT	0.0516	0.05	10.0	5.0
GlobalFiler™	89; TW-CS-P1-12H-EX1-RT	0.0178	0.02	10.0	5.0
GlobalFiler™	90; CP-CS-P1-12H-EX1-RT	0.0216	0.02	10.0	5.0
GlobalFiler™	91; BS-CS-P1-12H-EX1-RT	0.0232	0.02	10.0	5.0
GlobalFiler™	92; G-CS-P1-12H-EX1-RT	0.0563	0.06	10.0	5.0
GlobalFiler™	93; SS-CS-P1-12H-EX1-HT	0.0333	0.03	10.0	5.0
GlobalFiler™	94; TP-CS-P1-12H-EX1-HT	0.0709	0.07	10.0	5.0
GlobalFiler™	95; TW-CS-P1-12H-EX1-HT	0.0527	0.05	10.0	5.0
GlobalFiler™	96; G-CS-P1-12H-EX1-HT	0.0485	0.05	10.0	5.0
GlobalFiler™	97; SS-CS-P1-12H-EX1-LT	0.0273	0.03	10.0	5.0
GlobalFiler™	98; TP-CS-P1-12H-EX1-LT	0.058	0.06	10.0	5.0
GlobalFiler™	99; TW-CS-P1-12H-EX1-LT	0.0231	0.02	10.0	5.0
GlobalFiler™	100; G-CS-P1-12H-EX1-LT	0.0559	0.06	10.0	5.0
GlobalFiler™	101; SS-CS-P1-24H-EX1-RT	0.0306	0.03	10.0	5.0
GlobalFiler™	102; TP-CS-P1-24H-EX1-RT	0.0445	0.04	10.0	5.0
GlobalFiler™	103; TW-CS-P1-24H-EX1-RT	0.0227	0.02	10.0	5.0
GlobalFiler™	104; CP-CS-P1-24H-EX1-RT	0.0197	0.02	10.0	5.0
GlobalFiler™	105; BS-CS-P1-24H-EX1-RT	0.0346	0.03	10.0	5.0
GlobalFiler™	106; G-CS-P1-24H-EX1-RT	0.0541	0.05	10.0	5.0
GlobalFiler™	107; SS-CS-P1-24H-EX1-HT	0.0241	0.02	10.0	5.0
GlobalFiler™	108; TP-CS-P1-24H-EX1-HT	0.1034	0.10	9.7	5.3
GlobalFiler™	109; TW-CS-P1-24H-EX1-HT	0.0557	0.06	10.0	5.0
GlobalFiler™	110; G-CS-P1-24H-EX1-HT	0.0421	0.04	10.0	5.0
GlobalFiler™	111; SS-CS-P1-24H-EX1-LT	0.0587	0.06	10.0	5.0
GlobalFiler™	112; TP-CS-P1-24H-EX1-LT	0.0727	0.07	10.0	5.0
GlobalFiler™	113; TW-CS-P1-24H-EX1-LT	0.0182	0.02	10.0	5.0
GlobalFiler™	114; G-CS-P1-24H-EX1-LT	0.0692	0.07	10.0	5.0
GlobalFiler™	115; SS-CS-P1-168H-EX1-RT	0.0290	0.03	10.0	5.0
GlobalFiler™	116; TP-CS-P1-168H-EX1-RT	0.0513	0.05	10.0	5.0
GlobalFiler™	117; TW-CS-P1-168H-EX1-RT	0.0221	0.02	10.0	5.0
GlobalFiler™	118; CP-CS-P1-168H-EX1-RT	0.0189	0.02	10.0	5.0
GlobalFiler™	119; BS-CS-P1-168H-EX1-RT	0.0317	0.03	10.0	5.0
GlobalFiler™	120; G-CS-P1-168H-EX1-RT	0.0573	0.06	10.0	5.0
GlobalFiler™	121; SS-CS-P1-168H-EX1-HT	0.0093	0.01	10.0	5.0
GlobalFiler™	122; TP-CS-P1-168H-EX1-HT	0.1067	0.11	9.4	5.6
GlobalFiler™	123; TW-CS-P1-168H-EX1-HT	0.0746	0.07	10.0	5.0
GlobalFiler™	124; G-CS-P1-168H-EX1-HT	0.0133	0.01	10.0	5.0
GlobalFiler™	125; SS-CS-P1-168H-EX1-LT	0.0662	0.07	10.0	5.0
GlobalFiler™	126; TP-CS-P1-168H-EX1-LT	0.0638	0.06	10.0	5.0
GlobalFiler™	127; TW-CS-P1-168H-EX1-LT	0.0095	0.01	10.0	5.0
GlobalFiler™	128; G-CS-P1-168H-EX1-LT	0.1168	0.12	8.6	6.4

Legend:		
DNA to D1	transferred volume of DNA for dilution step 1	
Diluent to D1	volume of diluent for dilution step 1	

- CT; Control Sample
- P; Participant
- EX1; PrepFiler BTA
- Ex2; QIAamp Inve.
- P; Participant
- 3h; 3 hours
- 12h; 12 hours
- 24h; a day
- 168h; a week

- SS; Stainless Steel
- TP; Textured Plastic
- TW; Textured Wood
- CP; Copier Paper
- BS; Banana Skin
- G; Glass

- CS; Cotton Swab
- NS; Nylon Swab
- RT; Room Temp.
- HT; Hight Temp. (40c/50%)
- LT; Low Temp. (5c/78%)

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1
GlobalFiler™	73; SS-CS-P1-3H-EX1-RT	0.0241	0.02	10.0	5.0
GlobalFiler™	74; TP-CS-P1-3H-EX1-RT	0.0378	0.04	10.0	5.0
GlobalFiler™	75; TW-CS-P1-3H-EX1-RT	0.0199	0.02	10.0	5.0
GlobalFiler™	76; CP-CS-P1-3H-EX1-RT	0.0209	0.02	10.0	5.0
GlobalFiler™	77; BS-CS-P1-3H-EX1-RT	0.0309	0.03	10.0	5.0
GlobalFiler™	78; G-CS-P1-3H-EX1-RT	0.0617	0.05	10.0	5.0
GlobalFiler™	79; SS-CS-P1-3H-EX1-HT	0.0319	0.03	10.0	5.0
GlobalFiler™	80; TP-CS-P1-3H-EX1-HT	0.0517	0.05	10.0	5.0
GlobalFiler™	81; TW-CS-P1-3H-EX1-HT	0.0239	0.02	10.0	5.0
GlobalFiler™	82; G-CS-P1-3H-EX1-HT	0.0558	0.06	10.0	5.0
GlobalFiler™	83; SS-CS-P1-3H-EX1-LT	0.0284	0.03	10.0	5.0
GlobalFiler™	84; TP-CS-P1-3H-EX1-LT	0.0442	0.04	10.0	5.0
GlobalFiler™	85; TW-CS-P1-3H-EX1-LT	0.0241	0.02	10.0	5.0
GlobalFiler™	86; G-CS-P1-3H-EX1-LT	0.0584	0.06	10.0	5.0
GlobalFiler™	87; SS-CS-P1-12H-EX1-RT	0.0294	0.03	10.0	5.0
GlobalFiler™	88; TP-CS-P1-12H-EX1-RT	0.0439	0.04	10.0	5.0
GlobalFiler™	89; TW-CS-P1-12H-EX1-RT	0.0239	0.02	10.0	5.0
GlobalFiler™	90; CP-CS-P1-12H-EX1-RT	0.0173	0.02	10.0	5.0
GlobalFiler™	91; BS-CS-P1-12H-EX1-RT	0.0319	0.03	10.0	5.0
GlobalFiler™	92; G-CS-P1-12H-EX1-RT	0.0527	0.05	10.0	5.0
GlobalFiler™	93; SS-CS-P1-12H-EX1-HT	0.0309	0.03	10.0	5.0
GlobalFiler™	94; TP-CS-P1-12H-EX1-HT	0.0662	0.07	10.0	5.0
GlobalFiler™	95; TW-CS-P1-12H-EX1-HT	0.0378	0.04	10.0	5.0
GlobalFiler™	96; G-CS-P1-12H-EX1-HT	0.0412	0.04	10.0	5.0
GlobalFiler™	97; SS-CS-P1-12H-EX1-LT	0.0318	0.03	10.0	5.0
GlobalFiler™	98; TP-CS-P1-12H-EX1-LT	0.0518	0.05	10.0	5.0
GlobalFiler™	99; TW-CS-P1-12H-EX1-LT	0.0286	0.03	10.0	5.0
GlobalFiler™	100; G-CS-P1-12H-EX1-LT	0.0668	0.07	10.0	5.0
GlobalFiler™	101; SS-CS-P1-24H-EX1-RT	0.0317	0.03	10.0	5.0
GlobalFiler™	102; TP-CS-P1-24H-EX1-RT	0.0407	0.04	10.0	5.0
GlobalFiler™	103; TW-CS-P1-24H-EX1-RT	0.0214	0.02	10.0	5.0
GlobalFiler™	104; CP-CS-P1-24H-EX1-RT	0.0149	0.01	10.0	5.0
GlobalFiler™	105; BS-CS-P1-24H-EX1-RT	0.0277	0.03	10.0	5.0
GlobalFiler™	106; G-CS-P1-24H-EX1-RT	0.0567	0.06	10.0	5.0
GlobalFiler™	107; SS-CS-P1-24H-EX1-HT	0.0179	0.02	10.0	5.0
GlobalFiler™	108; TP-CS-P1-24H-EX1-HT	0.0791	0.08	10.0	5.0
GlobalFiler™	109; TW-CS-P1-24H-EX1-HT	0.0528	0.05	10.0	5.0
GlobalFiler™	110; G-CS-P1-24H-EX1-HT	0.0344	0.03	10.0	5.0
GlobalFiler™	111; SS-CS-P1-24H-EX1-LT	0.0487	0.05	10.0	5.0
GlobalFiler™	112; TP-CS-P1-24H-EX1-LT	0.0544	0.05	10.0	5.0
GlobalFiler™	113; TW-CS-P1-24H-EX1-LT	0.0173	0.02	10.0	5.0
GlobalFiler™	114; G-CS-P1-24H-EX1-LT	0.0814	0.08	10.0	5.0
GlobalFiler™	115; SS-CS-P1-168H-EX1-RT	0.0276	0.03	10.0	5.0
GlobalFiler™	116; TP-CS-P1-168H-EX1-RT	0.0458	0.05	10.0	5.0
GlobalFiler™	117; TW-CS-P1-168H-EX1-RT	0.0188	0.02	10.0	5.0
GlobalFiler™	118; CP-CS-P1-168H-EX1-RT	0.0211	0.02	10.0	5.0
GlobalFiler™	119; BS-CS-P1-168H-EX1-RT	0.0259	0.03	10.0	5.0
GlobalFiler™	120; G-CS-P1-168H-EX1-RT	0.0621	0.06	10.0	5.0
GlobalFiler™	121; SS-CS-P1-168H-EX1-HT	0.0058	0.01	10.0	5.0
GlobalFiler™	122; TP-CS-P1-168H-EX1-HT	0.0874	0.09	10.0	5.0
GlobalFiler™	123; TW-CS-P1-168H-EX1-HT	0.0587	0.06	10.0	5.0
GlobalFiler™	124; G-CS-P1-168H-EX1-HT	0.0276	0.03	10.0	5.0
GlobalFiler™	125; SS-CS-P1-168H-EX1-LT	0.0487	0.05	10.0	5.0
GlobalFiler™	126; TP-CS-P1-168H-EX1-LT	0.0676	0.07	10.0	5.0
GlobalFiler™	127; TW-CS-P1-168H-EX1-LT	0.0122	0.01	10.0	5.0
GlobalFiler™	128; G-CS-P1-168H-EX1-LT	0.0856	0.09	10.0	5.0

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1

- CT; Control Sample
- P; Participant
- EX1; PrepFiler BTA
- Ex2; QIAamp Inve.
- P; Participant
- 3h; 3 hours
- 12h; 12 hours
- 24h; a day
- 168h; a week

- SS; Stainless Steel
- TP; Textured Plastic
- TW; Textured Wood
- CP; Copier Paper
- BS; Banana Skin
- G; Glass

- CS; Cotton Swab
- NS; Nylon Swab
- RT; Room Temp.
- HT; Hight Temp. (40c/50%)
- LT; Low Temp. (5c/78%)

A5.4 QUANTIFICATION OF THE INFLUENCE OF SANDY SURFACES

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1
GlobalFiler™	129; SS-CS-P1-3H-EX1-HT-SND	0.0029	0.00	10.0	5.0
GlobalFiler™	130; TP-CS-P1-3H-EX1-HT-SND	0.0109	0.01	10.0	5.0
GlobalFiler™	131; TW-CS-P1-3H-EX1-HT-SND	0.0067	0.01	10.0	5.0
GlobalFiler™	132; G-CS-P1-3H-EX1-HT-SND	0.0076	0.01	10.0	5.0
GlobalFiler™	133; SS-NS-P1-3H-EX1-HT-SND	0.0324	0.03	10.0	5.0
GlobalFiler™	134; TP-NS-P1-3H-EX1-HT-SND	0.0437	0.04	10.0	5.0
GlobalFiler™	135; TW-NS-P1-3H-EX1-HT-SND	0.0221	0.02	10.0	5.0
GlobalFiler™	136; G-NS-P1-3H-EX1-HT-SND	0.0462	0.05	10.0	5.0
GlobalFiler™	137; SS-CS-P1-3H-EX2-HT-SND	0.0024	0.00	10.0	5.0
GlobalFiler™	138; TP-CS-P1-3H-EX2-HT-SND	0.0047	0.00	10.0	5.0
GlobalFiler™	139; TW-CS-P1-3H-EX2-HT-SND	0.001	0.00	10.0	5.0
GlobalFiler™	140; G-CS-P1-3H-EX2-HT-SND	0.002	0.00	10.0	5.0
GlobalFiler™	141; SS-NS-P1-3H-EX2-HT-SND	0.0216	0.02	10.0	5.0
GlobalFiler™	142; TP-NS-P1-3H-EX2-HT-SND	0.0367	0.04	10.0	5.0
GlobalFiler™	143; TW-NS-P1-3H-EX2-HT-SND	0.0127	0.01	10.0	5.0
GlobalFiler™	144; G-NS-P1-3H-EX2-HT-SND	0.0320	0.03	10.0	5.0
GlobalFiler™	145; SS-CS-P1-3H-EX1-HT-SND	0.0034	0.00	10.0	5.0
GlobalFiler™	146; TP-CS-P1-3H-EX1-HT-SND	0.0091	0.01	10.0	5.0
GlobalFiler™	147; TW-CS-P1-3H-EX1-HT-SND	0.0082	0.01	10.0	5.0
GlobalFiler™	148; G-CS-P1-3H-EX1-HT-SND	0.0062	0.01	10.0	5.0
GlobalFiler™	149; SS-NS-P1-3H-EX1-HT-SND	0.0345	0.03	10.0	5.0
GlobalFiler™	150; TP-NS-P1-3H-EX1-HT-SND	0.0462	0.05	10.0	5.0
GlobalFiler™	151; TW-NS-P1-3H-EX1-HT-SND	0.0245	0.02	10.0	5.0
GlobalFiler™	152; G-NS-P1-3H-EX1-HT-SND	0.0473	0.05	10.0	5.0
GlobalFiler™	153; SS-CS-P1-3H-EX2-HT-SND	0.0019	0.00	10.0	5.0
GlobalFiler™	154; TP-CS-P1-3H-EX2-HT-SND	0.0039	0.00	10.0	5.0
GlobalFiler™	155; TW-CS-P1-3H-EX2-HT-SND	0.0019	0.00	10.0	5.0
GlobalFiler™	156; G-CS-P1-3H-EX2-HT-SND	0.0028	0.00	10.0	5.0
GlobalFiler™	157; SS-NS-P1-3H-EX2-HT-SND	0.0235	0.02	10.0	5.0
GlobalFiler™	158; TP-NS-P1-3H-EX2-HT-SND	0.0381	0.04	10.0	5.0
GlobalFiler™	159; TW-NS-P1-3H-EX2-HT-SND	0.0118	0.01	10.0	5.0
GlobalFiler™	160; G-NS-P1-3H-EX2-HT-SND	0.0334	0.03	10.0	5.0
GlobalFiler™	161; SS-CS-P1-3H-EX1-HT-SND	0.0041	0.00	10.0	5.0
GlobalFiler™	162; TP-CS-P1-3H-EX1-HT-SND	0.0121	0.01	10.0	5.0
GlobalFiler™	163; TW-CS-P1-3H-EX1-HT-SND	0.0069	0.01	10.0	5.0
GlobalFiler™	164; G-CS-P1-3H-EX1-HT-SND	0.0059	0.01	10.0	5.0
GlobalFiler™	165; SS-NS-P1-3H-EX1-HT-SND	0.0333	0.03	10.0	5.0
GlobalFiler™	166; TP-NS-P1-3H-EX1-HT-SND	0.0475	0.05	10.0	5.0
GlobalFiler™	167; TW-NS-P1-3H-EX1-HT-SND	0.0231	0.02	10.0	5.0
GlobalFiler™	168; G-NS-P1-3H-EX1-HT-SND	0.0452	0.05	10.0	5.0
GlobalFiler™	169; SS-CS-P1-3H-EX2-HT-SND	0.0031	0.00	10.0	5.0
GlobalFiler™	170; TP-CS-P1-3H-EX2-HT-SND	0.0051	0.01	10.0	5.0
GlobalFiler™	171; TW-CS-P1-3H-EX2-HT-SND	0.0021	0.00	10.0	5.0
GlobalFiler™	172; G-CS-P1-3H-EX2-HT-SND	0.0029	0.00	10.0	5.0
GlobalFiler™	173; SS-NS-P1-3H-EX2-HT-SND	0.0228	0.02	10.0	5.0
GlobalFiler™	174; TP-NS-P1-3H-EX2-HT-SND	0.0359	0.04	10.0	5.0
GlobalFiler™	175; TW-NS-P1-3H-EX2-HT-SND	0.0138	0.01	10.0	5.0
GlobalFiler™	176; G-NS-P1-3H-EX2-HT-SND	0.0339	0.03	10.0	5.0

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1

- EX1; PrepFiler BTA
- Ex2; QIAamp Inve.
- P; Participant
- 3h; 3 hours
- SS; Stainless Steel
- TP; Textured Plastic
- TW; Textured Wood
- CP; Copier Paper
- CS; Cotton Swab
- NS; Nylon Swab
- HT; Hight Temp. (40c/50%)
- SND: Sand

A6. TOUCH DNA RECOVERY FROM HUMAN SKIN AND FABRIC

A6.1 QUANTIFICATION OF THE INFLUENCE OF COLLECTION TECHNIQUES ON TOUCH DNA COLLECTED FROM THE NECK

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluen t to D2
GlobalFiler™	1; CS-A-1-MxM	0.1864	0.19	5.4	9.6	0.0	0.0
GlobalFiler™	2; CS-B-1-MxM	0.3629	0.36	2.8	12.2	0.0	0.0
GlobalFiler™	3; CS-C-1-MxM	0.5503	0.55	1.8	13.2	0.0	0.0
GlobalFiler™	4; CS-A-2-MxM	0.3352	0.34	3.0	12.0	0.0	0.0
GlobalFiler™	5; CS-B-2-MxM	0.4461	0.45	2.3	12.7	0.0	0.0
GlobalFiler™	6; CS-C-2-MxM	0.2629	0.26	3.8	11.2	0.0	0.0
GlobalFiler™	7; CS-A-3-MxM	0.3933	0.39	2.6	12.4	0.0	0.0
GlobalFiler™	8; CS-B-3-MxM	0.4682	0.47	2.1	12.9	0.0	0.0
GlobalFiler™	9; CS-C-3-MxM	0.5914	0.59	1.5	13.5	0.0	0.0
GlobalFiler™	10; CS-A-4-MxM	0.1959	0.20	5.1	9.9	0.0	0.0
GlobalFiler™	11; CS-B-4-MxM	0.2924	0.29	3.4	11.6	0.0	0.0
GlobalFiler™	12; CS-C-4-MxM	0.2235	0.22	4.5	10.5	0.0	0.0
GlobalFiler™	13; NS-A-1-MxM	1.1872	1.19	1.0	9.0	8.5	6.5
GlobalFiler™	14; NS-B-1-MxM	1.3512	1.35	1.0	9.0	7.0	8.0
GlobalFiler™	15; NS-C-1-MxM	0.6029	0.60	1.7	13.3	0.0	0.0
GlobalFiler™	16; NS-A-2-MxM	0.8082	0.81	1.2	11.2	12.4	2.6
GlobalFiler™	17; NS-B-2-MxM	0.9607	0.96	1.0	9.4	10.4	4.6
GlobalFiler™	18; NS-C-2-MxM	0.7156	0.72	1.4	12.6	14.0	1.0
GlobalFiler™	19; NS-A-3-MxM	0.7187	0.72	1.5	12.4	14.0	1.0
GlobalFiler™	20; NS-B-3-MxM	0.7232	0.72	1.4	12.5	13.9	1.1
GlobalFiler™	21; NS-C-3-MxM	0.6717	0.67	1.5	13.5	15.0	0.0
GlobalFiler™	22; NS-A-4-MxM	1.2236	1.22	1.0	9.0	8.2	6.8
GlobalFiler™	23; NS-B-4-MxM	1.2987	1.30	1.0	9.0	7.7	7.3
GlobalFiler™	24; NS-C-4-MxM	0.8645	0.86	1.2	10.5	11.6	3.4

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

- CS; Cotton Swab
- NS; Nylon Swab
- A; Moist swab
- B; Dry swab
- C; Dry swab from moist skin
- CT; Control Sample
- Ref; Reference sample
- MxM; Male donor vs. Male receive

STR Kit	Sample Name	Quantity	Quantity Mean	T.Y	DNA to D1	Diluent to D1	D1 to D2	Diluen t to D2
GlobalFiler™	25; CS-A-5-MxF	0.2773	0.28	0.0232	3.6	11.4	0.0	0.0
GlobalFiler™	26; CS-B-5-MxF	0.3267	0.33	0.0245	3.0	12.0	0.0	0.0
GlobalFiler™	27; CS-C-5-MxF	1.2205	1.22	0.0126	1.0	9.0	8.2	6.8
GlobalFiler™	28; CS-A-6-MxF	0.2361	0.24	0.033	4.0	11.0	0.0	0.0
GlobalFiler™	29; CS-B-6-MxF	0.4954	0.50	0.0112	2.0	13.0	0.0	0.0
GlobalFiler™	30; CS-C-6-MxF	0.5817	0.58	0.0102	1.5	13.5	0.0	0.0
GlobalFiler™	31; CS-A-7-MxF	0.1619	0.16	0.0031	6.2	8.8	0.0	0.0
GlobalFiler™	32; CS-B-7-MxF	0.2836	0.28	0.0023	3.5	11.5	0.0	0.0
GlobalFiler™	33; CS-C-7-MxF	0.4758	0.48	0.0008	2.0	13.0	0.0	0.0
GlobalFiler™	34; CS-A-8-MxF	0.1782	0.18	0.0053	6.5	8.5	0.0	0.0
GlobalFiler™	35; CS-B-8-MxF	0.2773	0.28	0.0088	3.6	11.4	0.0	0.0
GlobalFiler™	36; CS-C-8-MxF	0.8372	0.84	0.0063	1.2	10.8	12.0	3.0
GlobalFiler™	37; NS-A-5-MxF	0.2871	0.29	0.0238	3.5	11.5	0.0	0.0
GlobalFiler™	38; NS-B-5-MxF	0.8509	0.85	0.0063	1.2	10.6	11.8	3.2
GlobalFiler™	39; NS-C-5-MxF	0.1184	0.12	0.0082	8.5	6.5	0.0	0.0
GlobalFiler™	40; NS-A-6-MxF	0.2011	0.20	0.0082	5.0	10.0	0.0	0.0
GlobalFiler™	41; NS-B-6-MxF	0.9214	0.92	0.0034	1.1	10.5	11.4	3.6
GlobalFiler™	42; NS-C-6-MxF	0.5367	0.54	0.0397	1.9	13.1	0.0	0.0
GlobalFiler™	43; NS-A-7-MxF	0.1025	0.10	0.0103	9.8	5.2	0.0	0.0
GlobalFiler™	44; NS-B-7-MxF	0.8217	0.82	0.005	1.2	11.1	12.2	2.8
GlobalFiler™	45; NS-C-7-MxF	0.5271	0.53	0.0237	1.9	13.1	0.0	0.0
GlobalFiler™	46; NS-A-8-MxF	0.4383	0.44	0.0118	2.3	12.7	0.0	0.0
GlobalFiler™	47; NS-B-8-MxF	1.2193	1.22	0.0321	1.0	9.0	8.2	6.8
GlobalFiler™	48; NS-C-8-MxF	0.2857	0.29	0.0042	3.5	11.5	0.0	0.0

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

- CS; Cotton Swab
- NS; Nylon Swab

- A; Moist swab
- B; Dry swab
- C; Dry swab from moist skin

- CT; Control Sample
- Ref; Reference sample
- MxF; Male donor vs. Female receiver

A6.2 INFLUENCE OF COLLECTION TECHNIQUES ON TOUCH DNA COLLECTED FROM THE NECK

CS					
sample no.	DNA con.	alleles called	avg. RFU	mixture ratio	PHR%
1; CS-A-1-MxM	0.1864	69	3194	1;1	83
4; CS-A-2-MxM	0.3352	69	1778	1;1	90
7; CS-A-3-MxM	0.3933	69	2023	1;1	92
10; CS-A-4-MxM	0.1959	68	827	1;1	85
25; CS-A-5-MxF	0.2773	48	3569	1;1	85
28; CS-A-6-MxF	0.2361	39	3441	1;2	75
31; CS-A-7-MxF	0.1619	49	3540	1;1	87
34; CS-A-8-MxF	0.1782	51	4107	1;2	71
2; CS-B-1-MxM	0.3629	69	2299	1;1	85
5; CS-B-2-MxM	0.4461	69	1798	1;1	79
8; CS-B-3-MxM	0.4682	69	2080	1;1	90
11; CS-B-4-MxM	0.2924	69	1773	1;2	72
26; CS-B-5-MxF	0.3267	60	1160	1;1	88
29; CS-B-6-MxF	0.4954	52	2874	1;3	70
32; CS-B-7-MxF	0.2836	55	2061	1;1	79
35; CS-B-8-MxF	0.2773	52	3659	1;2	74
3; CS-C-1-MxM	0.5503	69	1434	1;8	70
6; CS-C-2-MxM	0.2629	69	1237	1;2	74
9; CS-C-3-MxM	0.5914	69	1086	1;3	72
12; CS-C-4-MxM	0.2235	69	1503	1;3	77
27; CS-C-5-MxF	1.2205	62	1387	1;4	73
30; CS-C-6-MxF	0.5817	71	1644	1;8	70
33; CS-C-7-MxF	0.4758	58	3385	1;2	78
36; CS-C-8-MxF	0.8372	66	1332	1;4	72

- CS; Cotton Swab
- NS; Nylon Swab

- A; Moist swab
- B; Dry swab
- C; Dry swab from moist skin

- MxM; Male donor vs. Male receiver
- MxF; Male donor vs. Female receiver

NS					
sample no.	DNA con.	alleles called	avg. RFU	mixture ratio	PHR%
13; NS-A-1-MxM	1.1872	69	2276	1;4	80
16; NS-A-2-MxM	0.8082	69	2625	1;2	77
19; NS-A-3-MxM	0.7187	69	2237	1;1	72
22; NS-A-4-MxM	1.2236	69	2475	1;1	78
37; NS-A-5-MxF	0.2871	56	938	1;2	90
40; NS-A-6-MxF	0.2011	44	1710	1;1	88
43; NS-A-7-MxF	0.1025	72	2651	1;3	76
46; NS-A-7-MxF	0.4383	47	3789	1;2	87
14; NS-B-1-MxM	1.3512	69	2318	1;2	84
17; NS-B-2-MxM	0.9607	69	2523	1;1	81
20; NS-B-3-MxM	0.7232	69	1933	1;1	83
23; NS-B-4-MxM	1.2987	69	2340	1;1	86
38; NS-B-5-MxF	0.8509	47	4814	1;1	77
41; NS-B-6-MxF	0.9214	59	2231	1;2	83
44; NS-B-7-MxF	0.8217	61	3269	1;1	90
47; NS-B-8-MxF	1.2193	54	3423	1;1	82
15; NS-C-1-MxM	0.6029	69	1921	1;3	78
18; NS-C-2-MxM	0.7156	69	2065	1;3	82
21; NS-C-3-MxM	0.6717	69	1344	1;3	85
24; NS-C-4-MxM	0.8645	69	1807	1;1	81
39; NS-C-5-MxF	0.1184	71	1107	1;2	75
42; NS-C-6-MxF	0.5367	71	2454	1;2	78
45; NS-C-7-MxF	0.5271	62	3698	1;3	82
48; NS-C-8-MxF	0.2857	64	2857	1;3	85

- CS; Cotton Swab
- NS; Nylon Swab

- A; Moist swab
- B; Dry swab
- C; Dry swab from moist skin

- MxM; Male donor vs. Male receiver
- MxF; Male donor vs. Female receiver

A6.3 INFLUENCE OF TIME ON TOUCH DNA COLLECTED FROM THE NECK

Participant did not wash					
time	DNA con.	alleles called	avg. RFU	mixture ratio	PHR%
1h	0.2574	69	1680	1;2	77
1h	0.2959	69	3163	1;4	77
1h	0.2489	69	1985	1;2	83
1h	0.3212	69	2886	1;1	69
3h	0.2233	69	2610	1;6	68
3h	0.3636	69	2884	1;5	84
3h	0.4126	69	1985	1;3	73
3h	0.1932	69	3017	1;2	78
6h	0.5062	69	2629	1;4	71
6h	0.4518	69	3313	1;5	75
6h	0.2981	67	3178	1;9	68
6h	0.6494	69	2854	1;2	78
12h	0.8424	69	2947	1;6	77
12h	0.6974	68	2315	1;14	89
12h	0.9123	69	1986	1;4	79
12h	0.6228	69	3217	1;4	85
24h	0.5741	69	2845	1;14	69
24h	0.8246	69	2549	1;17	74
24h	0.4547	67	2105	1;15	70
24h	0.9247	69	3321	1;10	80
48h	0.4892	67	3021	1;14	76
48h	0.7581	69	1896	1;15	78
48h	0.9215	69	2987	1;11	72
48h	0.3219	69	3326	1;16	82
72h	0.4823	39	4879	0;0	80
72h	0.9456	39	3985	0;0	85
72h	0.5683	39	3365	0;0	81
72h	0.8562	39	4251	0;0	85

Participant did wash					
time	DNA con.	alleles called	avg. RFU	mixture ratio	PHR%
1h	0.2574	69	1780	1;2	75
1h	0.2959	68	2183	1;1	78
1h	0.1985	68	1942	1;3	71
1h	0.3487	69	2254	1;2	80
3h	0.2233	69	2315	1;3	70
3h	0.3636	69	2464	1;5	80
3h	0.2814	67	1914	1;2	75
3h	0.3317	69	3021	1;2	74
6h	0.3491	39	4178	0;0	80
6h	0.6128	39	3869	0;0	77
6h	0.5062	39	2629	0;0	74
6h	0.4518	39	3313	0;0	79

A6.4 INFLUENCE OF COLLECTION TYPE AND TIME ON TOUCH DNA COLLECTED FROM HANDS

hands vs. collection						
sample no.	method	DNA con.	alleles called	avg. RFU	mixture ratio	PHR%
1H	CS	0.2459	72	2311	1;3	79
2H	CS	0.4521	72	2371	1;9	74
3H	CS	0.3132	71	3059	1;12	76
4H	CS	0.3252	72	2365	1;10	69
5H	CS	0.5147	69	2874	1;9	80
6H	CS	0.2971	72	2254	1;5	69
7H	CS	0.3481	72	1978	1;11	77
8H	CS	0.1647	72	2995	1;9	75
9H	NS	0.0584	61	1025	1;3	80
10H	NS	0.0574	68	1315	1;2	73
11H	NS	0.0214	70	844	1;6	69
12H	NS	0.0312	72	745	1;7	88
13H	NS	0.0430	66	1439	1;11	81
14H	NS	0.0480	60	1042	1;4	85
15H	NS	0.0337	68	768	1;3	75
16H	NS	0.0263	72	678	1;3	79

hands vs. time						
sample no.	time	amount	alleles called	avg. RFU	mixture	PHR%
17H	3h	0.3781	66	3059	1;24	76
18H	3h	0.2567	61	1332	1;17	73
19H	3h	0.4871	59	2147	1;13	65
20H	3h	0.4124	67	2856	1;18	69
21H	3h	0.2987	62	1475	1;16	71
22H	3h	0.3625	67	1986	1;20	79
23H	3h	0.3712	64	2514	1;22	80
24H	6h	0.2978	39	3012	0;0	71
25H	6h	0.5715	39	2891	0;0	68
26H	6h	0.3145	39	2254	0;0	74
27H	6h	0.4812	39	3596	0;0	82
28H	6h	0.6012	39	2107	0;0	87
29H	6h	0.4153	39	3040	0;0	78
30H	6h	0.4821	39	2280	0;0	86

- CS; Cotton Swab
- NS; Nylon Swab

A6.5 QUANTIFICATION OF THE INFLUENCE OF COLLECTION AND EXTRACTION TYPES ON TOUCH DNA COLLECTED FROM FABRIC

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluen t to D2
GlobalFiler™	1; CS- FB1-P1-EXT1	0.0158	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	2; CS- FB1-P1-EXT1	0.0102	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	3; CS- FB1-P2-EXT1	0.309	0.31	3.3	11.7	0.0	0.0
GlobalFiler™	4; CS- FB1-P2-EXT1	0.521	0.52	1.9	13.1	0.0	0.0
GlobalFiler™	5; NS- FB1-P1-EXT1	0.0071	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	6; NS- FB1-P1-EXT1	0.0055	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	7; NS- FB1-P2-EXT1	0.0482	0.05	10.0	5.0	0.0	0.0
GlobalFiler™	8; NS- FB1-P2-EXT1	0.1017	0.11	9.5	5.5	0.0	0.0
GlobalFiler™	9; MT- FB1-P1-EXT1	0.2037	0.20	4.9	10.1	0.0	0.0
GlobalFiler™	10; MT- FB1-P1-EXT1	0.1344	0.13	7.5	7.5	0.0	0.0
GlobalFiler™	11; MT- FB1-P2-EXT1	2.2196	2.22	1.0	9.0	4.5	10.5
GlobalFiler™	12; MT- FB1-P2-EXT1	0.9564	0.96	1.1	9.5	10.5	4.5
GlobalFiler™	13; CS- FB1-P1-EXT2	0.0328	0.03	10.0	5.0	0.0	0.0
GlobalFiler™	14; CS- FB1-P1-EXT2	0.0126	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	15; CS- FB1-P2-EXT2	0.2204	0.22	4.6	10.4	0.0	0.0
GlobalFiler™	16; CS- FB1-P2-EXT2	0.1286	0.13	7.8	7.2	0.0	0.0
GlobalFiler™	17; NS- FB1-P1-EXT2	0.0168	0.02	10.0	5.0	0.0	0.0
GlobalFiler™	18; NS- FB1-P1-EXT2	0.0082	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	19; NS- FB1-P2-EXT2	0.2887	0.29	3.5	11.5	0.0	0.0
GlobalFiler™	20; NS- FB1-P2-EXT2	0.1685	0.17	6.0	9.0	0.0	0.0
GlobalFiler™	21; MT- FB1-P1-EXT2	0.0887	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	22; MT- FB1-P1-EXT2	0.1018	0.10	9.8	5.2	0.0	0.0
GlobalFiler™	23; MT- FB1-P2-EXT2	1.7473	1.75	1.0	9.0	5.8	9.2
GlobalFiler™	24; MT- FB1-P2-EXT2	0.9625	0.96	1.0	9.4	10.4	4.6
GlobalFiler™	CT-EXT1	0.0001	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	CT-EXT2	0.0000	0.00	10.0	5.0	0.0	0.0

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

- P; Participant
- EXT1; PrepFiler BTA
- EXT2; QIAamp Inve.
- CT; Control Sample
- FB; Fabric type
- CS; Cotton Swab
- NS; Nylon Swab
- MT; minitapes

A6.6 QUANTIFICATION OF THE INFLUENCE OF FABRIC TYPE ON TOUCH DNA

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluent to D2
GlobalFiler™	1; MT- FB2-P1-EXT1	0.0032	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	2; MT- FB2-P1-EXT1	0.0023	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	3; MT- FB2-P1-EXT1	0.001	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	4; MT- FB2-P1-EXT1	0.0007	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	5; MT- FB2-P2-EXT1	0.1118	0.10	9.8	5.2	0.0	0.0
GlobalFiler™	6; MT- FB2-P2-EXT1	0.2347	0.23	4.3	10.7	0.0	0.0
GlobalFiler™	7; MT- FB2-P2-EXT1	0.293	0.29	3.4	11.6	0.0	0.0
GlobalFiler™	8; MT- FB2-P2-EXT1	0.4542	0.45	2.2	12.8	0.0	0.0
GlobalFiler™	9; MT- FB1-P1-EXT1	0.2037	0.20	4.9	10.1	0.0	0.0
GlobalFiler™	10; MT- FB1-P1-EXT1	0.1344	0.13	7.5	7.5	0.0	0.0
GlobalFiler™	11; MT- FB1-P1-EXT1	0.0887	0.09	10.0	5.0	0.0	0.0
GlobalFiler™	12; MT- FB1-P1-EXT1	0.1018	0.10	9.8	5.2	0.0	0.0
GlobalFiler™	13; MT- FB1-P2-EXT1	2.2196	2.22	1.0	9.0	4.5	10.5
GlobalFiler™	14; MT- FB1-P2-EXT1	0.9564	0.96	1.1	9.5	10.5	4.5
GlobalFiler™	15; MT- FB1-P2-EXT1	1.7473	1.75	1.0	9.0	5.8	9.2
GlobalFiler™	16; MT- FB1-P2-EXT1	0.9625	0.96	1.0	9.4	10.4	4.6
GlobalFiler™	CT-FB1	0.0002	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	CT-FB2	0.0000	0.00	10.0	5.0	0.0	0.0

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

- P; Participant
- EXT1; PrepFiler BTA
- CT; Control Sample
- FB; Fabric type
- MT; minitapes

A6.7 QUANTIFICATION OF THE INFLUENCE OF AREA SIZE ON TOUCH DNA COLLECTED FROM FABRIC

STR Kit	Sample Name	Quantity	Quantity Mean	DNA to D1	Diluent to D1	D1 to D2	Diluent to D2
GlobalFiler™	1; CS- FB1-SZ2-P1-EXT1	0.2049	0.20	4.9	10.1	0.0	0.0
GlobalFiler™	2; CS- FB1-SZ2-P1-EXT1	0.1178	0.12	8.6	6.4	0.0	0.0
GlobalFiler™	3; MT- FB1-SZ2-P1-EXT1	0.0022	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	4; MT- FB1-SZ2-P1-EXT1	0.0006	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	5; CS- FB1-SZ2-P2-EXT1	0.6902	0.69	1.5	13.1	14.6	0.4
GlobalFiler™	6; CS- FB1-SZ2-P2-EXT1	0.3735	0.37	2.7	12.3	0.0	0.0
GlobalFiler™	7; MT- FB1-SZ2-P2-EXT1	0.1212	0.12	8.3	6.7	0.0	0.0
GlobalFiler™	8; MT- FB1-SZ2-P2-EXT1	0.2101	0.21	4.8	10.2	0.0	0.0
GlobalFiler™	9; CS- FB1-SZ1-P1-EXT1	0.0071	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	10; CS- FB1-SZ1-P1-EXT1	0.0102	0.01	10.0	5.0	0.0	0.0
GlobalFiler™	11; MT- FB1-SZ1-P1-EXT1	0.2037	0.20	4.9	10.1	0.0	0.0
GlobalFiler™	12; MT- FB1-SZ1-P1-EXT1	0.1344	0.13	7.5	7.5	0.0	0.0
GlobalFiler™	13; CS- FB1-SZ1-P2-EXT1	0.0369	0.04	10.0	5.0	0.0	0.0
GlobalFiler™	14; CS- FB1-SZ1-P2-EXT1	0.0658	0.07	10.0	5.0	0.0	0.0
GlobalFiler™	15; MT- FB1-SZ1-P2-EXT1	2.2196	2.22	1.0	9.0	4.5	10.5
GlobalFiler™	16; MT- FB1-SZ1-P2-EXT1	0.9564	0.96	1.1	9.5	10.5	4.5
GlobalFiler™	CT-FB1-SZ1	0.0000	0.00	10.0	5.0	0.0	0.0
GlobalFiler™	CT-FB1-SZ2	0.0000	0.00	10.0	5.0	0.0	0.0

Legend:	
DNA to D1	transferred volume of DNA for dilution step 1
Diluent to D1	volume of diluent for dilution step 1
D1 to D2	Transferred volume of mixed content of D1 to D2 for dilution step 2
Diluent to D2	volume of diluent for dilution step 2

- P; Participant
- EX1; PrepFiler BTA
- CT; Control Sample
- FB; Fabric type
- SZ; Fabric size
- CS; Cotton Swab
- NS; Nylon Swab
- MT; minitapes

A6.8 INFLUENCE OF DEPOSITION AREA AND TIME ON TOUCH DNA COLLECTED FROM FABRIC

A; chest area of the t-shirt (avg. of 15 samples)					
Time	DNA con.	Alleles called	avg. RFU	mixture ratio	PHR%
1h	0.68	69	2422.54	1;2	75
3h	0.41	68	2747.85	1;2	80
6h	0.19	69	2971.29	1;7	74
12h	0.08	69	1738.57	1;5	82
24h	0.03	69	1041.86	1;11	79

B; buttocks area of the pants (avg. of 15 samples)					
Time	DNA con.	Alleles called	avg. RFU	mixture ratio	PHR%
1h	0.14	69	2422	1;4	77
3h	0.06	66	2147.4	1;6	80
6h	0.02	39	2629.4	0;0	70
12h	0.01	39	3312.9	0;0	73
24h	0.01	39	3072.4	0;0	78

A7. DIRECT PCR AMPLIFICATION OF TOUCH DNA

A7.1 INFLUENCE OF COLLECTION TYPE

	No. of alleles recovered (avg. of 72 samples)							
	Stainless Steel (SS)	Textured Plastic (TP)	Textured Wood (TW)	Copier Paper (CP)	Fabric (FB)	Glass (G)	Alleles avg.	Recovery pct.
MicroFLOQ (MF)	43	43	43	35	39	43	41	95%
Mini Plastic Swab (MPS)	12	9	7	4	3	8	7	16%
Mini Cotton Swab (MCS)	15	17	12	11	10	21	14	33%
Minitape (MT)	43	37	40	43	43	43	42	98%
Cotton Swab (CS)	43	35	23	18	19	38	29	67%
4N6FLOQSwabs (NS)	43	26	16	12	16	31	24	56%
Recovery pct.	77%	65%	56%	49%	51%	72%		

A7.2 DIRECT AMPLIFICATION VS. EXTRACTION (SMALL AREA: 2.5 X 3.5 CM)

Average signal (RFU) per locus - Direct amp. (MT) (2.5 x 3.5 cm) (avg. of 18 samples)														
	Stainless Steel (SS)		Textured Plastic (TP)		Textured Wood (TW)		Copier Paper (CP)		Fabric (FB)		Glass (G)		Avg.	SD
D3S1358	3178	3058	7140	5698	2931	2596	3349	2703	8089	6979	5821	4510	4671	1984
vWA	3166	3255	7637	6124	3308	3090	3598	2995	13644	11811	4526	4096	5604	3629
D16S539	3610	3610	6481	6481	2740	2740	3987	3987	17784	17784	5832	5832	6739	5330
CSF1PO	891	897	1358	1193	377	303	640	516	4254	3491	1824	1020	1397	1243
TPOX	1033	1033	3042	3042	950	950	1300	1300	8166	8166	2559	2559	2842	2618
Yindel	13288	13288	29524	29524	23621	23621	29904	29904	27988	27988	19768	19768	24016	6239
AMEL	5744	7967	20016	18996	4906	5026	10619	10392	18029	17329	13437	12492	12079	5552
D8S1179	7675	6636	17510	15773	8967	9402	19634	14529	28729	28915	9735	7899	14617	7853
D21S11	2772	3298	5877	5931	904	811	2337	2095	12675	13392	4866	4186	4929	4148
D18S51	2832	2411	3852	2766	661	556	1076	735	5525	4760	3030	2621	2569	1619
DYS391	1215	1215	1218	1218	220	220	424	424	4045	4045	1260	1260	1397	1309
D2S441	8124	8124	13694	13694	11872	11872	12367	12367	28716	28716	10300	10300	14179	7031
D19S433	1250	1444	4661	3270	3017	2556	5993	4769	15584	13286	2697	2605	5094	4596
TH01	2071	1865	4791	3804	4626	4937	4429	4233	13611	12199	3141	2421	5177	3774
FGA	2281	997	3143	3100	2372	2617	3498	2703	11422	10280	2983	2312	3976	3281
D22S1045	6281	5278	12619	12182	8833	7490	11195	11191	15297	14043	8582	9645	10220	3078
D5S818	5518	5486	9479	8135	2608	2749	4971	3904	10199	9147	8154	6792	6429	2616
D13S317	3986	3968	4875	5118	679	651	2422	2044	8893	8355	3791	5174	4163	2604
D7S820	2470	2609	3291	2390	131	70	759	669	6488	5383	3736	2357	2529	2007
SE33	1010	1528	1034	1272	342	203	437	425	3726	3508	1239	1914	1387	1163
D10S1248	7340	5683	12405	11437	4472	3096	4368	3620	6054	5536	12473	9516	7167	3434
D1S1656	4041	4752	8373	7436	2458	1850	3194	2442	7959	7162	9249	5510	5369	2604
D12S391	3108	2516	5836	2748	1412	666	1842	1171	5101	4340	6349	3500	3216	1862
D2S1338	1752	1729	4294	2690	1863	1692	1918	1727	9612	8522	2660	2972	3453	2740

Average signal (RFU) per locus - Direct amp. (MF) (2.5 x 3.5 cm) (avg. of 18 samples)														
	Stainless Steel (SS)		Textured Plastic (TP)		Textured Wood (TW)		Copier Paper (CP)		Fabric (FB)		Glass (G)		Avg.	SD
D3S1358	820	838	6828	6960	2831	2496	3159	2823	6610	6014	11898	9759	5086	3501
vWA	5067	4042	7085	5876	3008	3190	2898	2795	7766	6444	17360	14704	6686	4720
D16S539	7588	7588	8495	8495	2241	2241	3497	3497	7662	7662	22419	22419	8650	6867
CSF1PO	568	433	3138	2679	317	283	547	506	1872	1830	5383	4696	1854	1781
TPOX	2029	2029	5857	5857	857	857	1103	1103	4031	4031	10332	10332	4035	3456
Yindel	26471	26471	18240	18240	16621	16621	20444	20444	29969	29969	28088	28088	23306	5323
AMEL	1380	1046	17461	12853	3906	3726	9049	9012	16115	14979	27095	25907	11877	8851
D8S1179	26395	25879	13237	12481	7957	7802	18034	15529	19422	20431	28574	28036	18648	7460
D21S11	2601	2004	8035	6977	784	801	2187	2005	5306	5153	16293	16308	5705	5475
D18S51	684	558	5608	5982	601	586	876	665	4175	3137	8359	7372	3217	2973
DYS391	781	781	5203	5203	187	187	224	224	1904	1904	5118	5118	2236	2239
D2S441	20838	20838	14599	14599	10772	10772	10457	10457	20105	20105	30209	30209	17830	7162
D19S433	11940	11089	6528	6055	2617	2496	5073	4889	7418	7020	17803	14439	8114	4741
TH01	8324	8238	6457	5397	4436	4337	4129	4013	7192	6962	14061	14308	7321	3555
FGA	7109	6780	4097	4471	2078	2287	3188	2693	7337	6038	14078	11841	6000	3778
D22S1045	7214	6513	8567	9008	7533	8110	10895	10141	12739	12009	20510	20593	11153	4784
D5S818	815	747	7975	7845	2558	2689	4431	3884	8475	7701	14491	14041	6304	4634
D13S317	396	378	7593	6525	578	491	2322	2174	4934	4831	12760	11905	4574	4394
D7S820	177	115	4805	4669	101	90	689	619	3073	2921	9602	8517	2948	3360
SE33	266	235	4832	4561	282	253	377	345	2079	1976	5874	4699	2148	2218
D10S1248	262	233	8364	7999	3172	2996	4189	3529	7338	6930	11002	9792	5484	3581
D1S1656	402	414	8704	8355	2288	1920	2994	2742	6454	5328	12857	11651	5342	4263
D12S391	514	397	6813	5183	1332	856	1548	1279	4001	2498	7992	6086	3208	2695
D2S1338	2628	2589	6276	5439	1483	1392	1458	1307	5884	4700	12260	11685	4758	3842

Average signal (RFU) per locus - Extraction (2.5 x 3.5 cm) (avg. of 36 samples)														
	Stainless Steel (SS)		Textured Plastic (TP)		Textured Wood (TW)		Copier Paper (CP)		Fabric (FB)		Glass (G)		Avg.	SD
D3S1358	2530	1912	2210	2266	2011	1985	1775	1376	5896	5269	742	526	2375	1617
vWA	1992	1912	2447	2114	2869	2746	1608	1442	4852	4458	3412	2221	2673	1078
D16S539	2305	2305	2947	2947	1874	1874	2042	2042	5745	5745	1878	1878	2799	1428
CSF1PO	1430	783	962	1268	245	229	810	602	3897	3589	214	187	1185	1266
TPOX	2085	2085	2285	2285	1278	1278	1366	1366	4215	3865	68	68	1854	1265
Yindel	7119	7119	6842	6842	6985	6985	3827	3827	25978	25978	28492	28492	13207	10453
AMEL	5491	7389	6272	5515	3278	3389	3298	3230	15829	16129	1327	1233	6032	5004
D8S1179	3198	3227	4707	5165	6985	6689	3083	2359	8956	7895	26471	21556	8358	7674
D21S11	2002	2110	2160	2495	1982	1798	1376	1495	9865	8745	504	452	2915	3059
D18S51	1618	1759	2038	1807	1298	1083	1450	984	4879	4689	1478	1254	2028	1323
DYS391	1381	1381	1452	1452	986	874	1268	1268	4187	3978	214	214	1555	1259
D2S441	4379	4379	5624	5624	6921	6921	4475	4475	9784	9784	16481	16481	7944	4420
D19S433	1363	1444	2915	2545	2189	2354	1982	1755	8749	8215	8091	6654	4021	2954
TH01	2254	1585	2841	2107	4187	3784	1825	1908	10787	9874	4604	3722	4123	3072
FGA	932	1011	1821	1709	2178	2088	1585	1062	8986	7968	1775	1544	2722	2726
D22S1045	4652	3462	3848	3865	5891	5742	1726	1637	11789	10788	9553	7858	5901	3401
D5S818	2661	2472	3144	2585	2274	2043	1675	1800	9654	9247	515	433	3209	3027
D13S317	2037	2227	2484	2146	387	296	1679	1247	7883	7369	57	57	2322	2632
D7S820	1877	1911	2619	2023	1781	1475	1426	1105	5897	5569	451	321	2205	1771
SE33	1113	1070	1237	1667	387	245	1167	1013	3259	3158	247	189	1229	1038
D10S1248	4871	4727	3673	3216	2987	2741	1809	1554	5489	5234	471	450	3102	1771
D1S1656	2400	2515	2657	2758	2178	2278	1573	1620	7458	7245	244	150	2756	2312
D12S391	2602	1731	2255	1265	1220	956	1436	1031	4569	4127	300	110	1800	1385
D2S1338	1362	1093	2864	2405	1088	965	1451	1596	8956	8458	208	202	2554	2975

A7.3 DIRECT AMPLIFICATION VS. EXTRACTION (LARGE AREA: 5 X 7 CM)

Average signal (RFU) per locus - Direct amp. (MT) (5 x 7 cm) (avg. of 18 samples)														
	Stainless Steel (SS)		Textured Plastic (TP)		Textured Wood (TW)		Copier Paper (CP)		Fabric (FB)		Glass (G)		Avg.	SD
D3S1358	2748	2685	7058	5578	2415	2236	4589	3489	8159	7489	6894	5515	4905	2168
vWA	3028	3174	7214	6421	2986	2478	3986	3649	12748	11879	4796	4189	5546	3463
D16S539	3471	3471	6235	6235	2546	2546	4487	4487	15965	15965	6596	6596	6550	4642
CSF1PO	748	701	1247	1078	301	289	968	1089	4895	3895	1796	1458	1539	1419
TPOX	984	984	2874	2874	876	876	1526	1526	7986	7986	3269	3269	2919	2546
Yindel	12078	12078	25489	25489	22198	22198	28915	28915	25987	25987	21892	21892	22760	5575
AMEL	4795	5124	19685	19044	4652	4965	11789	10789	17965	15879	12896	11748	11611	5741
D8S1179	6854	6478	16854	15243	8546	8856	14965	13269	22389	20147	10798	9652	12838	5188
D21S11	2478	2841	5712	5569	845	788	2596	2235	11891	10789	5236	4986	4664	3567
D18S51	2645	2310	3659	2815	596	523	1278	1129	5897	5248	3269	2965	2695	1702
DYS391	1074	1074	1079	1079	187	187	689	689	4178	4178	1485	1485	1449	1341
D2S441	7415	7415	11547	11547	9659	9659	14792	14792	27415	27415	11258	11258	13681	6819
D19S433	984	1147	4487	3581	2894	2639	6792	5539	14253	12748	2965	2689	5060	4284
TH01	1934	1802	4629	4121	4178	4269	4723	4361	12986	10789	3369	2697	4988	3404
FGA	1874	1542	2986	2812	2513	2633	3896	3263	9865	9108	2986	2564	3837	2712
D22S1045	5863	5321	11748	10218	7458	7236	12791	11745	13489	13179	9697	8236	9748	2888
D5S818	5236	5078	9352	8356	2413	2635	5139	4698	9896	9127	8695	7458	6507	2630
D13S317	3986	3752	4751	4968	594	536	3012	2869	8496	7489	4589	4236	4107	2337
D7S820	2289	2496	3142	2689	256	399	796	712	6689	5798	3895	2969	2678	2051
SE33	956	1120	986	1125	456	396	529	485	3649	3895	1859	2148	1467	1207
D10S1248	6596	5421	11579	9658	3846	3269	4695	3965	6187	5698	13691	9789	7033	3357
D1S1656	3965	4325	7854	7526	2196	1956	3695	2968	8147	7698	9369	7894	5633	2677
D12S391	2941	2415	4215	3269	1178	836	2017	1879	4965	4478	5895	4569	3221	1607
D2S1338	1458	1385	3958	3215	1796	1496	2369	1987	8465	8216	3269	3021	3386	2458

Average signal (RFU) per locus - Direct amp. (MF) (5 x 7 cm) (avg. of 18 samples)														
	Stainless Steel (SS)		Textured Plastic (TP)		Textured Wood (TW)		Copier Paper (CP)		Fabric (FB)		Glass (G)		Avg.	SD
D3S1358	1259	1895	6128	5894	4541	4158	3348	2874	6239	6695	7845	6594	4789	2097
vWA	5216	4789	6359	5984	3596	3345	3178	3241	5896	5548	12589	10458	5850	2921
D16S539	6956	6956	7845	7845	3210	3210	3685	3685	7569	7569	20145	20145	8235	5878
CSF1PO	698	589	2265	1987	415	695	651	512	1589	1365	2689	3521	1415	1012
TPOX	2269	2269	5549	5549	1423	1423	1425	1425	3356	3356	6598	6598	3437	2086
Yindel	27489	27489	17412	17412	19213	19213	21478	21478	27549	27549	26874	26874	23336	4329
AMEL	1587	1269	10245	11245	5589	4986	9236	8874	12478	11789	19658	18745	9642	5820
D8S1179	20489	22698	11874	10598	8569	7789	18425	16956	17894	15748	25698	22659	16616	5863
D21S11	3691	3125	7489	6987	1234	1895	2845	2236	5496	4986	12598	13548	5511	4038
D18S51	796	599	5236	4856	2153	1985	965	748	4214	3956	7845	6985	3362	2526
DYS391	859	859	4885	4885	1256	1256	336	336	2145	2145	4879	4879	2393	1923
D2S441	22598	22598	13594	13594	11589	11589	12748	12748	19841	19841	22589	22589	17160	4851
D19S433	12489	11890	5269	50129	3269	2847	5214	4789	6584	6123	15748	11549	11325	12899
TH01	8459	8874	6124	5986	5216	5014	4871	4215	7012	6859	12478	10245	7113	2473
FGA	7695	3965	3874	4123	4589	3845	3596	2946	7123	6541	11478	9865	5803	2744
D22S1045	6795	6587	9213	8549	8147	7745	11248	9865	10548	9856	19854	17849	10521	4162
D5S818	912	769	8012	7698	3956	3274	4745	3956	8147	7745	12478	11458	6096	3776
D13S317	523	498	7213	6698	1245	889	2556	2254	4712	3986	10148	9856	4215	3521
D7S820	226	187	5231	4758	541	365	789	845	3216	3012	8795	7859	2985	3066
SE33	589	784	4236	4487	754	602	458	412	1986	2125	5894	4485	2234	1994
D10S1248	698	985	7895	7485	3695	2948	4236	3856	6985	5986	9874	8745	5282	2996
D1S1656	789	896	8659	8124	2956	2136	3659	3124	6395	5489	11478	9856	5297	3594
D12S391	1256	1048	6598	5894	1574	1078	1784	1652	4189	3691	8126	7457	3696	2687
D2S1338	2895	3026	6147	5548	1986	1474	1369	1598	5298	4879	8749	7412	4198	2495

Average signal (RFU) per locus - Extraction (5x7cm) (avg. of 36 samples)														
	Stainless Steel (SS)		Textured Plastic (TP)		Textured Wood (TW)		Copier Paper (CP)		Fabric (FB)		Glass (G)		Avg.	SD
D3S1358	9506	8469	6787	6240	11773	9905	8421	7640	11792	10057	10840	9055	9207	1797
vWA	12097	11081	11349	9545	17654	14476	9636	9220	22746	18650	16880	14493	13986	4276
D16S539	16546	16546	16258	16258	24547	24547	13893	13893	32171	32171	22918	22918	21056	6521
CSF1PO	4522	4424	4291	4214	5709	5576	4865	4308	6274	5841	5338	4871	5019	705
TPOX	10322	10322	10601	10601	13149	13149	8716	8716	13897	13897	11247	11247	11322	1823
Yindel	29040	29040	29789	29789	28272	28272	29254	29254	27730	27730	28072	28072	28693	754
AMEL	17700	16697	13420	12300	27222	26129	17543	14708	26338	24276	23217	21295	20070	5334
D8S1179	23570	25677	28481	27840	28406	27611	21575	22550	26806	19725	28153	27358	25646	3021
D21S11	10607	11747	12601	11356	20948	21005	11983	10621	26010	25498	18574	18708	16638	5834
D18S51	7817	6773	5735	5148	8579	7572	6623	6506	7763	7157	6837	6250	6897	955
DYS391	6805	6805	5626	5626	7214	7214	7747	7747	6849	6849	5486	5486	6621	851
D2S441	26341	26341	27016	27016	30952	30952	24983	24983	27053	27053	30323	30323	27778	2237
D19S433	13711	12696	15056	14165	21841	19194	11668	10833	27106	30408	23098	20196	18331	6353
TH01	12826	13098	13104	12686	17215	17230	11177	11479	25288	25298	17863	18163	16286	4903
FGA	12734	11248	13770	12749	17202	15153	10186	8574	23786	21408	16862	14658	14861	4439
D22S1045	13303	13428	11539	10403	22255	21141	11777	11679	26073	23671	21012	18550	17069	5603
D5S818	9787	10174	7690	6940	16133	14847	10052	9244	15163	13758	13295	11922	11584	3040
D13S317	9573	9198	7792	7399	14059	12397	9571	8725	14359	13153	12410	11253	10824	2425
D7S820	8348	6602	6137	5721	11153	9599	7310	6483	10498	9082	8766	8170	8156	1752
SE33	7349	6642	5120	4632	6622	6161	8186	7129	6669	5883	5657	5012	6255	1050
D10S1248	7855	7054	4279	4048	9995	9355	7570	6648	7674	7133	7831	7617	7255	1721
D1S1656	9553	8789	5764	5750	12948	12146	9461	8765	10458	10375	10632	10033	9556	2157
D12S391	6446	5220	4307	3617	8751	6662	7123	5703	7545	6240	6773	5359	6146	1411
D2S1338	12352	12185	12165	9955	16174	14124	12562	11637	17870	15378	13773	12210	13365	2204

A7.4 INNOVATIVE SOLUTION

	No. of alleles recovered							
	Stainless Steel (SS)	Textured Plastic (TP)	Textured Wood (TW)	Copier Paper (CP)	Fabric (FB)	Glass (G)	Alleles avg.	Recovery pct.
MicroFLOQ direct amp. collected from cotton swab (MF*) (avg. of 12 samples)	43	41	25	30	43	43	38	88%
Minitape direct amp. collected from cotton swab (MT*) (avg. of 12 samples)	43	43	33	35	27	33	36	84%
Cotton Swab (CS) with Extraction (avg. of 24 samples)	43	43	43	43	43	43	43	100%

A8. TRACE DNA CASEWORK AT DUBAI POLICE FORENSIC DEPARTMENT

A8.1 COTTON SWAB (CS) SAMPLES

Samples Collection Sheet (A); Following this study protocol

Biology				DNA				
Case No.	**Item; Surface type**	**Collection type - No.**	**Sample No.**	**Ext. Quant. type**	**Quantity**	**Amp. dilution**	**STR Kit**	**Profile Type**
59809	Plastic bag - smooth	CS-1	3/59809	EXT 1 - Trio	0.1249	8.0 – 7.0	GF	FS
60691	Metal lock - smooth	CS-2	1/60691	EXT 1 - Trio	0.0008	10.0 – 5.0	GF	N
60691	Plastic spoon - smooth	CS-3	5/60691	EXT 1 - Trio	0.0034	10.0 – 5.0	GF	PM
61357	Metal Knife handle - smooth	CS-4	1A/61357	EXT 1 - Trio	0.0208	10.0 – 5.0	GF	FM
63132	Metal Knife handle - smooth	CS-5	3A/63132	EXT 1 - Trio	0.1724	6.0 – 9.0	GF	FM
63462	Plastic glove	CS-6	1A/63462	EXT 1 - Trio	0.0495	10.0 – 5.0	GF	FM
59683	Metal handcuff - smooth	CS-7	5A/59683	EXT 1 - Trio	0.0111	10.0 – 5.0	GF	FM
64864	Cutter – smooth metal	CS-8	4/64864	EXT 1 - Trio	0.0026	10.0 – 5.0	GF	PS
65765	Tennis racket handle – smooth plastic	CS-9	1A/65765	EXT 1 - Trio	0.0128	10.0 – 5.0	GF	FM
67247	Big metal cutter - smooth metal	CS-10	1/67247	EXT 1 - Trio	0.008	10.0 – 5.0	GF	FM

- CS; Cotton Swab
- NS; Nylon Swab
- MT; minitape
- MF; MicroFLOQ™
- EXT 1; PrepFiler BTA
- EXT 2; QIAamp Inve.
- GF; GlobalFiler
- FS; Full single profile
- PS; Partial single profile
- FM; Full mixture profile
- PM; Partial mixture profile
- N; Negative

Biology				DNA				
Case No.	Item; Surface type	Collection type - No.	Sample No.	Ext. Quant. type	Quantity	Amp. dilution	STR Kit	Profile Type
68474	Shoe - smooth leather	CS-11	1/68474	EXT 1 – Trio	0.0334	10.0 – 5.0	GF	FM
69826	Knife handle - smooth metal	CS-12	1A/69826	EXT 1 – Trio	0.1868	5.4 – 9.6	GF	FS
70105	Syringe handle - smooth plastic	CS-13	1/70105	EXT 1 – Trio	0.0068	10.0 – 5.0	GF	FS
69663	Knife handle - smooth metal	CS-14	1A/69663	EXT 1 – Trio	0.0281	10.0 – 5.0	GF	FM
70009	Hand bag – smooth leather	CS-15	1/70009	EXT 1 – Trio	0.0445	10.0 – 5.0	GF	FM
70103	Lamp head – smooth metal	CS-16	1A/70103	EXT 1 – Trio	1.9382	1.0 – 9.0 / 5.2 – 9.8	GF	FM
69807	Knife handle - smooth plastic	CS-17	1A/69807	EXT 1 – Trio	0.0242	10.0 – 5.0	GF	FM
69906	Knife handle - smooth metal	CS-18	1A/69906	EXT 1 – Trio	0.6419	1.6 – 13.4	GF	FS
70505	Baseball bat handle - smooth wood	CS-19	1B/70505	EXT 1 – Trio	0.0168	10.0 – 5.0	GF	FS
70570	Electric cigarette - smooth rubber	CS-20	6/70570	EXT 1 – Trio	0.5528	1.8 – 13.2	GF	FS
71863	iPhone - smooth metal	CS-21	4/71863	EXT 1 – Trio	0.0285	10.0 – 5.0	GF	FM
71863	Small bottle cover - smooth metal	CS-22	5/71863	EXT 1 – Trio	0.0108	10.0 – 5.0	GF	FM
71863	Small metal spoon	CS-23	9/71863	EXT 1 – Trio	0.0059	10.0 – 5.0	GF	FM
71843	Syringe handle - smooth plastic	CS-24	3A/71843	EXT 1 – Trio	0.3823	2.6 – 12.4	GF	FS
71890	Toy gun - smooth plastic	CS-25	2/71890	EXT 1 – Trio	0.0200	10.0 – 5.0	GF	FM
71890	toothbrush handle - smooth plastic	CS-26	3/71890	EXT 1 - Trio	0.0940	10.0 – 5.0	GF	FS

Samples Collection Sheet (B); Following Dubai Police lab protocol

Biology				DNA				
Case No.	**Item; Surface type**	**Collection type - No.**	**Sample No.**	**Ext. Quant. type**	**Quantity**	**Amp. dilution**	**STR Kit**	**Profile Type**
60691	Metal lock - smooth	CS - CS-2	1/60691	PrepFiler – quantiplex pro	Undetermined	X	GF	N
60691	Plastic spoon - smooth	CS - CS-3	5/60691	PrepFiler – quantiplex pro	0.004	X	GF	N
61357	Metal Knife handle - smooth	CS - CS-4	1A/61357	PrepFiler – quantiplex pro	0.0336	15.0 – 0.0	GF	FM
63132	Metal Knife handle - smooth	CS - CS-5	3A/63132	PrepFiler – quantiplex pro	3.1083	3.2 – 6.8 / 1.0 – 14.0	GF	FS
63462	Plastic glove	CS - CS-6	1A/63462	PrepFiler – quantiplex pro	0.00091	15.0 – 0.0	GF	FM
59683	Metal handcuff	CS - CS-7	5A/59683	PrepFiler – quantiplex pro	0.0009	X	GF	N
64864	Cutter – smooth metal	CS - CS-8	4/64864	PrepFiler – quantiplex pro	Undetermined	X	GF	N
65765	Tennis racket handle – smooth plastic	CS - CS-9	1A/65765	PrepFiler – quantiplex pro	0.0097	15.0 – 0.0	GF	FM
67247	Big metal cutter - smooth metal	CS - CS-10	1/67247	PrepFiler – quantiplex pro	Undetermined	X	GF	N
68474	Shoe - smooth leather	CS - CS-11	1/68474	PrepFiler – quantiplex pro	0.0045	15.0 – 0.0	GF	PM
69826	Knife handle - smooth metal	CS - CS-12	1A/69826	PrepFiler – quantiplex pro	2.8993	3.4 – 6.6 / 1.0 – 14.0	GF	FS
70105	Syringe handle - smooth plastic	CS - CS-13	1/70105	PrepFiler – quantiplex pro	0.0125	15.0 – 0.0	GF	FM
69663	Knife handle - smooth metal	CS - CS-14	1A/69663	PrepFiler – quantiplex pro	0.0141	15.0 – 0.0	GF	FM
70009	Handbag – smooth leather	CS - CS-15	1/70009	PrepFiler – quantiplex pro	0.8855	1.1 – 13.9	GF	FM

Biology				DNA				
Case No.	Item; Surface type	Collection type - No.	Sample No.	Ext. Quant. type	Quantity	Amp. dilution	STR Kit	Profile Type
70103	Lamp head – smooth metal	CS - CS-16	1A/70103	PrepFiler – quantiplex pro	0.2448	4.1 – 10.9	GF	FM
69807	Knife handle - smooth plastic	CS - CS-17	1A/69807	PrepFiler – quantiplex pro	0.0025	15.0 – 0.0	GF	N
69906	Knife handle - smooth metal	CS - CS-18	1A/69906	PrepFiler – quantiplex pro	0.0743	13.5 – 1.5	GF	FS
70505	Baseball bat handle - smooth wood	CS - CS-19	1B/70505	PrepFiler – quantiplex pro	0.9598	1.1 – 14.0	GF	FS
70570	Electric cigarette - smooth rubber	CS - CS-20	6/70570	PrepFiler – quantiplex pro	0.4202	2.4 – 12.6	GF	FS
71863	iPhone - smooth metal	CS - CS-21	4/71863	PrepFiler – quantiplex pro	0.0139	15.0 – 0.0	GF	FM
71863	Small bottle cover - smooth metal	CS - CS-22	5/71863	PrepFiler – quantiplex pro	0.0044	15.0 – 0.0	GF	PM
71863	Small metal spoon	CS - CS-23	9/71863	PrepFiler – quantiplex pro	0.0050	15.0 – 0.0	GF	PM
71843	Syringe handle - smooth plastic	CS - CS-24	3A/71843	PrepFiler – quantiplex pro	1.5829	6.3 – 3.7 / 1.0 – 14.0	GF	FS
71890	Toy gun - smooth plastic	CS - CS-25	2/71890	PrepFiler – quantiplex pro	0.0192	15.0 – 0.0	GF	FM
71890	toothbrush handle - smooth plastic	CS - CS-26	3/71890	PrepFiler – quantiplex pro	0.2717	3.7 – 11.3	GF	FS

A8.2 NYLON SWAB (NS) SAMPLES

Samples Collection Sheet (A); Following this study protocol

Biology				DNA				
Case No.	**Item; Surface type**	**Collection type - No.**	**Sample No.**	**Ext. Quant. type**	**Quantity**	**Amp. dilution**	**STR Kit**	**Profile Type**
60347	Duct Tape (outside) - textured	NS-1	1A/60347	EXT1 - Trio	0.0013	10.0 – 5.0	GF	FM
60347	Duct Tape (inside) - textured	NS-2	1B/60347	EXT1 - Trio	0.0761	10.0 – 5.0	GF	FM
60347	Duct Tape (outside) - textured	NS-3	2/60347	EXT1 - Trio	0.0005	10.0 – 5.0	GF	N
61645	Knife handle - textured wood	NS-4	1A/61645	EXT1 - Trio	0.0609	10.0 – 5.0	GF	FM
62003	Fake gun handle - textured metal	NS-5	1A/62003	EXT1 - Trio	0.0094	10.0 – 5.0	GF	FM
62786	Plastic Tape - textured	NS-6	2/62786	EXT1 - Trio	0.0619	10.0 – 5.0	GF	FS
63187	Knife handle - textured plastic	NS-7	1A/63187	EXT1 - Trio	0.0151	10.0 – 5.0	GF	FS
63403	Plastic cuff - textured	NS-8	2A/63403	EXT1 - Trio	0.0035	10.0 – 5.0	GF	FM
63403	Plastic cuff - textured	NS-9	6A/63403	EXT1 - Trio	0.0073	10.0 – 5.0	GF	FS
64411	Knife handle - textured plastic	NS-10	1/64411	EXT1 - Trio	0.01517	10.0 – 5.0	GF	FM

- CS; Cotton Swab
- NS; Nylon Swab
- MT; minitape
- MF; MicroFLOQ™
- EXT 1; PrepFiler BTA
- EXT 2; QIAamp Inve.
- GF; GlobalFiler
- FS; Full single profile
- PS; Partial single profile
- FM; Full mixture profile
- PM; Partial mixture profile
- N; Negative

Biology				DNA				
Case No.	Item; Surface type	Collection type - No.	Sample No.	Ext. Quant. type	Quantity	Amp. dilution	STR Kit	Profile Type
64349	Smoking pipe - textured wood	NS-11	2/64349	EXT1 - Trio	undetermined	10.0 – 5.0	GF	N
66344	Slippers - Textured plastic	NS-12	30/66344	EXT1 - Trio	0.0457	10.0 – 5.0	GF	FM
64264	Textured aluminium on glass bottle	NS-13	1B/64264	EXT1 - Trio	0.0019	10.0 – 5.0	GF	PS
64864	Sandy fabric glove	NS-14	6/64864	EXT1 - Trio	0.0037	10.0 – 5.0	GF	FM
66846	Toothbrush handle - textured plastic	NS-15	1/66846	EXT1 - Trio	0.0152	10.0 – 5.0	GF	FS
66846	hairbrush handle - textured plastic	NS-16	3A/66846	EXT1 - Trio	0.0752	10.0 – 5.0	GF	FM
70009	Hand bag - textured leather	NS-17	1/70009	EXT1 – Trio	0.0370	10.0 – 5.0	GF	FM
61903	Plastic cover - textured	NS-18	3/61903	EXT1 – Trio	0.0083	10.0 – 5.0	GF	PM
69724	Knife handle - textured plastic	NS-19	1/69724	EXT1 – Trio	0.1053	10.0 – 5.0	GF	FM
70505	Baseball bat handle - textured plastic	NS-20	1A/70505	EXT1 – Trio	0.0231	10.0 – 5.0	GF	FM
70683	Vegetable cutter - textured plastic	NS-21	1A/70683	EXT1 – Trio	0.0035	10.0 – 5.0	GF	N
70983	Bottle cover - textured plastic	NS-22	1/70983	EXT1 – Trio	0.0013	10.0 – 5.0	GF	PS
71890	Toy gun - textured plastic	NS-23	2/71890	EXT1 – Trio	0.0094	10.0 – 5.0	GF	FS
71890	toothbrush handle - textured plastic	NS-24	3/71890	EXT1 – Trio	0.1741	5.8 – 9.2	GF	FS
65499	car wheel handle cover - textured leather	NS-25	4/65499	EXT1 - Trio	0.1388	7.2 – 7.8	GF	FM

Samples Collection Sheet (B); Following Dubai Police lab protocol

Biology				DNA				
Case No.	**Item; Surface type**	**Collection type - No.**	**Sample No.**	**Ext. Quant. type**	**Quantity**	**Amp. dilution**	**STR Kit**	**Profile Type**
60347	Duct Tape (outside) - textured	CS - NS-1	1A/60347	PrepFiler – quantiplex pro	0.0446	15.0 – 0.0	GF	PM
60347	Duct Tape (inside) - textured	CS - NS-2	1B/60347	PrepFiler – quantiplex pro	0.3478	2.9 – 12.1	GF	FS
60347	Duct Tape (outside) - textured	CS - NS-3	2/60347	PrepFiler – quantiplex pro	0.0178	15.0 – 0.0	GF	PM
61645	Knife handle - textured wood	CS - NS-4	1A/61645	PrepFiler – quantiplex pro	0.0427	15.0 – 0.0	GF	FS
62003	Fake gun handle - textured metal	CS - NS-5	1A/62003	PrepFiler – quantiplex pro	0.0005	15.0 – 0.0	X	N
62786	Plastic Tape - textured	CS - NS-6	2/62786	PrepFiler – quantiplex pro	3.8284	2.6 – 7.4 / 1.0 – 14.0	GF	FS
63187	Knife handle - textured plastic	CS - NS-7	1A/63187	PrepFiler – quantiplex pro	0.0077	15.0 – 0.0	GF	PS
63403	Plastic cuff - textured	CS - NS-8	2A/63403	PrepFiler – quantiplex pro	0.0007	15.0 – 0.0	X	N
63403	Plastic cuff - textured	CS - NS-9	6C/63403	PrepFiler – quantiplex pro	0.0161	15.0 – 0.0	GF	FS
64411	Knife handle - textured plastic	CS - NS-10	1/64411	PrepFiler – quantiplex pro	0.0077	15.0 – 0.0	GF	FM
64349	Smoking pipe - textured wood	CS - NS-11	2/64349	PrepFiler – quantiplex pro	0.0006	15.0 – 0.0	GF	N
66344	Slippers - Textured plastic	CS - NS-12	30/66344	PrepFiler – quantiplex pro	0.1113	9.0 – 6.0	GF	FM
64264	Textured aluminium on glass bottle	CS - NS-13	1B/64264	PrepFiler – quantiplex pro	0.0163	15.0 – 0.0	GF	FM
64864	Sandy Knife handle	CS - NS-14	6/64864	PrepFiler – quantiplex pro	0.0025	15.0 – 0.0	GF	N

Biology				DNA				
Case No.	Item; Surface type	Collection type - No.	Sample No.	Ext. Quant. type	Quantity	Amp. dilution	STR Kit	Profile Type
66846	Toothbrush handle - textured plastic	CS - NS-15	1/66846	PrepFiler – quantiplex pro	0.0076	15.0 – 0.0	GF	FS
66846	Hairbrush handle - textured plastic	CS - NS-16	3A/66846	PrepFiler – quantiplex pro	0.0020	15.0 – 0.0	GF	N
70009	Hand bag - textured leather	CS - NS-17	1/70009	PrepFiler – quantiplex pro	0.8855	1.1 – 13.9	GF	FM
61903	Plastic cover - textured	CS - NS-18	3/61903	PrepFiler – quantiplex pro	0.0149	15.0 – 0.0	GF	FM
69724	Knife handle - textured plastic	CS - NS-19	1/69724	PrepFiler – quantiplex pro	0.0714	14.0 – 1.0	GF	FM
70505	Baseball bat handle - textured plastic	CS - NS-20	1A/70505	PrepFiler – quantiplex pro	0.1041	9.6 – 5.4	GF	FS
70683	Vegetable cutter - textured plastic	CS - NS-21	1A/70683	PrepFiler – quantiplex pro	0.0039	15.0 – 0.0	GF	PM
70983	Bottle cover - textured plastic	CS - NS-22	1/70983	PrepFiler – quantiplex pro	0.0071	15.0 – 0.0	GF	PM
71890	Toy gun - textured plastic	CS - NS-23	2/71890	PrepFiler – quantiplex pro	0.0192	15.0 – 0.0	GF	FM
71890	toothbrush handle - textured plastic	CS - NS-24	3/71890	PrepFiler – quantiplex pro	0.2717	3.7 – 11.3	GF	FS
65499	car wheel handle cover - textured leather	CS - NS-25	4/65499	PrepFiler – quantiplex pro	0.3659	2.7 – 12.3	GF	FM

A8.3 MINITAPES (MT) SAMPLES

Samples Collection Sheet (A); Following this study protocol

Biology				DNA				
Case No.	**Item; Surface type**	**Collection type - No.**	**Sample No.**	**Ext. Quant. type**	**Quantity**	**Amp. dilution**	**STR Kit**	**Profile Type**
59809	Medical mask - cotton	MT-1	1/59809	EXT 1 - Trio	0.0156	10.0 – 5.0	GF	FS
59809	Head cap - fabric	MT-2	2/59809	EXT 1 - Trio	0.2214	5.0 – 10.0	GF	FS
61244	A piece cloth (wazar) - fabric	MT-3	1/61244	EXT 1 - Trio	0.0523	10.0 – 5.0	GF	FM
61026	Small bag - fabric	MT-4	2/61026	EXT 1 - Trio	1.6358	1.0 – 9.0 / 6.0 – 9.0	GF	FS
62363	Medical mask - cotton	MT-5	2/62363	EXT 1 - Trio	0.1013	10.0 – 5.0	GF	FM
61646	Medical mask - cotton	MT-6	1A/61646	EXT 1 - Trio	0.0941	10.0 – 5.0	GF	FM
62364	Head cap - fabric	MT-7	1/62364	EXT 1 - Trio	0.0589	10.0 – 5.0	GF	FM
60423	Women black underwear	MT-8	3/60423	EXT 1 - Trio	2.3465	1.0 – 9.0 / 4.5 – 10.5	GF	FM
61509	Fabric glove	MT-9	1/61509	EXT 1 - Trio	0.0023	10.0 – 5.0	GF	PS
61509	Fabric glove	MT-10	3/61509	EXT 1 - Trio	0.0001	10.0 – 5.0	GF	N

- CS; Cotton Swab
- NS; Nylon Swab
- MT; minitape
- MF; MicroFLOQ™

- EXT 1; PrepFiler BTA
- EXT 2; QIAamp Inve.

- GF; GlobalFiler

- FS; Full single profile
- PS; Partial single profile
- FM; Full mixture profile

- PM; Partial mixture profile
- N; Negative

Biology				DNA				
Case No.	Item; Surface type	Collection type - No.	Sample No.	Ext. Quant. type	Quantity	Amp. dilution	STR Kit	Profile Type
63536	Medical mask - cotton	MT-11	4/63536	EXT 1 – Trio	0.2777	3.6 – 11.4	GF	FM
65699	Black men trouser	MT-12	4D/65699	EXT 1 – Trio	1.4589	1.0 – 9.0 / 6.9 – 8.1	GF	FM
66344	Scarf	MT-13	28/66344	EXT 1 – Trio	0.0178	10.0 – 5.0	GF	FM
64864	Sandy fabric glove	MT-14	3/64864	EXT 1 – Trio	0.0079	10.0 – 5.0	GF	PM
64864	Sandy fabric glove	MT-15	6/64864	EXT 1 – Trio	0.0068	10.0 – 5.0	GF	FM
64480	White candorah	MT-16	19A/64480	EXT 1 – Trio	0.1243	8.1 – 6.9	GF	FM
64480	White t-shirt	MT-17	20A/64480	EXT 1 – Trio	0.1035	9.7 – 5.3	GF	FM
64480	Black t-shirt	MT-18	21/64480	EXT 1 - Trio	0.2991	3.4 – 11.6	GF	FM
68474	Shoe - fabric	MT-19	1/68474	EXT 1 – Trio	0.0731	10.0 – 5.0	GF	FM
69704	Piece of a white cloth	MT-20	1A/69704	EXT 1 – Trio	0.5767	1.7 – 13.3	GF	FS
69704	Piece of a white cloth	MT-21	2F/69704	EXT 1 – Trio	0.8037	1.3 – 11.3 / 12.5 – 2.5	GF	FM
69747	White underwear	MT-22	3/69747	EXT 1 – Trio	0.4863	2.1 – 12.9	GF	FS
69627	Black glove - fabric	MT-23	2/69627	EXT 1 – Trio	0.0961	10.0 – 5.0	GF	FM
69627	White glove - fabric	MT-24	3/69627	EXT 1 – Trio	0.0384	10.0 – 5.0	GF	FM
68643	Fabric on a knife handle	MT-25	1/68643	EXT 1 – Trio	0.0415	10.0 – 5.0	GF	FM
65499	car wheel handle cover - fabric	MT-26	4/65499	EXT 1 - Trio	0.0732	10.0 – 5.0	GF	FM

Samples Collection Sheet (B); Following Dubai Police lab protocol

Biology				DNA				
Case No.	**Item; Surface type**	**Collection type - No.**	**Sample No.**	**Ext. Quant. type**	**Quantity**	**Amp. dilution**	**STR Kit**	**Profile Type**
59809	Medical mask - cotton	CS - MT-1	1/59809	PrepFiler – quantiplex pro	0.1709	5.9 – 9.1	GF	FS
59809	Head cap - fabric	CS - MT-2	2/59809	PrepFiler – quantiplex pro	1.5471	1.0 – 14.0	GF	FS
61244	A piece cloth (wazar) - fabric	CS - MT-3	1/61244	PrepFiler – quantiplex pro	0.3729	2.7 – 12.3	GF	PM
61026	Small bag - fabric	CS - MT-4	2/61026	PrepFiler – quantiplex pro	1.1156	9.0 – 1.0 / 1.0 – 14.0	GF	PS
62363	Medical mask - cotton	CS - MT-5	2/62363	PrepFiler – quantiplex pro	0.0616	15.0 – 0.0	GF	FM
61646	Medical mask - cotton	CS - MT-6	1A/61646	PrepFiler – quantiplex pro	0.0126	15.0 – 0.0	GF	FM
62364	Head cap - fabric	CS - MT-7	1/62364	PrepFiler – quantiplex pro	0.0555	15.0 – 0.0	GF	FM
60423	Women black underwear	CS - MT-8	3/60423	PrepFiler – quantiplex pro	0.0247	15.0 – 0.0	GF	FS
61509	Fabric glove	CS - MT-9	1/61509	PrepFiler – quantiplex pro	0.0001	15.0 – 0.0	GF	N
61509	Fabric glove	CS - MT-10	3/61509	PrepFiler – quantiplex pro	0.0005	15.0 – 0.0	GF	N
63536	Medical mask - cotton	CS - MT-11	4/63536	PrepFiler – quantiplex pro	0.1292	7.7 – 7.3	GF	FM
65699	Black men trouser	CS - MT-12	4D/65699	PrepFiler – quantiplex pro	0.0479	15.0 – 0.0	GF	FS
66344	Scarf	CS - MT-13	28/66344	PrepFiler – quantiplex pro	0.0280	15.0 – 0.0	GF	FM
64864	Sandy fabric glove	CS - MT-14	3/64864	PrepFiler – quantiplex pro	0.0008	15.0 – 0.0	GF	N
64864	Sandy fabric glove	CS - MT-15	6/64864	PrepFiler – quantiplex pro	0.0025	15.0 – 0.0	GF	N

Biology				DNA				
Case No.	Item; Surface type	Collection type - No.	Sample No.	Ext. Quant. type	Quantity	Amp. dilution	STR Kit	Profile Type
64480	White candorah	CS - MT-16	19A/64480	PrepFiler – quantiplex pro	0.4471	2.2 – 12.8 / 1.0 – 14.0	GF	FS
64480	White t-shirt	CS - MT-17	20A/64480	PrepFiler – quantiplex pro	0.1146	8.7 – 6.3	GF	FM
64480	Black t-shirt	CS - MT-18	21/64480	PrepFiler – quantiplex pro	0.1667	6.0 – 9.0	GF	FM
68474	Shoe - fabric	CS - MT-19	1/68474	PrepFiler – quantiplex pro	0.0045	15.0 – 0.0	GF	PM
69704	Piece of a white cloth	CS - MT-20	1A/69704	PrepFiler – quantiplex pro	5.5227	1.8 – 8.2 / 1.0 – 14.0	GF	FS
69704	Piece of a white cloth	CS - MT-21	2F/69704	PrepFiler – quantiplex pro	0.3122	3.2 – 11.8	GF	FM
69747	White underwear	CS - MT-22	3/69747	PrepFiler – quantiplex pro	0.4795	2.1 – 12.9	GF	FS
69627	Black glove - fabric	CS - MT-23	2/69627	PrepFiler – quantiplex pro	0.2508	4.0 – 11.0	GF	FM
69627	White glove - fabric	CS - MT-24	3/69627	PrepFiler – quantiplex pro	0.0540	15.0 – 0.0	GF	FM
68643	Fabric on a knife handle	CS - MT-25	1/68643	PrepFiler – quantiplex pro	0.0008	15.0 – 0.0	GF	N
65499	car wheel handle cover - fabric	CS - MT-26	4/65499	PrepFiler – quantiplex pro	0.3659	2.7 – 12.3	GF	FM

A8.4 DIRECT AMPLIFICATION SAMPLES

Samples Collection Sheet (A); Following this study protocol

Biology				DNA				
Case No.	**Item; Surface type**	**Collection type - No.**	**Sample No.**	**Ext. Quant. type**	**Quantity**	**Amp. dilution**	**STR Kit**	**Profile Type**
60691	Cigarette butt	MF-1	2/60691				GF	N
60691	Cigarette butt	MF-2	4/60691				GF	N
61357	Syringe handle	MF-3	1B/61357				GF	FS
61026	Syringe handle	MF-4	1A/61026				GF	PS
61645	Knife blade	MF-5	1B/61645				GF	PS
62363	Cigarette butt	MF-6	1/62363				GF	PS
60938	Syringe handle	MF-7	1A/60938				GF	PS
60423	Women black underwear	MF-8	3/60423				GF	N
63132	Metal knife handle	MF-9	3A/63132				GF	PM
63187	Plastic knife handle	MF-10	1A/63187				GF	FS

- CS; Cotton Swab
- NS; Nylon Swab
- MT; minitape
- MF; MicroFLOQ™

- EXT 1; PrepFiler BTA
- EXT 2; QIAamp Inve.

- GF; GlobalFiler

- FS; Full single profile
- PS; Partial single profile
- FM; Full mixture profile

- PM; Partial mixture profile
- N; Negative

Biology				DNA				
Case No.	Item; Surface type	Collection type - No.	Sample No.	Ext. Quant. type	Quantity	Amp. dilution	STR Kit	Profile Type
61743	Metal golf bat	MF-11	1A/61743				GF	N
64466	Cigarette butt	MF-12	1/64466				GF	FS
64349	Lighter	MF-13	1/64349				GF	N
64349	Plastic cover of a small bottle	MF-14	3/64349				GF	N
64303	Plastic safe cover with numbers	MF-15	1/64303				GF	N
63123	Pen	MF-16	1/63123				GF	PS
66344	Metal knife handle	MF-17	1A/66344				GF	N
66344	Metal hand ring	MF-18	25/66344				GF	N
66344	Metal hand ring	MF-19	26/66344				GF	PS
67191	Screwdriver – plastic handle	MF-20	1/67191				GF	N
65765	Tennis racket handle – smooth plastic	MF-21	2A/65765				GF	FM
67247	Big metal cutter - smooth metal	MF-22	1/67247				GF	N
66846	Toothbrush - textured plastic	MF-23	1/66846				GF	PS
66846	Hairbrush - textured plastic	MF-24	3A/66846				GF	FM
67523	unused bullet	MF-25	1/67523				GF	PS

Samples Collection Sheet (B); Following Dubai Police lab protocol

Biology				DNA				
Case No.	**Item; Surface type**	**Collection type - No.**	**Sample No.**	**Ext. Quant. type**	**Quantity**	**Amp. dilution**	**STR Kit**	**Profile Type**
60691	cigarette butt	Cut - MF-1	2/60691	PrepFiler – quantiplex pro	Undetermined	15.0 – 0.0	X	X
60691	cigarette butt	Cut - MF-2	4/60691	PrepFiler – quantiplex pro	0.0928	10.8 – 4.2	GF	PS
61357	Syringe handle	CS - MF-3	1B/61357	PrepFiler – quantiplex pro	0.0336	15.0 – 0.0	GF	FM
61026	Syringe handle	CS - MF-4	1A/61026	PrepFiler – quantiplex pro	0.1064	9.4 – 5.6	GF	FS
61645	knife blade	CS - MF-5	1B/61645	PrepFiler – quantiplex pro	0.1120	8.9 – 6.1	GF	FS
62363	cigarette butt	CS - MF-6	1/62363	PrepFiler – quantiplex pro	0.0452	15.0 – 0.0	GF	FS
60938	Syringe handle	CS - MF-7	1A/60938	PrepFiler – quantiplex pro	0.0018	15.0 – 0.0	X	X
60423	Women black underwear	CS - MF-8	3/60423	PrepFiler – quantiplex pro	0.0247	15.0 – 0.0	GF	FS
63132	Metal knife handle	CS - MF-9	3A/63132	PrepFiler – quantiplex pro	3.1083	3.2 – 6.8 / 1.0 – 14.0	GF	FS
63187	Plastic knife handle	CS - MF-10	1A/63187	PrepFiler – quantiplex pro	0.0077	15.0 – 0.0	GF	PM
61743	Metal golf bat	CS - MF-11	1A/61743	PrepFiler – quantiplex pro	0.0182	15.0 – 0.0	GF	FS
64466	Cigarette butt	CS - MF-12	1/64466	PrepFiler – quantiplex pro	1.1987	8.3 – 1.7 / 1.0 – 14.0	GF	FS
64349	Lighter	CS - MF-13	1/64349	PrepFiler – quantiplex pro	0.0005	15.0 – 0.0	GF	N
64349	Plastic cover of a small bottle	CS - MF-14	3/64349	PrepFiler – quantiplex pro	0.0001	15.0 – 0.0	GF	N
64303	Plastic safe cover	CS - MF-15	1/64303	PrepFiler – quantiplex pro	0.0025	15.0 – 0.0	GF	N

Biology				DNA				
Case No.	Item; Surface type	Collection type - No.	Sample No.	Ext. Quant. type	Quantity	Amp. dilution	STR Kit	Profile Type
63123	Pen	CS - MF-16	1/63123	PrepFiler – quantiplex pro	0.0382	15.0 – 0.0	GF	FM
66344	Metal knife handle	CS - MF-17	1A/66344	PrepFiler – quantiplex pro	0.0002	15.0 – 0.0	GF	N
66344	Metal hand ring	CS - MF-18	25/66344	PrepFiler – quantiplex pro	0.0280	15.0 – 0.0	GF	FM
66344	Metal hand ring	CS - MF-19	26/66344	PrepFiler – quantiplex pro	0.1093	9.2 – 5.8	GF	FS
67191	Screwdriver – plastic handle	CS - MF-20	1/67191	PrepFiler – quantiplex pro	0.0114	15.0 – 0.0	GF	FM
65765	Tennis racket handle – smooth plastic	CS - MF-21	2A/65765	PrepFiler – quantiplex pro	0.0087	15.0 – 0.0	GF	FM
67247	Big metal cutter - smooth metal	CS - MF-22	1/67247	PrepFiler – quantiplex pro	Undetermined	15.0 – 0.0	GF	N
66846	Toothbrush - textured plastic	CS - MF-23	1/66846	PrepFiler – quantiplex pro	0.0076	15.0 – 0.0	GF	FS
66846	Hairbrush - textured plastic	CS - MF-24	3A/66846	PrepFiler – quantiplex pro	0.0514	15.0 – 0.0	GF	FM
67523	Unused bullet	CS - MF-25	1/67523	PrepFiler – quantiplex pro	0.0017	15.0 – 0.0	GF	N

Samples Collection Sheet (A); Following this study protocol

Biology				DNA				
Case No.	**Item; Surface type**	**Collection type - No.**	**Sample No.**	**Ext. Quant. type**	**Quantity**	**Amp. dilution**	**STR Kit**	**Profile Type**
59809	Cotton swab -right handle of motorbike - plastic	CS+MF-1	4/59809				GF	FS
59770	Cotton swab - Knife handle - smooth plastic	CS+MF-2	5/59770				GF	N
59770	Cotton swab - glass bottle nozzle	CS+MF-3	6/59770				GF	N
59770	Cotton swab - Knife handle - smooth plastic	CS+MF-4	1/59770				GF	N
60347	Cotton swab - plastic bottle nozzle	CS+MF-5	6/60347				GF	N
60347	Cotton swab - plastic bottle nozzle	CS+MF-6	7/60347				GF	FS
60138	Cotton swab - Knife handle - smooth plastic	CS+MF-7	1/60138				GF	N
61357	Cotton swab- glass bottle	CS+MF-8	1B/61357				GF	FS
60587	Cotton swab - metal door handle from outside	CS+MF-9	2/60587				GF	N
60587	Cotton swab - metal door handle from inside	CS+MF-10	3/60587				GF	N

- CS; Cotton Swab
- NS; Nylon Swab
- MT; minitape
- MF; MicroFLOQ™
- EXT 1; PrepFiler BTA
- EXT 2; QIAamp Inve.
- GF; GlobalFiler
- FS; Full single profile
- PS; Partial single profile
- FM; Full mixture profile
- PM; Partial mixture profile
- N; Negative

Biology				DNA				
Case No.	Item; Surface type	Collection type - No.	Sample No.	Ext. Quant. type	Quantity	Amp. dilution	STR Kit	Profile Type
60083	Cotton swab - car steering wheel	CS+MF-11	1/60083				GF	N
59487	Cotton swab - around vagina (sexual assault)	CS+MF-12	1/59487				GF	PS
59487	Cotton swab - around anal (sexual assault)	CS+MF-13	2/59487				GF	FS
60185	Cotton swab - car steering wheel	CS+MF-14	1/60185				GF	PS
61645	Cotton swab - car steering wheel	CS+MF-15	1B/61645				GF	FS
61715	Cotton swab - watch case	CS+MF-16	1/61715				GF	N
62283	Cotton swab - under right hand fingernails	CS+MF-17	5/62283				GF	PM
62283	Cotton swab - under left hand fingernails	CS+MF-18	6/62283				GF	PS
62283	Cotton swab - vaginal swab	CS+MF-19	1/62283				GF	PS
62083	Cotton swab - car steering wheel	CS+MF-20	1/62083				GF	PS
61703	Cotton swab - Knife handle	CS+MF-21	1/61703				GF	N
61824	Cotton swab - right handle of motorbike	CS+MF-22	1/61824				GF	PM
63462	Cotton swab - plastic glove	CS+MF-23	1A/63462				GF	PM
59683	Cotton swab - metal handcuff	CS+MF-24	6A/59683				GF	FS
65699	Cotton swab - black men trouser	CS+MF-25	4D/65699				GF	PM

Samples Collection Sheet (B); Following Dubai Police lab protocol

Biology				DNA				
Case No.	**Item; Surface type**	**Collection type - No.**	**Sample No.**	**Ext. Quant. type**	**Quantity**	**Amp. dilution**	**STR Kit**	**Profile Type**
59809	Cotton swab -right handle of motorbike - plastic	CS+MF-1	4/59809	PrepFiler – quantiplex pro	0.3151	3.0 – 11.8	GF	FS
59770	Cotton swab - Knife handle - smooth plastic	CS+MF-2	5/59770	PrepFiler – quantiplex pro	0.0030	15.0 – 0.0	GF	PS
59770	Cotton swab - glass bottle nozzle	CS+MF-3	6/59770	PrepFiler – quantiplex pro	0.009	15.0 – 0.0	X	X
59770	Cotton swab - Knife handle - smooth plastic	CS+MF-4	1/59770	PrepFiler – quantiplex pro	0.0013	15.0 – 0.0	GF	N
60347	Cotton swab - plastic bottle nozzle	CS+MF-5	6/60347	PrepFiler – quantiplex pro	0.0079	15.0 – 0.0	GF	FM
60347	Cotton swab - plastic bottle nozzle	CS+MF-6	7/60347	PrepFiler – quantiplex pro	0.1429	7.0 – 8.0	GF	FS
60138	Cotton swab - Knife handle - smooth plastic	CS+MF-7	1/60138	PrepFiler – quantiplex pro	1.4932	1.0 – 14.0	GF	FS
61357	Cotton swab- glass bottle	CS+MF-8	1B/61357	PrepFiler – quantiplex pro	0.0336	15.0 – 0.0	GF	FS
60587	Cotton swab - metal door handle from outside	CS+MF-9	2/60587	PrepFiler – quantiplex pro	0.0086	15.0 – 0.0	GF	PM
60587	Cotton swab - metal door handle from inside	CS+MF-10	3/60587	PrepFiler – quantiplex pro	0.0162	15.0 – 0.0	GF	FM
60083	Cotton swab - car steering wheel	CS+MF-11	1/60083	PrepFiler – quantiplex pro	0.0965	10.4 – 4.6	GF	PM
59487	Cotton swab - around vagina (sexual assault)	CS+MF-12	1/59487	PrepFiler – quantiplex pro	0.0002	15.0 – 0.0	X	X

Biology				DNA				
Case No.	Item; Surface type	Collection type - No.	Sample No.	Ext. Quant. type	Quantity	Amp. dilution	STR Kit	Profile Type
59487	Cotton swab - around anal (sexual assault)	CS+MF-13	2/59487	PrepFiler – quantiplex pro	0.0001	15.0 – 0.0	GF	X
60185	Cotton swab - car steering wheel	CS+MF-14	1/60185	PrepFiler – quantiplex pro	0.0220	10.4 – 4.6	GF	PM
61645	Cotton swab - car steering wheel	CS+MF-15	1B/61645	PrepFiler – quantiplex pro	0.1120	8.9 – 6.1	GF	FS
61715	Cotton swab - watch case	CS+MF-16	1/61715	PrepFiler – quantiplex pro	0.008	15.0 – 0.0	GF	X
62283	Cotton swab - under right hand fingernails	CS+MF-17	5/62283	PrepFiler – quantiplex pro	0.0426	15.0 – 0.0	GF	FS
62283	Cotton swab - under left hand fingernails	CS+MF-18	6/62283	PrepFiler – quantiplex pro	0.0403	15.0 – 0.0	GF	FS
62283	Cotton swab - vaginal swab	CS+MF-19	1E2/62283	PrepFiler – quantiplex pro	0.0936	10.7 – 4.3	GF	FM
62083	Cotton swab - car steering wheel	CS+MF-20	1/62083	PrepFiler – quantiplex pro	0.2466	4.1 – 10.9	GF	FS
61703	Cotton swab - Knife handle	CS+MF-21	1/61703	PrepFiler – quantiplex pro	0.0025	15.0 – 0.0	X	X
61824	Cotton swab -right handle of motorbike	CS+MF-22	1/61824	PrepFiler – quantiplex pro	0.0848	11.8 – 3.2	GF	FM
63462	Cotton swab - plastic glove	CS+MF-23	1A/63462	PrepFiler – quantiplex pro	0.0091	15.0 – 0.0	GF	FM
59683	Cotton swab - metal handcuff	CS+MF-24	6A/59683	PrepFiler – quantiplex pro	0.0009	15.0 – 0.0	GF	N
65699	Cotton swab - black men trouser	CS+MF-25	4D/65699	PrepFiler – quantiplex pro	0.0479	15.0 – 0.0	GF	FS

A9. LIST OF PRESENTATIONS AND PUBLICATIONS

A9.1 PRESENTATIONS

1. Forensic & Applied Sciences (FAS) Research Poster Presentation, Foster building, University of Central Lancashire – UCLan, Preston, UK, 24th April 2019.

2. The 28th Congress of the International Society for Forensic Genetics (ISFG), Prague, Czech Republic, 9 - 13 September 2019.

3. Human Identification Solutions (HIDS) Virtual Conference, Thermo Fisher Scientific, 24 – 25 June 2020.

4. Human Identification Solutions (HIDS) Virtual Conference, Thermo Fisher Scientific, 19 – 20 May 2021.

5. World Police Summit (WPS), Forensic Science Conference, Dubai, UAE, 14 – 17 March 2022.

6. Human Identification Solutions (HIDS) Virtual Conference, Thermo Fisher Scientific, 11 – 12 May 2022.

7. The 29th Congress of the International Society for Forensic Genetics (ISFG), Washington, D.C., USA, 29 August – 02 September 2022.

A9.2 PUBLICATIONS

1. Alketbi, S.K. (2020) Collection of Touch DNA from rotten banana skin. *International Journal of Forensic Sciences*, 5(4), pp. 204-206.

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