IMPROVING METHODS TO OBTAIN DNA PROFILING RESULTS FROM CARTRIDGE CASES

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ABSTRACT

Crime associated with firearms has become more common and widespread in New Zealand and in the rest of the world. The continuous growth of firearm related crime has increased the forensic interest in improving the evidence collection from firearms and ammunition. A large number of studies are being conducted in various jurisdictions in maximising DNA profiling results from firearms and ammunition. However, locating and collecting DNA from firearms and ammunition remains challenging and expensive.

The aim of this study was to bring together expertise from ESR with diamond dye, firearms and DNA extraction and from the New Zealand Police Fingerprints team to explore the potential of using diamond dye to locate touch DNA on cartridge cases. At the same time, this project investigated alternative DNA extraction methods in an attempt to improve the success rates of profiling touch DNA and to generate better investigative leads for the New Zealand Police.

The research was divided into four objectives where the first two objectives focused on the visualisation of touch DNA from cartridge cases and the second two objectives focused on improving DNA profiling results. The effect of fingermark enhancement on cartridge cases was investigated and it was found that the fingermark enhancement technique vacuum metal deposition (VMD) will significantly impact subsequent visualisations of DNA using diamond dye. It was also found that the application of diamond dye prior to VMD gave good results for both visualisation and enhancement of fingermarks. The impact of the process of firing on the presence of touch DNA on fired cases was investigated as the second objective and it was found that the process of firing has a significant impact on the presence of touch DNA.

The second two objectives of this research investigated methods in improving DNA profiling results from cartridge cases. The DNA IQ™ extraction kit is used at ESR for the extraction of touch DNA samples. This research investigated two other extraction methods and compared these to DNA IQ™. The other two extraction methods used were QIAamp® DNA Mini kit coupled with a pre-extraction soaking method and QIAamp® DNA Mini kit coupled with direct lysis. The samples extracted using all these methods were amplified using Identifiler® Plus with standard PCR cycling conditions and Identifiler® Plus LCN with 34 cycles. In this objective it was found that the detection of allelic peaks was possible only with Identifiler® Plus LCN. Due to the very limited yield in DNA, the results obtained in this research cannot be interpreted quantitatively using statistics. However, a qualitative interpretation can be given,

with the number of alleles obtained from all extractions across all the samples to conclude that direct lysis coupled with Identifiler® Plus LCN yielded the greater number of alleles.

The quantity of touch DNA on a sample is generally very minimal and if it is subjected to multiple steps of sampling and extraction, the chances of DNA getting lost during each step is high. This disadvantage in the multiple steps of processing samples for DNA profiling led to the investigation of the success rate of direct PCR from cartridge cases. Direct PCR was performed using Identifiler® Plus (28 cycles), Identifiler® Plus LCN (34 cycles) and Minifiler®. No results were obtained using Identifiler® Plus (28 cycles) and Minifiler®. Allelic profiles were obtained only from the direct PCR coupled with Identifiler® Plus LCN (34 cycles), however the admissibility and validity of the LCN amplification techniques depends on the replication of the results which is not possible in direct amplification. With the poor yield and profiling of touch DNA from cartridge cases, this study highlights the necessity of having extensive research regarding this matter.

Keywords: cartridge cases, diamond dye, DNA extraction, DNA profiling, direct PCR, fingermarks, touch DNA

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LIST OF ABBREVIATIONS

µl: microlitre

0.22: .22 Long Rifle

0.223: .223 Remington

bp: base pairs

^{0}C: degree Celsius

cm: centimetres

C_T: cycle threshold

Diamond dye: Diamond™ Nucleic Acid Dye

DNA: deoxyribonucleic acid

DNA IQ™: DNA IQ™ system by Promega

DTT: dithiothreitol

epg: electropherogram

ESR: Institute of Environmental Science and Research

F: fired cartridge cases

Fingermarks: fingerprint impressions

HFE 7100: 3M™Novec™ Engineered Fluid 7100

Identifiler® Plus: Applied Biosystem™ AmpFLSTR™ Identifiler® Plus PCR Amplification Kit

IPC: internal PCR control

kv: kilovolts

LA: large autosomal

LCN: low copy number

mg: milligrams

Minifiler®: Applied Biosystem™ AmpFLSTR™ Minifiler® PCR Amplification Kit

ml: millilitre

mm: millimetre

ng: nanogram

NTC: non-template control

OL: off ladder

OMR: outside marker range

PCR: polymerase chain reaction

pg: picograms

QIAamp® DNA kit: QIAamp® DNA Mini Extraction kit by Qiagen

Quantifiler®: Quantifiler® Trio DNA Quantitation Kit

rfu: relative fluorescence unit

rpm: revolutions per minute

SA: small autosomal

Std: standard

STR: short tandem repeats

UF: unfired cartridges

VMD: vacuum metal deposition

CHAPTER 1

INTRODUCTION

1.1 Forensic Science

Forensic science is a discipline of science used in criminal investigations and the courtroom that cannot be described in a sentence or two. There are multiple definitions for forensic science as it has various facets. It can be explained as a convergence of multi-facets of science and technology that assist the criminal justice system (Roux et al., 2018). The knowledge of physics, chemistry, and biology is applied extensively in forensic science (Horncastle, 2016). Along with science, technology is also an essential attribute of forensic science in the 21st century. It is a collective of different inter-disciplines, such as forensic biology, forensic toxicology, ballistics, fingerprints, questioned documents, anthropology, odontology and crime scene investigation. (Roux et al., 2018). The roles of forensic science examination could be confined to recognition, identification, comparison, individualisation, and reconstruction that tries to establish a link between crime scenes, the complainant and suspect.

1.2 Forensic Biology and DNA

Forensic biology is a major part of forensic science that uses knowledge of biological sciences to link crime scene(s), the complainant(s) and suspect(s) to a crime. The study of DNA is one of the most advanced and familiar aspects of forensic biology.

Deoxyribonucleic acid (DNA), referred to as the genetic blueprint, is now an integral part of Forensic Science (Saferstein, 2001). While announcing the first draft of the Human Genome, President Bill Clinton (former president of the United States of America) mentioned DNA as the language in which God created life. DNA, the double helix structure, is composed of nucleotides. Nucleotides contain a phosphate group, nitrogenous base, and deoxyribose sugar. There are four types of nitrogenous bases in DNA called adenine (A), thymine (T), guanine (G) and cytosine (C). ‘A’ always binds with ‘T’ of a complementary strand using a double hydrogen bond, and ‘C’ binds to ‘G’ of complementary strands using a triple hydrogen bond. The arrangement of the nucleotides is unique among individuals (Butler, 2009; Saferstein, 2001). This established uniqueness is the basis for the use of DNA as a reliable and admissible forensic evidence which is used as a source of identification to individualise and identify people of interest.

The development and evolution of DNA profiling, as coined by Sir Alec Jeffreys in 1985, shook the scientific world by making individualisation possible using DNA (Butler, 2009; Saferstein, 2001). The first use of DNA in a criminal investigation was in 1986 by Sir Alec Jeffreys in solving a murder case in Leicestershire, England. DNA samples from two crime

scenes, which happened in 1983 and 1986, were analysed, and Jeffreys concluded that the DNA of the suspects in both the cases matched. Later the DNA from the two crime scenes were compared and matched to Collin Pitchfork, and he was subsequently convicted of both crimes (Butler, 2009; Saferstein, 2001). Further descriptions on DNA profiling are given in later chapters.

Nowadays, DNA is considered one of the important biological evidences, and intensive research is still ongoing to strengthen its reliability and validity. Along with the acceptance as conclusive evidence by the scientific community and judiciary, the admissibility of DNA is also facing solid criticisms. As the DNA profile of an individual is unique, it is used as a strong tool in identifying and proving wrongful convictions. The Innocence Project, an initiative in the United States to exonerate wrongfully convicted people, used DNA profiling to exonerate 375 people by 2020 (*Innocence Project - Help Us Put an End to Wrongful Convictions!*, n.d.). As a coin has two sides, DNA technology has advantages and disadvantages. The objects or areas of interest containing DNA will not always have DNA from one person. For example, in sexual assault cases, the chances of finding DNA from both the complainant and the suspect is common. The report published by the President's Council of Advisors on Science and Technology (PCAST) in 2016 identifies the gaps in the admissibility, reliability, and validity of DNA evidence when profiling complex mixtures. They recognised the necessity of conducting more empirical studies in analysing DNA samples from multiple contributors (*Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*, 2016).

DNA profiling is a live discipline in forensic science with a lot of ongoing research to solve the gaps. The source of DNA and the amount of DNA retrieved impacts on the success of DNA profiling. One of the progressions made while studying the methods to maximise the DNA retrieval from evidence is the discovery that DNA profiling results can be obtained from touch DNA.

1.3 Touch DNA

Fingerprint impressions (fingermarks) have been playing a significant role in solving crimes for approximately a century (Hefetz et al., 2019). Latent fingermarks are the most common, and sometimes the only, potential evidence retrieved from a crime scene (Martin et al., 2018). There are multiple ways to detect, visualise, collect and document fingermarks from a crime scene, as detailed in section 1.4.3. Apart from impression evidence, fingermarks also act as a

source of potential DNA evidence (Hefetz et al., 2019). The fact that DNA can be retrieved from fingermarks is of growing forensic interest due to the high chances of finding latent fingermarks at a crime scene.

DNA deposited on a surface by touch is termed 'Touch DNA' or trace DNA (Ah Van Oorschot et al., 2010) In other words, touch DNA is the deposition of DNA onto a surface due to the transfer of skin cells during the process of a person touching an object or surface (Alketbi, 2018). The term touch indicates possible chances of the deposition of fingerprints, and the presence of fingerprints indicates the potential deposition of touch DNA (Kanokwongnuwut et al., 2019).

Touch DNA has been very commonly used in criminal investigations for more than 15 years (Kanokwongnuwut et al., 2018). The deposition of touch DNA is always limited, and it is impacted by multiple factors (Elisha Prasad et al., 2022). Shedder status, the surface being contacted (surface type), and the nature of contact are the three main factors known to impact the deposition of touch DNA onto a surface (Tobias et al., 2017) . One of the factors affecting the amount of touch DNA deposition is the DNA shedder status of the individual. The DNA shedding ability differs from individual to individual as there are multiple factors affecting the DNA shedding ability of an individual. The activities the person was engaged in before the DNA deposition, including the habitual actions, routine tasks and daily activities, can affect the amount of DNA deposited (Alketbi, 2018). If a person has a habit of touching their face or other parts of their body they often might transfer DNA from that area to their hands (Alketbi, 2018).

As many factors affect the ability of a person in the transmission of DNA, multiple tests have to be conducted under different conditions to say if a person is a good DNA shedder or not (Alketbi, 2018). Since Touch DNA is not visible, the possible location of DNA deposition and how much DNA may have been deposited remains unknown until quantification (Kanokwongnuwut et al., 2018). Literature indicates that touch DNA has fewer PCR inhibitors than DNA from other biological sources since it is retrieved from the epithelial cells transferred due to the touching process (Prasad et al., 2022). The quality and quantity of deposited DNA determines the success rate of profiling (Alketbi, 2018). However, the wide use of touch DNA has never diminished the value of identification and comparison of individuals using fingermarks. This research revolves around this touch DNA and attempts to maximise the results from it.

1.4 Fingerprint Impressions

Fingerprint impressions (fingermarks) are the most used method for personal identification in forensic science. Literature suggests that fingermarks have been used legally, equivalent to a signature, since the seventh century starting with the T'ang dynasty in China (Margot, 2000). Even then, the archaeological evidence indicates the use of fingermarks in artistry and sculptures probably as a source of identification in the early sixth century (Margot, 2000). In the present 21st century, the progress in the knowledge, the application and interpretation of fingermarks have laid a strong foundation.

Fingermarks are impressions created by the friction ridge patterns on the surface due to a contact. Fingermarks are a common evidence type encountered at a crime scene and they are a main source of biometric identification (Pathan et al., 2019). Fingermarks are admissible evidence in the courtroom to aid in the criminal investigation and trial (Gialamas, 2000). According to Locard's principle of exchange, every contact leaves a trace; the contact of the skin's outer surface can leave impressions on the surface that has been contacted (Horsman-Hall et al., 2009). The outer layer of the skin has alternating ridges and furrows that form unique patterns in the skin surface of the palm, sole and digits that is, fingers and toes (Wertheim & Maceo, 2002). A fingerprint expert needs to know how these ridges are formed, how they are unique, and how they can be used to identify individuals.

1.4.1 Brief biology of fingerprints

The skin where the friction ridges and furrows are present is called volar skin. The friction ridges in the skin are entirely formed by the 18th week of embryonic development (Wertheim & Maceo, 2002). The skin proliferation, compression and regression in the volar skin during the embryonic development results in the formation of alternating ridges and furrows on the surface of the skin (Wertheim & Maceo, 2002). The volar skin consists of three layers named epidermis, dermis and hypodermis. The membrane connecting the epidermis to the dermis is the basement membrane. The cellular connection and attachment between these three layers are the reason for the permanence of the fingerprints, as the fingerprints are deeply rooted in the dermis through the basement membrane (Wertheim & Maceo, 2002). There are primary and secondary ridges in the fingerprints. Primary ridges in the basement layer of the volar skin relate to the protruding ridges on the surface of the skin. Primary ridges carry the ducts from the eccrine gland. The secondary ridges appear as the furrows on the surface of the skin

alternating between primary ridges (Maceo, 2003). *Figure 1* shows the arrangement of primary and secondary ridges through the layers of the skin.

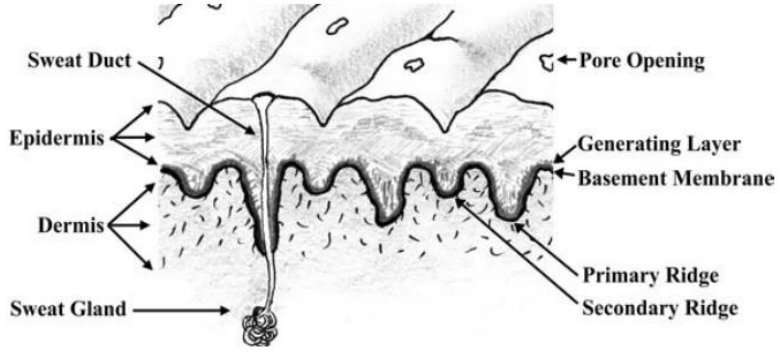

Figure 1: Arrangement of primary and secondary ridges in the layers of the skin (Wertheim & Maceo, 2002)

1.4.2 Principles of fingerprint examination

The fingerprint examination is based on three fundamental principles.

a. Principle of Individuality or the Principle of Uniqueness: No two fingers are proven to have the same fingerprint pattern (Saferstein, 2001). Fingerprints are individual characteristics that are not just unique among individuals but unique among fingers of the same individual. In the context of fingerprint examination, the individuality of fingerprints is determined by the ridge characteristics (Saferstein, 2001). The ridge characteristics, also known as minutiae, enclose multiple characteristics that fingerprints exhibit in general, but the location, size and numbers will differ from one fingerprint to another. Bifurcation, trifurcation, ridge endings, enclosure and ridge core are some of the ridge characteristics seen on fingerprints (Hutchins, 2013). *Figure 2* illustrates these ridge characteristics. Ridge characteristics formed during embryonic development are influenced by the environment in which a foetus develops and therefore cannot be duplicated (Hutchins, 2013). Every embryonic stage will exert unique pressure that strengthens the foundation of the principle of uniqueness.

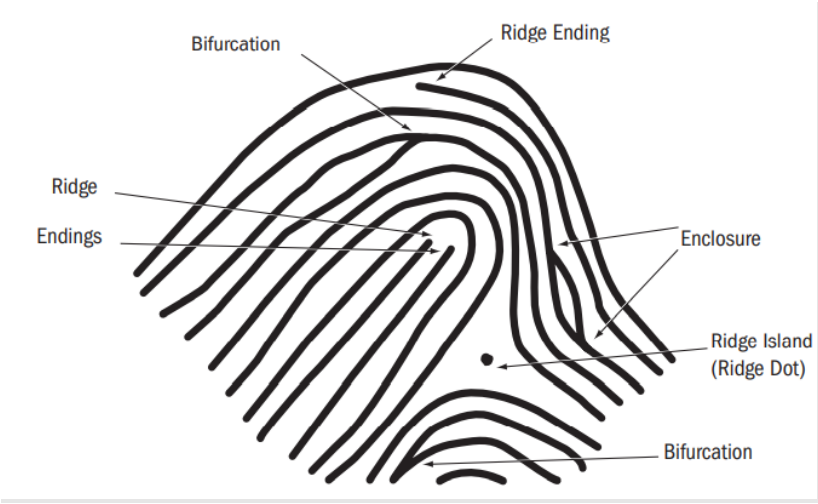

Figure 2: Ridge characteristics (Pathan et al., 2019)

b. Principle of permanence: As mentioned in the above sub-section. The ridge patterns on the epidermal layer of the skin are connected to the dermis using the basement membrane. Therefore, the pattern is not just formed on the superficial layer of the skin but is seen on inner layers as well (Maceo, 2003). Hence, any superficial layer injuries will not permanently remove or change the fingerprint pattern if the wound is less than 2 millimetres in depth. In some cases, the injury will leave a scar on the finger that remains as a unique feature (Maceo, 2005).
c. General ridge patterns: The fingerprint ridge patterns are formed systematically in humans. The ridge patterns fall under three different types. The different ridge patterns are arch, loop, and whorl. The finger ridge patterns are mostly either one of three or combinations of these three (Saferstein, 2001). Apart from the common patterns, unusual accidental patterns are also found. *Figure 3* is a pictorial representation of some of the fingerprint patterns.

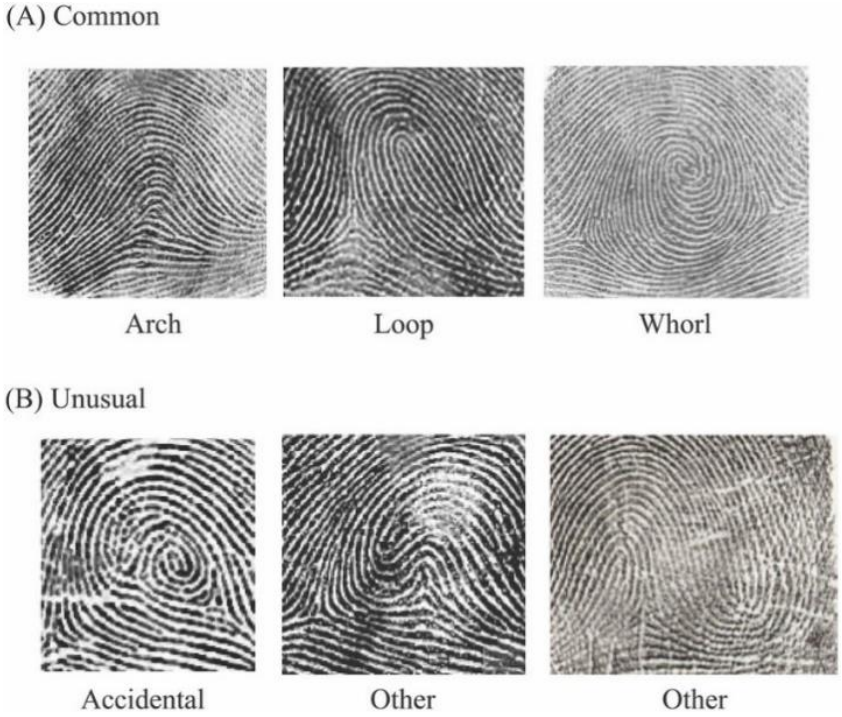

Figure 3: Different fingerprint patterns (Dermatoglyphic Patterns. (A) Arches, Loops, and Whorls Are Common... | Download Scientific Diagram, n.d.)

The above-discussed principles aid in the individualisation of people based on fingermarks.

1.4.3 Fingermark enhancement techniques

Fingermarks can be classified into latent, patent and plastic. Latent fingermarks are invisible impressions, mostly unnoticed and hard to see with the naked eye. Patent fingermarks are visible impressions that could be either positive or negative (Lennard, 2001). Plastic impressions can be described as three-dimensional impressions left on a flexible and malleable surface (Lennard, 2001). Latent fingermarks are of high forensic importance as they are mostly left at the crime scene unintentionally. Furthermore, the improvement and progress in the study of touch DNA has amplified the relevance of latent fingermarks.

There are multiple factors affecting the transfer and deposition of latent fingerprints. Surface type, environmental factors, nature of contact and the presence of any contaminants are the main factors affecting the transfer and persistence of fingermarks. The surface types can be classified into porous, non-porous and semi-porous.

The enhancement and documentation of fingermarks are very crucial steps in the examination and analysis of fingerprint evidence. The techniques employed for the detection, enhancement, collection and documentation primarily depend on the type of impression and the surface of

deposition (Lennard, 20001). In general, fingermark development can be done using four methods.

1. Optical enhancement of fingermarks
2. Physical enhancement
3. Chemical enhancement
4. Physio-chemical enhancement

Oblique lighting using alternate light sources is an example of optical enhancement of fingermarks. The use of fingermark development powders are common physical enhancement techniques used and Mikrosil® is one of the physical recovery techniques used. Vacuum metal deposition (VMD), a physical enhancement method, is employed widely on cartridges and cartridge cases. Different fluorescent dyes and fingermark development using ninhydrin are examples of chemical enhancement. The superglue method (cyanoacrylate) is an example of physio-chemical enhancement (Lennard, 20001).

Like the examination of other impression evidence, fingerprint examination also involves four steps. The four steps are: analysis, comparison, evaluation, and verification (Tierney, 2013). In the analysis step, the ridge patterns are identified and associated with the details of the pattern. The analysed ridge details are then compared with other known or unknown samples to obtain a link. The third step after comparison is evaluation. The conclusion of the examination is drawn in the evaluation procedure. The conclusion can be either inclusion, exclusion or inconclusive. Verification is a crucial step where another examiner reviews the identification of the ridge pattern without knowing the conclusion drawn by the primary examiner. The fact that the secondary examiner is unaware of the conclusion drawn by the primary examiner thereby reduces the chances of a biased conclusion (Tierney, 2013).

1.5 Firearms and Ammunition

Firearms are considered one of the most potent tools for committing a crime. Guns and the crimes associated with them are of great concern around the globe. A recent statistic shows a rise in firearms-related crimes in New Zealand even though ownership rules have been made stricter, especially after the Christchurch Mosque attack (Strang, 2021). The increasing rate of gun violence has raised concerns for the police, and they have previously issued a warning for the public to be cautious and vigilant (Burrell, 2021) (Church, 2022). In July 2021, several serious crimes were recorded in the country, from a police officer being shot to the police shooting the person who fired the gun (Burrell, 2021). Repeated gun violence makes the public

question the safety of living (Church, 2022). These increasing concerns reflect the necessity for advanced research in investigating crimes involving firearms. The predominant need for inexpensive, rapid, and effective techniques is the background of this research.

1.5.1 An Introduction to firearms and ammunition

The definition of firearms and ammunition will have minor variations across jurisdictions (Bolton-King & Schulze, 2016). In New Zealand, the legal interpretation of the term firearms is given under the Arms Act 1983. According to Section 2(1) of the Arms Act (1983), "firearms means anything from which any shot, bullet, missile, or other projectile can be discharged by force of explosive" (*Arms Act 1983 No 44 (as at 12 April 2022), Public Act – New Zealand Legislation*, n.d.).

There are multiple classifications for firearms and ammunition. Generally, based on the physical characteristics, firearms can be classified into handguns and long guns or shoulder firearms (Houck & Siegel, 2006). Handguns consist of revolvers and pistols that can be operated using one hand. Long guns or shoulder guns incorporate a broad range of rifles and shotguns (Saferstein, 2001). Long guns are operated by resting the gun on the front of the shoulder. Other than the above classification, firearms can also be classified into different categories based on the action of the weapon, traditional structure and portability (*Basics on Firearms and Ammunition*, 2019). Having a good understanding of different types of firearms and ammunition is very crucial in firearm examinations.

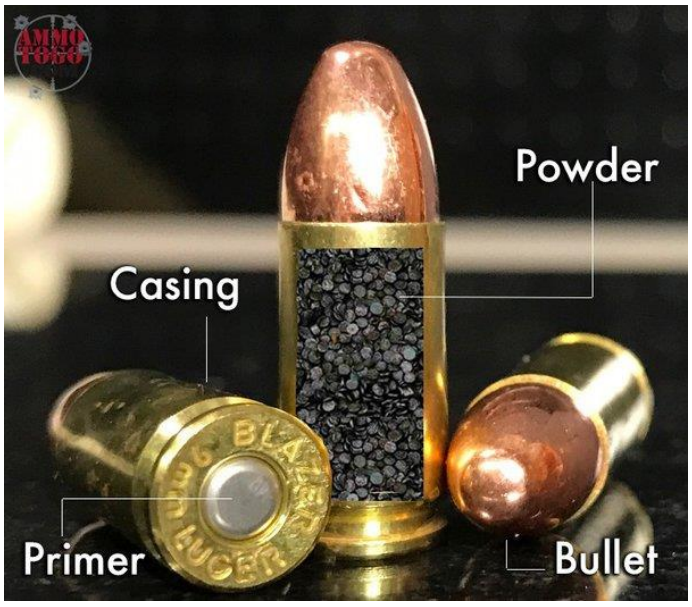

Figure 4: Different components of ammunition (Webb, 2019)

Ammunition is an important component of a firearm as the firearm is designed to fire this ammunition. The three components of a cartridge are projectile(s), propellant and primer as illustrated in *Figure 4*. In *Figure 4* the bullet is the projectile and the powder is the propellant. *Figure 5* shows the pictorial representation of the arrangements of these components into a cartridge. The primer ignites the propellant to initiate combustion, and thereby the projectile will be ejected (Saferstein, 2002). In handgun and rifle ammunition, the projectile is typically a single bullet. The projectiles called pellets. Primer is a pressure-sensitive material that ignites upon impact of the firing pin, which then subsequently ignites the propellent. The cartridge case holds the primer and propellant, respectively, and the projectile is housed in the mouth of the cartridge case (The Royal Society, 2021).

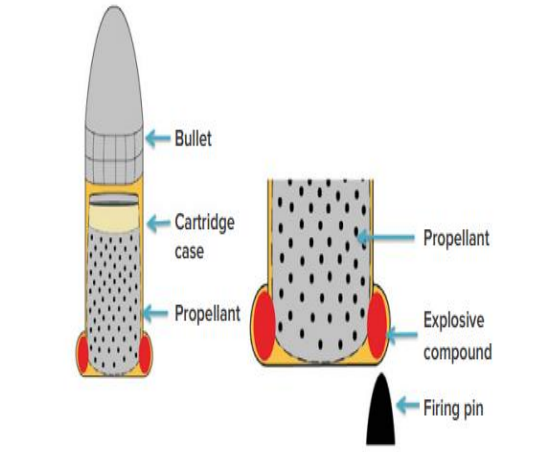

Figure 5: Arrangement of components of a cartridge (The Royal Society, 2021)

1.6 Diamond Dye

The presence of touch DNA (latent) on surfaces is of high importance in forensic science as it can act as evidence of identification. Visualising the presence of DNA on surfaces is highly challenging as there are not many scientifically reliable techniques. If visualisation of DNA is not possible, the presence or absence of DNA can only be identified after sampling and analysing the samples (Kanokwongnuwut et al., 2018). Sampling that is strongly reliant on assumptions about finding DNA can lead to a lot of mechanical, technical, and manual labour with limited positive outcomes, exploiting time and resources. If DNA evidence could not be retrieved even after all of the steps the hard work goes in vain.

Highly sensitive dyes exist that can bind to the DNA and emit green fluorescence when excited with lights of suitable intensity and wavelength (Haines et al., 2015). There are multiple binding sites for nucleic acid binding dyes, called NABD binding sites (*Figure 7*). Diamond™ Nucleic Acid Dye (diamond dye) is an external binder. Diamond dye is an inexpensive external groove binding dye that gives rapid results in its application (Haines et al., 2015). Diamond dye is a fluorescent nucleic acid binding dye that binds to DNA and RNA (Champion et al., 2019). Diamond dye emits fluorescence when excited with blue light of approximately 500nm if any DNA is present as represented in *Figure 6* (Haines et al., 2015). The fact that the visualisation of DNA using diamond dye is possible with the portable Dino-Lite microscope makes it appropriate to be used at a crime scene (Kanokwongnuwut et al., 2018).

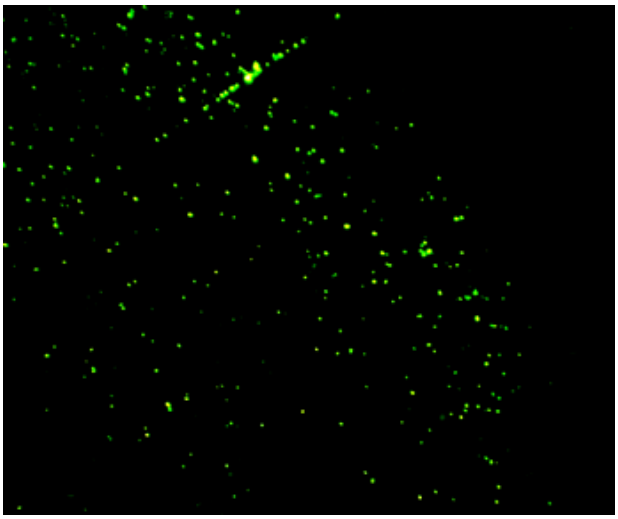

Figure 6: Fluorescence emitted by diamond dye when exited with Dino-Lite on a glass slide having fingermarks

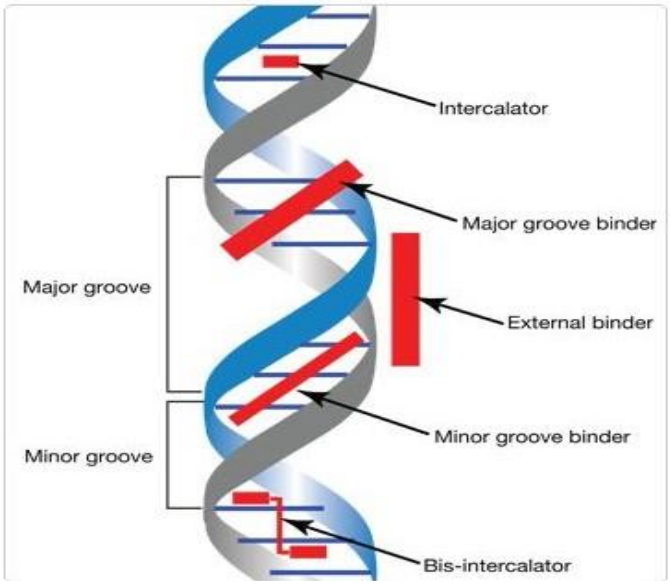

Figure 7: NABD binding cites (Royer et al., n.d.).

Diamond dye binds to the cellular material in approximately three seconds and is found to have little detrimental effect on subsequent DNA extraction (Haines et al., 2015). Diamond dye is proven to be highly sensitive and has a more negligible mutagenic effect as compared to other DNA staining dyes like Ethidium Bromide (Haines et al., 2015; Kanokwongnuwut et al., 2018). A study by Kanokwongnuwut et al did staining of cell nuclei using haematoxylin to confirm the fluorescence present after using diamond dye was from fingermarks (Kanokwongnuwut et al., 2018). Diamond dye can visualise any touch DNA deposited on surfaces even if the contact time is less than five seconds (Kanokwongnuwut et al., 2018) (Kanokwongnuwut et al., 2021). Research also shows that diamond dye can detect down to 0.5ng of DNA (Haines et al., 2015). Other cellular material might be present in the fingerprints, apart from human DNA. Studies mention that diamond dye exhibits bare minimum background fluorescence, especially from bacterial DNA. This was demonstrated by staining the Eukaryotic DNA using specific dyes (Kanokwongnuwut et al., 2018).

Touch DNA deposited on a surface might get transferred to another surface via a number of different methods. The transfer of touch DNA from the source to another surface is called primary transfer (Daly et al., 2012). The transfer of touch DNA from the primary transfer surface to one or more other surfaces is called secondary transfer (Daly et al., 2012). Most of the time, the amount of DNA getting deposited due to a primary transfer would be greater than the amount of DNA deposition due to a secondary transfer. Substrate type and contact type are the most common influences of transfer of touch DNA. It is possible to track and visualise transfer of touch DNA from one surface to another using diamond dye (Champion et al., 2019).

The visualisation of touch DNA using diamond dye could help to draw a conclusion as to whether the sample has sufficient DNA to perform an extraction to give a complete profile (Kanokwongnuwut et al., 2021). Locating and visualising touch DNA before subjecting it to extraction, also avoids unnecessary sampling. The extraction procedure could be more effective if the touch DNA could be visualised and located. The literature indicates the application of diamond dye on items of forensic interest and small areas. Most of the literature was indicative of intense research in staining touch DNA using diamond dye on non-porous surfaces, which is of forensic interest. Kanokwongnuwut et al. have addressed the challenges in using diamond dye by stating the gaps in the efficiency of diamond dye in visualising touch DNA from porous surfaces such as fabrics and papers (Kanokwongnuwut et al., 2018).

The visualisation of touch DNA is just an indicator of the possible presence of DNA on a surface. Therefore, just the visualisation of touch DNA cannot be used to identify and individualise a person. This DNA can then be used to identify an individual, this is discussed in the chapter below when the DNA is subjected to DNA profiling. The chapter below discusses the possible individualisation of DNA after subjecting the sample to DNA profiling.

1.7 DNA Profiling

DNA profiling is one of the most nuanced and most sensitive methods used to identify, compare, and individualise samples based on genetic information. As mentioned earlier, DNA is the genetic identity of a person, and it is one of its unique features. Apart from identical twins, no two-persons share the same genetic code (Butler, 2009). Thus, DNA evidence can be invaluable in a courtroom.

Since 1986, DNA profiling has been evolving as a pillar of criminal investigation (Butler, 2009). The identification of genetic markers and the evolution of DNA profiling are advances in science in general. Initially, techniques like restriction fragment length polymorphism (RFLP) and variable number tandem repeats (VNTR) were employed in terms of profiling.

Short Tandem Repeat (STR) markers have been widely used since the first use of STR markers in 1994 and are still used for DNA profiling. STRs are short repetitive sequences of 2 bp to 6 bp of DNA, 20 to 100 bp long and constituting roughly three percent of the human genome (Lareu, 2013) (Wyner et al., 2020). This short length helps in the amplification of the sequence using PCR (Lareu, 2013). STRs are non-coding gene expressions which are not allied with phenotypes. The alleles at STR loci are of different length in different people. Separating and analysing these alleles are the key to DNA profiling.

There are multiple steps in DNA profiling. Standard profiling of DNA starts with sample collection and ends with analysis. The procedure is done in sequential order as follows:

Sample Collection → DNA Extraction → DNA Quantitation →

DNA Amplification → Capillary Electrophoresis → Data Analysis

All these above-mentioned procedures are detailed in later sections starting with sample collection.

1.8 DNA Sample Collection

Before the extraction, sample collection is performed on substrates or objects, which may contain DNA. The collection method used will highly depend on the sample type and surface type. The collection methods used in touch DNA are mainly tape lifting, swabbing and soaking (Prasad et al., 2020). Direct lysis and direct PCR are other methods used to retrieve any touch DNA present (Moore et al., 2020). Even though there are multiple options, a study done by Alketbi (2018) opine that rigorous research is needed regarding the collection of touch DNA (Alketbi, 2018).

Swabbing, as the name suggests, is swabbing a surface using a cotton swab or a rayon swab. For touch DNA, swabbing is done to collect any epithelial cells, a possible source of DNA, that may have been left behind after the contact. Studies have already proven the application of double swabbing in recovering touch DNA (Prasad et al., 2020). Double swabbing is technically coupling wet and dry swabbing to collect any DNA. Firstly, the surface is swabbed using a moistened swab and followed with a dry swab. Maximising sample collection is very crucial in touch DNA evidence, and wet swabbing alone is unlikely to collect all of the epithelial cells on a surface (Pang & Cheung, 2007). Double swabbing is used in most jurisdictions to retrieve any touch DNA present. Recent studies demonstrate the low chances of obtaining DNA profiles from cartridge cases using traditional wet and dry swabbing techniques (Elisha Prasad et al., 2022).

Tape-lifting is another technique used for the collection of any DNA. Adhesive tape is stuck to the surface of interest and the tape is lifted. The method is useful for lifting samples from porous surfaces like fabrics. The sections of adhesive tape are typically small and are often called minitapes. Different minitapes of varying adhesive strength are presently in use and the adhesive strength is one of the criteria that decides the efficiency of tape lifting. Scenesafe FAST™ minitapes and Scotch® Magic™ are examples of the minitapes used to collect DNA. According to studies, adhesive strength of Scenesafe FAST™ is greater than the adhesive strength of Scotch® Magic™ minitapes (Verdon et al., 2014). Apart from the adhesive strength of the tape, the number of lifts also is an important factor that determines the efficiency of tape-lifting in the collection of DNA. More than one tape lift from the same surface recovered more DNA than single lifting (Verdon et al., 2014).

The techniques of swabbing and tape-lifting, use secondary objects such as the swab and adhesive tape respectively for the collection of DNA. Soaking involves directly soaking the

target objects in a buffer solution and using the solution in which the object was soaked for further extractions thereby merging sample collection and extraction (Dieltjes et al., 2011). Multiple literature articles indicate that not all of the DNA that is collected onto swabs and adhesive tapes is recovered from these sampling mediums during extraction. This knowledge has led to an increased focus on soaking as a method of DNA collection and recovery in the forensic community globally (Alketbi, 2018; Elisha Prasad et al., 2022; Verdon et al., 2014).

Direct lysis and direct amplification are also used in the recovery of DNA, however they cannot be completely classified as a sample collection method. Technically direct lysis can be explained as a method of merging the collection method with the extraction of DNA. The direct lysis, like soaking, omits a separate collection procedure by directly soaking the sample in the lysis buffer (Moore et al., 2020). The lysis buffer is a combination of appropriate volume of ATL buffer, Proteinase K and DTT.

Direct amplification of samples using PCR (direct PCR) is another technique used to profile touch DNA (Moore et al., 2020). Recent studies on direct PCR of samples omit the first two conventional steps, extraction and quantitation. Direct amplification is performed in order to minimise the chances of losing DNA during multiple sampling and extraction steps and to maximise the profiling results. Direct PCR is discussed in more detail in section 1.11.

A study conducted by Prasad et al. compared three prominent DNA collection techniques for the recovery of DNA from ammunition (Elisha Prasad et al., 2022). The study compared the techniques of swabbing, tape lifting, and soaking. For soaking, the samples were soaked in ATL buffer by Qiagen. The results showed that the soaking technique recovered more DNA from cartridges than the conventional double swabbing technique. Tape-lifting showed better DNA recovery than soaking, but the differences were not enough to create a comparable statistical distinction (Elisha Prasad et al., 2022).

1.9 DNA Extraction

Obtaining a complete DNA profile from cartridge cases is challenging. This can be impacted by the size or composition of the casings. DNA extraction from the cartridge case plays a crucial role in DNA profiling (Bille et al., 2020). The extraction procedure plays a significant role in successfully profiling DNA as it removes cell proteins and other impurities that inhibit the amplification of DNA using the polymerase chain reaction (PCR) (Butler, 2012).

1.9.1 An introduction to DNA extraction

DNA is recovered from the crime scene in a complex form which can have numerous impurities and inhibitors. The extraction procedures help in isolating the DNA by removing any contaminants and inhibitors, thereby increasing the probability of generating successful DNA profiles (Alonso, 2013). Based on the purification procedure, DNA extraction techniques can be divided into three techniques. These are: organic extraction, solid-phase DNA extraction methods and Chelex extraction (Butler, 2012).

Organic extraction, which is also called phenol-chloroform extraction, is one of the most used and accepted DNA extraction procedures in the forensic community. In the organic extraction, the product after cell lysis using Proteinase K and sodium dodecylsulfate is centrifuged with the phenol-chloroform solution. The resulting aqueous solution containing DNA is subjected to further purification (Alonso, 2013). In solid-phase extractions particles like silica are used so that the DNA will bind to it. These techniques are rapid, inexpensive and mostly automated as compared to the organic extraction. The purification and the removal of inhibitors happens while the DNA is bound to the solid particle. The purified and isolated DNA is then eluted from the solid particle. The DNA extraction kits DNA IQ™ and QIAamp® are examples of solid-phase extractions (Butler, 2011). The Chelex extraction is done using chelating resins that can bind to the DNA. The chelating resin deactivates nucleases. Thus, it resists the possible chances of any cleavage of the DNA molecule. In this method the solution is exposed to boiling temperature after adding the chelating resin and the DNA is collected from the supernatant (Butler, 2009). Due to the exposure to temperature, the quality of DNA extracted using chelating resins is generally lower than the quality of DNA extracted using organic and solid-phase extractions (Alonso, 2013).

Apart from the extraction methods mentioned earlier, there are other techniques like differential extraction that are implemented (Alonso, 2013). As discussed previously, the direct amplification method, that acts as a bypass of DNA extraction, is also being widely used. Choosing an extraction procedure is a very crucial step in DNA profiling, so a good understanding of the advantages and limitations of different extraction methods is necessary.

1.9.2 DNA IQ™ system

The Promega DNA IQ™ system (DNA IQ™) is a commonly used standard protocol for the extraction of DNA. Many forensic facilities around the world rely on the DNA IQ™ system for the extraction of DNA for casework, paternity testing and Databases (*DNA IQ™ System*—

Small Sample Casework Protocol, 2021). The kit comes with a well-explained protocol. Another advantage of DNA IQ™ is that it is certified DNA free therefore the kit has minimal risk of residual DNA being present. Compared to conventional DNA extractions like the Organic extraction, the DNA IQ™ kit has no hazardous components. DNA IQ™, in general, has a considerably good success rate in subsequent DNA profiling (Bogas et al., 2014).

Like any other extraction procedures, DNA IQ™ also focuses on removing any PCR inhibitors and isolating the DNA for subsequent amplification and interpretation using STR profiling (*DNA IQ™ System-Small Sample Casework Protocol Instructions for Use of Products DC6700 and DC6701*, 2021). DNA IQ™, designed explicitly for forensic and paternity purposes, uses paramagnetic beads to separate DNA from the parent material. The kit contains lysis buffer, resin containing the paramagnetic beads, wash buffer and elution buffer. Before beginning the extraction procedure, a calculated amount of dithiothreitol is added to the lysis buffer.

Dithiothreitol (DTT), a reducing agent that is added to the lysis buffer, helps in enzyme stabilisation. DTT, containing the compound thiol, protects DNA from damage during sensitive procedures like extraction by keeping the proteins in a reduced state (Fjelstrup et al., 2017). The use of DTT at optimal concentrations allows DTT to act as a DNA protective radical scavenger. However, excessive concentrations of DTT can be detrimental to DNA by causing chromosomal damage, which hinders the subsequent amplification and STR profiling of DNA (Fjelstrup et al., 2017).

1.9.3 QIAamp® DNA Extraction kit by Qiagen

The QIAamp® method using a silica column as a base for the separation and isolation of DNA is proven to yield good DNA profiling results (Greenspoon et al., 1998). QIAamp® is proven to have recovered a trace amount of DNA by successfully separating out the inhibitors (Castella et al., 2006). The QIAamp® DNA extraction kit is based on the solid-phase DNA extraction principle (Butler, 2011). The procedure uses the QIAamp® silica gel membrane to protect the DNA while the inhibitors and impurities are passing through. The DNA gets attached to the QIAamp® silica gel membrane in the spin column and the impurities are removed with two washes (Castella et al., 2006). After discarding the impurities, the purified and isolated DNA is eluted using QIAamp® elution buffer. The ATL buffer mixed with proteinase K is used for the primary lysis of the sample. The main component of the ATL buffer, sodium dodecylsulfate, denatures the protein and acts as a detergent in cell lysis. The AL buffer which contains guanidium chloride, a chaotropic agent, along with absolute ethanol helps in further

lysis and encourages the binding of the DNA to the silica gel (*Buffer AL - (EN) - QIAGEN*, n.d.).

Like DNA IQ™, the components of the QIAamp® extraction kit are also not as hazardous as the components of the organic extraction. The extraction kit comes with a well explained protocol and is proven to be successful in the purification and isolation of nuclear, viral and parasitic DNA.

1.10 DNA Quantitation

After extracting DNA from a sample, the amount of DNA in the extract has to be estimated. The quantitation of the amount of DNA after the extraction is an essential and crucial step in DNA profiling (Alonso, 2013). The performance of further DNA multiplexes depends highly on the input concentration and volume of DNA. An excessive amount of DNA which is more than the optimal concentration required for the specific DNA multiplex reaction might act as a PCR inhibitor. Thereby resulting in allele drop-out and allelic artefacts.

DNA quantitation acts as a bridge between DNA extraction and DNA amplification. Real-time PCR is used to quantify the amount of DNA in the extracted solution using PCR. In other words, real-time PCR is used to estimate the concentration of DNA in an extracted solution using the PCR of specific targets (Maddocks & Jenkins, 2017). Real-time PCR amplifies and measures the quantity of DNA using specific markers in a single thermocycler (Hoy, 2013). The real-time PCR assays use fluorescent signals at each amplification cycle in a PCR reaction to see the exponential progression of the quantity from the baseline (Alonso, 2013). Quantifiler® Trio DNA Quantitation Kit by Applied Biosystems® (Thermo Fisher Scientific Waltham, MA, USA) is one of the methods widely used to quantify the concentration of DNA in forensic casework samples.

1.10.1 Quantifiler® Trio real-time PCR

Quantifiler® Trio DNA Quantification Kit (Quantifiler® Trio) by Applied Biosystems® (Thermo Fisher Scientific Waltham, MA, USA) uses the 7500 real-time PCR instrument and the HID Real-Time PCR Analysis Software v1.2 for analysis and data review. Quantifiler® Trio is a highly sensitive, efficient, specific and accurate real-time PCR method for quantitation (*Quantifiler® Trio DNA Quantification Kit*, n.d.). The Quantifiler® Trio kit contains four components. The four components are Quantifiler® THP PCR reaction mix, Quantifiler® Trio primer mix, Quantifiler® THP DNA dilution buffer and Quantifiler® THP DNA standard. The

Quantifiler® Trio primer mix contains primers, FAM™ dye-labelled probes, and the Internal PCR Control (IPC) template.

The four assays or targets are small autosomal (SA), large autosomal (LA), Y chromosome (Y) and the internal PCR control (IPC). The SA, LA and Y targets are all human specific loci found in multiple copies across various autosomal chromosomes and the Y chromosome. LA and SA are targets of two different lengths which can be used to measure the degradation index. Compared to the LA, SA targets have a relatively small amplicon size of 80bp which helps in estimating if any of the DNA present is degraded. The length of the LA is 214bp. The degradation index is calculated by dividing the quantitation value obtained for the LA by the value obtained for the SA. The Y chromosome target, with an amplicon size of 75bp, is used in the quantitation of male DNA. The IPC is a synthetic DNA template which is 130bp in length that acts as a control to make sure the quantitation process happened without any default, especially for the reliability of the negative control (*Quantifiler™ Trio DNA Quantification Kit*, n.d.).

The sample is run in a 96-well plate in the 7500 instrument. After the assay, the values of the slope, Y intercept and R^2 of the standard curve are used as a criterion to check if the procedure was a success. The output table will be generated with the quantity, quantity mean, cycle thresholds and mean for the cycle threshold for every target of each sample. *Figure 8* and *Figure 9* are the pictorial representations of the standard curve and the output table respectively. Additional information about analysis and results are detailed in section 2.14.

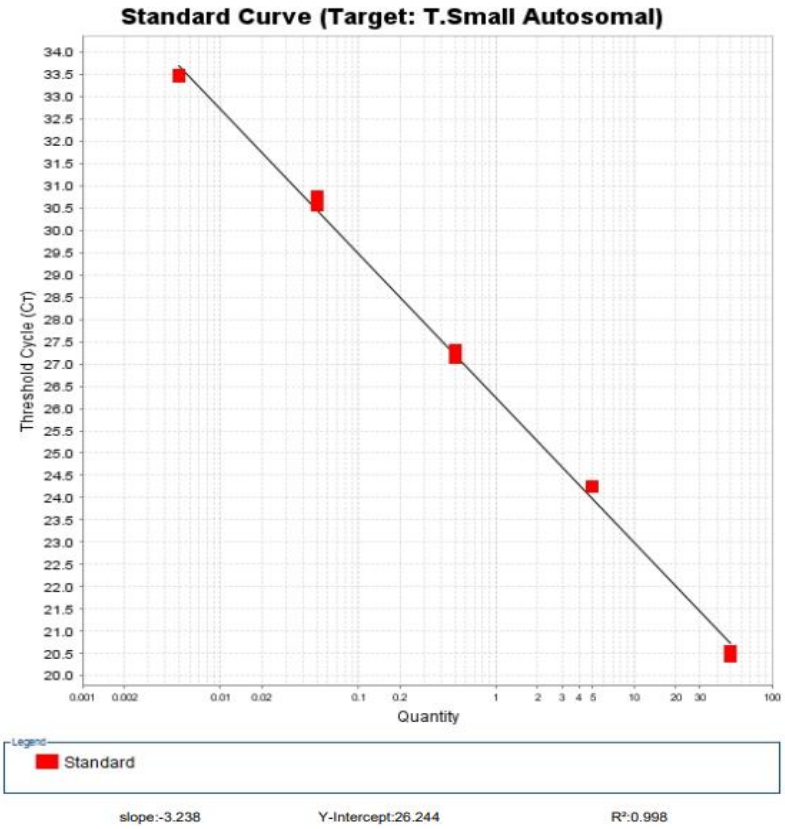

Figure 8: Standard curve graph

Results Table

Well	Sample	Target	Task	Quantity	Quantity (Mean)	Quantity (Std Dev)	CT	CT (Mean)	CT (Std Dev)
A1	Trio Standard 1	T.IPC	Unk				29.1623	29.08	0.12
A1	Trio Standard 1	T.Large Autosomal	Std	50			18.8565	18.88	0.03
A1	Trio Standard 1	T.Small Autosomal	Std	50			20.4367	20.49	0.08
A1	Trio Standard 1	T.Y	Std	50			20.3289	20.38	0.07
A2	Trio Standard 5	T.IPC	Unk				27.3327	27.37	0.06
A2	Trio Standard 5	T.Large Autosomal	Std	0.005			32.2656	32.49	0.32
A2	Trio Standard 5	T.Small Autosomal	Std	0.005			33.4306	33.47	0.06
A2	Trio Standard 5	T.Y	Std	0.005			33.6869	33.41	0.40
A3	IQ1E	T.IPC	Unk				27.5203	27.52	
A3	IQ1E	T.Large Autosomal	Unk				Undetermined		
A3	IQ1E	T.Small Autosomal	Unk	0.0001	0.00		39.0746	39.07	
A3	IQ1E	T.Y	Unk				Undetermined		
B1	Trio Standard 1	T.IPC	Unk				28.9921	29.08	0.12
B1	Trio Standard 1	T.Large Autosomal	Std	50			18.9051	18.88	0.03
B1	Trio Standard 1	T.Small Autosomal	Std	50			20.5501	20.49	0.08
B1	Trio Standard 1	T.Y	Std	50			20.4228	20.38	0.07
B2	Trio Standard 5	T.IPC	Unk				27.4149	27.37	0.06
B2	Trio Standard 5	T.Large Autosomal	Std	0.005			32.7114	32.49	0.32
B2	Trio Standard 5	T.Small Autosomal	Std	0.005			33.5093	33.47	0.06
B2	Trio Standard 5	T.Y	Std	0.005			33.1234	33.41	0.40
B3	IQ1F	T.IPC	Unk				27.2967	27.30	
B3	IQ1F	T.Large Autosomal	Unk				Undetermined		
B3	IQ1F	T.Small Autosomal	Unk	0.0001	0.00		39.2695	39.27	
B3	IQ1F	T.Y	Unk				Undetermined		
C1	Trio Standard 2	T.IPC	Unk				28.1138	28.15	0.05
C1	Trio Standard 2	T.Large Autosomal	Std	5			22.4651	22.48	0.02

Figure 9: Result table generated using HID Real-time PCR analysis software after Quantifiler® Trio

1.11 DNA Amplification

DNA amplification is the procedure of amplifying the short DNA fragments for the purpose of profiling. The amplification and replication of desired DNA strands is achieved using the polymerase chain reaction (PCR). The amplification involves rapid heating and cooling of the sample under different temperature conditions for a set number of cycles in a single thermocycler. The amplification makes multiple copies of the DNA sequences to get a complete profile (Butler, 2009). There are three steps involved in DNA amplification and they are denaturation, annealing and extension (Saferstein, 2001). The denaturation is the separation of double strands of DNA into single strands at high temperature. Annealing is the process involving multiple steps in which specific primers (short sequence of DNA) attach to the flanking region of the target strand. The process of extension happens in multiple cycles like annealing in which multiple copies of the targeted DNA is created (*PCR Basics | Thermo Fisher Scientific - NZ*, n.d.).

1.11.1 Amplification using Applied Biosystem™ AmpFLSTR™ Identifiler® Plus PCR Amplification Kit (Identifiler® Plus)

The Identifiler® Plus kit is a PCR amplification kit which enables the STR analysis of forensic samples. The amplicon size is up to 360 bp and uses AmpliTaq Gold DNA Polymerase for the reaction. Identifiler® Plus uses a five-dye identification system, 6-FAM™, NED™, PET®, VIC®, and LIZ® dyes, subsequently enabling high throughput. The Identifiler® Plus kit amplifies 15 STR loci, to give 30 alleles, and the Amelogenin locus in a single tube (*AmpFLSTR™ Identifiler® Plus PCR Amplification Kit*, n.d.). The amelogenin marker is the sex determining marker. The fifteen target STR loci of Identifiler® Plus are as follows:

D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA.

Among all the STR loci, D8S1179, D21S11, D7S820 and CSF1PO are labelled using a blue dye; D3S1358, TH01, D13S317, D16S539 and D2S1338 are labelled using a green dye; D19S433, vWA, TPOX and D18S51 are labelled using a yellow dye and D5S818 and FGA are labelled using a red dye.

Literature demonstrates the Identifiler® Plus kit as a validated protocol that has increased sensitivity and specificity compared to Identifiler® and the ability to overcome a lot of the inhibitors commonly encountered in forensic samples (Wang et al., 2012).

Low copy number (LCN) using Identifiler® Plus is a more sensitive version with increased cycle numbers (Forster et al., 2008). LCN uses the same Identifiler® Plus amplification kit and uses the same reaction volume. The only difference in LCN is the PCR cycle conditions. Standard PCR using Identifiler® Plus has 28 cycles and the number of cycles is increased to 34 for LCN. LCN is an ultra-sensitive amplification method where meaningful profiles can be generated when the amount of DNA is less than 100pg. The amplification using LCN is a more sensitive technique than the standard 28 cycle amplification and is subject to a number of stochastic effects which can result in allelic drop out and heterozygous imbalance. Hence in the majority of cases, the standard PCR cycle condition of 28 cycles using Identifiler® Plus is preferred (Budowle et al., 2009; Forster et al., 2008).

1.11.2Amplification using Applied Biosystem™ AmpFLSTR™ Minifiler® PCR Amplification Kit (Minifiler®)

AmpFLSTR™ Minifiler® PCR amplification kit is a sensitive protocol for the amplification of DNA that might be degraded or inhibited. Like Identifiler® Plus, Minifiler® also uses a five-dye system using 6-FAM™, NED™, PET®, VIC®, and LIZ® dyes (*AmpFLSTR™ MiniFiler® PCR Amplification Kit*, n.d.). The Minifiler® kit targets eight STR markers and the amelogenin marker all in a single tube. The eight target STR loci of Minifiler® are D21S11, D7S820, CSF1PO, D13S317, DI6S539, D2S1338, D18S51 and FGA. Literature indicates that the Minifiler® kit has given better profiles when other multiplexes have given zero or partial profiles (Mulero et al., 2008).

1.12 Capillary Electrophoresis

Capillary electrophoresis is a method used to separate the DNA fragments after amplification based on the fragment size. Capillary electrophoresis is a completely automated technique that enables high-throughput DNA fragment analysis. The DNA molecules, which are negatively charged, are attracted to a positively charged electrode (Shewale et al., 2012).

The amplified product, that is the DNA fragment, is injected into the capillaries of the Genetic Analyser using an electrokinetic injection of a desirable voltage. The injection parameters including the voltage and time are decided based on the amplification kit used and the PCR cycle conditions. The DNA fragments of different sizes that are labelled with different dyes emit fluorescence when they pass through a laser beam. The emitted fluorescent signals are converted into data that can be analysed.

The capillary electrophoresis unit, 3500xL Genetic Analysers by Applied Biosystems distributed by Thermo Fisher is used at ESR to perform the separation. The analysis can be performed using software such as GeneMapper® ID-X v1.4 software. The alleles will be demonstrated as peaks in an electropherogram (EPG) with peak heights in relative fluorescence unit (rfu).

1.13 Cartridge Cases and Touch DNA – A Brief Review

Cartridges cases are one of the sources from which touch DNA can be recovered (Montpetit, 2020). A study conducted by Horsman-Hall et al (2009) investigated the efficiency of the Minifiler® kit to obtain STR profiles from firearms and fired cases (Horsman-Hall et al., 2009). The presence of inhibitors, samples from more than a participant and the effect of the process of firing were the areas of interest. The samples were extracted using DNA IQ™. The study concluded that Minifiler® did give better results compared with Identifiler® and PowerPlex®, but the advancement is not enough to aid an investigation (Horsman-Hall et al., 2009).

Dieltjes et al (2011) introduced the use of QIAamp® DNA Mini kit coupled with a pre-extraction soaking method as a technique to collect DNA from ammunition for further profiling. The study involved the soaking of the ammunition in ATL buffer for 30 minutes to collect DNA from cartridge cases (Dieltjes et al., 2011). The success rate of using this method is given as 26.5% per case and an average success rate of 6.9%n is given per cartridge, bullet and cartridge case. The study addresses a challenge in using this method as the ATL buffer has an oxidation effect on the cartridge cases whilst they are directly soaked in it. This can impact on downstream analysis of striation marks on the cartridge case. They advise to wipe the cartridge case clean immediately after 30 minutes of soaking in order to protect other physical evidence such as striation marks on the surface of the cartridge case (Dieltjes et al., 2011).

Polley et al (2006) studied the success rate of getting DNA from firearms and ammunition; however, they raise concerns about the secondary and tertiary transfers of DNA (Polley et al., 2006). The DNA profiles generated from the firearms and ammunition, according to Polley et al., need not belong to the shooter, while the concerns of secondary and tertiary transfers exist. They raise questions on the identification of the shooter based on the DNA and the evidence of direct contact with the DNA donor and the surface. The fired cartridge cases would likely not have a considerable amount of DNA. The effects of the quality of DNA could lead to limitations in recovering DNA from cartridge cases since the cartridge is exposed to high heat, pressure, and other substrates during the firing process (Polley et al., 2006) (Hefetz et al., 2019).

A review published by Montpetit (2020), undertook a literature search on relevant literature available to obtain DNA from ammunition. The literature underlines the progress and limitations in the collection, purification and amplification of DNA (Montpetit, 2020). According to Montpetit, no one technique can be chosen to have a high success rate in DNA profiling from cartridge cases as there are multiple research projects showing successful DNA profiling using different techniques. The significant argument raised by the author is the difference in the definition of success making it difficult to choose one technique as the best. The collection methods including swabbing and soaking have proven to have successful DNA profiling. The literature also indicated the successful use of DNA IQ™ and QIAamp® as extraction techniques (Dieltjes et al., 2011; Martin et al., 2018; Montpetit, 2020). The review points out that the loss of DNA during extraction and purification steps is one of the challenges that needs to be addressed. Approximately 76% of touch DNA is lost during the extraction and purification procedures therefore the author promotes the evolving methods like direct PCR which bypass the extraction and purification steps (Montpetit, 2020).

The amount of touch DNA present on a surface is usually much lower than the amount of DNA recovered from body fluids and other tissues. Performing multiple steps before amplification might diminish the chances of recovering a sufficient amount of DNA (Moore et al., 2020). The chances of DNA getting lost at each stage cannot be avoided. The study conducted by Moore et al (2020) has introduced a semi-automatic method to recover DNA from fired and unfired ammunition (Moore et al., 2020). The study compared the swabbing technique with direct lysis and found that direct lysis yielded more DNA profiles than swabbing. The direct lysis method avoids the swabbing and soaking procedures by directly exposing the target object to the lysis buffer.

The history of having poor retrieval and profiling of DNA from numerous metal surfaces of forensic interest is a challenge put forward by the literature (Bonsu et al., 2020). Bonsu et al, undertook a literature review and summarised the reasons for the poor DNA profiling results from metal surfaces of forensic interest. One of the important reasons is the charge of the metal and the DNA. DNA is a negatively charged molecule and the metals and alloys used for the manufacture of ammunition mostly belong to the transitional group in the periodic table. The literature indicates that the transitional metals and alloys are proven to have an altering effect on the double helix structure of DNA which makes it difficult to amplify desirable strands (Bonsu et al., 2020).

Cartridge cases are rarely subjected to DNA extraction in most jurisdictions due to the history of very low success rates of obtaining DNA profiles from them. Collecting DNA from ammunition is an active area of research, and studies are still going on to maximise the chances of getting DNA profiling results from ammunition (Elisha Prasad et al., 2022). Studies were conducted by spiking the cartridge cases with saliva to increase the possibility of getting DNA profiling results from the cartridge case. The results from such studies cannot account for an actual crime scene scenario where the cartridge cases are likely to only have touch DNA. Studies show different methods to collect DNA from ammunition. Swabbing is the traditional method used to retrieve DNA, and the other techniques include tape-lifting, soaking, direct lysis and direct PCR as detailed in the previous sections (Dieltjes et al., 2011; Moore et al., 2020; Elisha Prasad et al., 2022).

Fingermarks exhibit different traits on different surfaces. Likewise, the persistence of DNA on objects of interest is also dependent on the surface type as well as the nature of contact and other environmental factors. The persistence of fingermarks need not to be considered as an indicator for the persistence of DNA. Hence the visualisation of DNA will be an advantage to know if any DNA is present on the fingermarks and to know if it is worthy to subject the sample for further DNA profiling.

Templeton et al studied the effect of dactyloscopic powders, white powder, silver aluminium, and black magnetic fingerprint development powder, on further DNA profiling using direct amplification across 160 samples (Templeton et al., 2017). 61% of the samples analysed, that is 98 out of 160, generated up-loadable DNA profiles according to the standards followed by the jurisdiction of their research. The direct PCR in this study was performed using AmpFLSTR™ NGM SElect^{TM} PCR amplification kit and concluded that the dactyloscopic powders investigated had no detrimental effect on profiling using direct PCR (Templeton et al., 2017).

The evidentiary value of latent fingermarks is high. Also, the success rate of enhancement of latent fingermarks is more than compared to the success rate of getting a complete DNA profile from fingermarks. Direct PCR from cartridge cases is a strong area of research in the forensic world. Many researchers advice the use of direct PCR to aid criminal investigations. The study used four different fingerprint enhancement powders including silver, white, black and magnetic black. The literature shows the efficiency of direct PCR in detecting alleles even after the use of dactyloscopic powders. In other words, the success rate of DNA profiling, according

to Templeton et al, is similar before and after the use of dactyloscopic powder (Templeton et al., 2017).

Thanakiatkrai and Rerkamnuaychoke (2019) investigated the efficiency of direct PCR in profiling DNA from fired and unfired cartridge cases. They used the Identifiler® Plus amplification kit to achieve their objective and found that direct PCR gave better allelic percentage than samples that were extracted. However, they state that there is not a statistically significant difference between the results (Thanakiatkrai & Rerkamnuaychoke, 2019). They also state the size and calibre of the ammunition had little effect on the results obtained which is also supported by other studies (Thanakiatkrai & Rerkamnuaychoke, 2019) (Prasad et al., 2020).

1.14 Experimental Design

The admissibility and reliability of the outcome of an experiment highly depends on the structure and design of an experiment (Weir, 2013). An experiment should be designed in such a way that the procedural treatment should be well defined to achieve the objectives and aims. Ideally 15-20 participants would be needed for the study however given time and cost factors the number of participants was finalised to four. This research is structured in such a way that it covers up the statistical blocks caused due to multiple biological and technical biases.

Technical bias results in consistent errors in the analysis which makes it difficult to reproduce the results. Technical bias can be caused due to artefacts of the equipment, reagents used and the defects in the protocol followed (Hunter, 2021). The calibration of the equipment used, having a validated protocol, quality assurance of the chemicals used and the use of negative and positive controls in the experiments will help to overcome this bias. This study is structured based on a completely randomised model to cover up biological bias. The samples from each participant were randomised after collection. These randomised samples were used for further experiments. Randomisation of sample reduces any chances of unintentional bias and ensures a fair treatment to every sample and replicates used for the study.

An ideal interpretation of the results is not possible by analysing just one replicate from a participant. Since the study uses DNA, there are multiple factors affecting the DNA shedding of an individual. Three replicates were randomly assigned per participant to cover up the variations in shedding of DNA from individual to individual. However, the statistical interpretation might not be as robust given the limited number of participants, and it resulted in a more qualitative interpretation rather than a quantitative interpretation.

1.15 Study Objectives

The Institute of Environmental Science and Research (ESR), a crown research institute that has expertise in multiple disciplines of forensic science, including crime scene investigation, firearm examination and forensic biology, have conducted many studies in the past to aid criminal investigation and evidence processing. The present study was outlined after an immense discussion with Jennifer Howarth (Senior Scientist, Forensic Biology, ESR) and Leah Tottey (Senior Scientist, Physical Evidence, ESR) as an effort to improve the possibility of collecting DNA evidence from cartridge cases.

This research is based on four objectives. The first two objectives are based on visualising touch DNA on cartridge cases using diamond dye. The third and fourth objectives are based on the extraction and profiling of touch DNA from cartridge cases.

Objective 1: To study the effect of visualisation of DNA using diamond dye on cartridge cases and the impact this has on further enhancement of fingermarks and vice versa.

This objective will be working with the New Zealand Police Auckland Fingerprint team to ensure that the diamond dye and subsequent sampling procedures carried out on ammunition do not have a detrimental effect on fingerprint enhancement techniques. Also, to investigate if the fingerprint enhancement techniques performed on ammunition affect the further visualisation of DNA using diamond dye.

Objective 2: To evaluate the effect of the process of firing on visualisation of DNA using diamond dye.

This objective will be investigated by spraying diamond dye on cartridge cases before and after firing. This objective includes comparing results between fired cartridge cases and unfired cartridges to study the impact of firing on the visualisation of DNA using diamond dye.

Objective 3: To evaluate alternative extraction methods to improve DNA profiling success from ammunition.

The aim of this objective is to investigate different extraction techniques in order to attain a higher success rate in profiling touch DNA from ammunition. The aim will be achieved by investigating the following extraction methods and direct PCR.

- DNA IQ™
- QIAamp® DNA Mini kit coupled with a pre-extraction soaking method

- QIAamp® DNA Mini kit coupled with Direct lysis.

DNA IQ™ is the kit currently used by ESR hence it will act as a baseline comparison kit. The QIAamp® DNA Mini kit coupled with a pre-extraction soaking method (Dieltjes et al., 2011) and QIAamp® DNA Mini kit coupled with Direct lysis (Moore et al., 2020) are the techniques demonstrated to have an improved DNA profiling ability from cartridge cases. The results from using the DNA IQ™ kit will be compared with the results obtained from the pre-extraction soaking and direct lysis coupled with the QIAamp® DNA mini kit.

Objective 4: To evaluate alternative DNA multiplexes.

This objective aims to study two DNA multiplexes, Identifiler® Plus and Minifiler®. DNA extracted using all of the methods from objective three will be amplified using Identifiler® Plus 28 cycles, Identifiler® Plus LCN 34 cycles and Minifiler® to investigate which amplification technique gives better results with each extraction. These will be investigated for Direct PCR of targeted areas as well.

CHAPTER 2

METHODOLOGY

PART I

Visualising Touch DNA on Cartridge Cases Using Diamond Dye

2.1 Introduction

The presence of fingermarks and the recovery of touch DNA are correlated, as fingermarks indicate the possible existence of touch DNA (Kanokwongnuwut et al., 2019). The first part of this research is focused on using diamond dye to locate touch DNA on cartridge cases. Fingermarks present on the cartridge case were enhanced by a Fingerprint Officer in the NZ Police before and after the application of diamond dye to determine whether or not diamond dye will have an effect on finger mark enhancement techniques. The impact of the enhancement technique and the firing process on the visualisation of touch DNA on cartridge cases was also studied.

2.2 Laboratory and Safety Considerations

Good care was taken with the collection and handling of the ammunition as live ammunition was used for the study. The firing was done at a designated firing range in the presence of an experienced firearm expert and a range officer. The firing range was set up following all of the safety protocols for controlled firing. Masks, gloves, safety glasses and ear protection were used while firing to ensure personal safety and to avoid possible chances of contamination.

The preparation and application of diamond dye was done in a Biosafety cabinet. The inside of the safety cabinet was cleaned using 1% virkon solution followed by 70% ethanol prior to use. All of the equipment used was cleaned before and after use with 70% ethanol. Proper personal protective equipment (PPE), including mask, gloves, safety goggles, hairnet and protective gown were used to ensure safety and to prevent contamination. Safety and disposal instructions were followed as per the Material Safety Data Sheet (MSDS) of all the chemicals used.

2.3 Preparation of Cartridge Cases

The study used cartridge cases of calibre .22 Long Rifle and .223 Remington. For the remainder of this document, these calibres will be referred to as 0.22 and 0.223 respectively. *Figure 10* is a pictorial representation of fired and unfired cartridge cases and cartridges of both the calibres.

Figure 10: Fired cartridge case and unfired cartridge of calibre 0.22 (left), unfired cartridge and fired cartridge case of calibre 0.223 (right)

2.3.1 Unfired cartridges

According to the study plan, unfired cartridges were required to study the effect of diamond dye on fingerprint enhancement. The reason behind the use of unfired cartridges, was to make sure that the firing process, which involves heat and pressure as well as combustion products, could be a controlled variable. However, due to safety reasons for handling and transporting live ammunition, cleaned fired cartridge cases were used as an alternative for unfired cartridge cases. The fired cartridges were cleaned using 70% ethanol to remove any residual DNA that may be present as well any combustion products and other debris that had been transferred from the firearm during the firing process. After cleaning, fingermarks were deposited, as detailed in section 2.4. Unfired live cartridges were used only in one experiment detailed later.

2.3.2 Fired cartridge cases

Fired cartridge cases were used to study the impact of firing on visualising DNA using diamond dye. The chamber of the firearm and the cartridges used were cleaned using 70% ethanol beforehand to minimise contamination.

Fingermarks were deposited onto 0.22 and 0.223 cartridges (show in Figure 10), as detailed in section 2.4. The cartridges were loaded into a firearm and fired. The two firearms used are shown in *Figures 11* & *12*.

Figure 11: .22 Long Rifle calibre - Ruger-brand, Model 10-22, semi-automatic rifle

Figure 12: .223 Remington calibre – Remington-brand, Model Seven, bolt-action rifle

2.4 Deposition of Fingermarks

Hands were washed using a foam handwash and cold water for 20 seconds. After 45 minutes, single fingermarks were deposited on both unfired and fired cartridge cases with medium pressure for 15 seconds. The fingermarks were deposited only from the thumb, index finger and middle finger of both hands. Single fingermarks from a finger were deposited on the surface of the cartridge case. The process was repeated until all of the cartridges and cartridge cases required for the study were covered. Activities during the 45-minute time interval between the handwash and fingermark deposition were noted.

2.5 Preparation of Diamond Dye

Diamond dye is a nucleic acid binding dye that fluoresces once bound to DNA. This property of the diamond dye allows for the visualisation of DNA present on surfaces. The diamond dye was purchased from Promega at 10,000x concentration and was diluted to 20x before being applied as a spray using a spray device (Section 2.6). Materials required to dilute the diamond dye were 3M™Novec™ Engineered Fluid 7100 (HFE 7100) and absolute ethanol. 100% HFE

7100 was diluted to 90% HFE 7100 in absolute ethanol. This was then used to dilute 10,000x diamond dye to 20x diamond dye. The prepared diamond dye solution was sprayed onto the cartridge cases to visualise any touch DNA present, as detailed in the section below

2.6 Application of Diamond Dye

Diamond dye was sprayed onto the cartridge cases and cartridges using an air brush-spray gun with a compressor (*Figure 13*), a continuous spraying device (Young & Linacre, 2020). The cartridge cases were held with tweezers making sure that the areas of potential DNA deposition were not affected. Diamond dye was sprayed at a distance of approximately 8-10cm from the sample whilst rotating it slowly using the tweezers to ensure a uniform coverage.

Figure 13: Air brush with compressor used as the spraying device

2.7 Visualisation

2.7.1 Dino-lite microscope

The Dino-lite microscope at 55x magnification was used to visualise touch DNA present, and the Dino-Capture software was used to control the microscope and capture the observations. With the Dino-lite microscope, diamond dye present on the surface of the cartridges and cartridge cases, that was bound to DNA, was excited using blue LED light at 510 nm. The Dino-lite was mounted to a stand using a clamp and placed approximately 1-2 cm away from the cartridge case. In order to not get background fluorescence from the lab bench, a black piece of cardboard was placed underneath the set-up. The brightness and contrast were

controlled using the Dino-Capture software. The images were saved for further analysis as outlined in Section 2.8.

2.7.2 Polilight

The cartridge cases sprayed with diamond dye were observed under a Polilight® to visualise any touch DNA. A DSLR camera, Canon EOS 550D, was mounted to a stand next to the Polilight® in a dark chamber. The cartridges were stabilised using a folded piece of paper and placed into the dark chamber (*Figure 14*). The cartridge cases were then excited with the Polilight® at 505 nm fine-tune 3. The resulting fluorescence was captured with the camera controlled by the computer software "DSLR Remote Pro for windows".

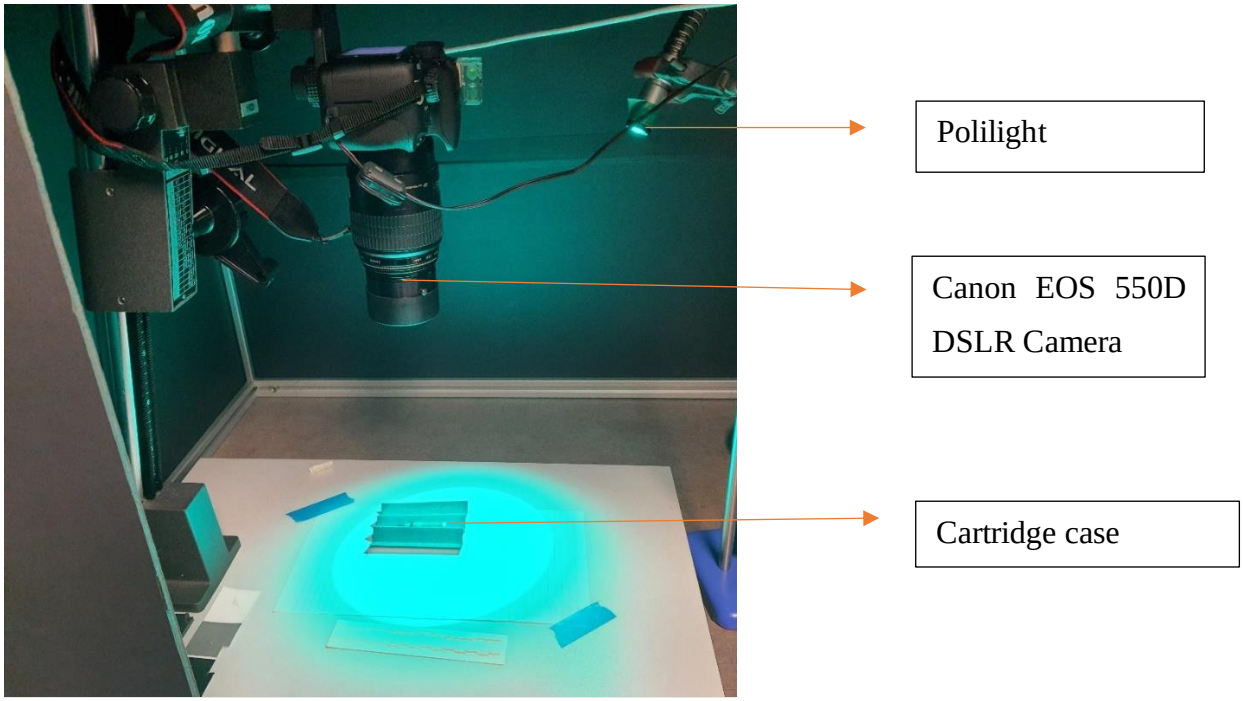

Figure 14: DSLR-Polilight set-up

The parameters used in this set-up were the following:

- Camera Model: Canon EOS 550D
- Lens used: 100 mm macro lens
- Distance of the camera from the surface: 25 cm
- Autofocus mode (AF mode): Manual Focus
- Shutter speed: 30"
- Aperture: 8.0
- Exposure: M
- Picture Style: Standard

2.8 Counting Fluorescence

Fluorescence on the cartridge case can indicate the presence of touch DNA. The amount of fluorescence was counted using the software ImageJ.

ImageJ is a software programme that enables the digital counting of the amount of fluorescence in images. The images were processed using the software downloaded onto the Windows operating system. Images were exported to the software, one at a time, and the number of fluorescent dots present were calculated.

The amount of fluorescence was calculated using ImageJ by following the steps below:

1. Opened the ImageJ software (*Figure 15*)

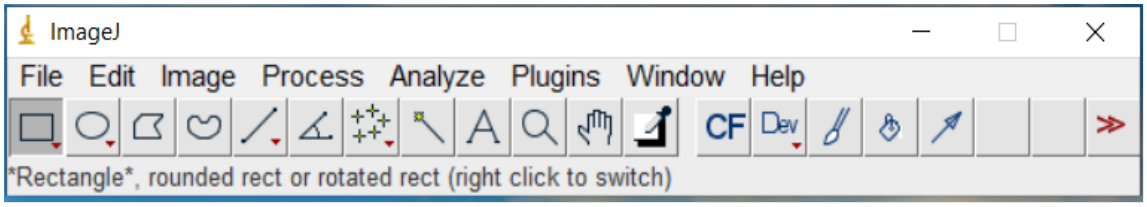

Figure 15: ImageJ software

2. Opened the image that was saved using the steps detailed in the section 2.7.

   File ⟶ Open ⟶ Selected the image

3. The image type was set (*Figure 16*)

   Image ⟶ Type ⟶ 8-bits

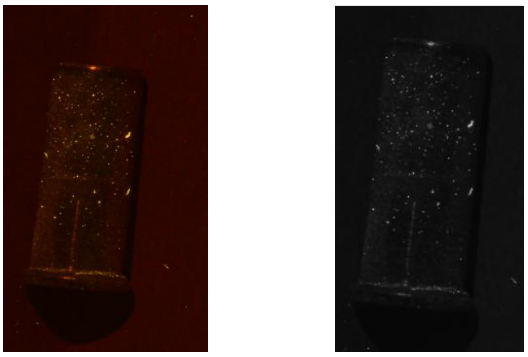

Figure 16: Normal image (left), converted to 8 bits (right)

4. The threshold was set as following:

   Image ⟶ Adjust ⟶ threshold ⟶ Set

   Lower threshold: 100/140

   Upper threshold: 255

The appropriate threshold was determined after manually comparing the results obtained from different thresholds.

5. Selected the cartridge case to avoid the interference of the background and analysed.

   Analyse ⟶ Analyze Particles (*Figure 17*)

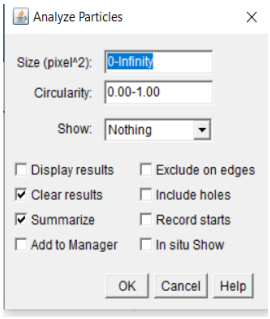

Figure 17: ImageJ particle analysis function

6. The output generated by the software was saved to a spreadsheet and that data was used for further interpretations (*Figure 18*)

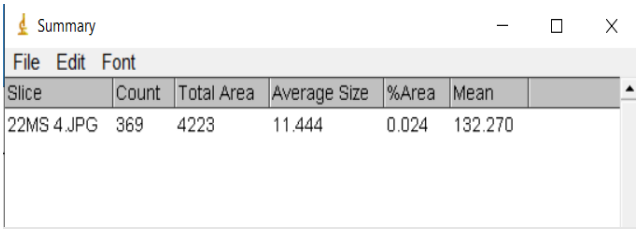

Figure 18: ImageJ analysis summary

2.9 Experiments

The study was divided into three different experiments based on three different objectives. The overall objective was to study the use of diamond dye to locate touch DNA on ammunition. The first and second objectives focused on working with the NZ Police Auckland fingerprints team to ensure that the diamond dye and subsequent enhancement procedures carried out on the cartridge case don't have a detrimental effect on their evidence and vice versa. The first objective was investigated using two experiments. The experiment one was to study the impact of visualisation of DNA using diamond dye on subsequent fingerprint enhancement techniques on cartridge cases. The second experiment was to study the effect of fingerprint enhancement techniques on the visualisation of DNA using diamond dye on cartridge cases. The second objective was to study the effects of the firing process on the presence of touch DNA using

diamond dye on cartridge cases. The study plan for objective 1 and 2 is illustrated in *Figure 19.*

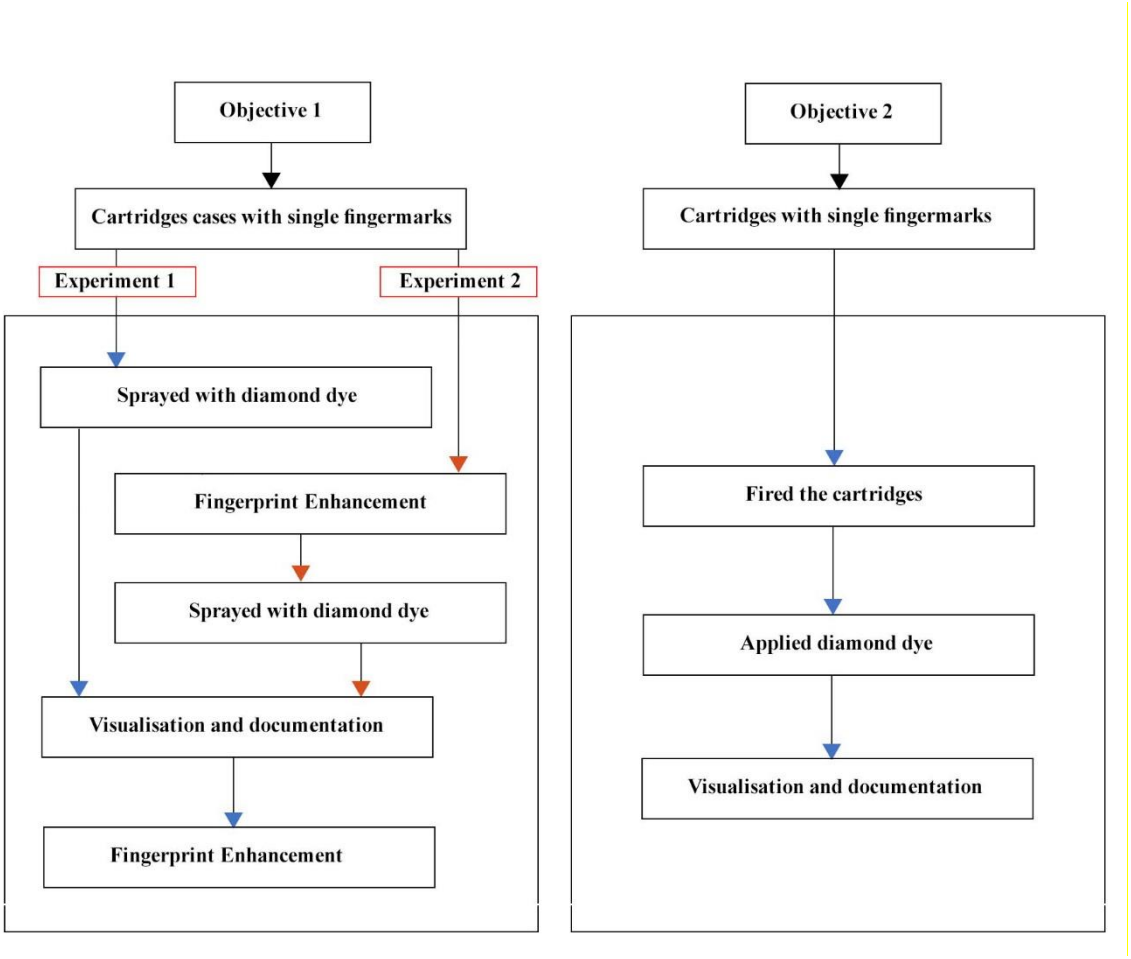

Figure 19: Study plan for objectives 1 and 2

2.9.1 Method development

Single fingermarks were deposited onto the cartridge cases as detailed in section 2.4. Diamond dye was prepared following the procedure described in section 2.5. The prepared diamond dye solution was sprayed onto the cartridge cases using the applicator as detailed in section 2.6. The cartridge cases were then observed under the Dino-lite microscope as described in section 2.7.1. The same procedure was repeated multiple times but with a change in the distance of application. This was done to find out what distance gave the best results. The counting of fluorescence was done manually using the grid method and also automatically using the ImageJ Software. The method development experiments helped in determining the best distance of application and counting of fluorescence.

2.9.2 *Objective 1: Visualisation of touch DNA on cartridge cases and fingerprint enhancement and vice versa*

2.9.2.1 Experiment 1

To study the effect of visualisation of touch DNA using diamond dye with subsequent enhancement of fingermarks on cartridge cases:

- Single fingermarks were deposited on five replicates each of cartridge cases of calibre 0.22 and 0.223, as detailed in section 2.4.
- Diamond dye was sprayed onto each cartridge case using the air brush-spray gun with compressor at ESR as detailed in section 2.6.
- An unfired cartridge with no fingermarks was sprayed with diamond dye and used as a negative control.
- The cartridge cases were then observed using the Polilight microscope as detailed in section 2.7.2 to visualise any cellular material present.
- The fluorescence was documented using a DSLR camera, as detailed in section 2.7.2.
- The amount of fluorescence spots was counted using the ImageJ software.
- All the replicates including the negative control were forwarded to Auckland Fingerprints to perform fingerprint enhancement techniques. The same procedure was followed for both calibres.
- After performing necessary enhancement procedures, the cartridge cases were returned to ESR.

2.9.2.2 Experiment 2

To study the effect of enhancement of fingermarks on the visualisation of touch DNA using diamond dye on cartridge cases:

- Single fingermarks were deposited on five replicates each of cartridge cases of calibre 0.22 and 0.223, as detailed in section 2.4.
- All five replicates were forwarded to Auckland Fingerprints to perform fingerprint enhancement techniques. The same procedures were followed for both of the calibres.
- After performing necessary enhancement procedures, the cartridge cases were returned to ESR.
- Diamond dye was sprayed onto each cartridge case using the air brush-spray gun with compressor at ESR following the procedure detailed in section 2.6.

- An unfired cartridge, with no fingermarks, was sprayed with diamond dye and used as a negative control.
- The cartridge cases were then observed using the Polilight microscope as detailed in section 2.7.2 to visualise any cellular material present.
- The fluorescence was documented using a DSLR camera, as detailed in section 2.7.2.
- The amount of fluorescence was counted using the ImageJ software.

2.9.3 Objective 2: The effect of firing on the presence of touch DNA on cartridge cases

To study the effects of the firing process on the presence of touch DNA using diamond dye on cartridge cases:

- Fingerprints were deposited as detailed in section 2.3 on six replicates of cartridges of calibre 0.22 and 0.223.
- Of the six replicates, three were fired, and the remaining three were used for comparison as controls.
- A cleaned and unfired cartridge having no fingermarks was sprayed with diamond dye as a negative control.
- Diamond dye was sprayed onto the cartridges and cartridge cases using the Airbrush-spray gun with compressor at ESR, as detailed in section 2.6.
- The cartridges and cartridge cases were then observed using the Polilight microscope as detailed in section 2.7.2 to visualise any cellular material present.
- The fluorescence was documented using a DSLR camera, as detailed in section 2.7.2.
- The amount of fluorescence was counted using ImageJ software.
- The amount of fluorescence on the fired cartridge cases and unfired cartridges, used as controls, were compared, and observations were noted.

PART II

Investigating and Comparing Different Extraction Methods for Touch DNA from Cartridge Cases

2.10 Introduction

The third and fourth objectives of the study focused on the extraction and profiling of touch DNA from cartridge cases. This part of the study used fingermarks from four participants to achieve the objectives. Touch DNA from cartridge cases were extracted using different techniques as detailed in section 2.13. The quantitation was done using Quantifiler® Trio Real-time PCR Quantitation Kit to quantify the amount of DNA present, as described in section 2.14. The samples were amplified using the techniques detailed in section 2.15. The amplified samples were processed using capillary electrophoresis and analysed using GeneMapper ID-X v1.4 software, as described in sections 2.16 and 2.17, respectively.

2.11 Laboratory and Safety

The cartridge cases were fired in a designated firing range in the presence of an experienced firearm expert and a range officer. The firing range was set up following all of the safety protocols for controlled firing. Proper personal protective equipment was used to ensure the safety of the sample collection.

All of the procedures and preparations were done in a Biosafety cabinet to ensure personal safety and to avoid any possible contaminations. The inside of the safety cabinet was cleaned using 1% virkon solution followed by 70% ethanol prior to use. After use, the cabinet was cleaned using 70% ethanol. The instruments were used in the designated instrument room and were all calibrated. The collection tubes and sample tubes were autoclaved to ensure that they were DNA free.

2.12 Sample preparation

2.12.1 Collection and cleaning

The cartridge cases were collected in a paper envelope from the firing range. The collected samples were taken to the laboratory and cleaned thoroughly using 70% ethanol to remove any gunshot residue and any persisting DNA. The cleaned cartridge cases were packed into paper envelopes and were added to the participant packs.

2.12.2 Participant pack

The components of the participant packs were:

1. Participant Information Sheet (PIS): A document containing the details of the study and the information regarding participant rights (Appendix I).

2. Participant Consent Form (CF): A document that was signed by the participant giving consent to take part in the study after understanding the plan, conditions, and participant rights (Appendix II).
3. Deposition Instruction sheet: The document that contained instructions for the participants on how to deposit their fingermarks on the cartridge cases provided (Appendix III).
4. A questionnaire: A questionnaire to note down the activities undertaken and time during sample deposition (Appendix IV).
5. Four sets of cartridge cases: Thirty cartridge cases (fifteen cartridge cases each of calibre 0.22 and 0.223) were given to each participant. The thirty cartridge cases were divided into four sets.
6. A buccal swab: A sterile swab to collect a buccal sample from the participant that was used as a reference sample.

2.12.3 Deposition of fingermarks

A deposition instruction sheet was attached to the participant pack to give proper instructions on sample deposition. Participants had four sets of cartridge cases as detailed in the previous sub-section. Participants were asked to wash their hands using a regular handwash procedure and to then wait for 45 minutes. After 45 minutes, they were asked to hold the cartridge cases with their fingers, one at a time, for 15 seconds with medium pressure. After depositing the fingermarks, the participants were asked to place the cartridge cases individually into the paper envelopes provided (all the cartridge cases were placed in separate envelopes). Participants were instructed to wash their hands using a regular handwash procedure and wait for 45 minutes before repeating the procedure for the next set.

2.13 DNA Extraction

The third objective of the study was to investigate three different techniques to extract touch DNA from cartridge cases. The extraction techniques investigated were the following:

1. DNA IQ™
2. Direct Lysis
3. Pre-extraction soaking coupled with QIAamp®

2.13.1 DNA IQ™

Three replicates each of both of the calibres (0.22 and 0.223) were used per participant for this extraction. A buccal swab from a known donor was used as an extraction positive. The

reference samples (buccal swab) from all four participants were extracted using the DNA IQ™ extraction kit following the extraction procedure detailed below.

a. Components of DNA IQ™ Kit by Promega

- Resin
- Lysis Buffer
- Wash Buffer
- Elution Buffer

b. Swabbing

- Wet and dry swabbing was used to collect any DNA present
- A sterile swab was moistened using sterile water, and the surface of the cartridge case was wiped using that.
- The swab head was removed from the shaft and put into a 1.5ml tube.
- The surface of the cartridge case was swabbed again using a dry sterile swab.
- The swab head was cut and added to the same 1.5ml tube that already had the wet swab head.
- The procedure was repeated for all of the cartridge cases that were extracted using DNA IQ™.

c. Extraction procedure

- The samples were placed on a tube rack in a labelled 1.5ml tube. Extraction positive and negative controls were added to every extraction.
- An appropriate volume of prepared lysis buffer was added to the sample tube. The amount of lysis buffer for each sample was calculated using the information in *Table 1*.
- The tube containing the sample and lysis buffer was vortexed to mix and then incubated at 70ºC for 30 minutes.
- After incubation, the swabs and all of the liquid were transferred into a spin basket that was attached to a newly labelled 1.5ml tube. The original tube was retained.
- It was centrifuged at maximum speed for 2 minutes in an Eppendorf 5804 R.
- The spin basket containing the swabs was removed and placed back into the retained original tube. These spin baskets containing the swabs were stored in the freezer.
- The stock resin bottle was vortexed to make sure that the resin was suspended in the solution. 7µL of resin was added to the tube containing the sample solution and vortexed for 3 seconds.

- The sample solution was then incubated at room temperature for 5 minutes. It was vortexed for 3 seconds once every minute during this five-minute incubation.
- After the five minutes incubation, it was vortexed briefly and was placed onto a magnetic stand.
- The resin pellet adhered to the side of the tube that was in contact with the magnet. The remaining solution (lysis discard solution) other than the resin pellet was pipetted out and discarded without disturbing the resin pellet.
- 100µL of prepared Lysis buffer was added to the resin pellet.
- The sample tube was removed from the magnetic stand and vortexed for 2 seconds. It was then placed back onto the magnetic stand.
- Again, the lysis discard solution was discarded without disturbing the resin pellet.
- 100µL of 1x Wash buffer was added. The sample tube was removed from the magnetic stand and vortexed for 2 seconds. It was then placed back onto the magnetic stand. The solution was discarded without disturbing the resin pellet. This step was repeated two times.
- After the third wash, the sample was air dried for 5-10 minutes with the lid open. 25µL of Elution buffer was added to each tube, and it was vortexed briefly. It was then incubated at 65ºC for 5 minutes. After incubation, it was vortexed for 2 seconds and pulse centrifuged. The sample tube was placed onto the magnetic stand immediately after the pulse centrifuge when the solution was still warm. The solution was transferred into a freshly labelled tube. This step was repeated.
- After the second elution, it was made sure that the final elution volume was greater than 45ul for every sample.
- The extract was stored at 4ºC for up to 5 days and at -20ºC for long term storage.

Number of samples	**Lysis buffer (µL)**	**DTT**
Control or no substrate = (A)	150 + 100 = 250 x A = (E)	(I/100) x 2.5µL = _____
Portion of swab size = (B)	150 + 100 = 250 x B = (F)	
1 swab size = (C)	250 + 100 = 350 x C = (G)	
2 swab size = (D)	400 + 100 = 500 x D = (H)	
	Total=(E)+(F)+(G)+(H) = (I)	

Table 1: Lysis buffer calculation for DNA IQ™ (Appropriate amount of lysis buffer was taken and was combined with the calculated amount of DTT)

Volume of other reagents used is given in the table below:

Reagent	Volume per sample (µL)
Resin	7
Wash buffer	300
Elution buffer	50

Table 2: Volume of other reagents required for DNA IQ™

2.13.2 Direct Lysis Coupled with QIAamp®

The extraction using direct lysis was performed using the QIAamp® DNA Mini Kit by Qiagen. Three replicates of cartridge cases of calibre 0.22 per participant were used for this extraction. Cartridge cases of calibre 0.223 were not used for this extraction due to the lack of availability of sample tubes that could fit the cartridge case and that also fitted into the thermal block.

a. Components of the kit:

- QIAamp® Mini Spin Columns
- Collection Tubes (2ml)
- Buffer ATL
- Buffer AL
- Proteinase K
- Buffer AW1 (Wash Buffer 1)
- Buffer AW2 (Wash Buffer 2)
- Buffer AE (Elution Buffer)

b. Procedure:

- The cartridge cases with fingermarks from a participant were placed into a 1.5ml tube.
- Lysis buffer was prepared according to the volumes in *Table 3*.
- 500µl of calculated Lysis Buffer was added to the tube and the tube was incubated at 56°C for 15 minutes while shaking at 900 rpm.
- The tube was centrifuged for 4 minutes at 2000g on an Eppendorf 5804 R.
- The sample and all of the solution was transferred to a spin basket in a freshly labelled 1.5ml tube. Then the tube was centrifuged at maximum speed for 1 minute.
- The spin basket containing the cartridge case was removed from the tube. The cartridge cases were wiped down using 70% ethanol to remove the traces of Lysis Buffer on the surface to prevent possible oxidation.
- The solution was incubated for 60 minutes at 56ºC while shaking at 800rpm.

- To the incubated solution, 400µl of AL Buffer was added, and it was vortexed for 10 seconds.
- The solution was then incubated for 10 minutes at 70ºC while shaking at 800rpm.
- To the incubated sample, 400µl of Absolute ethanol was added and was vortexed briefly.
- The solution was transferred into a freshly labelled mini spin column that was attached to a 2ml collection tube. The tube was centrifuged at 8000rpm for 1 minute.
- As the volume of the solution is more than the capacity of the mini spin column, this step was done in two steps. Half of the solution was first transferred into the mini spin column and was centrifuged. Then the rest was transferred into the mini spin column and centrifuged.
- The mini spin column was placed into a fresh 2ml collection tube. Then 500µl of wash buffer 1 (AW1) was added to it. The tube was centrifuged at 8000rpm for 1 minute.
- To the mini spin column, 500µl of wash buffer 2 (AW2) was added. The tube was centrifuged at 13000rpm for 3 minutes.
- The flow-through from the wash buffers was discarded, and the mini spin column was placed into a clean collection tube.
- 50µl of elution buffer (AE) was added to the tube. After incubating for one minute at room temperature, the tube was centrifuged for 1 minute at 8000rpm at room temperature.
- The mini spin column was removed, and the eluted sample was stored at 4ºC for up to 5 days and at -20ºC for long term storage.

Lysis Buffer calculation

Reagent	**Volume per sample (µL)**
ATL buffer	475
Proteinase K	25
DTT	20

Table 3: Lysis buffer calculation for direct lysis coupled with QIAamp®

Reagent	Volume per sample (µL)
AL Buffer	400
Absolute ethanol	400
Wash Buffer 1 (AW1)	500
Wash Buffer 2 (AW2)	500
Elution Buffer (AE)	50

Table 4: Other reagents required for direct lysis coupled with QIAamp®

2.13.3 Pre-Extraction soaking coupled with QIAamp®

Any DNA present was collected by soaking the cartridge cases in ATL buffer. The solution in which the sample was soaked was taken through the extraction procedure. The extraction was performed using the same QIAamp® DNA Mini Kit by Qiagen as used for Direct Lysis. Three replicates each of both of the calibres (0.22 and 0.223) were used per participant for this extraction.

a. Soaking

- The sample was placed into a 5ml screw cap tube.
- 400µl of ATL buffer was added to the tube containing the sample.
- The tube was incubated for 30 minutes at room temperature at a suitable angle. During this 30-minute incubation, the tube was rotated every 2 minutes. This was done to ensure that the entire sample was came into contact with the buffer.
- After incubation, the ATL buffer was transferred into a labelled 1.5ml tube.
- The cartridge case was taken out using sterile tweezers, and the surface of the cartridge case was swabbed dry using a dry sterile swab. The swab was then added to the same tube as the ATL buffer.

b. Extraction procedure

- The ATL buffer in which the sample was soaked and the swab in the ATL buffer were then incubated for 10 minutes at 85ºC while shaking at 800rpm.
- After the incubation, 30µl of proteinase K was added.
- The solution was then incubated for 60 minutes at 56ºC while shaking at 800rpm.
- After 60 minutes of incubation, 400µl of AL Buffer was added, and the solution was vortexed for 10 seconds.
- The solution was incubated for 10 minutes at 70ºC while shaking at 800rpm.

- After the incubation, 400µl of Absolute ethanol was added, and the solution was vortexed briefly.
- The swab and the solution were transferred into a spin basket attached to a 1.5ml tube. The tube was centrifuged at maximum speed for 2 minutes.
- The solution was transferred into a freshly labelled mini spin column that was attached to a 2ml collection tube. The tube was centrifuged at 8000rpm for 1 minute.
- The mini spin column was placed in a fresh 2ml collection tube. Then 500µl of wash buffer 1 (AW1) was added to it. The tube was centrifuged at 8000rpm for 1 minute.
- To the mini spin column, 500µl of wash buffer 2 (AW2) was added. The tube was centrifuged at 13000rpm for 3 minutes.
- The flow-through from the wash buffers was discarded, and the mini spin column was placed into a clean collection tube.
- To elute the sample, 50µl of elution buffer (AE) was added to the tube. After a minute, the tube was centrifuged for 1 minute at 8000rpm in room temperature.
- The eluted sample was stored at 4ºC for up to 5 days and at -20ºC for long term storage.

Reagent	**Volume per sample (µL)**
ATL buffer (Lysis Buffer)	400
Proteinase K	30
AL Buffer	400
Absolute ethanol	400
Wash Buffer 1 (AW1)	500
Wash Buffer 2 (AW2)	500
Elution Buffer (AE)	50

Table 5: Volume of reagents required per sample

2.14 DNA Quantitation

DNA quantitation of all of the samples was done using Quantifiler® Trio Real-time PCR Quantitation using the Quantifiler® Trio DNA Quantitation Kit by Applied Biosystems® (Thermo Fisher Scientific Waltham, MA, USA). The kit contains THP DNA standard, dilution buffer, Quantifiler® THP PCR Reaction mix, and Quantifiler® Trio Primer Mix. Using the components in the kit, the quantitation standards and reaction Mastermix were made.

2.14.1 Quantitation Standards

Five standards (STD1, STD2, STD3, STD4 and STD 5) and a negative control (NTC) were used as quantitation standards. A serial dilution was performed to make the standards using the THP standard and the dilution buffer in order to obtain DNA standards with known concentrations of DNA to create a standard curve. *Table 6* shows the amount of DNA in each standard and the volumes of THP standard and dilution buffer used to obtain the concentration.

Standard	**Concentration of DNA**	**Serial Dilution**	**Volume of Dilution Buffer (µL)**
Standard 1 (STD 1)	50ng/µL	100µL THP Standard	100
Standard 2 (STD 2)	5ng/µL	20µL STD 1	180
Standard 3 (STD 3)	0.5ng/µL	20µL STD 2	180
Standard 4 (STD 4)	0.05ng/µL	20µL STD 3	180
Standard 5 (STD 5)	0.005ng/µL	20µL STD 4	180
Negative Control (NTC)	0.00ng/µL	0	200

Table 6: Volumes required for the serial dilution of the quantitation standards

2.14.2 Quantitation of Samples

a. Preparation of Quantifiler® Trio Mastermix (Mastermix)

1µL of automation enhancer was added to a tube of the reaction mix to prevent the formation of bubbles that can inhibit and interfere with the quantitation process. The Mastermix was prepared by mixing an appropriate amount of THP reaction mix in which the automation enhancer was added and Primer Mix as given in the table below. Both the reagents were centrifuged for a minute before the preparation of the Mastermix.

Reagents for Mastermix	**Volume per sample**
Quantifiler® THP Reaction Mix	10µL
Quantifiler® Trio Primer Mix	8µL
Total Mastermix volume	18µL

Table 7: Calculation of Mastermix for Quantifiler® Trio

b. Sample preparation for quantitation using Quantifiler® Trio Real-time PCR

- Mastermix was prepared as detailed in the above sub-section.

- 18 µl of Mastermix was added into each well of a 96 well plate (*Table 8*). The number of wells used per quantitation depended upon the number of samples quantified. A 96-well plate layout was made as shown in *Table 7.*
- Two replicates of each of the five standards and the negative control were used to generate a standard curve for the quantitation.
- The prepared standards, as detailed in section 2.14.1, were vortexed for 10 seconds. 2µl of each standard were added in duplicate into the first 12 wells, respectively, following the printed layout.
- 2µl of each sample were added into each well as assigned.
- After adding the samples and Mastermix, the quantitation plate was sealed using the MicroAmp™ Optical Adhesive Film (*MicroAmp™ Optical Adhesive Film*, n.d.).

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1	STD 5										
B	STD 1	STD 5										
C	STD 2	NTC										
D	STD 2	NTC										
E	STD 3	Sample 1										
F	STD 3											
G	STD 4											
H	STD 4											Sample x

Table 8: Layout of the 96-well plate for Quantifiler® Trio

c. Quantifiler® Trio Real-time PCR

The sealed 96-well plate was centrifuged to remove any air bubbles that may be present. The plate was then run on the Applied Biosystems™ 7500 Real-Time PCR System using HID Real-Time PCR Analysis Software. After the run, the results were analysed using the software. The user manual was used to determine the validity of the results (*Quantifiler® Trio DNA Quantification Kit*, n.d.). The following parameters were checked to see if the quantitation passed or failed.

1. Standard Curve: Large Autosomal, Small Autosomal, and Y are the three targets for the assay. The R^2 value was checked to ensure that it was greater than or equal to 0.99. The slope of each target was checked to ensure it fell between the expected range. The

range for the slopes of the targets, small autosomal and Y, was -3.0 to -3.6, and large autosomal was -3.1 to -3.7.

2. Amplification Plot: The amplification plots were checked using the two lines in which the horizontal blue line depicts the C_T threshold for small autosomal, large autosomal and male targets, and the horizontal red line depicts C_T threshold for the IPC. The amplification plot was also checked to ensure it fell under the defined parameters as per the user manual.
3. Multicomponent Plot: The multicomponent plot was checked to ensure it was flat linear lines for the first 15-20 cycles and showed an exponential growth after that.

If the quantitation passed, the quantity estimated was used to inform how much sample was required for the amplification step. If the quantitation failed, it was performed again.

2.15 DNA Amplification

The fourth objective was to compare two DNA multiplexes to see which amplification technique gave better profiling results. The extracted solutions were amplified using Identifiler® Plus (28 cycles) and LCN (34 cycles) to see which cycle condition gave better results. Direct amplifications were also performed using Identifiler® Plus, LCN, and, Minifiler® and the results were compared for the study.

2.15.1 Dilution

Every amplification requires an optimal amount of DNA in the sample to ensure that the DNA profiling results are not over or under amplified. For amplification using Identifiler® Plus (28 cycles) the optimal amount of DNA is 1ng, for Identifiler® Plus (LCN 34 cycles) the optimal amount is 50-60pg of DNA and the optimal amount of DNA for amplification using Minifiler® is 0.2ng. An excessive amount of DNA might act as an inhibitor during amplification. In a sample that had a large amount of DNA, an aliquot of the sample was taken and diluted to obtain the optimal DNA concentration. The diluted sample was then used for further amplification. As the dilutions were performed on an aliquot of the solution, the DNA concertation of the extracted solution remained the same. In this study, dilutions were performed on 2µl of the sample extract. The diluted samples were then amplified following the procedure as detailed in the subsections below. The sample diluent used was TE Buffer. The dilution parameters are given in *Table 9.*

	Identifiler® Plus (half-reaction)	LCN using Identifiler® Plus (half-reaction)	Minifiler®
Optimal amount of DNA	1ng	50-60pg	0.2ng
Total reaction volume	12.5µl	12.5	25µl
Sample diluent	TE Buffer	TE Buffer	TE Buffer
DNA Volume	5µl	5µl	10µl
Volume of Mastermix	7.5µl	7.5µl	15µl

Table 9: The dilution parameters for different amplification kits

The volume of DNA and diluent is calculated using the equation below

$$c = n/v \longrightarrow v = n/c$$

where,

c = the concentration of the sample estimated using quantitation.

n = target amount or the optimal amount of DNA required after dilution. n= 1ng for Identifiler® plus and n = 0.2ng for Minifiler®

v = volume of DNA sample required

Dilution	DNA Volume	TE Buffer
1/5	2µl	8µl
1/10	2µl	18µl
1/100	2µl	198µl

Table 10: DNA dilution volumes

2.15.2Identifiler® Plus

AmpFLSTR™ Identifiler® Plus PCR Amplification Kit (Thermo Fisher Scientific Waltham, MA, USA), in short Identifiler® Plus, was used for the amplification of the samples. All of the samples were amplified using a half-reaction.

a. Components in the amplification kit.

- AmpFLSTR Identifiler® Plus Mastermix
- AmpFLSTR Identifiler® Plus Primer set
- AmpFLSTR Identifiler® Plus Allelic Ladder
- AmpFLSTR Identifiler® Plus Control DNA 9947A

The kit was stored following the manufacturer's protocol (Thermo Fisher Scientific Waltham, MA, USA). Prior to opening, it was stored at -20ºC. After opening, it was stored at 4ºC.

b. Preparation of Mastermix

The amount of Mastermix required for the amplification of samples, using Identifiler® Plus half-reaction, was calculated using the volumes as detailed in *Table 11.*

Reagent	**The volume needed for a half-reaction (µl)**	**Total volume (µl)**
Identifiler® Plus Master Mix	5	5µl x sample number = (A)
Identifiler® Plus Primer Set	2.5	2.5µl x sample number = (B)
Total Volume of Mastermix	5+2.7=7.5	(A) + (B)

Table 11: Calculation of Mastermix for Identifiler® Plus

c. Preparation of samples

- The procedure was performed in a clean biosafety cabinet.
- The sample was prepared in 0.2ml amplification tubes.
- The master mix was made in a 1.5ml tube following the calculation above.
- Dilution was calculated, as detailed in section 2.11.3, for the necessary samples.
- An appropriate amount of TE buffer was added to a labelled 0.2ml amplification tube.
- An appropriate amount of sample (or diluted sample) was added to the amplification tube.
- 5µL TE Buffer was used as the amplification negative control (ANEG), and the AmpFLSTR Identifiler® Plus Control DNA 9947A was used as the amplification positive control (APOS).
- The volume in each tube was 5µL after the steps above.
- 7.5µL of Mastermix was added to each 0.2ml sample tube
- The tube was placed into the 9700 PCR instrument and the lid of the tube was closed tightly using a roller.

d. PCR

- The sample was loaded to the PCR instrument 9700
- Programme: (9600 Emulation)

95°C, 11 minutes
94°C, 20 seconds
59°C, 3 minutes
60°C, 10 minutes
28 Cycles
4°C, indefinitely (hold)

12.15.3 Amplification using LCN

Amplification using LCN is a highly sensitive method to amplify DNA. LCN amplification uses the same Identifiler® Plus kit as detailed in section 12.15.2. Apart from the change in PCR cycling conditions, the amplification was undertaken following the same procedure as Identifiler® Plus, as detailed in section 12.15.2. PCR cycling conditions for LCN are the following.

95ºC, 11minutes
94ºC, 20 seconds
59ºC, 3 minutes
60ºC, 20 minutes
4ºC, indefinitely (hold)

34 cycles

12.15.4Minifiler®

AmpFLSTR MiniFiler® Amplification Kit (Thermo Fisher Scientific Waltham, MA, USA), in short Minifiler®, was used for the amplification of the samples.

a. Components in the amplification kit.

- AmpFLSTR Minifiler® Primer Set
- AmpFLSTR Minifiler® Master Mix
- AmpFLSTR Minifiler® Allelic Ladder
- AmpFLSTR Control DNA (Male 007)

The kit was stored following the manufacturer's protocol (Thermo Fisher Scientific Waltham, MA, USA). Prior to opening, it was stored at -20ºC. After opening, it was stored at 4ºC.

b. Preparation of Mastermix

The amount of Mastermix required for the amplification of samples using AmpFLSTR MiniFiler® was calculated using *Table 12.*

Reagent	**The volume needed for a full-reaction (µl)**	**Total volume (µl)**
AmpFLSTR MiniFiler® Master Mix	10	10µl x sample number = (A)
AmpFLSTR MiniFiler® Primer Set	5	5µl x sample number = (B)
Total Volume of Mastermix	10+5=15	(A) + (B)

Table 12: Calculation of Mastermix for Minifiler®

c. Preparation of samples

- The procedure was performed in a clean biosafety cabinet.
- The sample was prepared in 0.2ml amplification tubes.
- The Mastermix was made in a 1.5ml tube following the calculation above.
- Dilution was calculated, as detailed in section 2.11.3, for the necessary samples.
- An appropriate amount of TE buffer was added to a labelled 0.2ml amplification tube.
- An appropriate amount of sample (or diluted sample) was added to the amplification tube.
- 10µL TE Buffer was used as the ANEG, and the AmpFLSTR Control DNA (Male 007) was used as the APOS.
- The volume in each tube was 10µL after the steps above.
- 15 µL of Mastermix was added to each 0.2ml sample tube.
- The tube was placed into the 9700 PCR instrument and the lid of the tube was closed tightly using a roller.

d. PCR

- The sample was loaded to the PCR instrument 9700.

95°C, 11minutes
94°C, 20 seconds
59°C, 2 minutes
72°C, 1 minute
} 30 cycles
60°C, 45 minutes
4°C, indefinitely (hold)

2.16 Direct amplification using PCR (Direct PCR)

Under this study, Direct PCR was performed by swabbing the surface of the cartridge cases using micro applicator swabs. Direct PCR was performed using Identifiler® Plus, Identifiler® Plus LCN and Minifiler® to see which technique gave better results.

2.16.1 Direct PCR using Identifiler® Plus

Direct PCR using Identifiler® Plus used the same kit as detailed in section 2.12.1. The Master mix was made in a 1.5ml tube as in *Table 11*. The cartridge cases with fingermarks from a participant were swabbed using a micro applicator swab which was moistened using sterile water. The swab end was then cut and placed into a labelled 0.2ml amplification tube. 5µl of TE buffer was added to the tube. 5µL TE Buffer was used as the amplification negative control,

and the AmpFLSTR Identifiler® Plus Control DNA 9947A was used as the amplification positive control. 7.5µl of Mastermix was added. The tube was placed into the 9700 PCR instrument, and the lid of the tube was closed tightly using a roller. The PCR cycling conditions were the same as those detailed previously in subsection 2.15.2.

2.16.2Direct PCR using Identifiler® Plus LCN

Direct PCR using Identifiler® Plus LCN followed the same procedure as detailed in section 12.16.1 apart from the PCR cycle conditions. The PCR cycling conditions followed were the same as those detailed previously in subsection 2.15.3.

2.16.3Direct PCR using Minifiler®

Direct PCR using Minifiler® used the same kit as detailed in section 2.15.4. The Master mix was made in a 1.5ml tube as in *Table 12.* The cartridge cases with fingermarks from a participant were swabbed using a micro applicator swab which was moistened using sterile water. The swab end was then cut and placed into a labelled 0.2ml amplification tube. 10µl of TE buffer was added to the tube. 10µL TE Buffer was used as the amplification negative control, and the AmpFLSTR Control DNA (Male 007) was used as the amplification positive control. 15µl of Mastermix was added. The tube was placed into the 9700 PCR instrument, and the lid of the tube was closed tightly using a roller. The PCR cycling conditions were the same as those detailed previously in subsection 2.15.4.

2.17 Capillary Electrophoresis

The amplified samples were processed using capillary electrophoresis. This was performed using the 3500xL Genetic Analysers by Applied Biosystems. Amplified samples were prepared using the following steps to load to the Genetic Analyser. 1ml of Hi-Di™ Formamide was mixed with the size standard, GS-500 LIZ™, with the volumes used given in *Table 13.* The solution containing 1ml of Hi-Di™ Formamide and GS-500 LIZ™ size standard mix was added to the wells of a new 96-well plate following the plate record. The volumes used are given in *Table 14.* Samples and ladders were added to the plate following the plate record. Each column was covered using optical caps after adding the Hi-Di™ Formamide and GS-500 LIZ™ and the samples and ladder. The plate was centrifuged to remove any air bubbles then the optical caps were removed and replaced with rubber septa.

Kit	Volume of Hi-Di™ Formamide	Size standard	Volume of size standard (µl)
Identifiler® Plus (28 cycles)	1ml	GS-500 LIZ™	15
Identifiler® Plus LCN	1ml	GS-500 LIZ™	30
Minifiler®	1ml	GS-500 LIZ™	15

Table 13: Volumes of Hi-Di™ Formamide and size standard for each amplification kit

Kit	Hi-Di™ Formamide and size standard mix volume	Sample Volume	Ladder Volume
Identifiler® Plus (28 cycles)	9µl	1µl	1µl
Identifiler® Plus LCN	9µl	1µl	2µl
Minifiler®	9µl	1µl	1µl

Table 14: Input volumes of Hi-Di™ Formamide and size standard mix, sample and ladder for different amplification kits

The loaded plate was denatured in a PCR machine using the following cycle conditions: 95°C for 5 minutes, then 4°C for 2 minutes, followed by an indefinite hold at 4°C. After spinning the loading plate, it was loaded onto the moving autosampler of the 3500xL. The injection voltage for Identifiler® Plus (28 cycles), Identifiler® Plus LCN and Minifiler® was the same, however the time of injection varied. The injection parameters used are given in *Table 15*. Manufacturers protocols were followed to run the instrument and extract data from the samples.

Amplification Kit	Injection voltage (kv)	Injection time (sec)
Identifiler® Plus (28 cycles)	1.2kv	15
Identifiler® Plus LCN (34 cycles)	1.2kv	8
Minifiler®	1.2kv	24

Table 15: Sample injection parameters for different amplification kits for Capillary Electrophoresis

2.18 Data Analysis

The data obtained after capillary electrophoresis was analysed using GeneMapper® ID-X v1.4 software following the ESR's standard operating procedure. Firstly, the raw data was added to the software. Once the raw data was added, the following parameters were set. Each sample was assigned a sample type, and the sample types were set appropriately against the sample names before the analysis. There were four sample types used in the analysis. The sample types

were allelic ladder, positive, negative, and sample. The other parameters were set as given in *Table 16*.

Parameters	**Identifiler® Plus (28 cycles)**	**Minifiler®**	**Identifiler® Plus LCN**
Analysis method	Identifiler Plus Casework CWK	Minifiler – 3500 CWK	LCN ID Plus Casework
Panel	Identifiler Plus V1.3x	Minifiler V1.3x	Identifiler Plus V1.3x-dup
Size-standard	CE 05 HID GS500	CE 05 HID GS500	CE 05 HID GS500

Table 16: Analysis parameters for analysis using GeneMapper® ID-X for the different amplification kits

After setting the appropriate parameters, sample peaks were analysed. The sample peaks were checked one by one to omit any unwanted peaks and to retain the allelic peaks. The peaks that fell outside the ladder (OL) and outside the marker range (OMR) were deleted. The peaks that had uneven and pointy edges and were not allelic in morphology were also deleted. Pull-up peaks obtained due to the overlapping of the fluorescence emission spectra of the dyes used were omitted. After deleting all of the unwanted peaks, the peaks at each locus were cross-checked with the reference samples to see if they matched or not. Once the allelic peaks were obtained, an output table was created from the analysed data, which was saved as a text file. The analysed data was exported from the software and saved appropriately. The saved data in the generated output table was used for further statistical interpretations.

CHAPTER 3
RESULTS

3.1 Introduction

The research was conducted based on four objectives to detect and visualise touch DNA and to improve DNA profiling results from cartridge cases. As detailed in chapter two, the study was divided into two parts; the first part investigated the visualisation of touch DNA from cartridge cases using diamond dye, and the second part investigated the DNA profiling aspect of the research. In the second part, where the STR profiling from the cartridge cases used the fingermarks from the participants, samples from four participants were used for the analysis.

3.2 Objective 1: The effect of visualisation of touch DNA on cartridge cases on fingermark enhancement and vice versa

3.2.1 Experiment 1: Visualisation of touch DNA on cartridge cases using diamond dye prior to the enhancement of fingermarks

The purpose of experiment 1 was to investigate the effect of visualisation of touch DNA on cartridge cases on further enhancement of fingermarks. The cartridge cases with fingermarks were sprayed with diamond dye and visualised under the polilight for fluorescence as detailed in section 2.7.2. *Figure 20* is an example of the images documented during this process.

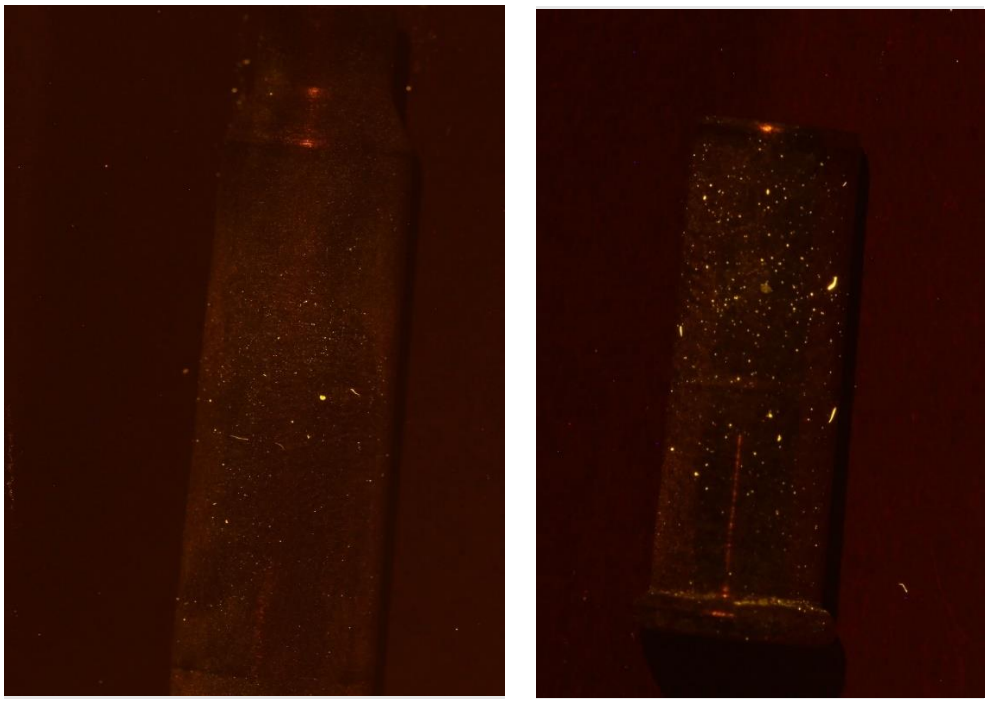

Figure 20: Cartridge cases of calibre 0.223 (left) and 0.22 (right) with fingermarks sprayed with diamond dye and visualised under polilight

The documented images were analysed using the ImageJ software. During the analysis using ImageJ, the images were converted to black and white where the photos became black dots with a white background format. The black dots represent the fluorescence.

Table 17 shows the amount of fluorescence obtained for five replicates of cartridge cases of calibres 0.22 and 0.223. The sample name is given in a 'calibre_replicate' format. *Table 18* gives the mean and standard deviation for both of the calibres.

Sample Name	Amount of fluorescence
0.22_1	**140**
0.22_2	**256**
0.22_3	**181**
0.22_4	**283**
0.22_5	**515**
0.223_1	**425**
0.223_2	**996**
0.223_3	**653**
0.223_4	**520**
0.223_5	**95**

Table 17: Amount of fluorescence observed on cartridge cases sprayed with diamond dye

Calibre	0.22	0.223
Number of replicates	5	5
Sum	1375	2689
Mean	275	537.8
Standard deviation	145.84	328.75

Table 18: Calculated mean and standard deviation of the amount of fluorescence observed on cartridge cases based on the values in Table 17.

There is a noticeable size difference between 0.22 and 0.223 cartridge cases. The fluorescence count is more for the 0.223 cartridge cases, which have more surface area compared to 0.22 cartridge cases. The differences in the mean value support this observation.

After the documentation using the ImageJ software, the cartridge cases were forwarded to the New Zealand police fingerprints section. After the analysis, it was found that some of the cartridge cases sprayed with diamond dye, when subjected to fingerprint enhancement using

VMD, showed an improvement in the quality of images. VMD is a fingerprint development technique that uses metal deposition on the surface of a metallic item to develop latent fingermarks. To confirm the findings, as requested by the fingerprint officer, five more replicates of cartridge cases of each calibre were sent for analysis. The revaluation of the cartridge cases also showed an improvement in the quality of developed fingermarks. *Figure 21* is the image documented after the enhancement of fingermarks using superglue followed with VMD after being sprayed with diamond dye. *Figure 22* is the image taken after the enhancement of fingermarks using VMD after being sprayed with diamond dye.

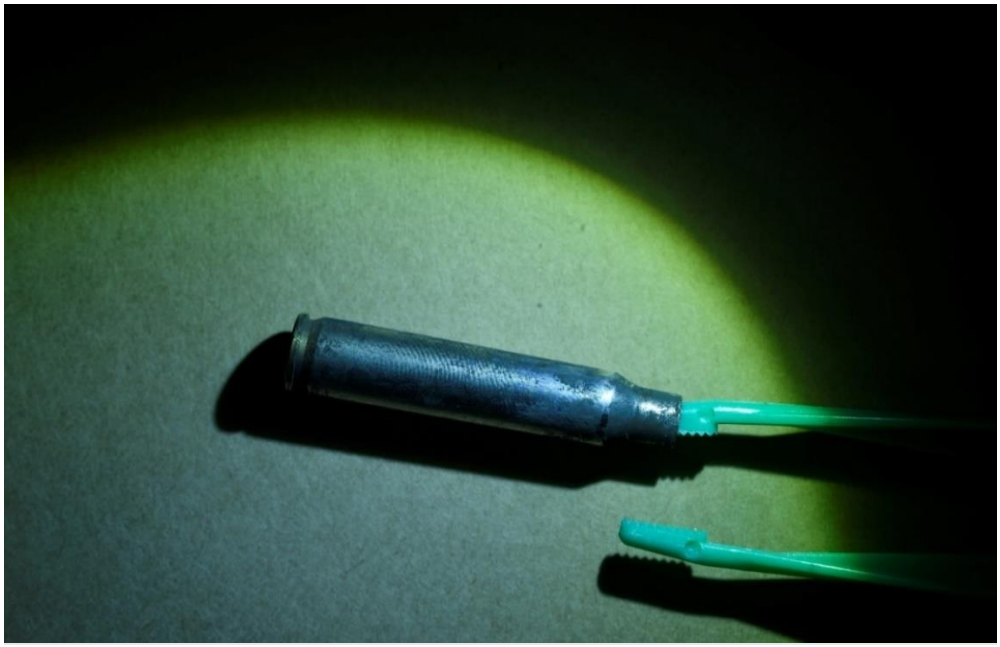

Figure 21: An example of a cartridge case of calibre 0.223 pre-treated with diamond dye and documented after fingermarks enhancement using superglue and VMD.

Figure 22: An example of a cartridge case of calibre 0.223 pre-treated with diamond dye and documented during the fingermarks enhancement using superglue.

3.2.2 Experiment 2: Visualisation of touch DNA on cartridge cases after fingermarks enhancement

The cartridge cases with fingermarks were forwarded to the New Zealand police fingerprint sections before the application of diamond dye. After performing the necessary enhancement using super glue and VMD, the cartridge cases were sprayed with diamond dye and observed and documented using the Polilight. *Figure 23* is an example of the images documented.

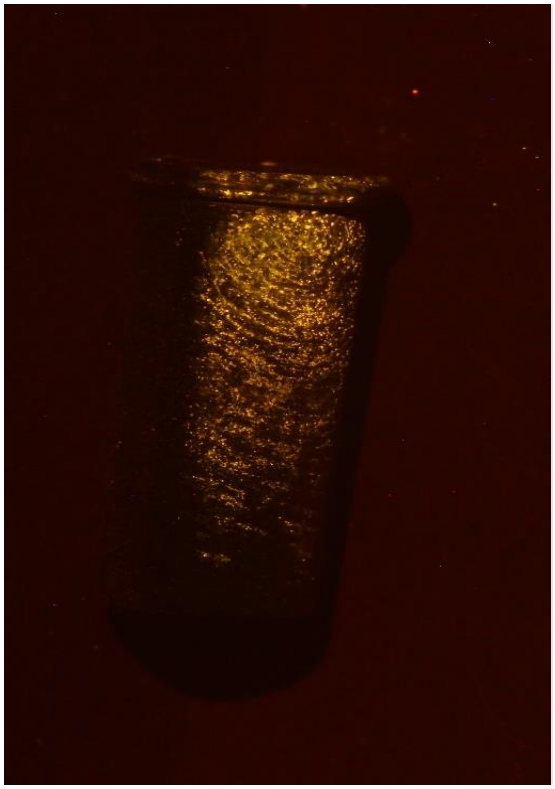

Figure 23: Cartridge case of calibre 0.22, fingermarks enhanced using VMD and then sprayed with diamond dye and visualised under Polilight

The fluorescence emitted by the cartridge cases reflected the entire ridge detail which were developed. In experiment 1, the fluorescence was concentrated on the area where the pristine fingermarks were deposited. The ridge detail on the cartridge cases were visible under Polilight but were not emitting any fluorescence. In experiment 2, the ridge detail was emitting fluorescence which resembled the fluorescence emitted by touch DNA in many cases. In experiment 2, the fluorescence emitted was smeary with no distinctive dots observed compared to what was observed in experiment 1. The differences in observation are visible in *Figures 20* & *23*. The amount of fluorescence was calculated from the samples of experiment 2 using ImageJ following the same parameters used for experiment 1 to make the uncertainty in the

results clear even though the entire fingermark is fluorescing which has nothing to do with the DNA.

The documented images were analysed using ImageJ to get the fluorescence count. The results obtained are given in *Table 19.* As compared to the results obtained for the previous experiment, the amount of fluorescence obtained for the replicates fluctuated irrespective of the calibre. The mean and standard deviation values were calculated with the values in *Table 19* as given in *Table 20.* The values obtained reflects the fluctuations in the results.

Sample Name	**Amount of fluorescence**
0.22_1	**318**
0.22_2	**534**
0.22_3	**872**
0.22_4	**586**
0.22_5	**1460**
0.223_1	**1243**
0.223_2	**306**
0.223_3	**873**
0.223_4	**237**
0.223_5	**72**

Table 19: Amount of fluorescence observed on cartridge cases sprayed with diamond dye after fingerprint enhancement

Calibre	0.22	0.223
Number of replicates	5	5
Sum	3770	2731
Mean	754	546.2
Standard deviation	441.32	492.56

Table 20: Calculated mean and standard deviation of the amount of fluorescence observed on cartridge cases based on the values in Table 19.

3.3 Objective 2: The effect of the process of firing on the presence of touch DNA on cartridge cases

The cartridges with fingermarks were fired, and the collected cartridge cases were sprayed with diamond dye and observed and documented under the polilight. The results obtained from the fired cartridge cases were compared with the unfired cartridges sprayed with diamond dye.

3.3.1 The amount of fluorescence obtained before and after firing on cartridge cases of calibre 0.22

The amount of fluorescence observed is given in *Table 21*. It was observed that the texture of the bullet and the paraffin wax on the bullet of the unfired cartridges of calibre 0.22 emitted fluorescence while documenting them (*Figure 24*). The differences in the amount of fluorescence are also included in *Table 21*. Since the fingermarks were only deposited on the body of the cartridge case, the value obtained from that was used to calculate the mean and standard deviation and the values are given in *Table 22*.

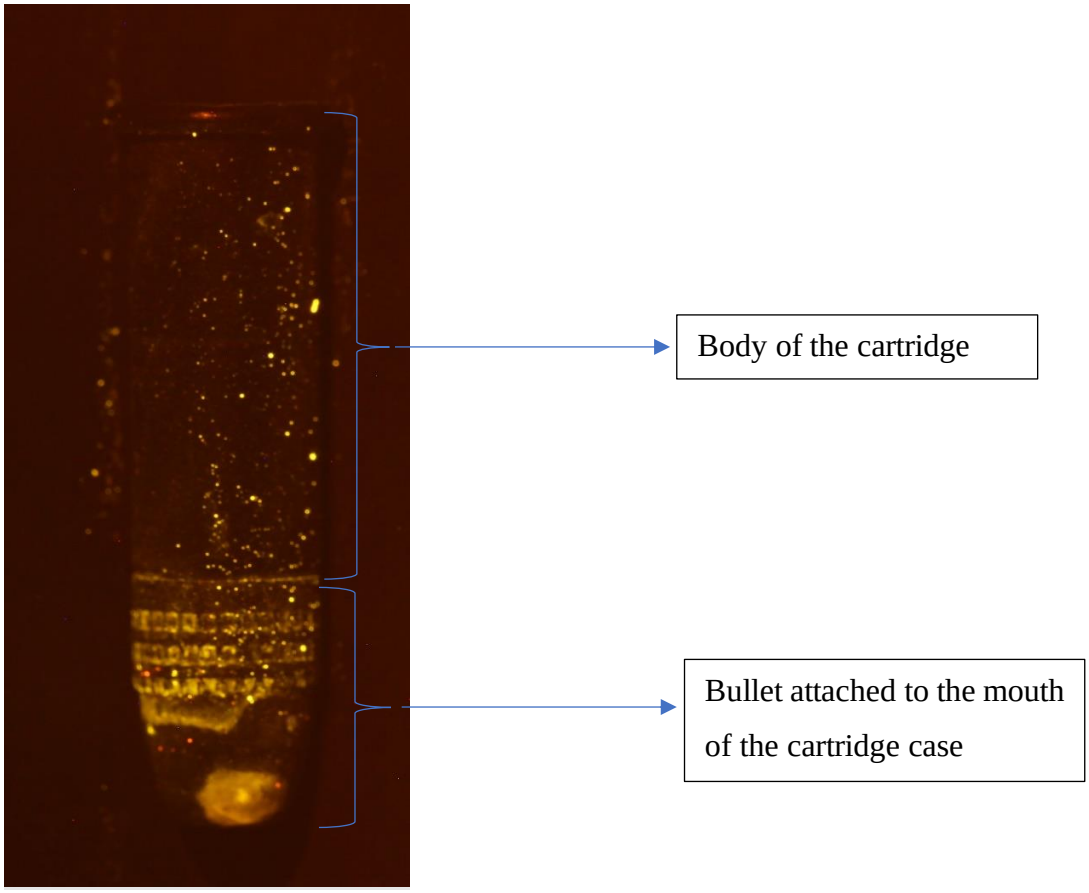

Figure 24: Unfired cartridge of calibre 0.22 sprayed with diamond dye and visualised under the polilight

Sample name	Calculated area	Amount of fluorescence
0.22_UF1.1	Entire cartridge	**233**
0.22_UF1.2	Just the body of the cartridge	**105**
022_UF2.1	Entire cartridge	**227**
022_UF2.2	Just the body of the cartridge	**92**
0.22_UF3.1	Entire cartridge	**261**
0.22_UF3.2	Just the body of the cartridge	**126**
0.22_F1	Entire cartridge case	**33**
0.22_F2	Entire cartridge case	**18**
0.22_F3	Entire cartridge case	**13**

Table 21: The amount of fluorescence observed on cartridges and cartridge cases of calibre 0.22 (UF: unfired cartridges; F: fired cartridge cases)

Calibre	0.22 unfired cartridges (Just the body of the cartridge)	0.22 fired cartridge cases
Number of replicates	3	3
Sum	323	64
Mean	107.66	21.33
Standard deviation	17.15	10.4

Table 22: Calculated mean and standard deviation of fired and unfired cartridge cases and cartridges of calibre 0.22

3.3.2 The amount of fluorescence obtained before and after firing on cartridge cases of calibre 0.223

Unlike 0.22 cartridges, the 0.223 bullets did not emit any fluorescence that interfered with the results. Since the fingermarks were deposited on the cartridge cases and not on the bullets, any

fluorescence on the bullet can be due to contamination. Hence the amount of fluorescence of the entire cartridge was analysed and the values obtained are given in *Table 23*. The calculated mean and standard deviation are given in *Table 24*.

Sample name	Calculated area	Amount of fluorescence
0.223_F1	Entire Cartridge case	**81**
0.223_F2	Entire Cartridge case	**376**
0.223_F3	Entire Cartridge case	**64**
0.223_UF1	Entire Cartridge	**235**
0.223_UF2	Entire Cartridge	**131**
0.223_UF3	Entire Cartridge	**94**

Table 23: The amount of fluorescence observed on cartridges and cartridge cases of calibre 0.223 (UF: unfired cartridge; F: fired cartridge cases)

Calibre	0.223 unfired cartridges	0.223 fired cartridge cases
Number of replicates	3	3
Sum	460	521
Mean	153.33	173.66
Standard deviation	73.1	175.43

Table 24: Calculated mean and standard deviation of fired and unfired cartridge cases and cartridges of calibre 0.223

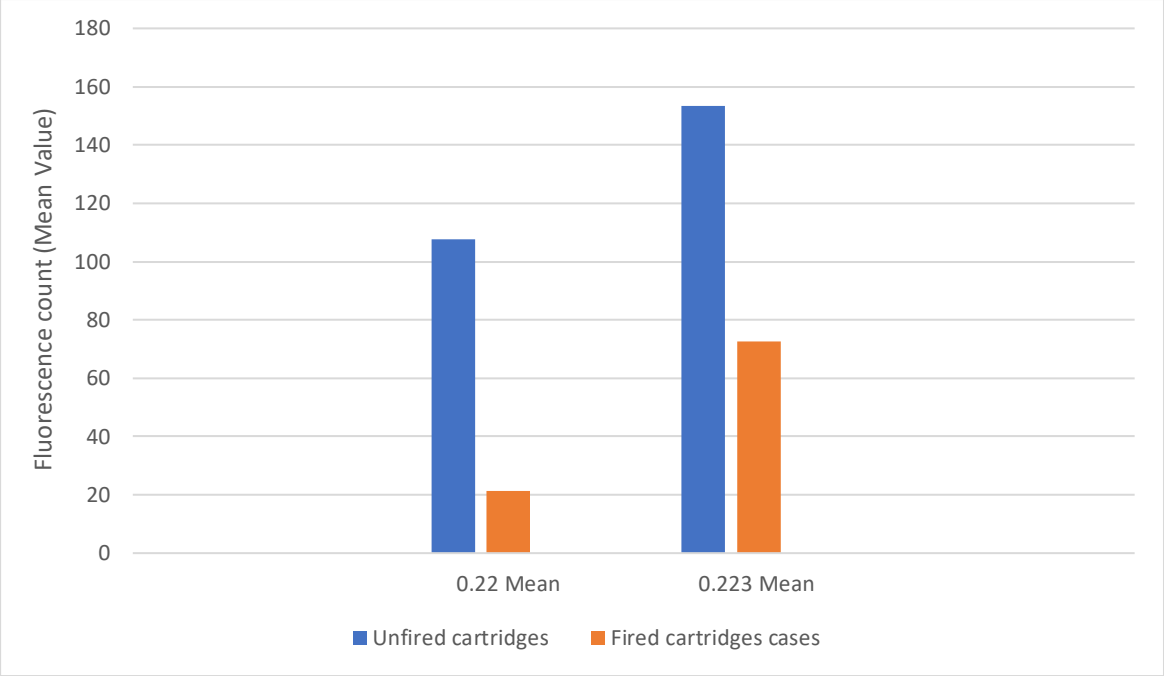

Figure 25: Graphical representation of the mean values of the amount of fluorescence observed on unfired cartridges and fired cartridge cases of calibres 0.22 and 0.223

Cartridge cases of calibre 0.22 showed a difference in the mean value of the amount of fluorescence however a difference was not that obvious initially in cartridge cases of calibre 0.223 (*Table 24*). One of the fired cartridge cases of calibre 0.223 showed an outlier in the amount of fluorescence compared to the other two replicates. That outlier, with a value of 376, has resulted in an overall increase of the sum. Hence, the calculations were done again after removing the value obtained for the outlier. After removing the outlier, the calculated mean value was 72.5 and standard deviation was 12.02. *Figure 25* illustrates the graphical representation of the mean values obtained for cartridge cases of calibre 0.22 and 0.223 in which the outlier observed is omitted. By analysing the graph, it is evident that the process of firing has a significant impact in the ability to visualise DNA on cartridge cases using diamond dye, however it should be noted that for 0.223 cartridge cases this is only based on two replicates.

3.4 Objectives Three and Four

The aim of objective three was to evaluate alternative extraction methods to improve DNA profiling success rates from cartridge cases. The aim of objective four was to evaluate alternative DNA multiplexes for the amplification of touch DNA including direct PCR. To obtain a better clarity of the results from these two objectives are discussed together from section 3.5 to section 3.7.

3.5 DNA Quantitation Results For The Three Extractions

The third objective of the study included the comparison of profiling results of direct lysis coupled with QIAamp® and pre-soaking extraction coupled with QIAamp® with the profiling results obtained for DNA IQ™ which is the standard procedure used at ESR. Following the standard procedure, the quantity of touch DNA in the extracts was estimated before amplification. The quantity was estimated using Quantifiler® Trio as detailed in section 2.14. The values obtained for each extraction is given in the below subsections.

3.5.1 DNA IQ™

The quantitation results obtained for DNA IQ™ given in *Table 25.*

Participant Number	Cartridge case_ Replicate	Quantity
Participant 1	0.223_1	0.0012
	0.223_2	0.0004
	0.223_3	0.0002
	0.22_1	UD
	0.22_2	0.0003
	0.22_3	UD
Participant 2	0.223_1	UD
	0.223_2	UD
	0.223_3	UD
	0.22_1	UD
	0.22_2	UD
	0.22_3	UD
Participant 3	0.223_1	UD
	0.223_2	UD
	0.223_3	UD
	0.22_1	UD
	0.22_2	UD
	0.22_3	UD
Participant 4	0.223_1	UD
	0.223_2	UD
	0.223_3	UD
	0.22_1	UD
	0.22_2	UD
	0.22_3	0.0001

Table 25: The quantity of DNA estimated using Quantifiler® Trio from all of the samples extracted with DNA IQ™; where UD stands for undetermined quantity

The amount of touch DNA of all replicates from participants 2 and 3 were undetermined. Four of the six replicates from participant 1 estimated some quantity. Validation studies of the Quantifiler® Trio Kit at ESR indicate that below 0.0005ng/µl the presence of human DNA cannot be distinguished from that of background noise. The quantity estimated with a high CT value might be an indication of background noise and not necessarily due to the presence of DNA.

3.5.2 Pre -extraction soaking coupled with QIAamp® DNA Mini Kit

No quantity was estimated from all of the replicates from participants 3 and 4. The quantities estimated from participants 1 and 2 were negligible like DNA IQ™ with high CT values. The quantitation results obtained are given in *Table 26.*

Participant Number	Cartridge case_ Replicate	Quantity
Participant 1	0.223_1	0.0002
	0.223_2	UD
	0.223_3	0.0002
	0.22_1	UD
	0.22_2	UD
	0.22_3	UD
Participant 2	0.223_1	UD
	0.223_2	UD
	0.223_3	0.0001
	0.22_1	UD
	0.22_2	UD
	0.22_3	UD
Participant 3	0.223_1	UD
	0.223_2	UD
	0.223_3	UD
	0.22_1	UD
	0.22_2	UD
	0.22_3	UD
Participant 4	0.223_1	UD
	0.223_2	UD
	0.223_3	UD
	0.22_1	UD
	0.22_2	UD
	0.22_3	UD

Table 26: The quantity of DNA estimated using Quantifiler® Trio from all of the samples extracted with pre-extraction soaking coupled with QIAamp®; UD stands for quantity undetermined.

3.5.3 Direct Lysis coupled with QIAamp® DNA Mini Kit

Only the cartridge cases of calibre 0.22 were subjected to direct lysis as the direct lysis was performed using 1.5ml Eppendorf tube. The cartridge cases of calibre 0.223 would not fit into a 1.5ml tube due to their large size. The quantitation results obtained are given in *Table 27.*

Participant Number	Cartridge case_ Replicate	Quantity
Participant 1	0.22_1	UD
	0.22_2	UD
	0.22_3	0.0002
Participant 2	0.22_1	UD
	0.22_2	UD
	0.22_3	UD
Participant 3	0.22_1	UD
	0.22_2	UD
	0.22_3	UD
Participant 4	0.22_1	0.0002
	0.22_2	UD
	0.22_3	0.0009

Table 27: The quantity of DNA estimated using Quantifiler® Trio from all of the samples extracted by direct lysis coupled with QIAamp® where UD stands for quantity undetermined,

3.6 DNA extractions coupled with Identifiler® Plus

As detailed in the previous chapter, the samples were extracted using DNA IQ™, pre-extraction soaking coupled with QIAamp® and direct lysis coupled with QIAamp®. The extracts were amplified using Identifiler® Plus under two different PCR conditions, that is, 28 cycles and LCN (34 cycles).

3.6.1 Amplification of reference samples using Identifiler® Plus (28 cycles)

The buccal swabs from the participants were extracted using DNA IQ™ to obtain a full reference profile. *Figure 26* shows a complete profile obtained from one of the participants. There are 15 STR loci, and each locus has two alleles.

Figure 26: A full profile obtained from the reference sample of participant 2

3.6.2 Amplification of sample extracts using Identifiler® Plus (28 cycles)

No alleles were obtained from the participants in the amplification with Identifiler® Plus (28 cycles) for extracts from all three extractions. Meanwhile the extraction positives of all three extractions gave a full profile and the extraction negatives gave a clear result. *Figure 27* shows the analysis in which no alleles were observed.

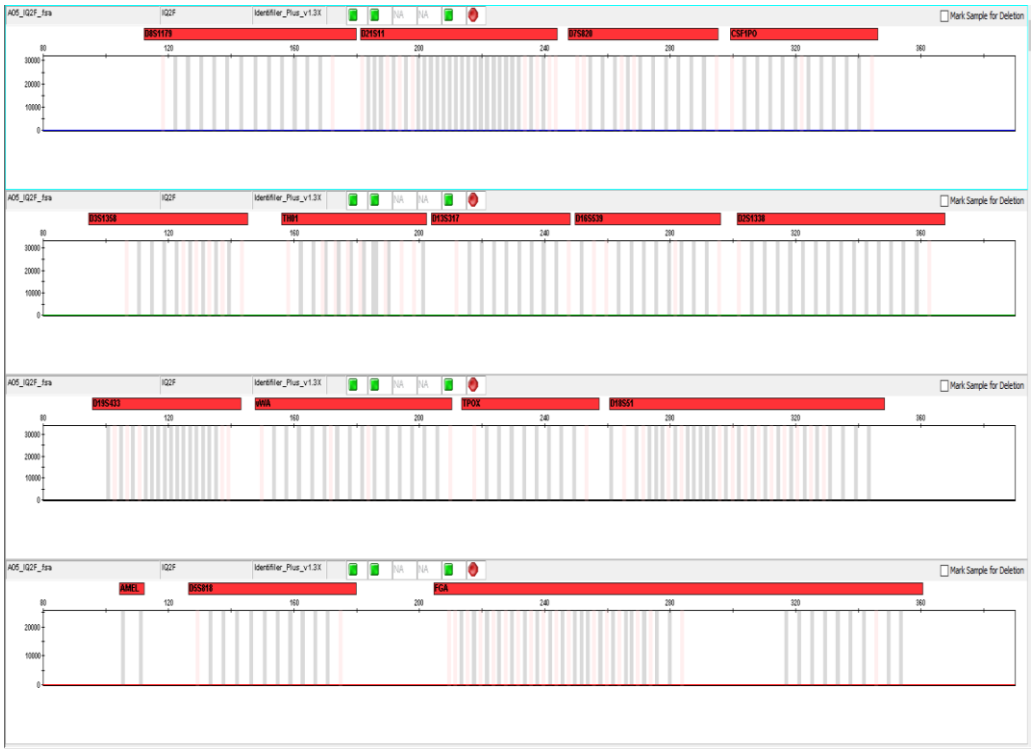

Figure 27: Example of no profile

3.6.3 *Amplification of sample extracts using LCN*

A few alleles were obtained from the amplification using the LCN conditions. The number of replicates per participant that provided alleles from each extraction is illustrated as a graph (*Figure 28*). The number of alleles obtained at each locus for each extraction across all participants are detailed as a graphical representation in *Figure 29*. *Figure 30* shows an example of alleles observed in a sample extracted using direct lysis from participant four. *Figure 31* is a graphical representation of the number of alleles obtained for each extraction per participant. No alleles were observed in any sample for participant number 3. From all the three extraction methods used, direct lysis coupled with QIAamp® gave the best results. Alleles were obtained from three participants out of four using direct lysis. Nine alleles from participant one, eight alleles from participant two and an allele from participant four were obtained across all the replicates analysed per participant using direct lysis coupled with QIAamp®.

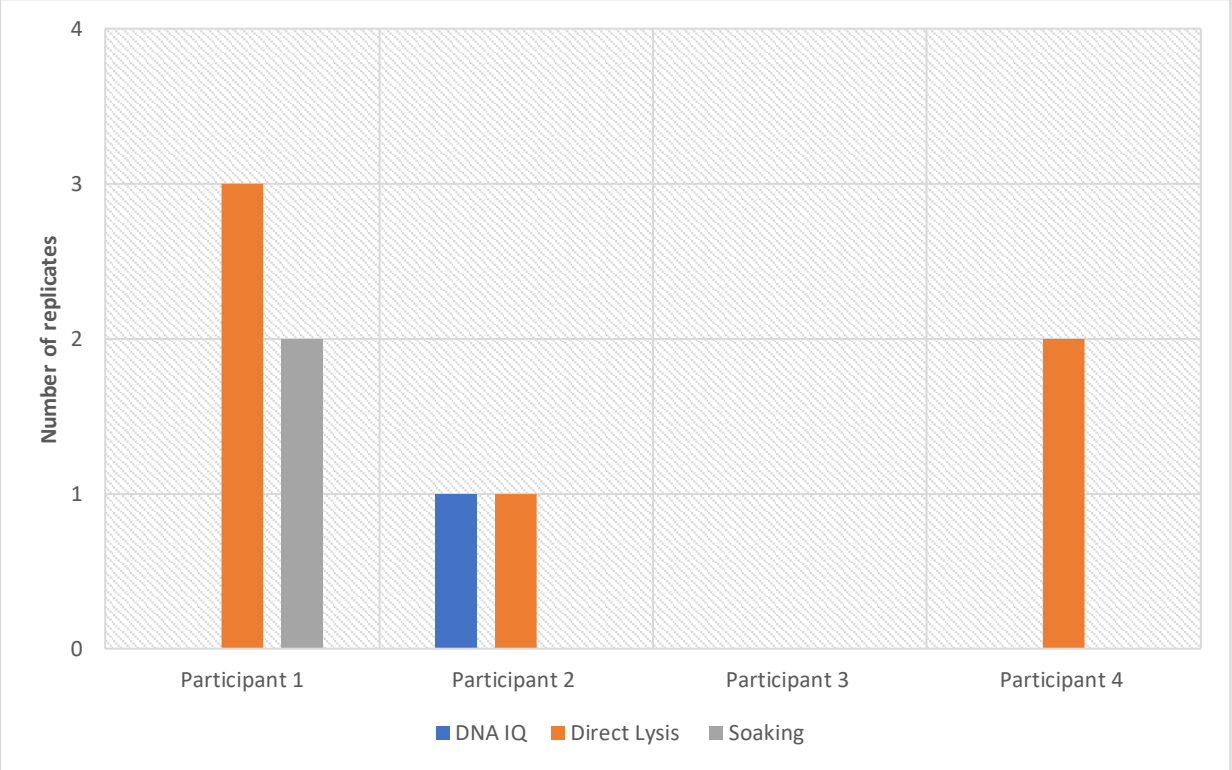

Figure 28 Graphical representation of the number of replicates that provided alleles for each extraction

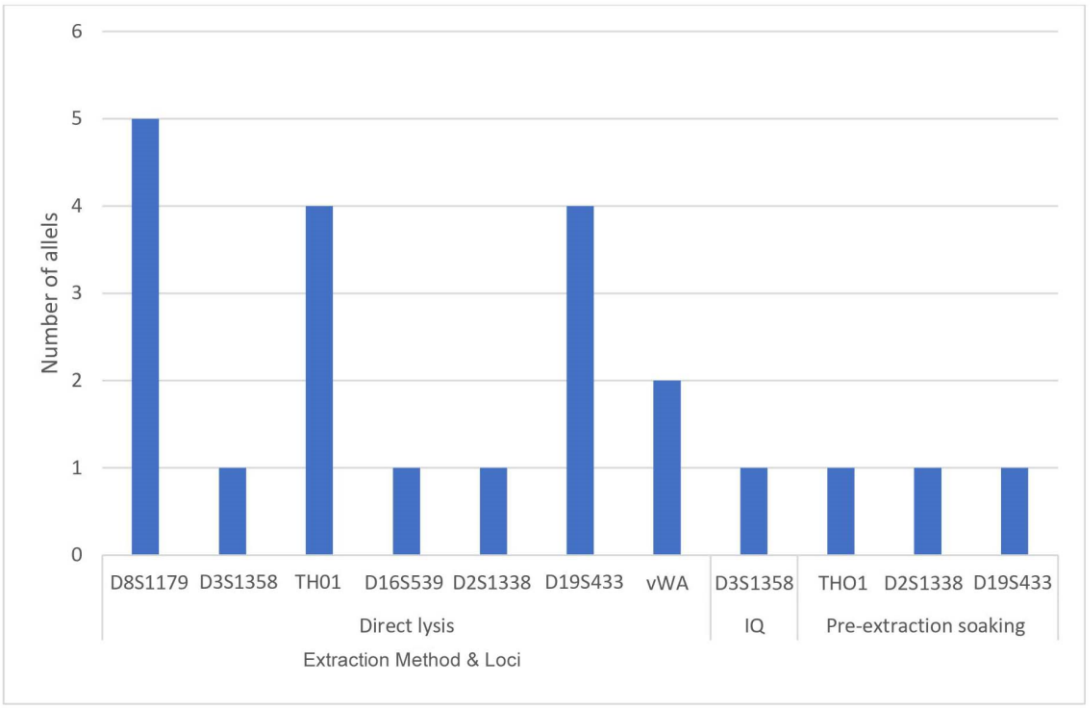

Figure 29: Graphical representation for the number of alleles obtained at each locus

Figure 30: Illustration of a partial profile obtained by LCN from a sample extracted using direct lysis

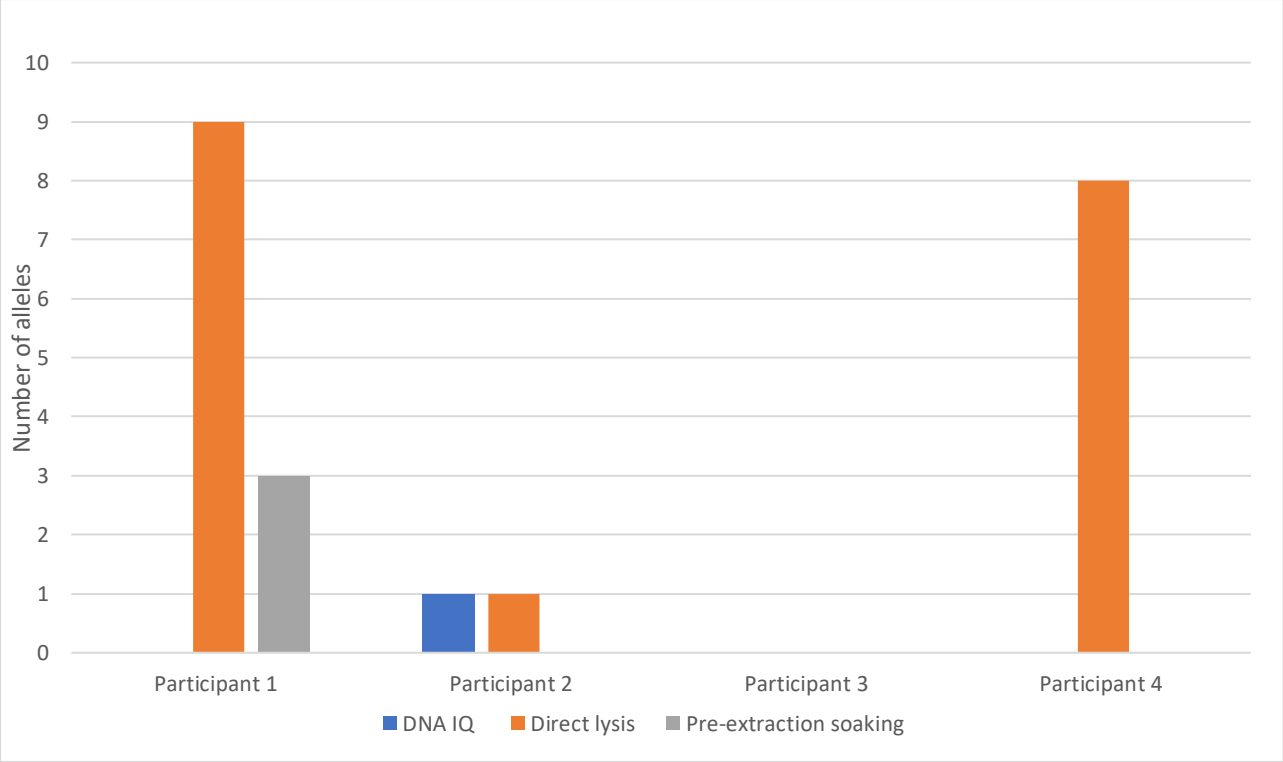

Figure 31: Graphical representation for the total number of alleles obtained per extraction across all the replicates for each participant

3.7 Direct PCR

Direct PCR was performed using three different methods to investigate different DNA multiplexes. The results obtained for each method are detailed in the following sub-sections.

3.7.1 Direct PCR using Minifiler®

No allelic peaks were obtained after the direct amplification of the samples using Minifiler®.

3.7.2 Direct PCR using Identifiler® Plus

No allelic peaks were obtained from the participants after the direct amplification using Identifiler® Plus. Non-allelic peaks from three non-human specific targets were observed irrespective of the participants. *Figure 32 and Figure 33* show the peaks that were detected. Non-allelic peaks at 115bp and 103bp, as shown in *Figure 32*, were observed at locus D3S1358. This non-allelic peak was found in 17 out of 24 replicates analysed. Two other non-allelic peaks were observed in the red dye region. One peak was outside the marker area of the FGA locus and another peak was found at the FGA locus at 201bp and 213bp respectively as per *Figure 33*. These peaks were observed on 11 out of 24 replicates. These peaks did not have the characteristic morphology of allelic peaks and fell outside of allelic bins and were thus easily categorised as non-allelic or artefact peaks.

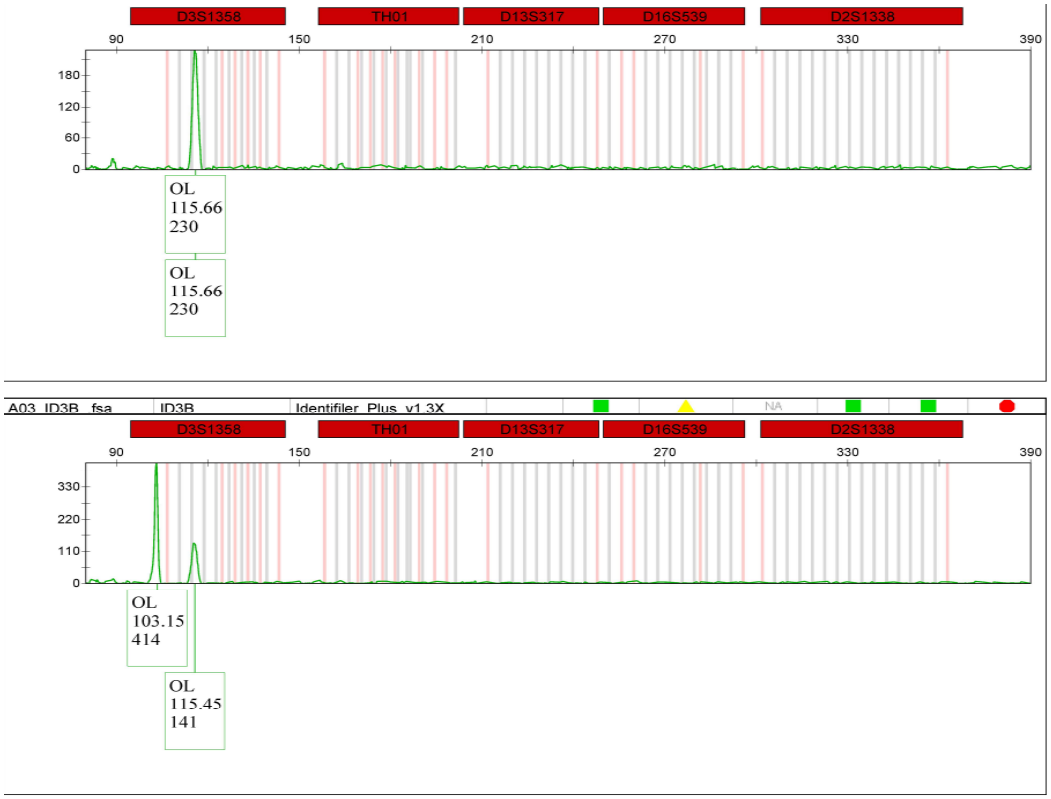

Figure 32: Off ladder peaks at the D3 locus obtained by direct PCR using Identifiler® Plus (28 cycles)

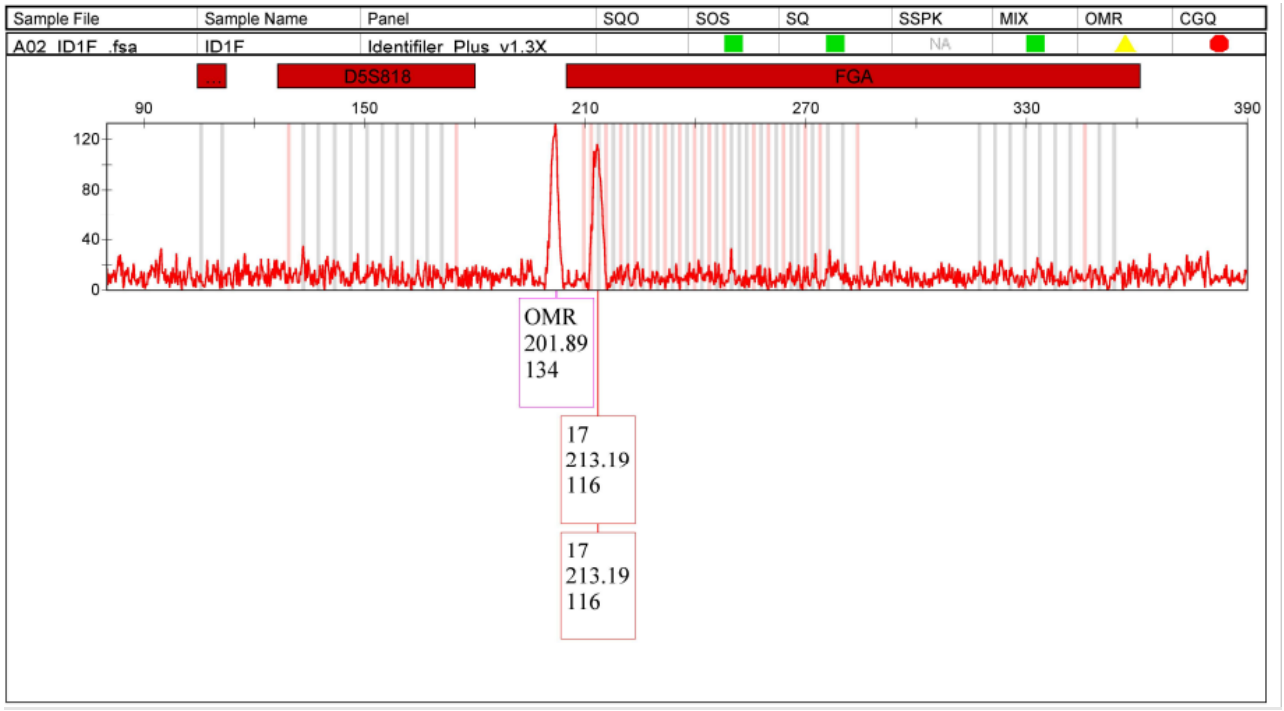

Figure 33: Two non-allelic peaks at FGA obtained by direct PCR using Identifiler® Plus (28 cycles)

3.7.3 Direct PCR using LCN

Due to the limited time for the completion of this research, direct PCR with LCN was performed with only two replicates per participant. Unlike the direct PCR with Identifiler® Plus

and Minifiler®, the LCN amplification detected a few alleles. The samples from participant 3 did not yield any DNA in direct PCR using the LCN method. For participant 4, only one allele at the amelogenin locus at 606rfu was detected. *Table 28* entails the alleles and peak heights obtained for each participant using LCN. An allele at the amelogenin locus was obtained in one replicate each of participants 1, 2 and 4. The peak height observed at each STR locus per replicate is illustrated in *Figure 34.* As discussed in the earlier sections, Identifiler® Plus is designed for profiling 15 STR loci and the sex determining amelogenin locus. In a full profile two alleles will be obtained per locus which makes a total of thirty alleles at STR loci and two alleles at the amelogenin locus.

Participant number_replicate	Loci	Allele 1	Allele 2	Height 1	Height 2
1_a	D8S1179	-	14	-	1124
1_a	D3S1358	15	16	1202	691
1_a	TH01	-	9.3	-	279
1_a	D19S433	12	15	803	527
1_a	vWA	14	-	771	-
1_a	AMEL	X	X	3865	3865
1_a	D5S818	12	13	394	275
1_b	D21S11	28	28	386	386
1_b	D7S820	10	10	393	393
1_b	D13S317	9	-	158	-
1_b	D19S433	12	15	487	762
1_b	vWA	14	-	1250	-
1_b	D5S818	-	13	-	409
1_b	FGA	24	24	396	396
2_a	D8S1179	-	13	-	571
2_a	D3S1358	15	17	298	283
2_a	vWA	15	15	327	327
2_a	AMEL	X	X	791	791
2_b	D8S1179	-	13	-	285
2_b	D18S51	14	-	522	-
2_b	AMEL	X	X	1093	1093
2_b	D5S818	11	11	520	520
4_a	AMEL	X	X	606	606

Table 28: STR profiling data obtained by direct PCR using LCN; a: replicate one and b: replicate 2 per participant

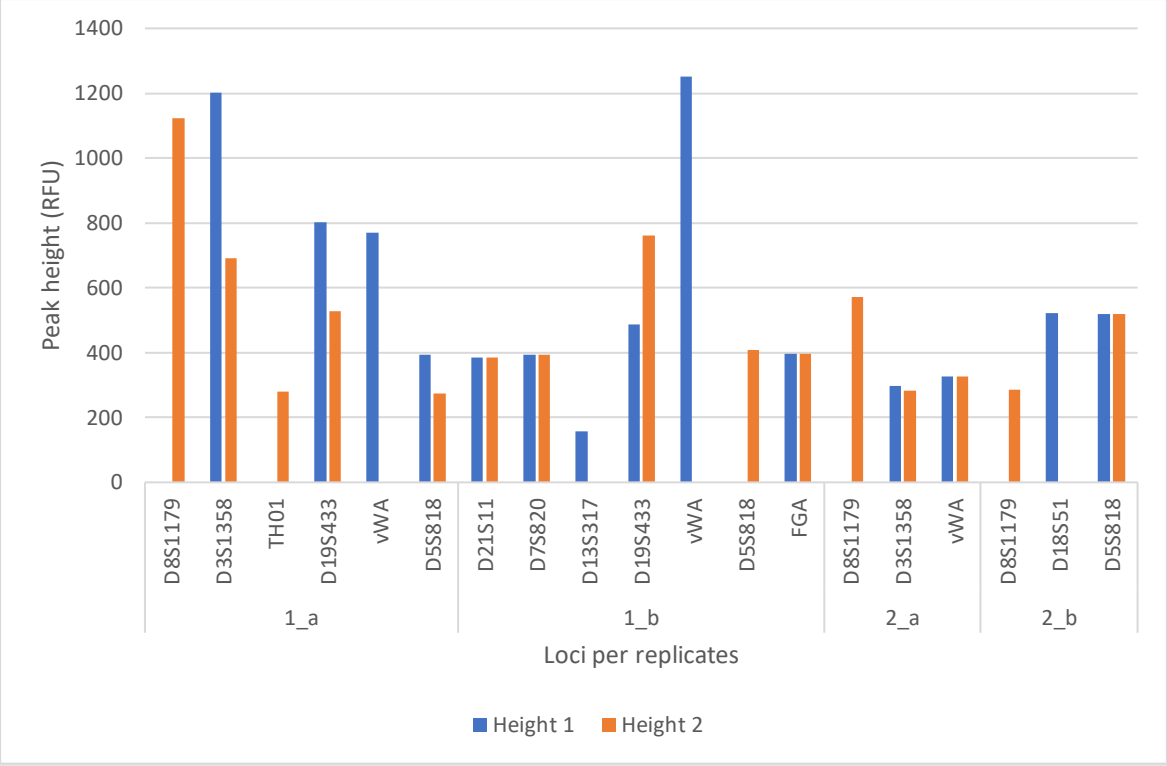

Figure 34: Graphical representation of peak heights obtained at each locus per replicate across all the participants; Height 1 and Height 2 stands for the height of allele 1 & 2 of each locus respectively.

CHAPTER 4

DISCUSSION

4.1 Introduction

Data published by the New Zealand police shows a large rise in firearm offences in 2021 in New Zealand. 1308 firearm offences were recorded in the country in 2021 (Church, 2022). The recorded number of cases in 2019, including the Christchurch Mosque attack, was 1142 (Church, 2022). This increase in gun violence has created distress among the public and police.

The firearm itself, ammunition and gunshot residue (GSR) are some of the main evidence types collected from firearm-related offences. Ammunition includes unfired cartridges and fired cartridge cases (*Basics on Firearms and Ammunition*, 2019). Cartridge cases are one of the main evidence types obtained from a crime scene after a gun related offence. The cartridge case obtained from a crime scene can provide multiple aspects of information during a criminal investigation. The finding of fingermarks on cartridge cases can indicate the possible presence of touch DNA on them, as detailed in chapter 1.

4.2 Visualisation of touch DNA on cartridge cases using diamond dye

4.2.1 The effect of visualisation of touch DNA on cartridge cases on fingermark enhancement and vice versa

The first objective of the research was to investigate the effect of visualisation of touch DNA using diamond dye on further fingermark enhancement on cartridge cases and vice versa. The study was conducted with the help of the New Zealand Police fingerprint section and the expertise of ESR. The cartridge cases that were sprayed with diamond dye and some that were not sprayed with diamond dye were forwarded to the New Zealand Police fingerprint section as detailed in section 2.9. The forwarded cartridge cases were analysed by a qualified fingerprint officer.

The amount of fluorescence or the fluorescence count on a cartridge case after the application of diamond dye was obtained using the ImageJ software following all the parameters as detailed in section 2.8. The differences and similarities in the amount of fluorescence are used to interpret the results. The quality of the fingermarks developed by the fingerprint expert also had a key role in the interpretation and the conclusions drawn.

Figure 35, gives a graphical representation for the comparison of the mean of the amount of fluorescence obtained for experiment 1 and experiment 2. This comparison provides an answer for the research question raised in objective 1.

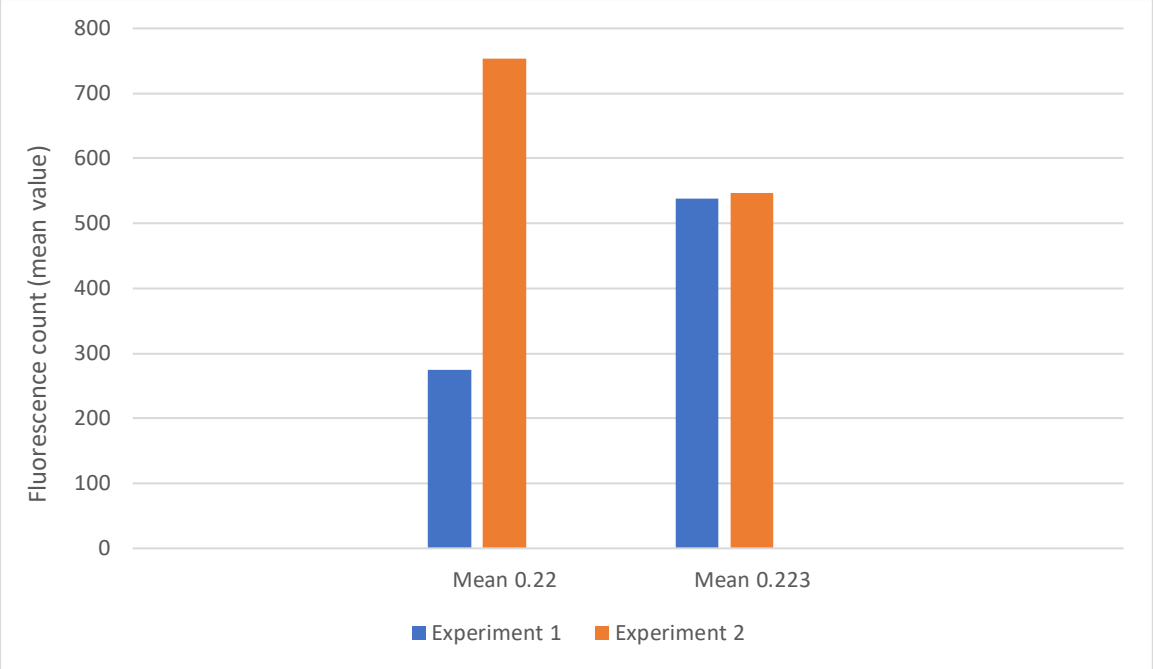

Figure 35: Graphical representation depicting the differences of mean values obtained during the visualisation of touch DNA on cartridge cases using diamond dye before and after the enhancement of fingermarks (Experiment 1 and experiment 2)

The size difference of the cartridge cases leads to a difference in the amount of fluorescence obtained in experiment 1. The cartridge case of calibre 0.223 is bigger in size and surface area and had more area of contact for the deposition of pristine fingermarks. The cartridge cases of calibre 0.22, which are considerably smaller in size and surface area, had less area of contact for the deposition of pristine fingermarks. The mean values reflect the differences in the amount of fluorescence observed on cartridge cases of both the calibres. The fluorescence emitted by the diamond dye on cartridge cases showed the location of the deposition of fingermarks. The rest of the areas on the cartridge cases without fingermarks did not emit any fluorescence.

In experiment 2, fingermarks already developed on cartridge cases were subsequently sprayed with diamond dye and then visualised. The fluorescence emitted was observed in the pattern of developed ridge detail as smears rather than dots. The fluorescence was observed on the entire fingermark deposited. The similarity in the calculated mean values of the fluorescence count observed on cartridge cases of calibres 0.22 and 0.223 is mainly due to the fluctuations in the individual values. *Figure 36* shows the differences in the images documented for experiment 1 and experiment 2.

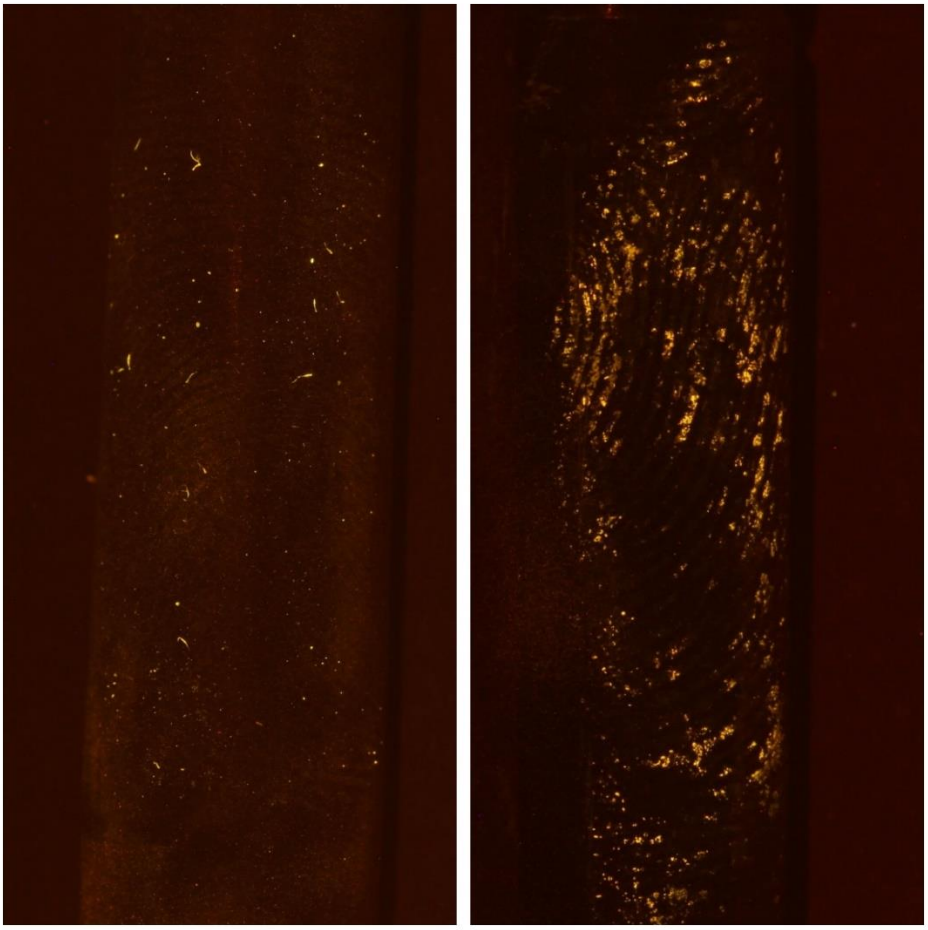

Figure 36: An example of images documented displaying the differences in observation for experiment 1 (left) and experiment 2 (right). (In experiment 1 fluorescence is observed as distinctive dots and in experiment 2 it is observed as smears along the enhanced ridges).

The first objective investigated the possibilities of visualising touch DNA before and after the enhancement of fingermarks. As per the information obtained from the fingerprint expert, as detailed in section 3.2, some of the cartridge cases pre-treated with diamond dye showed better ridge details in the subsequent enhancement of fingermarks using VMD and superglue with VMD. This was not the case for all of the cartridge cases analysed. The uncertain nature of fingermarks and the inconsistencies in the transfer of ridge details are some of the main reasons for the varying results.

The visualisation of DNA using diamond dye after the development of fingermarks did not give a reliable fluorescence count due to the smeary non-dotted nature of the fluorescence. The fluorescence count of all the replicates in experiment 2 fluctuated, and variable values were obtained using ImageJ, especially for cartridge cases of calibre 0.22. The differences in the standard deviation calculated from the amount of fluorescence of experiment 1 and experiment 2 supports this inconsistency (*Figure 37*). The inconsistencies in the values diminish the

reliability of the count. In an effort to improve the reliability of the visualisation, it would be better to visualise the DNA prior to fingerprint enhancement. This is possible as diamond dye had little impact on enhancement of fingermarks on cartridge cases using VMD.

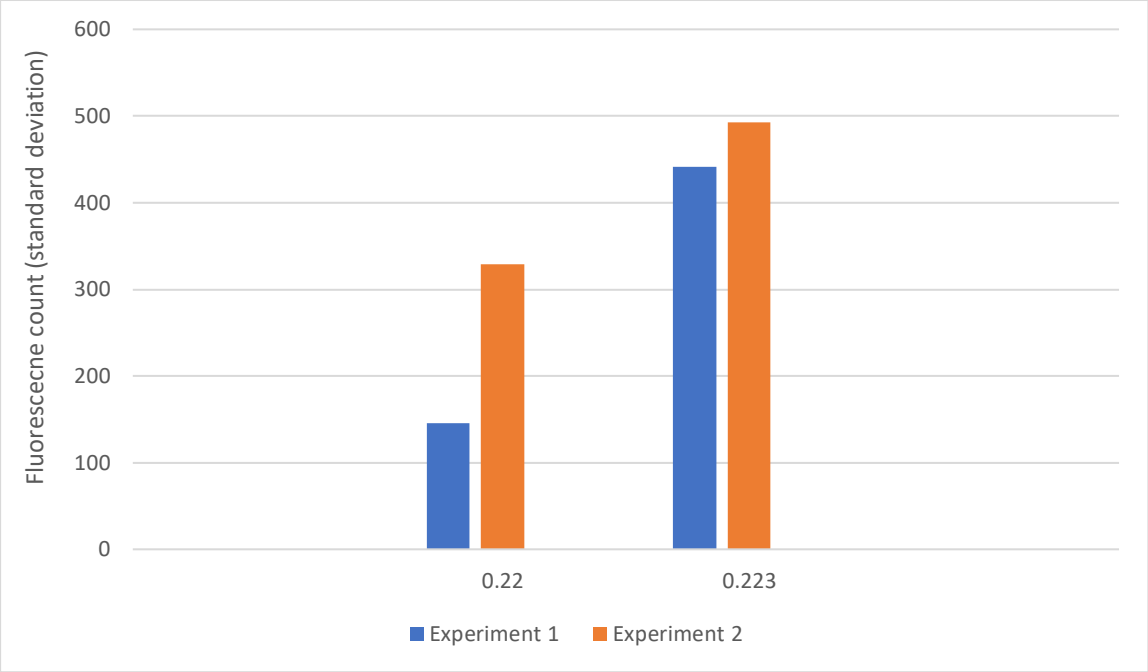

Figure 37: Graphical representation depicting the differences in standard deviation obtained during the visualisation of touch DNA on cartridge cases using diamond dye before and after the enhancement of fingermarks

It is not possible to recreate the same ridge detail on all of the samples. The quality of ridge detail on fingermarks depends on multiple factors and these factors often lead to inconsistencies in the deposition of fingermarks. The quality of the fingermarks deposited by the same finger on multiple locations will show variations based on multiple factors such as the surface of deposition and the pressure. This inconsistency in the quality of the recreation of fingermarks makes it difficult to generalise the findings. This finding is applicable to all of the samples analysed for this study as per the findings shared by the fingerprint expert.

The visualisation of cellular material using diamond dye can only act as an indicator to show the presence of touch DNA. The visualisation alone is not conclusive evidence for the identification of the person, DNA profiling is required. Further DNA profiling has to be conducted to confirm that the cellular material obtained is of human origin and to identify the source of the sample (Kanokwongnuwut et al., 2018). The literature has indicated that bacterial DNA does not interfere with visualisation of eukaryotic DNA using diamond dye (Kanokwongnuwut et al., 2018).

The existing literature has shown the use of diamond dye to visualise touch DNA before and after the use of aluminium and white powders for the enhancement of fingermarks (Kanokwongnuwut et al., 2019). The surfaces used for the study were glass slides, debit cards, bank notes, soda cans and cardboard. Hence the findings of the above-mentioned literature cannot be directly applied to cartridge cases.

4.2.2 The effect of the process of firing on the presence of touch DNA on cartridge cases

The second objective of this research investigated the effect of the process of firing on the presence of DNA on cartridge cases using diamond dye. To determine the effect, unfired cartridges and fired cartridge cases, both with fingermarks, were sprayed with diamond dye and observed for fluorescence. In the results it can be observed that the process of firing has reduced the amount of fluorescence considerably apart from replicate two for cartridge case of calibre 0.223 (*Figure 38*).

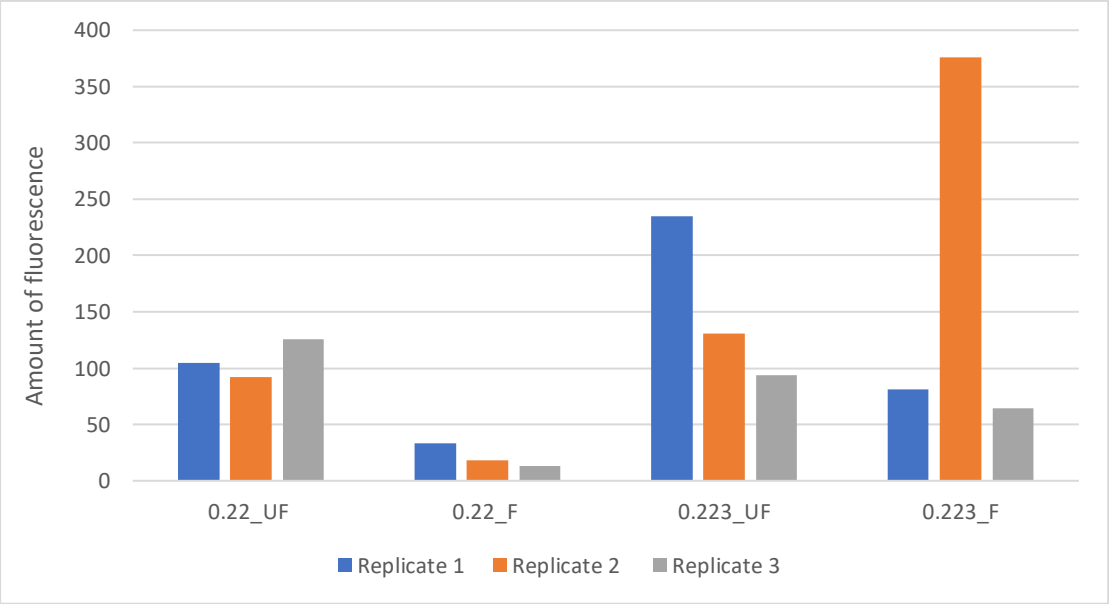

Figure 38: Graphical representation of the amount of fluorescence obtained across all the replicates analysed for objective 2; UF: unfired cartridges and F: fired cartridge cases.

The process of firing exposes the cartridges to high temperatures and pressure. The outer surface of the cartridge cases will also come into contact with multiple surfaces during the processes of loading it into the firearm to discharging after firing. The friction, high temperature, high pressure and the exposure to the primers and propellent will diminish the chances of finding good quality DNA evidence from the fired cartridge cases (Malanio et al., 2020). The environmental factors of the crime scene from which cartridge cases have been

collected can also have an effect on the persistence of DNA on them. The humidity and atmospheric temperature of the crime scene are some of the environmental factors that affects the persistence of DNA on a surface (Poetsch et al., 2022). The time period from handling the cartridge case that resulted in the deposition of touch DNA and the visualisation could also impact on the results. Transfer and persistence of touch DNA are some of the important determining factors that aids the visualisation using diamond dye. The metal surface of the cartridge case and the environmental factors together have a detrimental effect on the persistence of touch DNA. The results obtained from investigating objective two clearly indicates the differences in the amount of fluorescence obtained before and after firing.

The second replicate analysed for fired cartridge cases of calibre 0.223 shows a significant difference compared to the other results obtained. Despite the large amount of existing literature, it is hard to find out one particular reason for the variation in DNA shedding ability of an individual as it is affected by many factors (Alketbi, 2018; Tobias et al., 2017). But if the spike in the value was due to variation in natural shedding, the fluorescence should have been concentrated where the fingermark was deposited.

In this case the fluorescent dots were scattered across the entire cartridge case rather than concentrated just where the fingermarks were deposited, as shown in *Figure 39*. This supports the possibility of contamination occurring at some point. The use of a negative control, a cartridge case with no fingermarks treated with diamond dye, gave a zero-fluorescence count. The zero count of the negative control demonstrates that the diamond dye is unlikely to have been contaminated.

The chamber of the firearm was cleaned using a swab moistened with 70% ethanol before the first fire. This spike in the count could be due to the accumulation of DNA on the chamber due to the firing of multiple cartridge cases. After firing, the cartridge cases were ejected onto a piece of clean white paper placed on the ground. Any contamination on the paper and accidental contact of the cartridge case to the ground might have also caused this potential contamination of the cartridge case. Since the fluorescence was distributed across the entire cartridge case and the value is significantly greater as compared to the other replicates, the fluorescence observed might be an unexplained outlier. Hence, this value was omitted from further analysis. The replicated were randomised and that might be the reason for not finding this outlier on other replicates.

Figure 39: Fired cartridge case observed under polilight that presented as an outlier

Apart from the one replicate that gave a higher amount of fluorescence after firing, the results obtained support the existing literature. Existing literature points out that the amount of touch DNA is considerably decreased during the process of firing (Elisha Prasad et al., 2020, 2022). This has an impact on visualisation and STR profiling.

Visualisation of touch DNA using diamond dye can clearly indicate if the amount of touch DNA present on a surface is sufficient to perform DNA profiling (Piyamas Kanokwongnuwut et al., 2021). Research by Kanokwongnuwut et al, concluded that the amount of fluorescence of 1000 using diamond dye would result in 9-25 alleles in further STR profiling (Kanokwongnuwut et al., 2021). The fluorescence amount of 40-100 would likely result in one allelic peak. With the amount of fluorescence obtained for this research, where the highest value obtained was 996, as detailed in chapter 3, the chances of getting more than eight alleles is limited.

The visualisation of touch DNA using diamond dye can act as a cue for the possible recovery of DNA (Kanokwongnuwut et al., 2021). Under standard amplification protocols 500pg, that is 80 diploid cells, can give a complete STR profiling result based on the sample type. In some sample types, such as blood, less than 500pg could give a complete profile. The visualisation of DNA aids in this matter. With the smallest of chances of finding any DNA on fired cartridge cases, as illustrated in *Figure 37,* attempting DNA profiling might lead to a wasted effort.

4.3 Extraction and Amplification of Touch DNA From Cartridge Cases

The third objective of this research compared DNA IQ™, which is the standard extraction protocol used at ESR, with direct lysis and pre-extraction soaking, both coupled with QIAamp®. The fourth objective was to investigate different DNA multiplexes for STR profiling. The research investigated Identifiler® Plus (28 cycles), Minifiler® and Identifiler® Plus LCN (34 cycles) for direct amplification. The samples that were extracted were amplified using Identifiler® Plus (28 cycles) and LCN (34 cycles).

The extraction of DNA using DNA IQ™ kit is the standard protocol as used at ESR. Hence, the DNA IQ™ kit acts as a baseline for the comparison of other methods investigated under this research. As per the results obtained, only one allele was obtained using DNA IQ™ across all the replicates from all four participants used. The standard protocol of DNA IQ™ uses double swabbing (wet and dry) for the collection of the DNA. Existing literature has described the disadvantages of the swabbing techniques due to the ability of swabs to retain DNA (Elisha Prasad et al., 2022). While extracting trace amounts of DNA, it is advisable to minimise the loss of DNA during the extraction procedure. The results obtained from the DNA IQ™ extraction will be compared with the results of pre-soaking extraction and direct lysis, both coupled with QIAamp®. While the DNA IQ™ extraction kit uses paramagnetic beads for the separation and isolation of DNA from inhibitors, the QIAamp® kit uses a silica spin column to achieve the same.

4.4 Pre-extraction Soaking

The pre-extraction soaking coupled with DNA extraction using QIAamp® followed the procedure set out by (Dieltjes et al., 2011).In the research undertaken by Dieltjes et al, the cartridge cases were soaked in ATL buffer for 30 minutes. DNA extraction from the ATL buffer in which the cartridge cases were soaked was extracted using QIAamp® (using a silica spin column). The amplification of STR loci was achieved using Powerplex® 16. Dieltjes et al investigated the reproducibility of the profiling results. A profile was called reproducible, according to their study, if the genotypes of an STR loci were reproduced on a minimum of two independent PCR reactions, up to five PCR reactions (Dieltjes et al., 2011).

As mentioned earlier, the current research was structured by referring to Dieltjes et al and followed the same soaking and extraction procedure. The amplification, unlike Dieltjes et al, was performed using standard Identifiler® Plus (28 cycles) and LCN (34 cycles). The same soaking time of 30 minutes was followed as per the reference (Dieltjes et al., 2011).

During the 30 minutes soaking of cartridge cases of calibre 0.223, the colour of the ATL buffer changed to blue from colourless as observed by Dieltjes et al. The blue colour is caused due to the oxidation of the cartridge case due to a reaction with the ATL buffer. The intensity of the blue colour was different for the two different cartridge cases of calibre 0.22 and 0.223. The ATL buffer changed to a slight greenish tint with blue when cartridge cases of calibre 0.22 were soaked. According to Dieltjes et al, the differences in the blue colour change could be due to the differences in the alloys used for the manufacture of the different cartridges (Dieltjes et al., 2011).

The oxidation on the surface of the cartridge cases might damage evidence such as striation marks and firing pin impressions. To avoid the possible destruction of this evidence, the cartridge cases were swabbed using a sterile swab after soaking and were then air dried and rinsed using 96% ethanol. *Figure 40* shows the microscopic comparison of the head stamp impression of a 0.22 cartridge case that was not rinsed with ethanol compared to a cartridge case that was rinsed with ethanol after soaking. No significant difference was observed in the microscopic detailing of the firing pin impressions when the rinsing with 96% ethanol step was either completed or not, but the ethanol rinse significantly reduced the dulling of the metal. Prasad et al (2019) conducted a study to investigate the effect of soaking in ATL buffer in striation marks on cartridge cases and concluded that the ATL buffer has no detrimental effect on the microscopic detail of the striation marks (Prasad et al., 2019). The results obtained in this study support the findings from Prasad et al.

Figure 40: Head stamp impression of cartridge cases after soaking, observed under the microscope (left: subjected to soaking with no rinsing step, right: rinsed with 96% ethanol after soaking)

The DNA profiling results obtained by Dieltjes et al showed a success rate of 6.9 % per item and a number of 10.9 alleles per profile on average. They also concluded that reproducible results, as per their interpretation, were obtained from 40-60pg of DNA without changing PCR standard conditions.

In this research, the quantitation of the sample extracts was done using Quantifiler® Trio and amplification was done using Identifiler® Plus (28 cycles) and LCN (34 cycles) using the Identifiler® Plus kit. The quantitation results obtained from the sample extracts showed a very low amount of DNA. Only two replicates out of six from participant 1 and one replicate out of six from participant 2 gave values upon the analysis in Quantifiler® Trio. The quantity of DNA on all the other replicates was undetermined. Even though the three replicates showed some value in the quantity of DNA, the values obtained were less than 0.0005ng/µl. According to the validation studies of the Quantifiler® Trio Kit at ESR, if the quantity of DNA is less than 0.0005ng/µl the presence of human DNA cannot be distinguished from that of background noise. The amplification using Identifiler® Plus (28 cycles) following the standard PCR cycle conditions as per the manufacturer's protocol gave zero alleles. The amplification using LCN gave three alleles, two from the first replicate of participant 1 and one allele was obtained from replicate five of participant 1. In short, only two replicates from participant 1 gave allelic peaks using LCN across all the replicates from all the participants. By comparing the amplification result with the quantitation values, it is observed that only one replicate that showed a quantity of 0.0002ng/µl ended up showing allelic peaks. But still the quantitation value obtained cannot be used to distinguish the presence of human DNA on its own as it is below the cut-off and the other replicates that reflected the same value did not give any alleles upon amplification.

While comparing the results obtained for this research with the conclusions of Dieltjes et al, it can be observed that the improvement reflected in the reference article is not observed in the current research (Dieltjes et al., 2011). No statistically significant results were obtained in this current research to at least do a percentage comparison. The comparison of the number of alleles obtained in the soaking and DNA IQ™ methods cannot be statistically distinguished and interpreted. Also, Dieltjes et al used cartridges, bullets and cartridge cases (CBC) from actual cases for their study while this research was done with just cartridge cases having fingermarks from participants.

4.5 Direct Lysis

Direct lysis was one of the techniques investigated under this research. The idea was drawn by referencing the study published by (Moore et al., 2020). The study compared the conventional double swabbing technique with a semi-automated extraction using direct lysis. The current research followed the same procedure of direct lysis from Moore et al (Moore et al., 2020).

The article by Moore et al was used as the reference article for the preparation of the lysis buffer. The reference literature used an automated Hamilton® Microlab STAR® AutoLys® robot for the extraction after lysis (Moore et al., 2020). As there was no access to a Hamilton® Microlab STAR® AutoLys® robot this current research undertook a manual extraction, after the direct lysis from cartridge cases, using QIAamp®. According to the procedure, the cartridge cases were incubated at 56°C in the lysis buffer for 15 minutes. During the incubation it was observed that the colour of the lysis buffer changed to greenish blue, the same as the colour change observed for the soaking of cartridge cases of calibre 0.22 in ATL. This colour change is due to the oxidation of the metal (Dieltjes et al., 2011). To avoid any possible damage to the metal, after the direct lysis, the cartridge cases were wiped down with a paper towel and rinsed using 96% ethanol. After soaking the surface of the cartridge cases were swabbed and that swab was added to the lysate according to the procedure followed. In direct lysis the cartridge cases were centrifuged using a spin basket. Spinning it down removed all the lysis buffer hence the surface was wiped using a paper towel just to remove any residue.

Figure 41 shows the microscopic comparison of the head stamp impressions of a 0.22 cartridge case that was not rinsed with ethanol after direct lysis and a cartridge case that was rinsed with ethanol after direct lysis. No significant difference was observed in the microscopic detailing of the firing pin impressions when the rinsing with 96% ethanol step was completed compared to when it wasn't; however, as observed with the soaking step, the ethanol rinse has significantly reduced the dulling of the metal. In summary, the ethanol rinse of the cartridge cases after direct lysis and soaking will help to maintain the quality of the cartridge cases by preventing oxidation.

Figure 41: Head stamp impression of cartridge cases after direct lysis observed under the microscope (left: subjected to soaking with no rinsing, right: rinsed with 96% ethanol after soaking)

Moore et al, using direct lysis, compared the DNA profiling results obtained from unfired and fired cartridge cases. According to Moore et al, quantitation of the sample extracts was done using the PowerQuant™ system and the amplification was done using PowerPlex® ESI 17 Fast System, both by Promega (Moore et al., 2020). Their research compared the results obtained from both unfired and fired cartridge cases using direct lysis with the results obtained from swabbing. The results obtained by Moore et al conclude that interpretable DNA profiles, allelic peak of 40rfu or more, were obtained from unfired and fired ammunition where unfired cartridges gave better results. They also conclude that the direct lysis method can give better results than swabbing.

In this current research, only one replicate out of three from participant 1 and two replicates out of six from participant 4 gave values after quantitation using Quantifiler® Trio. From the obtained values only the quantity of one replicate (third replicate) of participant four crossed the ESR cut-off of 0.0005ng/µl. The amplification of sample extracts using Identifiler® Plus did not detect any alleles. Through the amplification using LCN eighteen alleles were detected across all the replicates from all four participants. In a complete profile 30 alleles from 15 STR loci and 2 alleles from the sex determining amelogenin locus are obtained. But in this research, the maximum number of alleles obtained from one profile was seven alleles. For the replicate in which a value of 0.0009ng/µl was detected in quantitation, upon amplification only one allele was detected.

A direct comparison of results obtained for this current research and Moore et al cannot be done due to the differences in systematic approaches. Moore et al used touch DNA samples on 9mm cartridges and cartridge cases for their study while this research used Touch DNA samples cartridge cases of calibre 0.22. The current research did a manual extraction and amplified the sample extracts using Identifiler® Plus (28 cycles and 34 cycles) while Moore et al used PowerPlex® ESI 17 Fast System. The differences in the sensitivity and specificity in the amplification kits makes it hard to do a direct comparison. A comparison of results can be done within this research with direct lysis coupled with QIAamp® and swabbing coupled with DNA IQ™ which is used as the baseline for comparison. The result obtained from DNA IQ™ and direct lysis cannot be interpreted statistically in this research due to the low yield in DNA.

4.6 Direct Amplification from Cartridge Cases

In total this research investigated three different amplification techniques for direct amplification which were Identifiler® Plus (28 cycles), LCN (34 cycles) using Identifiler® Plus and Minifiler®. The Identifiler® Plus amplification kit is designed to profile fifteen STR loci and the Minifiler® kit is designed to profile eight STR loci.

As detailed in section 3.6, three artefact peaks were observed in the direct amplification results using Identifiler® Plus using standard cycling conditions. These peaks were not seen in the LCN amplification that used the same Identifiler® Plus kit for direct PCR. The peaks observed have peak heights of less than 200rfu which is below the analytical threshold for templates. The analytical threshold is the minimum peak height to call a peak an allele rather than baseline. This value is obtained from validation studies. At ESR100rfu is the detection threshold for standard analysis and for LCN it is 150rfu for the blue and green dyes and 250rfu for the red and yellow dyes. These peaks are not listed in the artefacts by the manufacturer so further research would need to be conducted to further interpret the results obtained. These results cannot be replicated as the direct PCR don't have samples to go back to.

As detailed in the results in chapter 3, alleles were obtained using the LCN amplification method only. The detection of a low template profile is possible using LCN as the optimal quantity of DNA required to obtain a full profile is 50-60pg. The results obtained are not sufficient for a quantitative statistical interpretation, similar as to the results obtained for the extraction procedures. The peak heights of the alleles obtained (*Figure 34*) demonstrates that the results obtained are subjected to stochastic effects. The relatively low peak heights raise questions related to the reproducibility of the alleles which is generally required for LCN

analysis to confirm that an allele is inherent to a sample and is not drop in. This reproduction, or replication, of results is however not possible with direct amplification. Further validation is needed to admit the reliability of the allelic profile obtained using LCN amplification which uses more cycle numbers than the standard procedure. Some of the issues associated with using an LCN amplification is detailed in the section below.

The literature points out many challenges in using LCN as an amplification method. Some of the challenges associated with LCN amplification are stochastic effects, reproducibility and contamination (Forster et al., 2008). The inconsistencies in the allelic profile obtained among replicates can be defined as stochastic effects in amplification. This can be due to allelic drop-out, heterozygous imbalance and the detection threshold. Allelic-drop out is a major phenomenon associated with amplification using LCN. In allelic drop-out, the samples that were expected to be amplified are not amplified due to the low template observed. The challenges in reproducing the same alleles in multiple amplifications will question the scientific validity of the result. The high sensitivity of the LCN method can detect internal and minute contaminations. The allelic drop-in is a form of contamination that might interfere with the results (Forster et al., 2008; Gill, n.d.).

4.7 Factors that may have resulted in the low yield of DNA profiles from cartridge cases

The results obtained in this research show a poor recovery of DNA from cartridge cases. The reason for the results cannot be confined to one factor. The multiple factors that may have resulted in the low yield of DNA profiles across all the participants are briefly discussed in this section. The variation in the DNA shedding of the individuals, as discussed earlier, will cause variations in the amount of DNA being deposited onto the cartridge cases. Degradation of DNA is another factor that might have caused the poor recovery. The degradation index can be calculated by dividing the quantities of Large Autosomal by the quantity of Small Autosomal estimated using Quantifiler® Trio (Elisha Prasad et al., 2022). The quantitation values obtained in this study were not used to calculated degradation index as the values were below the cut-off.

The packaging material used for the collection and transportation of the objects for the recovery of DNA has significant impact on the transfer and persistence of DNA on that surface (Goray et al., 2012). Cartridge cases, after deposition of fingermarks, were collected in a paper envelope. The constant contact of the surface of the cartridge case with the paper might have

caused transfer of DNA from the surface to the paper. This could have been minimised by collecting the samples from the participants directly into the extraction tube but that would not be a true reflection of real crime scene scenarios.

The interaction of the negatively charged DNA with the metal surface can cause significant degradation of DNA making it hard to detect. Metal surfaces are demonstrated to have less persistence of touch DNA due to the non-porous surface not trapping the DNA as much compared to the porous surfaces. The less persistence and degradation might be an increase in the intensity of DNA loss (Bonsu et al., 2020). Studies have been conducted by spiking the cartridge cases with saliva and cell lines to improve DNA profiling results from cartridge cases but that cannot be applied to criminal investigations (Elisha Prasad et al., 2022).

CHAPTER 5

CONCLUSION

The spike in the rate of gun violence increased the necessity of having improved research in the collection of reliable evidence from firearms and ammunition. DNA, which is the biological identity of a person would aid the criminal investigation by identifying its source. The recovery and profiling of touch DNA from cartridge cases are an active area of research for many jurisdictions. This research was structured based on four objectives and the results observed after investigating those four objectives are used to draw the following conclusions.

The first objective of the research was to investigate the effect of visualisation of DNA using diamond dye on cartridge cases on further enhancement of fingermarks and vice versa. The objective was studied using two experiments where the first experiment studied the effect of visualisation of touch DNA using diamond dye with subsequent enhancement of fingermarks on cartridge cases. The second experiment investigated the effect of enhancement of fingermarks on the visualisation of touch DNA using diamond dye on cartridge cases. After conducting necessary analysis, it is concluded that it is better to visualise the presence of DNA with diamond dye prior to the enhancement of fingermarks using VMD as the enhancement of fingermarks using VMD had a significant impact on the visualisation of DNA with diamond dye.

The second objective of this research investigated the effect of the process of firing on visualisation of touch DNA on cartridge cases using diamond dye. The results obtained by analysing the cartridge cases before and after firing shows that the cartridge cases observed after firing have a lesser amount of fluorescence compared to the unfired cartridges. Hence it is concluded that the process of firing has significant impact on the presence of touch DNA on cartridge cases. This conclusion can be validated further by increasing the number of participants and by increasing the number of replicates analysed for each participant.

The third and fourth objectives of this research were structured to improve the DNA profiling results from cartridge cases using three different extraction techniques and direct PCR. The three extraction methods investigated under this study were swabbing coupled with DNA IQ™, pre-extraction soaking coupled with QIAamp® and direct lysis coupled with QIAamp®. The samples extracted using all the techniques were amplified using Identifiler® Plus and LCN using Identifiler® Plus. Under this study allelic peaks were only obtained for samples amplified using Identifiler® Plus LCN. Zero alleles were obtained in amplification using Identifiler® Plus following the standard PCR cycling conditions.

The results obtained for the three techniques of swabbing, soaking and direct lysis from this research cannot be interpreted quantitatively using statistics due to the very limited yield in DNA. A qualitative interpretation can be given with the number of alleles obtained from all extractions across all the samples from all the participants. The results obtained across all the replicates from all of the participants using swabbing (wet and dry) coupled with DNA IQ™ yielded one allele, pre-extraction soaking coupled with QIAamp® yielded three alleles and the direct lysis coupled with QIAamp® yielded eight alleles.

The efficacy of direct PCR in the recovery of DNA from cartridge cases was investigated using Identifiler® Plus, Minifiler® and LCN using Identifiler® Plus. No alleles were obtained from direct PCR using Identifiler® Plus and Minifiler®. 29 STR alleles were obtained from direct PCR using Identifiler® Plus LCN across all the replicates from all the participants.

In summary, with the results obtained after investigating objective one it can be concluded that the visualisation of DNA using diamond dye on cartridge cases will give better results if done before the enhancement of fingermarks. After investigating objective two it can be concluded that the process of firing has an impact on visualisation of DNA using diamond dye on cartridge cases. With the results obtained after investigating objectives three and four, by comparing the results obtained from extraction it can be concluded that a greater number of alleles were obtained using direct lysis and a lesser number of alleles were obtained using swabbing even though the values are not sufficient to statistically distinguish the results. With the results of direct PCR from cartridge cases using Identifiler® Plus, Minifiler® and Identifiler® Plus LCN it can be concluded that alleles can be obtained using LCN. But the alleles obtained cannot be reproduced as the direct PCR uses the entire swab for amplification and that is the main drawback of direct amplification, especially for the LCN method that demands replication as a factor of admissibility.

CHAPTER 6

FUTURE RESEARCH

As discussed in earlier chapters, this research was structured based on four objectives. The first two objectives focused on the visualisation of touch DNA using diamond dye on cartridge cases. The third and fourth objectives were structured to improve DNA profiling results from cartridge cases. There are a few aspects that could not be included in the research due to time and resource constrains. Hence there were a few gaps identified which would lend themselves for future research. The results obtained also pointed out areas for future research. This chapter entails potential areas of future research that can be undertaken for the visualisation of touch DNA on cartridge cases using diamond dye and the profiling of touch DNA from cartridge cases.

1. ***Further research on the STR profiling of touch DNA on cartridge cases after visualisation using diamond dye after the enhancement of fingermarks***

This research investigated the visualisation and STR profiling of touch DNA on cartridge cases as two independent areas of research. Existing literature on the effect of fingerprint development methods on further DNA profiling was used to draw conclusions. The results obtained draw attention to the possibility of obtaining better results for the enhancement of ridge details on cartridge cases pre-treated with diamond dye. Hence, further research could be conducted on improving the DNA profiling results from cartridge cases pre-treated with diamond dye and subjected to enhancement of fingermarks using VMD.

2. ***The deposition, visualisation and enhancement of fingermarks at various time intervals***

This research followed a consistent time interval between handwash and deposition of fingermarks on cartridge cases. This research did not account the change of results based on the time of observation as the same time interval was used for all participants. The same study on visualisation of touch DNA on cartridge cases using diamond dye could be structured as a time period study. Thereby, investigating all the objectives of this study under different time intervals and seeing if a large period of time between deposition and enhancement has an impact on the amount of fluorescence detected.

3. ***Further research associating the amount of fluorescence observed with STR profiling***

As detailed in the discussion, the amount of fluorescence indicates the presence of touch DNA. There are only limited studies associating the amount of fluorescence observed using diamond dye on cartridge cases on further STR profiling. Undertaking research in this area would help in understanding the variations in results with different fluorescence counts.

4. ***Further research on the first two objectives with more participants and replicates***

The research on the first two objectives based on the visualisation of touch DNA on cartridge cases was conducted with the fingermarks deposited by the researcher. The study could be expanded by increasing the number of participants and replicated in order to decrease the impact the variation in the DNA shedding ability of individuals has on the results. Hence the study will be applicable across a large group of the population.

5. ***Necessity of conducting further research on the packaging materials used for the collection and packaging of cartridge cases.***

When it comes to cartridge cases that already haves a poor record in the recovery of DNA, it is very important to protect the maximum amount of recoverable evidence from the time of collection. As discussed in the previous chapter, the contact of the outer surface of the cartridge case with the packaging material might result in the transfer of DNA from it. Further studies need to be conducted to improve the collection and transportation of cartridge cases to maximise the result.

6. ***Further research on direct lysis using QIAamp® coupled with LCN amplification.***

Compared to all the three extractions investigated under this study, direct lysis coupled with LCN amplification gave slightly better results. Even though the procedure seemed to have given slightly better results, a statistical interpretation of the results obtained was not able to be undertaken. So, further research could be conducted to improve the profiling results from cartridge cases using direct lysis coupled with an LCN amplification.

7. ***Further research to validate the results using a greater number of participants***

Objectives three and four were investigated using four participants. Further validation studies can be conducted by increasing the number of participants and increasing the number of replicates. A greater number of participants and samples would help to generalise the results with more admissibility.

CHAPTER 7

APPENDICES

APPENDIX I

SCIENCE SCHOOL OF CHEMICAL SCIENCES

SCHOOL OF CHEMICAL SCIENCES
Faculty of Science Centre,
Building 301
23 Symonds St
Auckland
Ph. (09) 373 7599

Participant Information Sheet

Improving methods to obtain DNA profiling results from cartridge cases.

Research Team

Student Researcher: Meghna Suresh - Candidate for the School of Chemical Sciences MSc in Forensic Sciences at the University of Auckland

Principal Investigator: Dr Douglas Elliot - Director Forensic Science Program at the University of Auckland and Team Leader, AFSC at ESR Ltd.

Co-supervisor: Jennifer Howarth - Senior Scientist, Forensic Biology at ESR Ltd.

Co-supervisor: Leah Tottey - Senior Scientist, Physical Evidence team at ESR Ltd.

Invitation and Background

This is an invitation to participate in a research project of importance to forensic science. You have received this invitation after reading the summary that was emailed to you where you have decided that you have an interest in participating.

Firearms and cartridge cases themselves constitute a portion of potential evidence in a criminal investigation. It could be striation marks, firing pin marks, fingerprints and even touch DNA. Fingerprints are also an important piece of evidence like DNA and, getting both without one affecting the other is important. DNA deposited onto a surface by touch is termed as either 'Touch DNA' or trace DNA. Since this touch DNA is not visible, it is hard to know the possible location of DNA deposition and how much DNA may have been deposited.

DNA extraction plays an important role in the successful profiling of DNA. There are different standard and emerging techniques for DNA extraction. This study will also be investigating different amplification techniques to find which amplification technique gives better results

with different extractions. This project will investigate alternative DNA extraction methods to improve the success rates of DNA profiling and minimise wasted effort and generate better investigative leads for the NZ Police.

Participant Procedures

Please wash your hands thoroughly 45 minutes before opening the packaging. Clean any surfaces that might meet the samples and packaging before and after collection. The total sample collection will not take more than 2 hours. You might be asked to repeat the sample if necessary.

By participating in this study and signing the consent form, you are agreeing to wash your hands, wait for up to 45 minutes, deposit your finger marks onto cartridge cases given in the participant pack. Also, you are agreeing to deposit the fingermarks on to the cartridges and place the cartridges into the chamber of the firearm that is with the firearm expert. In order to maintain your anonymity, an assigned third person will collect the consent form and reference sample and will give you a unique alpha numerical code. This is to ensure that the link between you and your DNA profile remains anonymous. You will be provided with a deposition instruction sheet, buccal swab, cartridges, cartridge cases and a questionnaire. Kindly follow the instructions on the deposition instruction sheet and deposit your finger marks onto the given samples. A questionnaire will be given to record the time of handwash and the surfaces of contact before the deposition of the finger marks. After the procedure return the samples as instructed.

Participant rights

Each participant in the study will be randomly assigned an identification number so that the information you provide and the deposited biological fluid samples, can be kept together without identifying you. This information will only be accessible by an independent person. Consent forms will be kept in a locked cabinet at the Institute of Environmental and Science Research Ltd. for six years. At the end of the six years, they will be destroyed by the Principal Investigator (Douglas Elliot).

Information and samples given by you will not be used for any additional research purposes beyond the scope of this project. Any samples that are supplied by you will be destroyed during the course of the research, no later than November 2022.

Data generated in this research will be used for the completion of a MSc thesis, and may subsequently be published in a scientific journal and/or be used in the development of a commercial service but will be untraceable to you.

Participation in this research does not carry any significant risk, although if there is an adverse reaction to any of the surfaces halt the experiment immediately and wash your hands with soap and water. Contact details of the researchers are provided if you need further assistance. Skin cells will be collected from the fingermarks to extract DNA for the purpose of the research. The loci that are being used for DNA profiling will not uncover potential health problems or diseases, therefore, there is very little risk of incidental findings.

As a voluntary participant, you have the right to withdraw from this project at any stage without explanation **up to three months** after sample collection. If you do this, donated sample(s) and any subsamples such as DNA extracts will be destroyed. All information acquired from the sample(s) and questionnaire will also be destroyed.

Participation is voluntary, so non-participation will in no way affect your relationship with the Institute of Environmental and Science Research Ltd. The General Manager Forensic, John Bone, assures this in writing. Due to the voluntary nature of this project, no compensation will be offered or given for participation.

Upon completion of this research study, the summary of the results will be available to all participants, and you may choose to request a copy by ticking the appropriate box and providing your email address on the consent form. In addition, results will be found in the final thesis of Meghna Suresh, which will be available for viewing at both the University of Auckland and the library located at the Institute of Environmental and Science Research Ltd., Mt Albert.

Spiritual/Cultural Beliefs:

You may hold beliefs about a sacred and shared value of all or any tissue samples you agree to provide, and the data generated from them. The cultural issues associated with this should be discussed with your family/whanau as appropriate. There are a range of views held by Māori around these issues; some iwi disagrees with the collection and storage of samples citing whakapapa and advise their people to consult before participating in research where this occurs. However, it is acknowledged that individuals have their right to choose. If you are Māori and would like to request a specific tikanga (Māori custom) process, please feel free to talk with the research team.

Thank you for taking the time to read over this participation information sheet and for your consideration in voluntarily taking part in this research. In the event you have any further questions or concerns regarding your participation in this study, please feel free to contact one of the researchers below.

Researcher: Meghna Suresh

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Co-supervisor: Jennifer Howarth

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For concerns of an ethical nature, you can contact the Chair of the Auckland Health Research Ethics Committee at ahrec@auckland.ac.nz or at 373 7599 x 83711, or at Auckland Health Research Ethics Committee, The University of Auckland, Private Bag 92019, Auckland 1142.

Approved by the Auckland Health Research Ethics Committee on 16.11.2021 for three years.

Reference number AH23043

APPENDIX II

THE UNIVERSITY OF AUCKLAND
Te Whare Wānanga o Tāmaki Makaurau
NEW ZEALAND

SCIENCE SCHOOL OF CHEMICAL SCIENCES SCHOOL OF CHEMICAL SCIENCES

Faculty of Science Centre,
Building 301
23 Symonds St
Auckland
Ph. (09) 373 7599

Participant Consent Form

[This will be held for a period of six years]

Improving methods to obtain DNA profiling results from cartridge cases

Research Team

Student Researcher: Meghna Suresh – Candidate for the School of Chemical Sciences MSc in Forensic Sciences at the University of Auckland

Principal Investigator: Dr Douglas Elliot – Director Forensic Science Program at the University of Auckland and Team Leader, AFSC at ESR Ltd.

Co-Supervisor: Jennifer Howarth – Senior Scientist, Forensic Biology at ESR Ltd.

Co-Supervisor: Leah Tottey – Senior Scientist, Physical Evidence team at ESR Ltd.

Consent is sought for the following:

1. Consent for a reference buccal swab
2. Consent for finger marks to be collected, sampled and used in research.

Please read the following and fill in the information needed if that applies to you

- I have read the Participant Information Sheet describing this research project, and I understand its contents.
- I have had the opportunity to ask questions and have had them answered to my satisfaction.
- I understand that I have the ability to ask further questions and have my questions answered by any of the following researchers listed above.
- I agree to take part in this research.

- I understand that my participation is completely voluntary and if appropriate, I have consulted with my family/ whanau.
- I understand that non-participation as a staff member or student will in no way affect my relationship with the Institute of Environmental and Science Research Ltd. The General Manager Forensic, John Bone (ESR Ltd.), assures this in writing.
- I have been informed of my right to withdraw my participation without explanation up to three months after sample collection, and I have been given information on how to make an application to withdraw my sample and any data associated with my samples.
- I understand that by withdrawing my participation, my donated sample(s) and any subsamples such as DNA extracts will be destroyed. All information acquired from the sample(s) and questionnaire will also be destroyed.
- I understand that this research will require the deposition of finger marks onto cartridge cases and that DNA profiles produced from this will be used in this research.
- I understand that the skin cells will be collected from fingermarks to obtain data for this research.
- I understand that my identity as a participant will remain confidential and that all of my samples will be destroyed at the completion of the thesis.
- I understand that the data generated from these samples may be used for the publication of scientific papers in peer-reviewed journals, at conferences and the production of MSc theses directly related to this project. However, I understand that no data or information will be published that is identifiable back to me.
- I understand that the samples obtained in this research will be used for only this named project.
- I understand that a summary of the results from this study may be used in the development of a commercial service in the future.
- I understand that any samples submitted for this research will not be used for any future research.
- I understand that as part of this project the data from my samples may be shared with collaborators that are not currently named on the participant information sheet and may be from outside of University of Auckland and ESR Ltd, but that my identity will remain confidential. The anonymised data may also be shared or deposited onto a public database.

- I understand that the research data obtained from my samples will be stored electronically at ESR indefinitely.

Please tick the box if you consent to the following

☐ I want to receive a summary of the findings, which can be emailed to me at the address provided below.

☐ I consent to being contacted via the email address provided below, if there are further samples needed for this research project only.

Full Name (printed clearly):

Signature: Date:

Email Address (if you have ticked the box):

Approved by the Auckland Health Research Ethics Committee on 16.11.2021 for three years. Reference number AH23043

APPENDIX III

THE UNIVERSITY OF AUCKLAND
Te Whare Wānanga o Tāmaki Makaurau
NEW ZEALAND

SCIENCE SCHOOL OF CHEMICAL SCIENCES

SCHOOL OF CHEMICAL SCIENCES
Faculty of Science Centre,
Building 301
23 Symonds St
Auckland
Ph. (09) 373 7599

Deposition Instructions Sheet

Please wash your hands before and after providing the sample.

Buccal Swab

1. Take the buccal swab out of the tube.
2. Swab the inside of your cheek for 30-60 seconds, rotating the swab as you go so that the whole swab has touched your cheek.
3. Remove the swab from your mouth, making sure not to touch the swab tip, and place it into the collection tube.
4. Place the collection tube inside the envelope provided and seal it.

Finger marks for DNA extraction

1. Wash your hands with soap and water and wait for 45 minutes (Note down the time you washed your hands).
2. After 45 minutes, hold the cartridge cases with your fingers, one at a time. Make sure you are applying a medium pressure and the cartridge cases are in contact with your fingers for 15 seconds. Make sure that you are touching the Headstamp of the cartridge cases. Alternate between left and right hands.
3. After depositing the fingermarks, place cartridge cases individually into the paper envelopes provided (all the cartridge cases in separate brown envelopes).
4. Wash your hands with soap and wait for 45 minutes after each set. Repeat the procedure for the remaining cartridge cases.

5. Make sure that you do not place the cartridge cases on any other surfaces other than the envelope provided. Remove the cartridge case from the envelope and return it to the envelope immediately after handling it.
6. Once you have completed this, make sure to wash your hands.
7. Sampling can be done over 2 days. Make sure to wash your hands and wait for 45 minutes before sampling. Note down the activities in that 45 minutes in the sheet provided.

Approved by the Auckland Health Research Ethics Committee on 16.11.2021 for three years.

Reference number: AH23043

APPENDIX IV

THE UNIVERSITY OF AUCKLAND
Te Whare Wānanga o Tāmaki Makaurau
NEW ZEALAND

SCIENCE SCHOOL OF CHEMICAL SCIENCES

SCHOOL OF CHEMICAL SCIENCES
Faculty of Science Centre,
Building 301
23 Symonds St
Auckland
Ph. (09) 373 7599

Questionnaire

Improving methods to obtain DNA profiling results from cartridge cases

Researcher to fill out

Participant number:

Date of sample collection:

Date of extraction:

Date of amplification:

Questions for the participants

1. Time of handwashing
2. Time of Deposition of the sample
3. Activities between handwashing and deposition

Approved by the Auckland Health Research Ethics Committee on 16.11.2021 for three years. Reference number AH23043

CHAPTER 8

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