Experimental Approaches to Improving Trace DNA Recovery from Developed Fingerprints

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Abstract

The identification of individuals through the analysis of DNA deposits has progressed rapidly in the last three decades since its inception. It is possible, ever since that inception, to identify an individual with high degree of certainty on the basis of DNA containing materials found at scenes of crime. The enhanced sensitivity of the methodology allows the analysis of biological deposits originating from the interaction of human skin with other surfaces.

The result presented herein of DNA recovery from combined middle and ring fingers deposited onto glass slides showed that recovered amount of DNA was highly variable both between and within individuals. The efficiency of DNA extraction methods varied with the E.Z.N.A.® Blood DNA kit significantly outperforming both Chelex and IQ system in recovering DNA from fingerprints.

Experimental work into the relative DNA shedding propensity of palmar and fingertip surfaces demonstrated that the quantity of DNA shed from the surface of two fingertips is significantly higher than from the entire palm. This information will inform the prioritising of evidence collection, so that when palmar marks are found at crime scenes, every effort should be made to recover friction ridge detail to use as identification metric with collection for DNA analysis performed afterwards.

The research went further to examine systematically of the effects of different fingerprint enhancement materials was undertaken. With ninhydrin, cyanoacrylate, magnetic and aluminium powders, no PCR inhibition was observed. Ninhydrin was found to impact on the availability of profile-competent DNA, while cyanoacrylate, magnetic and aluminium powders interfered with DNA recovery.
Optimization of evidence recovery from ninhydrin treated office paper was carried out. Incubation of paper with 1% Triton™ X-100 prior to DNA extraction was sufficient to increase yield of DNA significantly. Sample treated with 1% Triton™ X-100 was found to be compatible with direct PCR protocols of DNA deposits allowing visualization of fingerprints and increased profiling success rates. Similarly, tape lifting using post it Tape Flags® was shown to be successful in recovering DNA from paper without damaging the paper surface.

These experimental approaches can be integrated into the normal multidisciplinary workflow of forensic evidence by establishing the best recovery techniques for each surface type, which should be available to criminal scene investigators.
Dedication

This Dissertation is dedicated with eternal love

To:

The soul of my Father, the first to teach me

My loved Mother, for her prayers to me;

My loving and caring wife Muna

My beloved children Ahmed, Rawan and Yousif
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1 Introduction

“Wherever he steps, whatever he touches, whatever he leaves, even unconsciously, will serve as silent evidence against him. Not only his fingerprints or his footprints, but his hair, the fibres from his clothes, the glass he breaks, the tool mark he leaves, the paint he scratches, the blood or semen he deposits or collects - all these and more bear mute witness against him. This is evidence that does not forget. It is not confused by the excitement of the moment. It is not absent because human witnesses are. It is factual evidence. Physical evidence cannot be wrong; it cannot perjure itself; it cannot be wholly absent. Only its interpretation can err. Only human failure to find it, study and understand it can diminish its value”.

Edmund Locard 1934

(Translation by: Kirk 1953)
Obtaining DNA profile from a touch has gained the attention of forensic scientists during the last two decades. The forensic scientists seized the scientific developments to push the frontiers of the amount of information, which could be obtained from ever smaller amounts of trace amounts of DNA evidence such as these deposited in fingerprints.

Generating a DNA profile from a fingerprint for the purpose of human identification is found to be beneficial in resolving a broad spectrum of criminal investigations, ranging from robbery to crimes of terrorism (Hoffmann et al. 2012) considering that full DNA profiles might be produced from fingerprints in about 80% of the general population (i.e. 18.6% heavy shedders plus 60.5% intermediate shedders (Allen et al. 2008). Experimental results have shown that the amount of DNA found in fingerprints is subject to a wide variety of factors such as shedding propensity of individuals and activities prior touching (Dominick et al. 2009; Quinones 2011).

In order for DNA analysis from fingermark evidence to be regarded as an evidential tool with confidence, it is important to further the current knowledge of the factors that govern the deposition of touch DNA evidence. Previous research studies carried out investigating factors affecting DNA deposition in fingerprint neglected the anatomical location as an additional factor which impacts on the DNA amount deposited. One of the current research aims was to identify the potential of different parts of the volar surface, namely the palm and the distal phalanx of the fingers, to shed DNA.

Since fingerprints might be found at crime scene in a latent form. In order to make latent fingerprints visible to the naked eye, several enhancement techniques are used to visualize these latent prints (Bowman 2004). After developing the fingerprint,
the quality of ridge information should be examined and a decision should be made whether it is good enough for classical comparison or not. Enhanced fingerprints may lack adequate details as these could be smudged, malformed, or overlapped and if so, then efforts should be made to generate a DNA profile from them to aid personal identification. Many researchers reported that DNA profiles could be obtained from enhanced latent fingerprints after visualization, without impacting subsequent DNA profiling process (Zamir et al. 2000; Lee and Gaensslen 2001; Pesaresi et al. 2003; Norlin et al. 2013). The visualization technique, whether chemical or physical, may still have an impact on the ability to obtain a usable DNA profile. Generally, it is unclear if this impact is a result of removal, dilution of, or damage to DNA/DNA containing materials (Bandey 2014).

A second area of research effort was to systematically examine the impact of some fingerprint enhancement methods on the DNA recovery process and then attempt to improve DNA recovery. Also, attempts were made to maximize the retrieval of DNA from some substrates to assist in improving methodologies for recovering DNA from developed fingermarks, which should help to improve the usefulness of this type of evidence.

1.1 Forensic Genetics

With its rapid development, forensic DNA has become a discipline in its own right. Due to the broad range of applications of forensic DNA such as cold cases; touch DNA approach; missing person identification, this discipline has a larger influence than any other previous scientific development on the criminal justice system (Walsh 2007).
ABO blood typing is considered as the first form of human identification through the analysis of body fluids; which was the result of Landsteiner’s research in 1900 to improve the success of blood transfusions, (cited by Yamamoto et al. 1990). In Italy, the human ABO blood grouping was the first based genetic tool used in court for resolving a paternity case on the basis of their different blood groups (Gaensslen 1983), which was established in the 20th Century. Even though it uses a limited technique from a forensic perspective due the high biological materials that required to conducting the analysis process, the implementation of ABO blood grouping was considered as revolutionary by law enforcement agencies. This, ABO grouping, has paved the way for investigators to exclude conclusively a suspect as having contributed a biological stain to a crime scene. Although definite positive identification was not possible during that period since the ABO system results in only a few phenotypes (1 in 10) (Bruns et al. 2007); this was an established as a significant achievement in the forensic biology discipline (Jobling and Gill 2004).

ABO grouping was applied on a wide scale for this purpose throughout the 20th century until the early 1970s; in which an examination of alternative protein and enzyme markers emerged, which was identified by the forensic scientists in the New Scotland Yard laboratory in London. The study was conducted by Spencer et al. (1964), on phosphoglucomutase (PGM), a phosphotransferase that found to be involved in glucose metabolism and exists in most mammalian tissues. PGM is an isomerase of which six different isozymes were identified. PGM, erythrocyte acid phosphatase (EAP) and haptoglobin were observed using protein gels for human identification purposes due to their higher degree of polymorphism. However, the approach of isoelectric focussing of the protein isomers was found to be a labour and time consuming process yielding relatively low variability and information (Murch and
Budowle 1986).
Figure 1-1: A Historical Timeline of Forensic Genetics Development.

- 1900: ABO blood discovered
- 1915: First use of ABO in the court
- 1930: Use of other blood groups and serum proteins
- 1984: Discovery of electrophoresis variants of red cells enzymes
- 1986: First use of DNA fingerprinting by Jeffrey in criminal case
- 1991: First publish on using PCR in forensic case
- 1992: Forensic STR characteristics kit developed
- 1995: National DNA database established in the UK
- 1997: DNA profiling from touched objects and single cells demonstrated
- 2001: minISTRs introduced to deal with degraded DNA
- 2005: The European Network of Forensic Science Institutes (ENFSI) and the European DNA Profiling Group (EDNAP) published recommendation of a new STR multiplex DNA 17
- 2007: First minISTR commercial kit
- 2014: DNA 17 chemistry launched in UK and National DNA Database upgraded to take that into account.
The use of ABO blood grouping and protein polymorphism typing was not very informative in determining a precise linkage required to carry out kinship analyses because the probability of a coincidental match is large. Thus, while the ABO blood groups are useful for excluding an individual from being the source of crime scene stain, the technique is not appropriate when an inclusion has to be made, for instance in cases where two closely related individuals were involved (Aitken 1995).

In 1984, the breakthrough molecular biology revolution took place, when Sir Alec Jeffreys first described that certain regions of DNA contained repeated sequences next to each other and that repeated sections could differ from individual to individual. He developed a novel hybridisation technique to examine the variation in repeat unit number and resulting length variation of repetitive DNA (Jeffreys et al. 1985). These DNA repeat regions were known as Variable Number of Tandem Repeats (VNTRs). These repeats were examined using a technique called Restriction Fragment Length Polymorphism (RFLP). This technique involves the use of restriction enzymes to make a cut within the sequences flanking the VNTRs. This technique was termed “DNA fingerprinting” (Jeffreys et al. 1985). This technique provided very high powers of discrimination $1 \times 10^{11}$ (Jeffreys 1985).

The RFLP technique requires high amount of human DNA 10-50ng as well as intact molecular weight $>12$ DNA molecules (Budowle and Baechtel 1990), so the technique was limited to only relatively fresh samples present in large quantities, such as blood and semen, that could be tested successfully (Evett and Gill 1991). This limitation restricted the VNTR analysis to crime scene investigation as forensic samples forensic are often challenging samples (degraded or minute) (Butler 2006).

This drawback was resolved in the early 1990s with the adoption of the
polymerase chain reaction (PCR) in forensic DNA analysis (Reynolds et al. 1991). The reaction was first described in the mid 1980s, and it allows small sections of DNA to be replicated myriad times, effectively amplifying the template material up to a workable level (Mullis and Faloona 1987; Mullis et al. 1992). By employing the temperature sensitivity of DNA, the molecule is denatured and then cooled down to allow primers to bind to the single-stranded DNA. The primers are short oligonucleotide sequences synthesised to target a complimentary sequence of DNA template that is being analysed. As the primers bind to an area flanking the desired region, a DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction to produce a copy of the strand. The previous process is repeated for a number of times; after the second cycle and during each cycle, the number of DNA molecules theoretically doubles (Mullis and Faloona 1987). Following the introduction of the PCR, successful DNA profiles were recovered from crime scene samples such as cigarette butts (Hochmeister et al. 1991a) and hairs (Higuchi et al. 1988).

Another distinguishable advance in forensic DNA analyses was achieved with the use of short tandem repeats STRs (Edwards et al. 1991), which are composed of short repeated units of DNA. STRs used by forensic scientists consist ideally of repeat units which are only four or five base pairs long and the alleles of which have significant length variability within human populations (Jin et al. 1994). The small size of these loci has given them a more applicability than VNTRs for use in forensic where degraded DNA is common and they can be analysed three or more at a time (Carracedo and Lareu 1998) and up to 26 loci such as a 26plex Autosomal STR (Hill et al. 2009). The small size of STR alleles (100-400bp) compared to that of minisatellite VNTRs
alleles (400 to 1000bp) allows STR profiles to successfully analyse samples from older mixtures and more degraded material (Whitaker et al. 1995; Shutler et al. 1999). The estimated number of STR loci distributed in the human genome is more than a million, approximately 3% of the entire human genome (Lander et al. 2001). Among the different types of STR, tetranucleotide repeat loci are the more common STRs system used in forensic DNA typing than di-, and trinucleotide repeats. Tetranucleotide STRs are less likely to yield stutters (15%) during the amplification process compared to 30% or more produce by di and trinucleotides (Carracedo and Lareu 1998). Furthermore, the heterozygotes alleles are differentiated more easily with four bases rather than with two or three bases.

DNA typing, from a forensic point of view, indicates the generation of DNA profiles made up of genotypes from a number of highly variable loci in the human genome. By increasing the number of variable loci genotyped, the resulting discrimination potential will also increase accordingly (Green et al. 2013). The development and validation of multiplex systems, which can simultaneously amplify several STR loci on the same genome prior to analysis has improved the ability to produce highly distinguishable profiles from minute amounts of crime scene materials.

In order to conduct comparison of profiles in a standardized fashion, which is applicable internationally within and between jurisdictions, the same set of STR markers should be analysed. The international forensic community has defined, and recommended the use of, a number of core STRs (Green et al. 2013). The STR marker selection was based on their high degree of polymorphism, which allows for greater discrimination, stability (low mutation rates), robustness and reproducibility, and smaller allelic length (Butler 2007). Several commercial kits now available that have
been validated for forensic use include AmpFlSTR® SGM Plus™ (Applied Biosystems), an 11 locus kit used in the United Kingdom, and AmpFlSTR® Identifiler (Applied Biosystems) and PowerPlex® 16 (Promega Corporation), which are both 16 loci kits, allows for more discrimination between individuals.

In order to meet the continual expansion of national DNA databases across Europe as well as the recent cross-border data-sharing initiatives of the Prüm Treaty (Baldaccini 2008). The European Network of Forensic Science Institutes (ENFSI) and the European DNA Profiling Group (EDNAP) published their recommendations for improved next-generation multiplexes. The recommendations particularly called for the inclusion of additional STR loci with shorter amplicons, better able to amplify degraded or otherwise compromised sample types, as well as highly polymorphic loci with better discriminatory power (Gill et al. 2006). Commercial kits such as PowerPlex® ESI 17 Fast System and Life Technologies AmpFlSTR® NGM™ Kit are now available to accommodate these recommendations. These kits were improved to deal with degraded DNA samples by using mini STRs and it was targeted new loci that less susceptible to effects (SWGDAM 2014). These kits incorporate all of the 10 loci of SGM Plus®, amelogene, 5 European Standard Set (D10S1248, D22S1045, D2S441, D1S1656, D12S391) and the highly discriminating SE33 locus (Andrade et al. 2011). In 2014; ESI17 FAST and NGMSE were accredited by the United Kingdom Accreditation Service (UKAS) to be used for comparison across England and Wales (Crown Prosecution Service 2015).
1.2 Formation of the Volar Skin

A widespread belief of fingerprint formation is that they are formed in the womb and are unique and unchanging throughout a human’s life, only increasing in size from child to adulthood (Kucken 2007) unless there is mutilation of the hands either deliberate, accidental or through disease (warts) or occupation, which can affect the fine ridge detail of fingerprints (Shetty et al. 2009). Studies have shown that during the early stages of embryo development, the hand undergoes significant alterations in its topographical features (Wertheim 2011). During the 5\textsuperscript{th} and 6\textsuperscript{th} gestation weeks, the hand becomes evident as a ventrodorsally flattened plate (O'Rahilly and Gardner 1975). There is a progressive contouring of the margins of the hand plate such that the plate appears to have a crenate margin, indicating the appearance of fingers. By 6 to 7 weeks of gestation, the hand plate begins to differentiate into cartilaginous bone structure and the external morphology of the hand demonstrates the continued formation of fingers. Also within this period volar pads first appear on the volar surface of the palm (Error! Reference source not found.) (Tuan 2004).

\textbf{Figure 1-2: Fetal Growth and Formation of Hand in Womb.} A - Paddle form, B - Finger separation, C - Volar pads, D - Hand at 8 weeks (Wertheim 2011).
In periods of 7 to 8 weeks of gestation, the fingers start to separate and the process of ossification begins. At the week 8 of gestation, the joints start to form between the bones of the hand, and the outer hand morphology appears similar in proportion to that of an infant (Hale 1952).

Under the epidermis of the both palms and soles of the human fetus, the volar pads are emerged as a transient swelling tissue called mesenchyme (Figure 1-3) (Wertheim 2011).

These pads differ in number, size, and shape among mammals (Hamrick 2003). Initially, in human embryos, the first pads form as a berry-shaped build-up of mesenchyme connective tissue on the ventral proximal parts of the fingers. Subsequently, the interdigital thenar and hypothenar regions of the palms and soles will be formed. In the beginning, volar pads seem to be disconnected elevations on the palm around 8.5 weeks gestation age followed on the digits by apical pads, which are completed one week later. Then they shift and spreads at the top of fingers are the sites of papillary ridge development (Pospisil et al. 1989; Babler 1991).

Figure 1-3: Schematic of Volar Pads on the Developing Hand. 1 to 5 (volar pads on the ventral apical regions of the digits; I to IV (interdigital volar pads on the palm); Hd (proximal hypothenar); Hp (distal hypothenar); Th (Thenar pad) (Wertheim 2011, p. 3-5 and 3-6).
During the period from 8 to 10 weeks of gestation, thumb rotation is achieved (Lacroix et al. 1984). The pads stay well rounded in their rapid growth around 9–10 weeks of gestation, afterward they start to show some individual variation in both shape and position (Cummins 1929; Burdi et al. 1979; Babler 1987). At about 11 weeks of gestation the distal transverse flexion crease in the palm begins formation. Additionally during the week 11 of gestation the volar pads of the palm start to retrogress, followed closely by the volar pads of the fingers. The distal finger pads are developed at their pinnacle during the 12th week, while the proximal transverse flexion crease of the palm begins formation at about 13 weeks (Kimura 1991).

Consequently, the growth of the volar pads starts to slow; their contour appears progressively less distinct on the more rapidly growing superficial area (Figure 1-4). This process is known as “regression” (Lacroix et al. 1984); it is crucially important to acknowledge that the pad is not actually shrinking; rather the volar pad size is affected by the quick growth of the larger surrounding surface. Mergence along with contours of volar pads (palms, fingers, sole) will be completed by the 16th weeks of gestation (Cummins 1929).

Figure 1-4: A Schematic Representation Development of the Volar Pad. Formation from initial to complete regression, excluding growth of the size of the finger. (Wertheim 2011, p. 3-5 and 3-6) measured by gestation weeks.
During the 10–10.5 weeks of gestation, the basal cells of the epidermis start to split rapidly (Babler 1991). The proliferation of volar epidermal cells would form shallow primary ridges known as “ledges” (Hale 1952), visible on the underside of the epidermis. These ledges depict the overall patterns that will be formed permanently on the volar surfaces several weeks later (Babler 1991; Evatt 1906). Therefore, primary ridges will become the first apparent evidence of interaction between the dermis and epidermis and eventually form continuous ridges.

At about 14 weeks of gestation, sweat glands anlagen appear in intervals with the apices of the ridges. At approximately 15-17 weeks of gestation, several sequential events occur during the epidermal ridge development. At 15 weeks, the Stratum Corneum (SC) starts to be visible along with initial keratin deposition. Accordingly, at the end of 17 weeks epidermal ridges become visible on the volar surfaces as fingerprints. From 17 to 24 weeks, secondary ridges continue to proliferate until they fit one to one with primary ridges. The secondary ridges develop in a manner similar to that of primary ridges. At 24 weeks, the epidermal ridge system has an adult morphology (Babler 1991).

The epidermal-dermal fit establishes the unique patterns of ridges and grooves, which exist on the surface of the plantar and palmar epidermis. The pattern of the epidermal ridges on our fingers, palms and soles, colloquially called fingerprints, is a part of our everyday life (Cummins 1929).

1.3 The DNA of the Skin

The predominant organ of the body, the skin, is considered as the largest organ, which forms up to 7-15% of the total body weight (Marieb and Hoehn 2007). The skin
consists of a number of cells with a density of 500 000 cells for every square centimeter of skin (Marieb and Hoehn 2007). Those cells undergo constant regeneration and destruction i.e., shedding process (Butler et al. 2004). With the number of cells, which are shed daily by each individual estimated to be 400 000 cells per person per day, DNA seems to be easily spread in a case of a crime scene (Wickenheiser 2002). Therefore skin shedding helps to broaden the use of biological traces to determine the identity of a person that was involved in the crime scene from their DNA.

The pitfall of the above is that, during the shedding process, skin cells are often denucleated (Fuchs 2007). Denucleation is a process, which takes place at the skin’s two regions: the epidermis, which is on the outer layer and lower dermis, which is on the inner layer of the skin, of a considerably tougher texture enriched with fibrous connective tissue. The epidermis is mostly composed of keratinocytes; and is distinguished by its proliferative features. Being proliferative allows the cells to renew through mitosis whenever skin shedding occurs.

Keratinocyte cells produce keratin, which is a protein responsible for creating protective coating for inner organs of the body (Fuchs 2007). Keratinocytes, in the skin, are usually found in the basal layer of the epidermis, the very first layer of the epidermis. Keratinocytes as living cells are also exposed to the aging process, which occurs in every cell in the body (Fuchs 2007). As aging commences, keratinocytes denucleate and progress through the layers of the skin. The keratinocytes will be flattened due to disintegration of their organelles and nuclei as a result, in part, of the cells’ deoxyribonuclease action. When the denucleated cells reach the surface of the skin, they are considered to be ‘dead’ and ready for shedding. The process might be accelerated by external factors. Those factors can be physical interaction as in friction,
for instance palm and soles are subjected to higher friction, thus cell production and keratin formation are accelerated (Fuchs 2007). This process, on maximum, takes up to 39 days however it can be shorter due to the factors mentioned earlier (El Gammal et al. 2007).

The epidermis has also Langerhans cells to protect the skin from external conditions such as being exposed to ultra-violet radiation. These are part of the immune system (Marieb and Hoehn 2007). Merkel cells are also present, which act as sensory receptors (Marieb and Hoehn 2007). Passing through the epidermis are the ducts for sweat and sebaceous glands. The sweat glands are physically spread within the skin layers. Sweat glands are distributed throughout the skin of the entire body except for the nipple and genital areas (Fuchs 2007). Those glands are of two types: eccrine and apocrine. The first produces a filtrate of the blood by exocytosis. Eccrine secretions consist of 99% water with salts and metabolic wastes (Marieb 1998). The second type of sweat glands, are apocrine glands, present in the axillary and anogenital areas of the body. Apocrine glands produce a fluid similar to that released by the eccrine glands but they appear on the hair follicles. The only difference in the apocrine sweat is that the sweat contains fatty substances and protein (Marieb 1998). The dermis holds small appendages called sebaceous glands. These glands are distributed overall the human body except palm and sole areas. The glands secretion is called sebum, which mainly consists of lipids. The sebum helps in preserving the body temperature by preventing sweat evaporation (Yamashita and French 2011).

The DNA, theoretically, stems from the aforementioned fragments carried to the surface by sweat and sebaceous glands, however the source has not yet been conclusively identified (Van Oorschot et al. 2010).
A few studies have attempted to explore the source of DNA recovered from fingerprints by depositing fingerprints on glass slides followed by staining and observing fingerprints under microscope. Balogh et al. (2003) reported that the majority of the epithelial cells, which were found in latent prints, were denucleated corneocytes. Also, they reported there was some occurrence of DNA containing material, which was enough for generating DNA profiles. On the other hand, Alessandrini et al. (2003) have reported results that mirror the above by observing a sample of fingerprints applied to glass slides. During their observation they found that there was an average of 5 nucleated cells or stripped nuclei in a sample. Additionally, they mentioned that the number of cells was found to depend not only on the size of the print, but also on the donor shedding status. However, some individuals tended to deposit more DNA containing material than others (Alessandrini et al. 2003; Quinones 2011) amply discussion in section 1.4.1.

In reality, the secreted sebum present on the forehead can transport DNA containing material to the surface of the face, which in turn is transferred to the person’s fingers as results of wiping their foreheads (Färber et al. 2010). Recently, a study conducted by Zoppis et al. (2014) showed that shedding sebaceous fluid on the skin surface contributed to some of the DNA-containing material left by a touch. They also reported that fragmented single stranded DNA was detected in most of cells forming the sebaceous gland using an immunohistochemistry technique and it was absent in the epidermis layers, so they came to the conclusion that sebaceous fluid represents an important source of DNA. Furthermore they found that the individual propensity of shedding sebaceous fluids is dependent on age, hormones, and skin health conditions (Zoppis et al. 2014). Also, rubbing eyes with fingertips might provide more DNA as the cells of the eyeball are nucleated and regenerated every 6-24h (Duke-Elder
Biting fingernails is also, conceivably, more likely to load the fingers with DNA from saliva (Wickenheiser 2002). Furthermore, DNA genotype analysis was achievable from DNA extracted from dandruff particles (Herber and Herold 1998). The above disorder is estimated to occur in 50% of adults (Schwartz et al. 2012) which helps to accelerate the turnover of keratinocytes. The results of the incomplete keratinization leave nucleated cells in the corneal layer.

Herber and Herold (1998) have found an average of 4.7ng of DNA per dandruff particle. Another study by Lorente et al. (1998) increased the average to 72.5-183.3ng of DNA from 1-1.5mg of dandruff, dependent on the method of extraction. As well as an optimization of the extraction method to increase the amount of recovered DNA, a condition such as psoriasis also helps to increase the number of nucleated cells in the outer layers of the epidermis (De Bersaques 1966). Further, it has been suggested that, in a normal epidermis, DNA is estimated to be 0.1% weight in comparison to 0.55% in the psoriatic epidermis.

A study by Kita et al. (2008) attempted to conclusively examine the source of DNA present on and its location in skin. In their study an immuno-electron microscope technique was used. In their study they found single stranded DNA in both the corneal layer of the skin and in swabs from skin. This study suggested that DNA from touched surfaces is secreted from the corneal layer, sloughed from the surface and passes out through the sweat. The research also highlighted that the DNA is degraded as it leaves the skin surface.

Although, research has been done with regard to identifying the origin of the cell type from recovery of touch DNA, as mentioned above, which may be evidentially useful, this is likely to be a laboratory based solution and will not assist crime scene
investigators in the initial identification of areas that will provide good recovery or profiling rates (Zoppis et al. 2014).

1.4 Factors Affecting DNA Deposition

A simple interaction, such as touching, has been proven to be able to lead to the transfer of DNA; this was demonstrated in early studies of the features of DNA deposition (van Oorschot and Jones 1997; Kisilevsky and Wickenheiser 1999). The latter research demonstrates that during the process of a brief interaction, the transfer occurs as the touching or physical interaction commences and that the interaction provides most of the DNA deposited at first contact, it does not however increase the DNA transfer if the interaction is prolonged or the method of interaction altered.

The DNA deposition process is affected by a number of factors, which influence the amount of the DNA as well as its suitability for analysis. These factors include the propensity of the individuals to shed DNA, the activities that the individual was engaged in prior to depositing DNA, the surface from which DNA is recovered and the nature of the physical contact of the DNA deposition (Lowe et al. 2002; Raymond et al. 2004; Phipps and Petricevic 2007; Allen et al. 2008; Cowell 2011).

Two studies were conducted to assess DNA transfer upon touching surfaces (Lowe 2001; Phipps and Petricevic 2007). It was determined by both studies that individuals deposit different amounts of DNA and the ability to transfer DNA relies heavily on the factors detailed in the next paragraph. In the research carried out by Lowe, et al. (2002), it was suggested that people can be classified into “good” or “poor” based on their propensity to deposit DNA on 50ml plastic tube and the likelihood of
obtaining a full DNA profile 15min after hand washing. Following this study, there was a subsequent yet more comprehensive study by Phipps and Petricevic (2007), the results have shown the possibility of obtaining full DNA profiles from touched plastic tubes, but their results were not successful in proving the existence of good and poor shedders. None of the 60 participants produced a full profile with both hands. The aim of the second study outlined that the status of each shedder was variable and irregular. It was suggested, as a result of the study, that dividing individuals into only two groups might be an oversimplification of the true nature of DNA deposition (Phipps and Petricevic 2007).

The factors impacting DNA deposition are several, but in general it can be categorized into three main groups, which are detailed next.

Pre contact factors, this group represents factors that already exist before contact occurs.

### 1.4.1 Shedding Status

It is widely accepted that there are inter- and intra-individual differences in the amount of the DNA deposited. Although the differences were determined by the aforementioned experiments, the reason behind this is still controversial. However, Murray et al. 2001; Lowe et al. 2002; Phipps and Petricevic 2007; Allen et al. 2008 and Quinones 2011 considered the possibility of the variation between individuals in terms of their ability to deposit or ‘shed’ DNA onto a surface. Murray et al. (2001) examined a group of 29 participants, and established that a typical ‘good shedder’ (their definition) left a complete profile on the surface of a plastic tube after contact of 10sec. A ‘poor shedder’ left only a partial profile with only few alleles. After testing 22
subjects, Lowe et al. (2002) found the significant difference between shedder types occurred at a time interval of 15 min after hand washing. They used this time interval to define a classification of shedder type: good shedders deposit a full profile 15 min post washing upon plastic tube, whilst poor shedders deposit a partial profile. On the contrary, Phipps and Petricevic (2007) reported that shedder status was less easy to classify. Five individuals were tested over five days using a similar method to Lowe et al., with and without prior hand washing. The difference within and between volunteers was found to be similar, with no donor providing consistently strong profiles. This study also conducted on a large scale where 60 volunteers were recruited; the results demonstrated that no ‘good’ shedders were identified. This study established that many other factors are involved in the deposition of trace DNA other than the shedding ability of the individual, such as hand dominance and environmental effects. However, Allen et al. (2008) tested single fingerprints from the unwashed hands of 129 individuals deposited onto glass slides, and found that 82% of the individuals were either ‘heavy’ (depositing over 300 pg of DNA) or ‘intermediate’ (depositing between 50-300 pg of DNA) shedders, with the minority depositing less than 50 pg of DNA. The study also found significant differences between the hands of individuals, with more DNA resulting from the non-dominant hand of volunteers. Variation was also noticed between the sexes, where males more likely to be grouped as heavy shedders; however, this variation was not statistically significant. In another study, Dominick et al. (2009) found no link between a donor’s DNA shedding ability and the quality of the fingerprints they deposited. A poll of five volunteers was recruited in a study of Quinones (2011). On different occasions, volunteers were asked to do one of the following post an hour of hands washing: to deposit five fingerprints on glass slides, to grab onto cut pieces of DNA free cotton fabric where the fabric was pulled sliding
through the person’s grip, and to rub 10 g of glass beads between their hands. Their findings confirmed that different amounts of DNA could be recovered from different individuals (0ng to 4.8ng). Their results were found to in supporting of the notion of “good” and “poor” shedders (Quinones 2011).

These findings might reflect the natural variability in the propensity to shed cells, as well as the variation in the DNA analysis conditions of the sample such as the multiplex STR chemistry used (Prinz et al. 2006), extraction methods (Sewell et al. 2008) and the time between DNA deposition and recovery (Raymond et al. 2009).

1.4.2 Condition of Skin

As mentioned previously, the amount of DNA deposited from different types of shedders can increase due to environmental, behavioral and health conditions (see 1.3). It has been observed that skin condition might affect the DNA deposition, as those individuals with relatively dry hands were hypothesized to be more likely to shed or flake more than others, increasing the amount of DNA shed (Bright and Petricevic 2004). This hypothesis was further supported in that individuals, with flaky skin conditions on their hands, such as atopic dermatitis, neurodermitis and psoriasis, deposited more DNA resulting in better quality DNA profiles than those without such disorders (Kamphausen et al. 2012). However, the quality and quantity of the deposited DNA were reduced as a result of prolonged treatment of those skin disorders.

1.4.3 Activities Prior to Touching and Time since Those Activities

The quality of DNA profiles deposited by touch was monitored at various time intervals after hand washing, which was set to help to determine the shedder type
(Lowe et al. 2002). They demonstrated that different results in terms of DNA profile completeness were obtained 15min after hand washing. They also reported that all individuals examined deposited full DNA profiles six hours post hand washing. Quinones (2011) demonstrated that there is a statistically significant relationship between time interval between hand washing and sample deposition and increased DNA deposits. Volunteers (n=91) were participated in this study; the results showed that over 1ng of DNA was obtained in samples taken as soon as 5min after hand washing and after over 2hour. Also this study reported that some neglectable amount of DNA was recovered from volunteers who had not washed their hands for either a few minutes or several hours; this experienced variation might be attributable to the variation in hand washing regimes of different volunteers (Quinones 2011). A more recent research of Zoppis et al. (2014) reported that 10min after deep hand washing with antiseptic soap and air drying resulted with no DNA profiles in all attempts compared to that with no hand washing where full DNA profiles were generated with some incidence of contamination.

Building on the truism that DNA mainly is responsible for the quality of profile; less DNA might be released with shorter intervals of hand washing due to the removal of DNA containing materials. Hand washing therefore could act as an influential factor that could result in the reduction of available DNA for shedding (Meakin and Jamieson 2013).

It was observed that repeated touching of pieces of plastic reduced the amount of DNA deposited when subsequent pieces were touched (Farmen et al. 2008). They observed that one person categorized as a poor shedder, showed the most consistent DNA deposition after three subsequent handling of same-type glass beaker, compared
to medium and good shedders whose deposited amounts of DNA decreased with subsequent contacts (Farmen et al. 2008). It has also been noted that personal items are a good source of DNA, when eight bags and twelve purses or wallets of 20 volunteer were swabbed for DNA. DNA was recovered from every item, with an average of 7.5ng and ranging from 0.9ng to 28.1ng. Although every profile recovered was found to have the majority of alleles in common with the owner of the item following 28 cycles standard STR approach, DNA from the offenders of simulated robberies could still be detected in usable quantities (10.9ng-15.4ng) on these items (Raymond et al. 2008).

Conflicting opinions have been published with regards to DNA deposition patterns by different hands. Bright and Petricevic (2004), in their study, have established no significant difference in the DNA levels deposited by either donor hand. In contrary, a study of Allen et al. (2008) found significant differences between the hands of individuals, with more DNA resulting from the non-dominant hand of volunteers. Likewise, based on an eight volunteers, Dominick et al. (2009) showed that three of eight individuals showed a statistically significant difference in DNA yields from each hand, for instance the averages for donor 7 for the left and right hand are 27.27% and 100.00%, respectively. Furthermore, it has been found that the amount deposited from different fingers from the same hand is not subject to substantial variation (Dominick et al. 2009). However, this observation has not been supported by the subsequent study of Quinones (2011) where 20 volunteer participated, the differences in DNA amount recovered from both hands of the same volunteer were not significant. Differences in DNA yields originating from different hands were attributed to be as a result of dominant hands being exposed to a higher level of activity and therefore friction; generating loose cells which are therefore deposited more readily (Phipps and Petricevic 2007). Consequently, more work should be carried out before
higher DNA yields can be attributed to dominant hands.

### 1.4.4 Substrate and Type of Surface on Which DNA is Deposited

Wickenheiser (2002) suggested that rough porous surfaces retain more DNA than smooth non-porous ones due to the abrasive nature during interaction with a rough surface being more likely to dislodge cells and will encourage DNA retention. In contrast to this, one experiment conducted by Pesaresi et al. (2003), showed that more DNA was recovered from smooth non-porous surfaces, such as glass, than from rough porous surfaces, such as untreated wood. These findings attributed to that more DNA deposited on smooth non-porous surfaces as a result of increasing perspiration during the interaction, which could explain the comparatively higher yields detected. They also emphasized that more DNA could be deposited, as suggested by Wickenheiser (2002), but the lower recovery observed might be attributed to ineffective recovery processes from the rough surface. However a study of Goray et al. (2010) demonstrated that amounts of retrievable DNA deposited on cotton substrate (rough surface), average of 11.68ng, is significantly greater than for plastic (smooth surface) 0.40ng. This study suggested that preferential DNA deposition on rough surfaces would be advantageous, since DNA persistence is highest on porous primary substrates, which are less likely to surrender DNA deposits than non-porous surfaces. However the pitfall of above studies is all was based on a small sample size, so further work is needed considering a larger sample size.

### 1.4.5 Nature of Contact

A common assumption is that more DNA will be deposited on a surface when the time and friction applied to that surface is increased. van Oorschot and Jones (1997)
reported that length of contact during deposition was found to be a non-significant factor, as the DNA would transfer at first contact. Another study confirmed this finding in which similar amounts of DNA were obtained from different objects regardless of the length of time it was held (Balogh et al. 2003); fingerprints were deposited from four donors on white office paper. The handling time ranged from 1 to 60s. Full AmpF/STR®Profiler Plus STR profiles were generated during 1, 2, 50, 60sec handling time periods. These findings concurred results of van Oorschot and Jones (1997) in which most of DNA transfers occurred at the first contact. On the other hand, Linacre et al. (2010) reported that only 5sec were needed to obtain full DNA profiles using SGM Plus® (Applied Biosystems, CA, USA) from a range of fabrics rubbed between volunteers thumb and first finger utilizing direct PCR. This does not preclude increased friction increasing the amount of DNA deposited through touch. Goray et al. (2010) established that manner of contact significantly increased the transfer of skin cells from one surface to another using passive or pressure contact. Although it was not in all instances, the results demonstrated that transfer rates were about double from passive contact (average of 11.05%) to pressure (average of 5.88%) and increased further with friction (average of 20.95%).

1.4.6 Time between Deposition and Recovery

Fregeau et al. (2000) have suggested that more DNA is recovered from fresh fingermarks rather than those that have been stored over long time of periods. Murray et al. (2001) found that full profiles were recovered from a plastic tube after contact of only 10 seconds by good shedders after four months, when kept at room temperature. However a notable decrease in the amount of DNA recovered from poor shedders. The time between deposition and collection of DNA has been identified as a contributing
factor for the decline of recovered DNA (Bille et al. 2009) where a significant decrease in the amount of DNA recovered was observed between samples collected and analysed within seven days (average 0.34ng/µl) compared with the samples collected and analysed within ninety days (average 0.038ng/µl). Similarly, Raymond et al. (2009) highlighted that the amount of DNA deteriorated over time, but highly dependent on the conditions that touched object were exposed to. In the same vein, Li and Harris (2003) reported that the likelihood of contamination was less when shorter time intervals had passed between deposition and recovery. Both studies came to the same conclusion that minimizing the time taken for the DNA sample collection is a crucial factor to ensure highest DNA yields.

1.4.7 Environmental Factors

In Australia, an experimental study revealed that interpretable DNA profiles were actually obtained from an outside surface, such as a window frame, which were recovered two weeks post deposition [the average temperature and relative humidity were 24.1°C, 63% (day) and 18°C, 71% (night)], and DNA profiles can be recovered from a glass slide stored in a cool and dark location (laboratory), for up to six weeks from deposition (Raymond et al. 2009). They attributed the difference in the lifetime of both cases to environmental factors such as high temperature, humidity and exposure to UV-light, as weather conditions and moisture surrounding the surface can impact on the likelihood of DNA persistence. DNA in a damp environment is subject to hydrolytic cleavage and oxidative base damage. Hydrolytic cleavage primarily targets the glycosidic base sugar bond, resulting in base loss through depurination and subsequent nicking of the DNA (Poinar 2003). The rate of hydrolytic cleavage is increased with heat and humidity, causing direct strand cleavage of the DNA due to drying (Poinar
Oxidative damage of DNA, whereby oxidation of carbon bonds in pyrimidines and imidazole rings in purines results in ring fragmentation (Lindahl 1993). UV irradiation (e.g., sunlight) of DNA can lead to cross-linking of adjacent thymine nucleotides, preventing passage of the DNA polymerase during PCR (Lindahl 1993). Traces of any biological material that are exposed to humid environments have shown to be reduced over time due to environmental attrition (Raymond et al. 2008). Although moisture in a sample can accelerate degradation, the moisture might enhance DNA transfer, a study showed that only 0.36% of the total DNA available is carried over, when dry biological samples are being transferred from one surface to another. In comparison, 50-96% of a sample is typically transferred when the source sample is fresh (Goray et al. 2010).

1.4.8 Type of Sampling Method Employed

Recovering DNA deposited through a touch can be conducted using various techniques such as mini-taping, swabbing, cutting-out the area of interest for direct extraction or amplification; the choice of which is usually dependent on the surface from which the DNA is to be recovered (Williamson 2012). DNA can be recovered from solid and smooth surfaces with common wet and dry swabs (Sweet et al. 1996), for instance, recovering DNA from knife handles (Goray et al. 2010). Mini-tapes can be used for fabric, glass and wood (Daly et al. 2012). Some tapes were found to be superior to others in recovering DNA (Verdon et al. 2014). In this study four substrates namely polyester strapping, cotton flannelette, cotton drill woven fabric and polyester/cotton plain woven fabric were rubbed by a good DNA shedder. Significantly more DNA was extracted, and a higher proportion of alleles detected, from Scenesafe FAST™ tape than from Scotch® Magic™ tape. They attributed DNA high recovery with
Scenesafe FAST™ tape due to the stronger adhesion properties of the tape. However, the DNA recovery is hugely influenced by the sampling technique. For instance, when sampling shoe insoles, tape lifting showed comparatively higher DNA recovery than swabbing and soaking methods (Bright and Petricevic 2004). Hansson *et al.* (2009) compared the Scenesafe FAST™ minitape (Scenesafe, UK) to three different swab types (cotton, flocked and foam) and found tape to be more efficient (range concentration of recovered DNA 0.1ng/µl-0.48ng/µl) than the three swabs (range concentration of recovered DNA 0.0ng/µl-0.075ng/µl) for sampling touch DNA from a single type of cotton shirt material. Similarly, taping cotton flannelette, cotton drill woven fabric and polyester/cotton, with Scenesafe FAST™ collected more DNA than swabbing and, for the three substrates, generated a greater median number of donor alleles (Verdon *et al.*2014). Contrariwise, de Bruin *et al.* (2012) established that double swab and stubbing methods showed roughly the same distribution in simulating strangulation experiment among 50 couples (50 male offenders and 50 female victims). Small differences were observed in the number of full profiles. Overall, for stubbing, 49 out of 50 DNA profiles were obtained with at least 1 allele. For double swab, each of the 50 DNA profiles showed at least one allele. Plaza *et al.* (2015) evaluated the efficacy of three DNA collection techniques from latent fingerprints deposited on various paper substrates for STR profiling. These techniques are Electrostatic Detection Apparatus (ESDA®), dry swabbing, and substrate cutting. The substrates examined were resume paper, cotton paper, magazine paper, currency, copy paper, and newspaper. All samples were extracted using the Qiagen® EZ1® DNA Investigator Kit on the EZ1 Advanced Instrument (Qiagen, Venlo, Limburg, The Netherlands), and amplified with AmpF™STR® Identifiler® Plus PCR Amplification Kit (Life Technologies, Carlsbad, CA). This study results demonstrated that both the ESDA® and
dry swabbing techniques outperformed the methodology of substrate cutting, where more DNA was recovered from all five substrates, the average recovery among all substrates ranged 0.17ng - 0.36ng with ESDA®, and 0.267ng-1.049ng for dry swabbing technique, while the average range for the cutting method was 0.0ng- 0.677ng. Furthermore more full DNA profiles were generated from samples that recovered with ESDA® and dry swabbing techniques compared to that DNA profiles obtained by cutting method (Plaza et al. 2015).

It would be beneficial to broaden the current knowledge of the impact of sampling methods in recovering touch DNA by conducting a comparison study of the collection methods of touch DNA from a variety of substrates.

1.4.9 Efficiency of Extraction of DNA from Sample

The process of DNA profiling, in most cases, starts with extracting DNA from the substrate. The efficiency of extraction highly depends on the techniques used, with some superior methods, 5% Chelex outperformed organic method in recovering DNA from heels and toes (Bright and Petricevic 2004). In addition, the DNeasy® plant mini kit (QIAGEN®) was found to improve DNA recovery from paper by over 150% compared with the QIAamp® mini kit (Sewell et al. 2008), and on the nature of substrate from which the DNA has been recovered (Daly et al. 2012). For instance, certain substances like bleach traces in different types of papers are proposed to hinder the DNA extraction process (Sewell et al. 2008). Also van Oorschot et al. (2003) highlighted that significant DNA was wasted as swabs retain some DNA (24% and 52% of 100ng in 100µl extract was recovered when dry and wet swabs used respectively). As a result, improvements are continuously needed to develop DNA recovery. Linacre
et al. (2010) successfully omitted extraction steps using 30 cycles for Powerplex 16 (Promega, WA, USA) or 28 cycles for SGM Plus® (Applied Biosystems, CA, USA). This improved the DNA profiling, as full profiles obtained by direct amplification from all substrates (cotton, colored polyester, nylon, denim - light and dark colored, and wood stick cotton swabs) that amplified with SGM Plus®. However, this is only possible if the surface can be cut and placed directly in the PCR tube, for instance on an item of clothing. Generating a DNA profile directly from fabric was found to provide better quality profiles in comparison to those obtained by removal and extraction of DNA (Linacre et al. 2010).

1.5 Latent Fingerprint Development

The term ‘latent’ is used to describe a fingerprint as invisible. Further development with some physical or chemical reactions or illumination is required to achieve visualization (Yamashita and French 2011). Ideally, the techniques utilized for developing latent fingerprints have been targeted towards specific components present in fingerprints. Both the content and quality of a latent fingerprints are affected by three stages relative to deposition: prior, during and after deposition (Yamashita and French 2011). First, prior deposition, factors are related to donor characteristics (i.e. age, sex, diet, lifestyle etc.); these factors are very difficult to consider when recovering crime scene marks. Secondly, mid-deposition factors, which indicate the dynamics of deposition and the substrate properties such as texture, shape and topography. The effect on print quality of contact conditions such as pressure applied and lateral movement during deposition would be revealed only after development, although, the surface texture is always important for development method choice (Bowman 2004; Bandey and Britain 2007; Yamashita and French 2011). Finally, factors related to the
effects of certain post-deposition conditions, such as ambient temperature, exposure to water, environmental contamination, surface chemistry, and surface porosity can potentially be anticipated for the optimization of development (Champod et al. 2004; Yamashita and French 2011).

### 1.5.1 Dry Powders

The classic example, powder dusting, is one of the oldest and most consistent of any kind of technique (Lee and Gaensslen 2001). As powder dusting is a fast and low-cost method for developing latent fingerprint on non-porous surfaces, it has been used to develop fingerprints for over a century (Galton 1892) and, during this period, a great number of formulations have been developed (Bowman 2004). Generally, the developed formulae are based on the general principle of a pigment component (e.g. metal oxides, sulphates and carbonates) for contrast combined with a resinous component (e.g. rosin, corn starch and gum Arabic) for adhesion to moist and oily fingerprints residues (Yamashita and French 2011). The powder is applied through brushing onto a surface with a fine haired brush made from synthetic, natural or fibreglass material; a method which is simple yet potentially destructive to fragile marks (Bandey and Britain 2007). Some modifications on the traditional model have been implemented such using fluorescent powders, which are effective on surfaces where more contrast is needed (Home Office 2014). Magnetic powders also are used in which a magnetic wand replaced the brush, thus eliminating the possibility of brushing destruction (Champod et al. 2004). Powder dusting methods are most effective on smooth, dry, non-porous substrates and despite the longevity as a technique, these are now typically restricted to surfaces that cannot be removed from a crime scene for more sensitive laboratory development (Champod et al. 2004).
1.5.2 Ninhydrin

Ninhydrin was discovered by chance in 1910 by Siegfried Ruhemann (Ruhemann 1910). Ninhydrin, also known as triketohydrindene hydrate, is both water and alcohol soluble. Ninhydrin reacts with the small and detectable amount of amino acids of fingerprint residues (Hansen and Joullié 2005). When ninhydrin reacts with fingerprints’ amino acids, a dark purple product, the ammonium salt of Ruhemann’s purple is produced (Ruhemann 1910), and has been used to develop fingerprints since 1954 (Odén and Von Hofsten 1954).

As the eccrine products of fingerprints stay relatively stable in porous surfaces, the technique has become widespread for such substrates, particularly with very old marks (Champod et al. 2004). Ninhydrin solution can be applied by dipping, spraying or brushing after being prepared in a solution with acetic acid (for optimum pH conditions) and various stabilizing and carrier solvents (Champod et al. 2004). In ideal conditions, if sufficient amine is available, with contrasting background and minimal background reaction, the technique is found to be very effective; however, these conditions rarely exist operationally and so a considerable number of ninhydrin analogues and contrast enhancement steps have been introduced since the compound was first used for fingerprint development (Almog 2001).

1.5.3 Cyanoacrylate Fuming

The cyanoacrylate fuming technique is used widely as the process, is quick and provides superior development and visualization of the fingermark (Fisher 2008; Ulery et al. 2011; Penrose 1968). Generally, it is used for smooth non-porous surfaces and involves the selective polymerization of cyanoacrylate (CA) ester monomers on
fingerprint residues. The cyanoacrylate fumes adhere to the fingerprint residue (Penrose 1968) make latent fingerprints turn white and become visible. The Japanese National Police Agency developed this technique in 1978 (Lee and Gaensslen 1984), the process requires an isolated and controlled environment (or fuming chamber) for application and involves heat induced vaporization of CA solution in the presence of an object (Lee and Gaensslen 2001). Also, a high humidity condition is vital to ease the polymerization initiation (Bandey and Britain 2007). The consequences of the above conditions are used in initialization and spread of solid white cyanoacrylate covering the fingerprint. Commercial chamber units are available and preferred for forensic application as such provide a sealed controlled environment of heat and humidity, the latter has been reported to be important in development effectiveness (Champod et al. 2004; Paine et al. 2011). When examining a surface where the white colored development product presents a contrast issue, print can be enhanced further with an optical technique or stained with a colored or luminescent dye before optical enhancement (Home Office 2014). The fingerprint components involved to initiate CA polymerization and then carry out good quality development still need more investigation to be fully understood (Wargacki et al. 2007; Paine et al 2011; Yamashita and French 2011). Such conclusions were based on examining the microscopic morphology of the polymerized structures which has been shown to differ with fingerprint type (sebaceous or eccrine) and age, which in turn impacts on the enhancement of ridge detail by CA polymerization (Lewis et al., 2001; Paine et al. 2011). The process was found to be complex, with multiple physical and chemical steps (Czekanski et al. 2006). The CA technique was reported to be less effective on fingerprints over two weeks old, due to water loss, which is frequently established as a critical component for polymerization initiation, although, Wargacki et al. (2007)
reported that amines and carboxylic acid alone are sufficient for polymerization to occur. Moreover, the vapour of cyanoacrylate also adheres to residues of other biological materials (blood, saliva, semen, or sweat), and as such may or may not be detected through initial examination; these are potential sources of biological evidence that contain DNA (von Wurmb et al. 2001; Wargacki et al. 2007).

1.5.4 Effects of Fingermark Enhancement on DNA Analysis

Several fingerprint enhancement techniques have been used successfully in visualizing latent fingermarks such as dusting powders, vacuum metal deposition, ninhydrin 1, 8-diaza-9-fluorenone (DFO) and CA fuming, all of which are recognized as pioneering techniques to visualize latent fingerprints (Bowman 2004). Much research has been conducted in order measure the influence of each enhancement technique on subsequent DNA profiling as discussed in the next paragraph.

In a study conducted by Stein et al. (1996), in which they used blood and saliva stains on different substrates. They determined the influence of several enhancement techniques have on recovered DNA from high DNA containing samples. The study involved CA fuming on razor blades and plastic foils Gentian violet on the sticky side of an adhesive tape, ninhydrin on paper and carbon fingerprint powder on glass slides. The findings of this study showed that the chemicals used in the visualisation process had no impact on the DNA extraction as well as DNA quality and typing. They came to a conclusion that fingerprinting can actually be conducted before DNA collection, which as a result would not damage the DNA. Their study neglected that the quantity of DNA from fingerprints is limited compared to that recovered from blood and saliva.
Additionally, adhesive tape was used by Zamir et al. (2000) in studying DNA collection from fingermarks visualised using CA fuming followed by Basic Yellow 40 and crystal violet staining. Although they reported that the number of loci was reduced, it was possible however to recover the donor’s DNA profile prior and post enhancement from three out of four donors. Their results support findings of Stein et al. (1996). In another study, Bhoelai et al. (2011) concluded that DNA profiles were successfully generated from DNA recovered from fingermarks deposited on chlorine-free paper and plastic sheets and visualized by vacuum metal deposition and CA fuming. They also found that combination of CA fuming with either basic yellow 40 or safranin resulted in reducing DNA recovery where greater than 50% of the Relative Fluorescence Units (rfu) of the donor alleles was decreased compared to untreated ones.

A study of Zamir et al. (2000) investigated DFO; Schulz et al. (2004) studied ninhydrin while Sewell et al. (2008) investigated both techniques. All previous studies have established that DNA was successfully recovered from the paper-based substrates investigated. As noted by Zamir and Geller (2000), although DFO was established to be “a destructive reagent in DNA profiling”, they were still able to recover DNA profiles. Another study was conducted by Schulz et al. (2004), in which they were successfully genotyped DNA obtained from ninhydrin enhanced fingerprint deposited upon wallpaper in a criminal case. Although, that the result of their study did not help to resolve the case, it was shown that generating a DNA genotype was still achievable after ninhydrin treatment. In a study by Sewell et al. (2008), on stationery related paper such as business cards and office paper, the amount of the DNA, recovered from a particular surface, was reduced with 80gsm office paper (City PaperTM, UK) and Whatman® filter paper. Schulz et al. (2004) demonstrated that amplification was not impacted upon to a great level, and profiles were obtained. Bhoelai (2011) obtained
results that mirror Schulz et al. (2004), but they were experienced some DNA contamination with samples obtained from ninhydrin and DFO fingerprints. von Wurmb et al. (2001) and Bille et al. (2009) studied the effects of CA fuming on DNA recovered from glass slides and pipe bombs respectively. Likewise, von Wurmb et al. (2001) examined the effect of CA fuming on DNA recovered from saliva and bloodstains but not fingermarks. Both established that DNA recovery and profiling were achievable. von Wurmb et al. (2001) findings demonstrated CA enhanced samples extracted with Chelex were 60% lower than the controls. They also reported that the DNA extraction method used could impact on the effectiveness of amplification process; using the Invisorb forensic kit (Invitek, Berlin, Germany) produced more amplifiable DNA compared to that obtained by Chelex. Bille et al. (2009) showed a significant reduction in DNA when 2µl of 500cell/µl cell suspension was planted on areas on pipe bombs. They performed CA visualization. Up to 90% of the DNA amount was lost when a three month interval between blast and collection were examined. Although they allowed ample time between the collection and the blast, the CA did not appear to possess a “measurable effect” on the collected DNA.

Shalhoub et al. (2008) explain that DNA was recovered successfully after CA processing of fingermarks that were deposited on several substrates such as a drinks can, plastic bottle, waxed paper cup etc. Also, they examined Isomark™ (Isomark Ltd., Nuneaton, Warwickshire, UK), casting material, which was then coated over the marks and left for 24h, then recovered and subsequently treated both the cast material and the substrate with CA, before swabbing for DNA. DNA extraction was conducted by QIAamp® Mini extraction kit, DNA quantified with Quantifiler™ Human DNA Quantification Kit (Applied Biosystems, CA, USA), and then amplified with AmpFISTR® SGM Plus Kit®. All in all, the DNA was recovered from the substrates.
More DNA was recovered from the non-porous smooth substrates such as the aluminium can. In calculating the DNA collected, it was found that approximately 42% of the substrates tested gave enough DNA to be profiled and 82% of these gave full profiles.

The effect of DNA recovery after vacuum metal deposition (VMD) on fingerprints visualisation was examined. Murray et al. (2001) experimented with several enhancement techniques and concluded that DNA profiles could be generated from 33% to 100% of the samples, depending on the technique and time of DNA collection. Evidently, 100% of the donor profile was recovered from a freshly enhanced print compared to 0% from one that had been left for 100 days at room temperature when tested using VMD.

Fingerprint dusting brushes and powder from kits were found to be source of cross contamination. van Oorschot et al. (2005) examined brushes and fingerprints dusting powders (White and black powders, Optimum Technology) for DNA, showing a full AmpF/STR SGM Plus profile from one of the brushes. In addition, brushes used in casework were reported to play a role in DNA transfer between crime scenes. Accumulated DNA from brushed surfaces can redeposit DNA containing material on a number of subsequently brushed surfaces. This could result in increased contamination when powdering biological samples, such as blood, saliva, skin, or fresh fingerprints (van Oorschot et al. 2005). The latter noted that there was variation between brushes in terms of their potential for causing DNA cross contamination. However table 1-1 details several researchers that examined the effects of fingerprints enhancements on DNA analysis.
<table>
<thead>
<tr>
<th>Fingerprint enhancement</th>
<th>DNA source and Substrate</th>
<th>DNA analysis</th>
<th>Impact on DNA</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superglue, ninhydrin, gentian violet</td>
<td>Latent fingerprints were taken on the surfaces of razor blades, plastic foils, glass slides, sticky surface of an adhesive tape and ground wood-free white paper.</td>
<td>Extraction: Organic method. Quantification: slot blot method using alphoid probe D17Z1. DNA profiling: AMPLF analysis (D1S80 and ApoB) three STRs VWA, TPOX, TH01.</td>
<td>No affect the subsequent DNA typing where high molecular weight DNA was recoverable from both treated and untreated samples. Amplification was successful for both typing systems were used.</td>
<td>(Stein et al.1996)</td>
</tr>
<tr>
<td>Argon ion laser, Polilight UV, Polilight green, Superlite, and shortwave UV</td>
<td>Dried bloodstains on glass were exposed for up to 30min</td>
<td>Extraction: Organic method. Quantification: no quantification. DNA profiling: quadruplex PCR system.</td>
<td>No amplifiable DNA were obtained from bloodstain exposure to shortwave UV light for more than 30sec</td>
<td>(Andersen and Bramble 1997)</td>
</tr>
<tr>
<td>1) White powders: a) BVDA (International, Amsterdam, The Netherlands). b) Sirchie indestructible white. c) Faurot white (Youngs-ville, NC, USA); 2) Black powders: a) BVDA blower black. b) BVDA special black. c) BVDA concentrated black. d) Sirchie volcano.</td>
<td>Five fingerprints were deposited on a glass and a wooden plate</td>
<td>Extraction: Chelex method. Quantification: no quantification. DNA profiling: multiplex of four STR loci (CD4, TH01, D21S11 and SE33)</td>
<td>The intensity of peaks in the DNA profile obtained from the fingerprints on glass was five times lower than those obtained from wood.</td>
<td>(Van Hoofstat et al. 1998)</td>
</tr>
</tbody>
</table>
### Magnetic Powders

3) Magnetic powders:
   a) BVDA magnetic black.
   b) BVDA magnetic gray.

4) Metal powders.
   a) BVDA special sliver.
   b) Faurot aluminium bronze.

### Other Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Licked Stamps</th>
<th>Extraction</th>
<th>DNA Extraction</th>
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<th>Comments</th>
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<tbody>
<tr>
<td>DFO</td>
<td>Licked stamps</td>
<td>Organic method.</td>
<td>No quantification.</td>
<td>Multiplex of Six STR loci</td>
<td>DNA was successfully extracted, amplified and typed for six STR loci (Zamir, Oz and Geller 2000)</td>
</tr>
<tr>
<td>Cyanoacrylate</td>
<td>Bloodstains on glass slides</td>
<td>Chelex method and Invisorb Forensic kit (Invitek, Berlin, Germany)</td>
<td>Photometrically</td>
<td>Profiler Plus kit (PE, Applied Biosystems)</td>
<td>The presence of CA had a negative effect on the signal intensity as shown by mixing experiments. The amount of the PCR product from 10ng male DNA served as a 100% amplification control. It could be shown that adding 9μl of CA containing solution to 10ng DNA led to a signal that was about 50% smaller than the controls, suggesting a strong inhibitory effect of CA (von Wurmb, Meissner and Wegener 2001)</td>
</tr>
<tr>
<td>CNA (in and out of a vacuum), aluminium powder, metal deposition, DFO, ninhydrin and physical developer</td>
<td>Fresh and 100day post enhancement single fingerprint on acetate (nonporous) paper (porous)</td>
<td>Not mentioned</td>
<td>No quantification</td>
<td>STR profiling</td>
<td>100% DNA profile from untreated acetate samples Fresh (F) and 77% DNA profile 100day post enhancement (100d)</td>
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<td>a. F: 100% CNA – 100d: 68%</td>
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<td>b. F: 49% CNA (vacuum) - 100d: 86%</td>
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<td>c. F: 77% Aluminium powder -100d: 77%</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>d. F: 100% Metal deposition</td>
</tr>
</tbody>
</table>

### Additional Comments

- Murray et al. 2001
<table>
<thead>
<tr>
<th>Compound</th>
<th>Method</th>
<th>Extraction</th>
<th>Quantification</th>
<th>DNA Profiling</th>
<th>Notes</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2-indanedione formulations (acidic and neutral) both HFE7100 base</td>
<td>Licked stamps</td>
<td>Organic</td>
<td>No quantification</td>
<td>AmpF/STR Profiler Plus PCR Amplification Kit</td>
<td>No differences were observed between the STR profiles obtained from treated and untreated stamps and envelopes.</td>
<td>(Azoury et al. 2002)</td>
</tr>
<tr>
<td>Magnetic powder, soot powder.</td>
<td>Four fingerprints on glass</td>
<td>InViSorbTM Forensic Kit I (InViTek GmbH, Berlin)</td>
<td>No quantification</td>
<td>Singleplex PCR of Hum FGA</td>
<td>From 48 directly swabbed fingerprints, 14 could be successfully amplified and typed. Eight of these were visualized with soot and 6 with magnetic powder. From 48 scotch tape-archived fingerprints, nine could be successfully amplified and typed. Four of these were visualized with soot and five with magnetic powder.</td>
<td>(Schulz and Reichert 2002)</td>
</tr>
<tr>
<td>Cyanoacrylate, aluminium powder, iodine, ninhydrin, metal deposition and physical developer</td>
<td>Fingerprint from interior of latex gloves, the grips and hafts of tools, drinking glasses and clothes</td>
<td>Not mentioned in the research</td>
<td>DNA profile: 100d: 0%</td>
<td>From 48 scotch tape-archived fingerprints, nine could be successfully amplified and typed. Four of these were visualized with soot and five with magnetic powder.</td>
<td>It was noted that the yield of DNA varied from 33% to 100% of donor DNA profile and the recovery of DNA depends on chemical reagents used. It was also observed that when the time period between the fingerprint</td>
<td>(Lowe et al. 2003)</td>
</tr>
</tbody>
</table>

2. 74% DNA profiles from untreated paper samples Fresh (F) and 76% DNA profile 100 day post enhancement (100d)
   a. F: 79% DFO -100d: 35%
   b. F: 44% Ninhydrin -100d: 29%
   c. F: 33% Physical developer -100d: 2%
<table>
<thead>
<tr>
<th>Methodology</th>
<th>Description</th>
<th>DNA Extraction</th>
<th>DNA Quantification</th>
<th>DNA Profiling</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>White and black powders</td>
<td>Dried Saliva stains on plastic sheets</td>
<td>Extraction: 5% Chelex and QIAquick® (Qiagen). Quantification: Quantiblot® (Applied Biosystems) DNA profiling: AmpF/STR® Profiler Plus</td>
<td>In all instances when Chelex extraction failed to remove fingerprints powders from DNA extract, no DNA profiles were obtained.</td>
<td>(van Oorschot et al. 2005)</td>
<td></td>
</tr>
<tr>
<td>Amido black for bloody fingerprints while cyanoacrylate fuming followed by rhodamine 6G</td>
<td>Bloody fingerprints, sweat fingerprints and oily fingerprints were deposited on brass, nickel-plated brass and aluminium</td>
<td>Extraction: Organic Quantification: not mention. DNA profiling: AmpF/STR® Profiler Plus</td>
<td>The use of fingerprint reagent that significantly reduce the amount of DNA available for analysis where only 3 DNA profiles were obtained, all of which were obtained from bloody fingerprints</td>
<td>(Spear et al. 2005)</td>
<td></td>
</tr>
<tr>
<td>1, 2-indanedione</td>
<td>Thermal and carbonless paper</td>
<td>Extraction: Chelex and Qiam (Qiagen). Quantification: Quantiblot® Human DNA profiling: AmpF/STR® Profiler Plus</td>
<td>1,2-Indanedione did not adversely affect the DNA profiles obtained from the treated fingerprints. On thermal paper, only 4 out of 100 samples generated full DNA profiles. DNA profiles from fingerprints deposited on carbonless substrate had more correct alleles for each of the sample tested.</td>
<td>(Yu and Wallace 2007)</td>
<td></td>
</tr>
<tr>
<td>Ninhydrin DFO</td>
<td>Five fingerprints on filter paper Office paper Magazine card Newspaper</td>
<td>Extraction: QIAmp DNA mini kit DNeasy® plant mini kit Quantification: Quantifiler™ Human DNA DNA profiling: AmpF/STR® Profiler</td>
<td>No DNA was recovered from office paper following all fingerprint enhancement treatments. 60% decrease was noted in DNA recovery in all paper types following fingerprint enhancement treatment compared to untreated samples.</td>
<td>(Sewell et al. 2008)</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>Description</td>
<td>Extraction</td>
<td>Quantification</td>
<td>DNA Profiling</td>
<td>Notes</td>
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<tr>
<td><strong>Cyanoacrylate fuming</strong></td>
<td>Fingerprints deposited on cold aluminum can, plastic water bottle, £2 coin, wax paper cup, and a hard plastic mobile phone case (Nokia 3330).</td>
<td>Extraction: QIAmp DNA Mini Kit</td>
<td>Quantification: Quantifiler™ Human DNA</td>
<td>DNA profiling: AmpF/STR SGM Plus Kit.</td>
<td>The DNA recovery was found to be highly variable and ranging from 0.1 to 2.3 ng. Cyanoacrylate fuming was found to have no significant effect on the amount of DNA recovered. Full profiles were obtained from 82% of the samples were examined (n=50), with the rest yielding partial profiles. No mixed profiles were obtained, with all alleles matching the donor’s. (Shalhoub et al. 2008)</td>
</tr>
<tr>
<td><strong>Magnetic powder and black fingerprint powder.</strong></td>
<td>Fingerprints on skin</td>
<td>Extraction: Organic method and NucleoSpin® Tissue XS kit.</td>
<td>Quantification: No quantification.</td>
<td>DNA profiling: Eight STRs were used (Amelogenin, TH01, VWA, FGA, D3S1358, D8S1179, D21S11, and SE33).</td>
<td>Magnetic powder (18.4%) yielded better results than black fingerprint powder (13.6%) for the recovery of fingerprints. DNA was successfully extracted from the powdered and lifted latent fingerprints in one-third of the cases. Black fingerprint powder gave better results with the rate of 2.2% for full DNA profiles and profiles useful for inclusion as compared 1.8% for magnetic powder. (Färber et al. 2010)</td>
</tr>
<tr>
<td><strong>Red, green, and yellow fluorescent powder</strong> (Lynn Peavey, USA)</td>
<td>Glass plates, glossy magazine papers, and plastic sheets</td>
<td>Extraction: Chelex and QIAmp® DNA Mini Kit (Qiagen, USA)</td>
<td>Quantification: NanoDrop™ 1000 Spectrophotometer</td>
<td>DNA profiling: AmpF/STR® Profiler Plus™</td>
<td>The number of loci generated from processed fingerprints was comparable to those obtained from the undusted fingerprint samples. (Thammurak et al. 2011)</td>
</tr>
<tr>
<td><strong>Cyanoacrylate followed by basic yellow or safranin staining</strong></td>
<td>Chlorine-free paper or plastic sheets</td>
<td>Extraction: QIAmp-based DNA isolation (Qiagen; standard protocol).</td>
<td>Quantification: no quantification</td>
<td></td>
<td>Cyanacrylate and VMD (and the combination of both) did not affect subsequent DNA profiling. Cyanacrylate combined with safranin or basic yellow staining resulted in less</td>
</tr>
<tr>
<td><strong>Magnetic powder and black fingerprint powder.</strong></td>
<td>Fingerprints on skin</td>
<td>Extraction: Organic method and NucleoSpin® Tissue XS kit.</td>
<td>Quantification: No quantification.</td>
<td>DNA profiling: Eight STRs were used (Amelogenin, TH01, VWA, FGA, D3S1358, D8S1179, D21S11, and SE33).</td>
<td>Magnetic powder (18.4%) yielded better results than black fingerprint powder (13.6%) for the recovery of fingerprints. DNA was successfully extracted from the powdered and lifted latent fingerprints in one-third of the cases. Black fingerprint powder gave better results with the rate of 2.2% for full DNA profiles and profiles useful for inclusion as compared 1.8% for magnetic powder. (Färber et al. 2010)</td>
</tr>
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<td>Glass plates, glossy magazine papers, and plastic sheets</td>
<td>Extraction: Chelex and QIAmp® DNA Mini Kit (Qiagen, USA)</td>
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<td>DNA profiling: AmpF/STR® Profiler Plus™</td>
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</tr>
<tr>
<td><strong>Cyanoacrylate followed by basic yellow or safranin staining</strong></td>
<td>Chlorine-free paper or plastic sheets</td>
<td>Extraction: QIAmp-based DNA isolation (Qiagen; standard protocol).</td>
<td>Quantification: no quantification</td>
<td></td>
<td>Cyanacrylate and VMD (and the combination of both) did not affect subsequent DNA profiling. Cyanacrylate combined with safranin or basic yellow staining resulted in less</td>
</tr>
<tr>
<td>VMD</td>
<td>DNA profiling: AmpF/STR® Profiler Plus</td>
<td>donor DNA was retrieved. Ninhydrin or DFO treatment did not affect subsequent DNA profiling. Physical developer was found to be deleterious for DNA profiling.</td>
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</tbody>
</table>
| DFO | Black and white powders on glass, cyanooacrylate on non-porous surface, DFO on paper | Extraction: Nucleomag 96 Blood kit (Macherey-Nagel, Düren Germany)  
Quantification: qualitatively with the amplification of the beta-globin gene (KM38 and PC03 10X primers and QIAGEN Multiplex PCR MasterMix), and two more specific methods: RT-PCR using “Quantifiler Human” kit (Applied Biosystems, Foster City CA USA) and the NanoDrop spectrophotometer (Thermo Scientific NanoDrop1000 Spectrophotometer).  
DNA profiling: PowerPlex ESX 17 System” (Promega)  
No differences were noticed between samples enhanced and unenhanced samples. Comparable DNA profiles were obtained from processed and unprocessed samples. (Gino and Omedei 2011) |
| Ninhydrin physical developer | Glass, plastic, aluminium, plastic-coated paper, pantyhose and paper (white, colored, recycled) | |
| Black and white powders on glass, cyanooacrylate on non-porous surface, DFO on paper | Glass, plastic, aluminium, plastic-coated paper, pantyhose and paper (white, colored, recycled) |
| No differences were noticed between samples enhanced and unenhanced samples. Comparable DNA profiles were obtained from processed and unprocessed samples. (Gino and Omedei 2011) |
| Wet powder, magnetic powder, black powder, gel lifters, silver nitrate, cyanooacrylate, physical developer and ninhydrin | Tape, lab bench, wood, plastic bag, brown paper envelope, white paper envelope | Extraction: Wizard Genomic DNA Purification Kit (Promega, Madison, WI)  
Quantification: Real-Time PCR using TaqMan Universal PCR Mix with probe (RBI-2727) labelled with FAM for nuclear DNA amplification.  
DNA profiling: STR without mentioned which chemistry has been used.  
Physical developer and silver nitrate eliminated DNA completely.  
In general, we observed a large difference in the DNA amounts extracted after treatment with different techniques. As expected, untreated prints on paper and tape contained more DNA than most treated prints. (Norlin et al. 2013) |
| Super Black Powder obtained from Lynn | Glass | Extraction: Organic method  
DNA recovery from enhanced fingerprints was low (concentration of |
| Steadman et  |
| Pea-vey (Lenexa, KS) | Quantification: Quantifiler™ Duo quantification kit (Applied Biosystems) DNA profiling: PowerPlex™ 16 kit | recovered DNA ranged from undetermined to 4.130 pg/µl) compared to unenhanced fingerprints (concentration of recovered DNA ranged from undetermined to 643 pg/µl). 1-2 alleles were generated from all 8 samples underwent STR profiling. | al. 2015 |
1.5.5 Touch DNA Sample Collection

Generally, crime scene investigators and laboratories collecting touch DNA using the wet / dry swabbing method, namely the double swab technique (Sweet et al. 1996). By employing this swabbing method, the surface of the object is rubbed with a wet cotton swab, followed by a dry cotton swab in an effort to collect possible skin cells/DNA. The double swab method is recommended for hard, non-porous items such as metal, glass or plastic; which can easily be conducted at the crime scene with limited risk of contamination with exogenous DNA (e.g. from the person collecting the sample, or from nearby surfaces/objects (Koblinsky et al. 2005; Pang and Cheung 2007).

Swabbing an area requires a wet swab to traverse the whole targeted area several times along with some pressure and rotation of the swab (Sweet et al. 1996), allowing the full surface area of the swab to contribute to collection. However, a moist cotton swab does not pick up all of the available biological material from the surface and, in many cases; it may pick up less than half the available sample (van Oorschot et al. 1998). Multiple swabs for a particular surface and the co- extraction of these swabs have been used to facilitate overall retrieval of DNA (Pang and Cheung 2007). It is now considered standard to perform a double swabbing technique.

Mainly, swabs are moistened with water, but some laboratories use 0.01% Sodium dodecyl sulphate (Prinz et al. 2006) or isopropanol (Collopy 2008). Less attention has been given to research optimising the solution for the retrieval of touch DNA. Studies have shown that some surfaces are more difficult to collect samples from than others and the use of different moistening agents for different surfaces may enhance collection (Templeton and Linacre 2014).
Currently, several types of cotton swabs are utilised in trace DNA collection but other swab types such as foam are also in use (Hansson et al. 2009). The choice between the several types of swabs can be a matter of convenience, for instance if the swab was successfully used to collect other sample types or is related to financial issues. Limited research has been undertaken to examine the suitability of the swab type used to collect touch/trace samples (van Oorschot et al. 2010). van Oorschot et al. (2010) suggested optimal collection methods and swab types for particular samples on certain substrates would be advantageous and assurance of DNA-free sterile swabs is vital to preclude the problems of contamination.

Some variation in DNA retrieval between different swab types has been noticed, nylon flocked swab produced significantly higher yields of DNA (control DNA recovery 65%) than cotton swabs (control DNA recovery 35%) when both were extracted using the QIAamp DNA investigator extraction kit (Brownlow et al. 2012). These variations in DNA yield between nylon flocked and cotton swabs depending on the extraction method used, where combination cotton swab /QIAcube automated extraction and nylon / QIAamp DNA investigator extraction kit (manually conducted) yielded the highest quantities of DNA with percentage recovery values of 64.5 ±8.0% and 59.3 ± 10.0%, respectively (Brownlow et al. 2012).

Leaving loaded swabs to dry out before conducting the DNA extraction, results in less DNA yield; immediate processing of swabs gave considerably larger amounts (van Oorschot et al. 2003). Preserving swabs in -20°C or -80°C post collection, rather than drying it prior to extraction results in DNA recovery rates close to that of a moist swab (van Oorschot et al. 2003). Directly freezing swabs is now routinely in some forensic laboratories (van Oorschot et al. 2010). Cotton swabs have been reported to contain

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inhibitory factors or introduce profiles that were difficult to interpret (van Oorschot et al. 2010), even more by introducing some undesirable substances into the extraction tube, such as isopropanol residues (swabbing solution), was seen through inhibition generating DNA profiles from the self-saturating swab extracted by Chelex (Hansson et al. 2009).

With certain types of surfaces, swabbing was not found to be the best choice; for example lifting trace evidence including DNA attached to the surface of worn clothing (Hansson et al. 2009). For further forensic investigation, the use of adhesive tape has become a commonly used recovery approach in a broad range of forensic disciplines (Wickenheiser 2002; Hansson et al. 2009). For DNA recovery, the adhesive end of a tape strip is repeatedly pressed against the material to be examined. The most recently deposited material, with fewer inhibitory factors, will be transferred and accumulated on the tape surface (Franco and Goetz 2006). Some laboratories are using tape or mini tapes to collect biological material not only from clothing, but also from other touched surfaces (Hansson et al. 2009). Generally, some precautions can prevent DNA contamination, for instance tape pre-treated in a UV cross-linker to ensure no extraneous DNA can be profiled. There are some obstacles with tape lifting; for instance the need to use more than one tape strip when dealing with a large surface area may pose downstream processing difficulties. The adhesives can interfere with the DNA extraction process. Such obstacles meant examiners had to validate their extraction technique, so that they can successfully get rid of the adhesive without affecting the DNA yield (Gunnarsson et al. 2010).
1.5.6 Extraction DNA from Touch Samples

Throughout the 1990s, forensic examiners have placed reliance on Chelex 100 (Bio-Rad, CA, USA: (Walsh et al. 1991) and organic solvent methods (Sambrook et al. 2001) to extract DNA from forensic specimen. The widespread use of commercial kits; most of which are based on the use of silica-matrix columns; and methodology, optimized for specific types of samples, have become commonplace during the last three decades (Greenspoon et al. 1998). Recently kits have been developed which utilize silica-coated magnetic beads capturing DNA from the cell lysate (i.e. Promega’s DNA IQ) such kits were optimized and recruited successfully in automated systems (Greenspoon et al. 2004; Frégeau et al. 2010).

Although a number of DNA extraction methods have been utilized by forensic examiners to recover DNA samples, up to 75% (organic extraction) of DNA loss can occur depends on the extraction methods are used (Frégeau et al. 2010; van Oorschot, et al. 2003). The substrate on which the sample is deposited was found to contribute towards some DNA loss but mainly is attributed to the method itself. The fundamentals that magnetic bead procedures are based upon is binding DNA molecules to silica coated magnetic beads, then beads would be held aside using a magnetized rack or tube holder, and washing off other components of the sample (Greenspoon et al. 2004). Although validation studies of bead based kits were successful in the extraction of DNA from small stains deposited on difficult substrates, such as denim, leather, and soil, the systems have a low binding capacity (over 0.1-0.5ng) and the remaining DNA is discarded (Schiffner et al. 2005; Hudlow et al. 2011). However, as a result of what was mentioned previously, there is a focus on optimizing extraction techniques specifically for low DNA content samples.
In order to avoid or limit the possibility of obtaining non reliable DNA results, forensic examiners have tried to conduct replicate tests to improve the reliability of the analysis of low template samples (Budowle et al. 2009). However, when dealing with a trace sample, the elution of the DNA in a relatively large volume could be limiting. In some occasions, the concentration of the DNA extract is required, by reducing extract volume. Such a concentration step would limit the replication of the test as a result of reduction of the final volume of DNA extract. Moreover using concentration and clean up devices was reported to cause DNA loss (van Oorschot et al. 2003). This loss found to be reduced by adding PolyA RNA or salmon sperm DNA to the concentration device, such additives would occupy areas on the devices on which desired DNA could adhere (Schiffner et al. 2005).

In general majority of extraction methods lack of efficiency in DNA recovery (van Oorschot et al. 2010), several recommendations by van Oorschot et al. (2010) were proposed to improve methodology specifically for such challenging evidentiary samples such as (a) minimizing DNA loss and collecting as much as the all possible DNA; (b) purifying DNA from inhibitors without the loss of DNA; (c) when dealing with extreme low DNA quantities, utilizing all of the extracted DNA for amplification might be a good choice for success as more DNA might available to be amplified, when the entire sample was amplified with 34 PCR cycles, 67% of the loci were complete and correct, 25% were heterozygous loci that showed only one allele, 5% showed complete locus drop out and 3% showed allele drop compared to that consensus profiles, which showed a notable increase in allele and locus drop out (Grisedale et al. 2012) (d) avoiding DNA transfer by minimizing extraction steps and in some occasions adding amplification components to the tube containing the DNA; and (e) direct PCR is promising approach to overcome obstacles of losing DNA during extraction and
purifying steps, it has gained increasing interest over the last few years. Newer, more robust amplification kits claim to perform direct amplification better and faster than kits previously available in the forensic science community such as PowerPlex® 16, 16 HS, 18D, and 21 Systems from Promega Corporation, and AmpFlSTR® Identifiler® Direct, Identifiler® Plus, and Identifiler® PCR Amplification kits from Applied Biosystems.

1.5.6.1 Chelex Extraction

An alternative and inexpensive method for DNA extraction from a wide range of samples, during the 1990s it has been become popular among forensic scientists is the use of the chelating-resin suspension that can be added directly to the samples. In 1991, Walsh et al. (1991) introduced Chelex 100 resin to the forensic community as the least expensive method that can be used in DNA extraction. Although phenol-chloroform purification is known as a powerful method for removing organic solvent soluble PCR inhibitors (Hochmeister et al. 1991b) phenol is highly toxic and corrosive, and may have a negative effect on subsequent PCR, so it is being replaced by other methods. Chelex is a chelating polymer that binds polyvalent metal ions that may otherwise catalyse DNA degradation at high temperatures and low ion content (Walsh et al. 1991). Additionally, Chelex extraction is usually conducted in a single tube, no sample transfer or removal of reagents is needed, thus reducing DNA waste and minimizing the risk of contamination and sample mix-ups. Disrupted cells are pelleted early in the process, and water-soluble PCR inhibitors are removed. Nonetheless, disrupted cells and free DNA may be lost, possibly lowering the yield. In order to achieve cell lysis, samples are heated to 56°C then boiled to denature and degrade proteins. Care should be taken to avoid introducing Chelex beads into the PCR vessels, as they would chelate
vital Mg$^{+2}$ ions. However, the extracts are generally not pure and amplification may be obstructed, especially when less inhibitor-tolerant DNA polymerases are used (Hedman et al. 2011a; Hedman et al., 2011b). The combination of Chelex extraction with proteinase K digestion mediates cell lysis, and eases degradation of proteins that would hinder the PCR amplification (McHale et al. 1991).

1.5.6.2 Silica Based Columns

One of the pioneering steps in DNA extraction was the solid-phase extraction method that has been developed in the last three decades in formats that enable high-throughput DNA extractions. This approach is considered the most effective, as silica is selective in allowing nucleic acids to adsorb to the silica support, such a small glass beads. The addition of highly concentrations of chaotropic salt for instance guanidine hydrochloride, 6M guanidine thiocyanate, sodium iodide, 6M sodium chloride, sodium perchlorate (Vogelstein and Gillespie 1979; Boom et al. 1990; Duncan 2003) during or after cell lysis disrupts the hydrogen-bonding network of proteins, thereby make denatured proteins and nucleic acids more thermodynamically stable than their correctly folded or structured counterparts (Tereba et al. 2004).

In solutions with a pH of 7.5, DNA adsorption to the silica is estimated to be around 95% and unwanted impurities can be washed away (Hanselle et al. 2003). In alkaline conditions along with low salt concentrations, the DNA will elute from the silica material (Hanselle et al. 2003). Successfully, the solid-phase extraction method was automated by performing the centrifugation or vacuum manifolds in single tube or 96-well plate formats (Yasuda et al. 2003). Several automated platforms commercially available to process Qiagen DNA extractions such as QIAcube (Qiagen), EZ1
(Promega), and JANUS® Forensic Workstation (PerkinElmer). The solid-phase extraction is also being developed into formats that will work on microchip (Wolfe et al. 2002).

1.5.6.3 DNA IQ® System

Another solid phase extraction approach is the DNA IQ™ system (the IQ stands for “isolation” and “quantitation”) is based on salting-out and binding DNA molecules to silica coated beads which added directly into the extraction tube (Tereba et al. 2004). A magnet is used to draw the silica-coated beads to the bottom or side of the extraction tube leaving any impurities in solution. The solution of impurities can easily be pipetted out of the extraction tube. Subsequently the DNA-magnetic bead complex is washed and the DNA removed under low salt and high pH conditions (Tereba et al. 2004). Nevertheless, impurities such as denim dyes and proteins will initially compete with DNA for the binding sites on the magnetic beads (Poon et al. 2009). Any impurity is then removed during the washing steps yielding, in the final extract, purified DNA molecules suitable for downstream amplification analysis. The DNA IQ™ system requires the binding of DNA to the magnetic beads; chemicals and/or conditions that deter either the binding of DNA or the elution of DNA from the beads will have a negative influence on the performance of DNA recovery using this procedure (Poon et al. 2009).
1.5.7 Touch DNA Quantitation

Although in case of casework samples it is necessary to quantify DNA (FBI Quality Assurance Standard 9.4) (Date 2008), it may not always needed for trace DNA samples (Caddy et al. 2008); when the expected concentration of DNA is low, a prior knowledge of the DNA template concentration would be useful when interpreting the DNA profile results (van Oorschot et al. 2010), because too little DNA can lead to non-detection of alleles (allelic dropout), allelic drop-in, enhancement of stutter product amount and heterozygote peak imbalance as a result of stochastic PCR amplification (Gill 2001). However, obtaining a negative quantitation result should not prohibit subsequent amplification and downstream processing of trace DNA samples should be carried out. In spite of the sensitivity of quantitative PCR (qPCR), with certain kits its down to <1 pg/μl such as Investigator Quantiplex Kit (Qiagen), some research has established that STR profiling results can be obtained even when no measurable DNA is found (Cupples et al. 2009). These results demonstrated that partial or even complete 9 locus STR profiles (AmpF/STR® Profiler Plus® PCR Kit) obtained from samples that gave negative DNA quantities with one of the most commonly used forensic quantification kit, the Quantifiler Human DNA Quantification kit where its DNA detection limit down to 16pg/μl (Applied Biosystems, Foster City, CA, USA) (Cupples et al. 2009).

Assuming touch DNA samples always contain only low DNA quantity is erroneous, since the DNA quantity depends on multiple factors, such as the substrate nature, frequency and duration of the contact, together resulting a highly variable range of recovered DNA in the region of 0-100ng (Castella and Mangin 2008; Daly et al. 2012; Verdon et al. 2013). Determination of the amount of DNA of all samples would be useful in preventing replication analyses of samples that needed to confirm results
(Caddy et al. 2008). Generally, any quantitation result of very low amounts of DNA must be considered as an indication of the concentration, rather than as an absolute measurement of the input amounts (van Oorschot et al. 2010).

1.5.8 **Touch DNA Amplification**

Without the ability to make copies of DNA molecules, many forensic specimens would be impossible to examine. Such potential was realised by van Oorschot and Jones (1997) who reporting on the production of DNA profiles from the minute amounts of DNA present in a fingermark. In addition, it has been demonstrated that DNA profiles for clinical analysis could be obtained from a single cell by increasing the number of cycles to 34 in the PCR reaction; a feat not possible through standard amplification protocols (Findlay et al. 1997).

In the UK, a low template protocol was first published in forensic context in 1999, where it was established that the production of profiles from less than 100pg of DNA was most ideally performed by increasing the thermal cycling for SGM Plus from 28 cycles to 34 cycles which was named ‘Low Copy Number (LCN)’ DNA protocol (Gill et al. 2000).

Since its inception, the application of LCN technology has been vital to many cases, several of which were confined to a “cold case” category. For instance, Antoni Imiela was charged as the rapist and murderer of Marion Crofts, by the recovery of a DNA profile from minute traces of semen recovered from a 20 year old microscope slide (Forensic Science Service 2005). The addition of extra cycles improved the sensitivity of PCR product by 64-fold. The extra product allows detection of decreasing
amounts of DNA but produces increasing incidences of artefacts (Gill et al. 2000). The Forensic Science Service pioneered the invention of LCN in the United Kingdom. The LCN approach would involve the 34cycle amplification of samples thought likely to contain trace amounts of DNA, excluding the typical quantification step in order to save template DNA (Caddy et al. 2008). Additional approaches have been applied in order to increase the detection of PCR product of low template DNA samples, for instance increasing the PCR product injected for capillary electrophoresis as well as purifying PCR product, Roeder et al. (2009) reported that compared to the results from non-enhanced PCR products, the number of scorable alleles increased by 60% (AmpF/STR® SGM Plus®) and 62% (AmpF/STR® Identifiler®) after adding 1µl of purified PCR product to 9µl Hi-Di™ formamide/internal lane standard mixture, while adding 9µl to 11µl of Hi-Di™ formamide/internal lane standard mixture resulted in 75% increasing of scorable alleles in both assays. Concentrating PCR product, in a study of Forster et al. (2008), where 49ml of PCR product from each sample amplified (AmpF/STR® SGM Plus®) under the 28-cycle conditions were subsequently purified and concentrated with the Qiagen MinElute PCR purification kit (Qiagen Ltd., UK). Cleaned PCR product was eluted in 10ml of the included Elution Buffer resulting in an approximate assumed 5× concentration of the PCR product. A 64% increase in the mean of peak high area was noticed in samples underwent Post-PCR purification compared to that non-purified. There are a number of advantages for trace DNA samples in progressing from the standard 28-cycle process to the post-PCR processing method as compared to 34-cycle PCR method, including reduced sample consumption, reduced number of PCR amplifications required, and a staged approach to sample processing and profile interpretation (Forster et al. 2008).
1.5.9 DNA from Touched Objects Used Within Simulation & Criminal Investigations

With the modern technology of DNA analysis, a simple act of picking up an object or touching a surface can lead to the identification and apprehension of a criminal. The analysis of touch DNA samples is no longer an exception to the rule but it is a norm. In the last two decades, both the number of touch DNA evidence items being submitted to the lab for analysis, and the number of research articles concerning touch DNA and DNA transfer (both primary and secondary) have risen steeply.

Several workers in the field of touch DNA have conducted research in response to casework scenarios, and some examples are considered below:

In Belgium, researchers recovered profiles from tools, bag grips (chisel, screwdriver, hammer), clothing (glove, trousers, hat, shirt) and bags with considerable success (Van Hoofstat et al. 1998). Evidence items first examined for fingerprints using powders and spray (no details was provided about fingerprinting enhancement materials were used). Samples for DNA fingerprinting were taken using a sterile scalpel or swab. DNA was extracted Chelex extraction. DNA profiling was conducted at first with in house STRs multiplex system in which 7 multiplex of 7 STR loci (CD4, TH01, D21S11, SE33, D16S539, D8S1179 and D18S51) was used. In addition two commercial STR kits were used Powerplex™ and Profiler™. PCR reaction has been optimized. Although problems were encountered in obtaining good DNA fingerprints, when the objects were powdered or sprayed for regular fingerprint analysis, the results of DNA profiles obtained from clothes gave the best results when 40 cycles used. It was possible to obtain a complete DNA profile of all loci without extra amplification. Both commercial kits gave comparable results although the intensity of the observed peaks
was smaller than in house 7 STRs multiplex PCR. This difference was more seen in the DNA profiles obtained from tools and grips of bags. 40 cycles were found necessary to obtain full profiles. However it was early research that demonstrated the ability to acquire a useful DNA profile from a touched evidence items.

In Canada in a murder case, assailant broke into a family residence and removed two steak knives from the kitchen drawer. He then entered the bedroom of two eight year old twin girls, and subsequently stabbed one girl with a single fatal thrust. The murder weapon and the second knife were then dropped on the deceased's bed. Knives were processed for fingerprints with cyanoacrylate and metal deposition. No fingerprints were located. Blood was identified on the blade of one of the knives. No blood was identified on the blade of the second knife. The blade of first knife and handles of each knife were swabbed with distilled water. DNA was extracted from these swabs using a single-step phenol/chloroform, followed by Microcon 100 purification. The DNA was then quantified using ACES 2.0+ Human DNA Quantification System (Gibco BRL, Gaithersburg, MD). The DNA was amplified using three separate PCR multiplex systems, which referred as STR1, STR2, and STR3. The amplified DNA was then analysed using an ABI Prism® 377 gene sequencer (ABI Prism®, Foster City, CA). The swab from the blood identified on the blade of first knife was yielded 392ng DNA. The swab from the handle of first knife yielded 10ng DNA. The swab from the handle of second knife yielded 49ng of human DNA. The DNA profile produced from the swab of the handle of the first knife matched the deceased, with a trace male profile. The handle of second knife, found on the bed of the deceased, provided a DNA profile. This DNA profile matched that of the known suspect blood sample (Wickenheiser and Challoner, 1999).
Thirty-eight lip cosmetics were obtained from eleven subjects; DNA was extracted from the swabs with Chelex (BioRad, USA) and quantitated using the QuantiBlot kit (Applied Biosystems) DNA profiling was performed with the AmpF/STR® Profiler Plus™ PCR Amplification Kit (Applied Biosystems). No full profiles were recorded, 44.8% of the profiles were partial with 10 loci (Webb et al. 2001). In this research the detail was scant with regard to in which way the cosmetic materials interfered with DNA analysis process.

Another study by Bright and Petricevic (2004) sought to recover DNA from footwear insoles in which 11 items of footwear were collected from two donor wearers and the insoles removed for DNA analysis. Three areas of the insole were examined using three different collection methods. Tape lifts were collected from the heel and toe regions of each shoe and pooled. The heel and toe areas were sampled using the double swab technique. Further areas of the heel and toe were cut out and extracted using the soaking method. Shoe insole samples were extracted using the organic extraction method. DNA quantification was conducted by ACES™ 2.0+ Human DNA Quantitation (Life Technologies). AMPF/STR® SGM Plus™ was used in profiling DNA samples. DNA yield ranging from 0.2 to 2.0ng of DNA, were obtained from 8 of the 11 tape lifted shoe insole samples, indicating the presence of human DNA. Five of the 12 swabbed samples yielded DNA ranging from 0.2 to 0.5ng; while 6 of the 9 soaked samples gave results ranging from 0.16 to 0.8ng of recovered DNA. Full DNA profiles were obtained from 8 of the 10 shoe insoles examined. Six of these were taken from sports shoes and two from leather dress shoes. DNA profiles were not obtained from two of the insoles from sports shoes, despite showing positive quantification results. However, in this research it would appear that only two volunteers contributed footwear for DNA analysis, which limited the value of their results.
Saravo et al. (2004) reported on a strangulation case where the victim was assaulted with a steel cable put around the neck, but was able to run away, and the contact was only for a few moments. Police found a 3m long cable, which was 0.6 cm in diameter, at the suspect’s house and immediately submitted it to DNA analysis. The specimen cables were at first cut into 10 portions and each portion was washed for 3h in an ultrasonic bath in 1ml of a lysis buffer in the presence of proteinase K (20mg/ml) in a single tube. The supernatant was concentrated using Microcon 100 Amicon. DNA extraction was carried out by Chelex procedure. No quantification step was reported. The amplification of extracted DNA was performed by AmpFLSTR Identifiler kit (Applied Biosystem) following the 28 cycle protocol. This crime prompted further experimentation to prove that DNA transfer was possible with a limited contact of 30sec for this material type. The findings state that prolonged time of contact with the cable may lead to stronger profiles. The research emphasises the immediate availability and preservation of the item for examination along with additional lab experiments to support the analysis outcomes.

Unused single white flat bed sheets (polyester cotton) were provided to five volunteers. These new sheets were placed on the volunteers’ beds. Volunteers slept on the sheet for one night, by themselves. The next morning, approximately 3cm² of fabric were cut from the upper shoulder, mid body and foot areas of each lower sheet. The sheet samples were cut into smaller pieces. DNA extraction was conducted by organic method, DNA concentration was estimated using the QuantiBlot® Human DNA Quantitation system (Applied Biosystems). DNA profiles were generated by AMPF/STR® SGM Plus™ (Applied Biosystems) with 28 PCR cycles. The results show that DNA can be obtained from bedding after one night of sleeping in a bed. More DNA recovered from the upper shoulder compared to mid body and foot area. 1.6ng,
1.6ng, and 1.5ng recovered from three volunteers from the upper shoulder while DNA recovered from only one of the five volunteers from mid body and foot. The profiling results mirrored that of DNA quantifications, profiles were obtained mainly from upper shoulder area. Furthermore when volunteers asked to sleep for one night in somebody else’s ‘bed’ in which they had never previously slept, the results demonstrated that DNA profiles were obtained for both, the owner of the bed and another occupant (Petricevic et al. 2006). This research provides some knowledge with regard to crime scene location and recovery and what the probabilities are for achieving a successful outcome.

In Denmark a survey of crime case in which 241 car interior sample swabs were collected in the period of 2003–2004. 6 areas were targeted (wheel, gearshift, handbrake, door handle, window and headrest). DNA was extracted from the swabs using the Chelex method and concentrated using Centricons®. DNA concentration of the extracts was quantified with a slot blot method. DNA profiling was performed using the AmpF/STR® SGM Plus kit with 28cycles PCR. The results showed differences in the DNA retrieval from various locations of the interior of the car. Only 23% of the samples had a DNA concentration 0.02ng/µl. The highest DNA amount recovered from the wheel (65ng) while the lowest amount was recovered from the door handle (5ng). The overall average success rate obtaining full interpretable DNA profile of casework swabs from vehicles was 22% and a match between the profiles was found. Since more DNA was recovered from steering wheels and gearshifts compared to the other part of the interior cars that emphases and these parts are the most used parts during car driving, the types of the contact in real forensic exhibits have a significant effect on the obtained results (Lenz et al. 2006). These results are reinforced by more recent research conducted by Metropolitan Police, UK (2010), which revealed successful DNA profiles
are often obtained from stolen mobile phones (30%) and burgled houses (20%) due to the several touches. In the same vein, trace DNA evidence is shown to be easily recoverable from the personal items of the user, respectively wearer and more DNA was recovered from objects that were handled frequently by the same person for extended periods of time (Raymond et al. 2008). Furthermore, less accessible areas, such as the grooves in mobile phones, were shown to be useful (Tokutomi et al. 2009).

Another case, which occurred in Italy, as described by Barbaro et al. (2006), where DNA profiles were successfully obtained from marker pens and a comb, which were known to be personal possessions of the ‘suspect’. The DNA extracted with Chelex. All extracts were quantified by the Quantifiler Human DNA Quantification kit (Applied Biosystems), less than 100pg was recovered, a modified LCN protocol was carried out to generate DNA profiles as the number of cycles was increased to 35, one more than LCN DNA profiling within the UK using AmpFLSTR® Identifiler® kit (Applied Biosystem). Mixed and partial DNA profiles were found on the material from the pens and ‘cells’ on the comb. It does not specify whether the DNA profile on the comb was full, partial or mixed, only that it matched the scene stain. It is also not stated to what degree it matched the scene stain. However, it does further demonstrate the ability to acquire a useful DNA profile from a touched item. An explanation is not given as to why LCN DNA profiling was used on the suspects’ personal possessions as opposed to obtaining a routine AmpFLSTR® Identifiler DNA sample from him and compared directly with the scene stain. Also there was no mention whether the DNA profile obtained from comb was full, partial or mixed; just matching was recorded with the scene stain. However, the research further establishes that acquiring a useful DNA profile from touched items is achievable.
Another case reported by Staiti et al. (2008) where an old woman was found dead in her apartment in the south of Italy. Her arms were tied behind her back with a nylon rope. No fingermark or any biological evidence was found, few centimetres of the knot and of the portions not in direct contact with the victim were separately collected. Samples were washed with sterile distilled water. The obtained solutions were then submitted to several concentration/filtration steps by means of Microcon™ YM-100 (Amicon, Millipore). DNA extracted using Chelex method. DNA was quantified using Quantifiler™ Human DNA quantification kit (Applied Biosystem). DNA extracted from all the samples were amplified according to the AmpF/STR Identifiler™ and Y-filer™ PCR kit (Applied Biosystem) using 28 and 30 cycles. No differences in outcome were noted between 28 and 30 cycles. In this case a male DNA profile was obtained from the knotted portions of the rope, which was then compared with DNA profiles obtained by some relatives of the alleged offender who had in the meantime settled abroad. The haplotype profiling ultimately assisted with the successful prosecution of an offender. This case emphases that care should be taken during the sampling of the rope to avoid areas that had come into direct contact with the victim, as that would help in avoiding obtaining mixed profiles.

Branch (2011) examined whether it is possible to generate human DNA profiles from fired and unfired cartridge cases, and from gun grips, under varying conditions relevant to forensic casework. Seven common cartridge cases were examined, including 0.22 calibre brass cases, 0.38 calibre brass, aluminium and nickel cases; and 9mm brass, steel, aluminium and nickel cases. Gun grips made of wood, plastic and rubber, with either textured or smooth surfaces, were also sampled. A total of 639 swabs were collected and analysed, 501 for the cartridge case studies and 138 for the gun grips studies. The DNA from the samples was extracted on the BioRobot® EZ1 using the
trace protocol. Quantitation was achieved using Quantifiler™ Human DNA Quantification kits (ABI, Foster City CA). The Applied Biosystems AmpF™STR Minifiler™ system was used to genotype all of the samples. Samples producing Minifiler™ profiles with 6 or more alleles and containing at least 0.1ng of DNA were also genotyped with AmpF™STR Identifiler™ system Identifiler™. Overall, 135 of the 639 samples yielded “full” or “partial” profiles with Minifiler™, 63 from the cartridge cases and 72 from the gun grips. Of the samples that also underwent additional AmpF™STR Identifiler™ profiling, 24 produced profiles. One full profile was produced from a cartridge case and six “full profiles” were produced from gun grips. In addition, four “partial profiles” were produced from the cartridge cases and 14 were produced from the gun grips.

In the research presented by Hoffmann et al. (2012), a new approach was sought for identifying assemblers of explosive devices namely improvised explosive devices (IEDs). 10 of UV crosslinked 600D polyester backpacks were randomly distributed to eight volunteers who used them for 11 days. An additional backpack was served as a positive control in which, predeflagration, a participant handled the 11 targeted areas (five zippers, the top handle, the left and right strap, the neck region, the front middle region, and the front tab) three times a day for 3 days. Backpacks loaded with pipe bombs and deflagrations were conducted in the facility’s smoke room. Following deflagration, bomb and backpack fragments were collected and placed in a new paper bag. Backpacks were individually processed; each backpack was swabbed with sterile cotton swabs (Puritan Medical Products, Guilford, ME) in the 11 targeted areas. DNA was extracted with the organic solvent method. DNA yields from each backpack region were estimated using a Quantifiler Human DNA Quantification Kit (Applied Biosystems). Extracted DNA was amplified using an AmpF/STR®MiniFiler Kit. The
highest yield was 1.25ng/µl from the neck region of backpack 4 whereas the lowest was 0.0014ng/µl for the front tab of backpack 10. The profiling results reported that 46 of the 74 regions analysed had the handlers’ alleles at all nine loci. The top handle was the most effective area for recovering DNA, producing the volunteers’ full profiles in all instances. As a result, a full profile of the handler was obtained from every backpack except at a single locus, providing a feasible method to analyse deflagrated IEDs. The research broadened the applicability of using touch DNA in solving broad range of crimes.

Although a considerable literature has been published on touch DNA, as mentioned previously, and these studies prove the ability to recover DNA from a skin contact has been clearly demonstrated, routine collection of trace DNA evidence remains limited by the ability of the forensic investigator to find and collect the appropriate evidence. Trace DNA analysis is mostly considered for use in more serious cases in context of which the offender is not have been wearing gloves. Usually collection for DNA analysis takes place following the unsuccessful enhancement of fingerprints or smudged marks (Harbison et al. 2008). However, unlike DNA from blood or semen, it is difficult to precisely determine the source and amount of DNA transferred from skin, which raises the question, “which cells are contributing the DNA and how much can a single skin contact transfer?”
1.6 Aims and Objectives

This research study aims to examine experimental approaches to improve the efficiency of DNA recovery from trace DNA deposited in fingerprints.

The literature review that has been done with regard to this research project revealed that there is a lack of information available on the likelihood of different parts of the volar surface to shed DNA on a touched surface. The current research seeks to examine the propensity of palm and fingers to shed DNA. This would help to prioritize evidence recovery as either fingerprint pattern evidence or touch DNA evidence, which is paramount for the forensic evidence recovery plan, as recovery processes for either evidence type can negatively impact on the other (Bandey 2014).

As underlined in the Fingermark Visualization Manual (Home Office 2014), the impact of fingerprint visualization processes on DNA recovery and analysis still needs more research to be carried out to increase the forensic scientists understanding when dealing with such type of evidence. So this research will attempt to provide a better understanding of the influence of some fingerprint enhancement techniques on the trace DNA recovery from fingerprints by evaluating systematically that impact and at which DNA analysis stage occurs. Also, the derived protocols provide approaches to overcome obstacles that might results from using enhancement techniques and those problems resulted from substrates on which fingerprints are deposited.

In order to meet these aims the following objectives will be covered:

- To determine the most suitable extraction methods of E.Z.N.A.® Blood DNA kit, Chelex-100 and the IQ system for DNA extraction from fingerprints.
- To evaluate the propensity of volar surface namely palmar and finger surfaces to
deposit DNA and thus define a new factor that affects deposition;

- To systematically evaluate the impact of different fingerprint development processes namely ninhydrin, aluminium and magnetic powders as well as cyanoacrylate fuming on DNA yield, the mechanism by which influence occurs of fingerprint enhancements on DNA analysis process will be investigated to ascertain whether the impact on DNA recovery can be predicted and used to maximize the chances of yielding successful identification of individuals using DNA analysis-based approaches with this evidence type;

- To maximize DNA recovery from fingerprints deposited upon white office paper with and without ninhydrin development, consequently maximizing the likelihood of obtaining DNA profiles;

- To investigate the applicability of ‘post-it’ tape lifting in collecting DNA containing material from office paper, thus develop a novel method of improving evidence collection from paper surfaces.
2 Materials and Methods

This chapter details the experimental design and methods carried out throughout the entire course of the research.

2.1 Reagents and suppliers Product

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Supplier</th>
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<tr>
<td>Acetone ≥99.5%</td>
<td>Sigma-Aldrich</td>
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<tr>
<td>Aluminium Powder</td>
<td>Tetra Scene of Crime</td>
</tr>
<tr>
<td>Amicon Ultra-0.5 mL Centrifugal Filter</td>
<td>Millipore</td>
</tr>
<tr>
<td>AmpFLSTR® SGM Plus® PCR Amplification Kit</td>
<td>Life Technologies corp.</td>
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<tr>
<td>Chelex® 100</td>
<td>BioRad</td>
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<td>Boots Baby Cotton Buds</td>
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<tr>
<td>Cyanoacrylate</td>
<td>3M</td>
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<tr>
<td>Diothiothreitol (DTT) ≥99%</td>
<td>Sigma-Aldrich</td>
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<td>Promega</td>
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<tr>
<td>DNA Z1 Human control 20ng/μl</td>
<td>Qiagen</td>
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<td>Omega Bio-Tek</td>
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<td>Fisher</td>
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<tr>
<td>Ethylenediamine tetra – acetic acid (EDTA) Di Sodium salt</td>
<td>Fisher</td>
</tr>
<tr>
<td>Fairy washing-up liquid</td>
<td>Procter &amp; Gamble</td>
</tr>
<tr>
<td>GeneScan – 500 ROX size standard</td>
<td>Life Technologies corp.</td>
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<tr>
<td>Glacial Acetic Acid ≥99.7%</td>
<td>Sigma-Aldrich</td>
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<td>Haematoxylin biological stain</td>
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Hexadecyltrimethylammonium bromide (CTAB) for molecular biology ≥99.0 %
HFE 7100
Hi-Di™ Formamide
Hydrogen Chloride ≥99.8%
Investigator® Quantiplex Quantification Kit
Magnetic powder
Methanol HPLC, ≥99.9%
Ninhydrin
Phosphate buffered saline
PHS washing up liquid
POP-4™
Post It Tape Flags®
Proteinase K BioUltra, ≥30 units/mg
Sodium Chloride ≥99.0 %
Sodium Dodecyl Sulphate (SDS) >99%
Sodium Hydroxide ≥97.0 %
Tris-Base ≥99.9%
Triton™ X-100 for molecular biology

Sigma-Aldrich
Sigma-Aldrich
Life Technologies corp.
FLUKA
Qiagen
Tetra Scene of Crime
Sigma-Aldrich
Sigma-Aldrich
Sigma-Aldrich
PHS Direct
Life Technologies corp.
3M
Sigma-Aldrich
Fisher
Fisher
Sigma-Aldrich
Sigma
2.2 Ethics

Ethical permission has been confirmed by the Life Sciences Ethics Committee – Wolverhampton University on 31 March 2011 to volunteers into a study entitled “Experimental Approaches to Improving Trace DNA Recovery from Developed Fingerprints” The samples were anonymised and all samples were destroyed at the end of the study.

2.3 General Sample Handling

DNA Samples recovered from fingerprints were expected to fall below recommended thresholds of 100pg of DNA template (Gill 2001), so extra care has been considered to prevent DNA cross-contamination originating throughout the analysis process, which may be experienced either from the consumables or mishandling of exhibits, thus ensuring the authenticity of the DNA under analysis. Personal Protective Equipment such as gloves, clean lab coats, facemasks were worn and all experiments were conducted in a linear process, in which the DNA analysis was performed within two major stages, the pre-PCR stage (sample preparation and PCR preparation) and the post-PCR stage (PCR execution and analysis) that were spatially separate and utilized separate sets of dedicated equipment. All work surfaces, including balances, were decontaminated prior to processing samples with 10% washing up liquid (10ml Fairy washing up liquid up to 100ml with distilled H₂O) followed by 70% ethanol. Gloves were changed between samples to prevent cross contamination. Only one type of analysis (fingerprint deposition, DNA extraction or addition of template DNA for PCR) was performed at any given time and the benches were completely decontaminated after the completion of each procedure. Negative controls consisting of nuclease free
water PCR grade in place of the DNA extract were included in all sample-handling procedures to monitor the occurrence of contamination. Other negative controls such as blank swabs, glass slides, glass plates, office paper, and enhancement materials were routinely carried out to serve as a guideline in case of encountering contamination. Positive controls, containing a sample or DNA extract of known quality and reliability, were also included in all tests to examine the performance of the quantification or amplification procedures. All tools required for any stage of analysis (e.g. pipettes, boxes of pipette tips, stainless steel scissors, tweezers, plastic tube racks, container of reaction tubes, marker pens, beakers etc.) were wiped with 10% washing liquid then 70% ethanol before exposing each side of the tool to UV-irradiation for a minimum of 30min in a sealed UV chamber (CL-1000, UVP, USA).

2.4 Hand Washing Procedure

Volunteers were asked to wash their hands with water and liquid soap (No-germs™ alcohol free hand sanitiser, PHS Direct) to remove any foreign cellular material. The hand washing was applied to avoid the interference of DNA secondary transfer (intra- and inter-individual DNA transfer), this has been reported as one of contributors encountered towards DNA deposited through a touch, fingerprints can be loaded with DNA containing materials via grooming process from other parts of the body or by touching other others (Quinones 2011). Volunteer were asked to sit in the lab lifting their hands up to air dry for at least 60min prior to testing without wearing gloves or touching any surface. Hand washing was carried out throughout all experiments during the course of this research.
2.5 Glass Surface DNA Decontamination

Plain glass slides 76 × 26mm, 0.8 to 1.0mm thick (Fisher Scientific FB58620, UK.) and glass plates 200×150×4mm (Purchased from local glass merchants) were washed prior to use with plate washing up liquid, cleaned with tap water, rinsed with distilled water and ethanol respectively and left to air dry.

2.6 Office Paper Cotton Buds DNA Decontamination

Commercial office printer paper was used as the starting material for all experiments. A commonly used paper type was selected, a recycled paper with 80gm⁻² mass and 77–78% white ISO 9706. First of all, 30mm × 40mm sections of paper were prepared for all experiments using cleaned scissors. The paper sections were decontaminated using a UV crosslinker for 30min and turned over during the decontamination period.

Commercial cotton buds was used to collecting DNA containing materials from glass. Cotton buds were crosslinked with a UV crosslinker for 40min and turned around two times, 20min intervals. Throughout this research the term cotton swabs will be used instead of cotton buds.

2.7 Fingers and PalmprintDeposition

A glass microscope slide was placed on a balance (Quintix, Sartorius) and set to zero. Volunteers were asked to touch with Combined Middle and Ring Fingers
(CMRFs) a single slide for 30sec, each to an estimated force of 2N (equivalent to 200g on balance reading).

Palm print deposition was conducted by placing a cleaned glass plate on a balance (Quintix, Sartorius) and set to zero. Volunteers were asked to touch with their palm a single glass plate for 30sec, each on estimated force of 20N (equivalent to 2000g on balance reading).

The deposition on paper was carried out by asking volunteers to touch the prepared paper sections as described in section 2.6 for 30sec with their CMRFs with an estimated force of 2N.

2.8 Calculating Surface Area of CMRFS and Palmprints

The surface area of both palm and fingers were calculated by depositing a fingerprint on a glass slide for each one of the fingers, then enhancement using aluminium powder, photography and then surface calculations were performed using Adobe Photoshop software CS3 version 10.0 2007 (Agley et al. 2012). Using the selection tool, the mark area was selected. Then the colour range opened, from the selection preview, black matt option has been chosen. After that, while the shift button was held, either the palm or fingers prints were selected. The fuzziness button was dragged to the right till the all mark area had been selected. From histogram numbers of pixels were recorded. Along with mark pixels measurement, number of pixels of white squared paper (2 × 2cm) was recorded as a reference. Finally to convert pixels to physical units, the number of mark pixels divided by the number of pixels of references time by the area of references white paper (4cm²) to obtain mark areas. The latter
protocol was obtained from Agley et al. (2012) with some modification. The mean of five measures of superficial area of each CMRFs and palm were collected from two volunteers has been calculated and then used in determining the applied force per squared millimetre to be exerted during deposition of CMRFs and palm.

The glass slides or plate were placed on the top of a top pan balance, then volunteers were asked to touch decontaminated slides for 30sec with their CMRFs. Volunteers were asked to exert a roughly constant pressure equivalent to 230g on balance reading for the middle finger and 170g for ring finger. Palm print deposition was conducted using the same deposition instructions as described above but using decontaminated glass plate instead of microscope slides with a force equivalent to 3500g on balance reading.

2.9 Applying Force

In order to examine the effect of applied force during fingerprint deposition on glass, either a DNA decontaminated glass slide (CMRFs) or glass plate (palm) were placed on the top of a digital balance (Quintix, Sartorius) and then set to zero. Two volunteers participated in this experiment. DNA was collected at five sampling events, with each force monitored at different sampling events. Volunteers were asked to wash their hands as previously described (see 2.4), then deposit CMRFs on a single slide, each to approximate forces of 1, 2.5, 5, 7.5 and 10N (equivalent to masses of 100, 250, 500, 750 and 1000g). The forces for palm prints were equivalent to those used with fingers, as follow 10, 25, 50, 75 and 100N (equivalent to 1000 2500, 5000, 7500 and 10000g). The equivalent forces were calculated by dividing the applied forces over the
surfaces area of middle and ring fingertips. To obtain the equivalent forces to be examined against palm, forces applied per each squared millimetre of fingers were multiplied by the total surface area of palm.

2.10 Control DNA Preparation

Control DNA Z1 and QuantiTect (Qiagen) nucleic acid dilution buffer were thawed at room temperature. All solutions were mixed thoroughly before use to avoid localized concentrations of salt. Fresh serial dilutions of the control DNA Z1 were prepared in two concentrations as indicated 1.25ng/µl and 0.312ng/µl prior to each experiment. Prepared dilutions were vortexed for at least 5s and centrifuged each dilution briefly before removing an aliquot for the next dilution. A new pipette tip for each dilution was used between dilutions.

2.11 The Influence of Different Solutions on DNA Recovery from CMRFs

Because the keratinized cells of interest in this study are collected using a cotton swab, it has been hypothesized that a method to remove these nucleated cells from the swab before submitting them to cellular and nuclear lysis protocols using number of surfactants solutions would increase DNA yield. Improving the release of cells from the substrate would theoretically increase the DNA available for extraction.
2.11.1 1% Triton™ X-100 Solution

100ml 1% Triton™ X-100 solution was made at working concentration by mixing 1ml of Triton™ X-100 with 99ml of filtered dH₂O using a stirrer (water filtered with 0.2µm). The solution was filtered with 0.45µm filter, and then stored at 4°C before use.

2.11.2 0.5 % SDS Solution

100ml 0.5% SDS (Sodium Dodecyl Sulphate) solution was made at working concentration by dissolving 0.5g of SDS in 20ml filtered dH₂O with 80ml up with filtered dH₂O. The solution was filtered with 0.2µm filter, and then stored at 4°C before use.

2.11.3 2 % CTAB Solution

100ml 2% CTAB (hexadecyltrimethylammonium bromide) solution was made at working concentration by dissolving 2g of CTAB in 20ml and with 80ml of filtered dH₂O. The solution was filtered with 0.2µm filter and stored at 4°C before use.

2.12 Reference Samples

The participants’ reference samples were collected using buccal swabs and DNA extraction has been conducted using the E.Z.N.A® Blood DNA Kit (Omega Bio-Tek) following the manufacturer’s instructions for buccal swab protocol.
2.13 Direct Swabbing

Either middle and ring distal phalanx or the whole palm of four volunteers was swabbed directly according to double swab technique (Sweet et al. 1996). The wet swabs were prepared by loading a sterile cotton swab with 50µl of filtered dH$_2$O. The whole surface area of fingers and palm was swabbed with a wet swab followed by a dry swab with the same swabbing protocol. The surface of each of the two fingers and palm was swabbed for about 15s using moderately strong pressure and circular motions. The swabs were rotated along its long axis allowing every side of the swabs to come into contact with finger and palm surface. Both swabs heads were cut off from the stem using sterilised and cleaned scissors and were placed into a sterile 2ml microcentrifuge tube.

2.14 Fingerprint Detection Techniques

2.14.1 Dusting with Aluminium Powder (AL) (Bowman 2004)

Application was done by sprinkling flakes on CMRFs deposited on glass slide and shaking the surface manually as described by Pesaresi et al. (2003). A brush was not used to apply flakes, as it removes DNA (van Hoofstat et al. 1999). The developed slide was left at room temperature and DNA extraction immediately conducted.

2.14.2 Dusting with Black Magnetic Powders (MG) (Bowman 2004)

The magna brush was placed into the container of black magnetic powder, which produced a bristle-like effect at the end of the wand when withdrawn. A gentle circular motion was applied to the surface being examined that contained CMRFs or palmprint;
an important consideration was that only the magnetic powder should touch the surface, not the wand. After the print has been developed, the wand was held over a new container and the control rod withdrawn. The magnet was disengaged and released the powder. Then the magnet re-engaged and the clean wand passed over the developed latent print and the surrounding area to remove any excess powder. This process always used fresh powder to minimise secondary DNA transfer. The developed slide was left at room temperature and DNA extraction immediately conducted.

2.14.3 Ninhydrin Acetone Base (Bowman 2004)

0.6g of ninhydrin crystal was dissolved in 20ml acetone with minimum stirring as required to get complete dissolution, then made up to 100ml with acetone and stored in a dark container.

2.14.4 Ninhydrin HFE 7100 Base

The ninhydrin – HFE7100 was prepared following the procedure described by the Manual of Fingerprint Development Techniques (Bowman 2004). 25g of ninhydrin crystal were placed into 500ml beaker and then 225ml of absolute ethanol was added to the beaker and stirred at room temperature until the ninhydrin crystals have completely dissolved. During stirring, 10ml of ethyl acetate was added, followed by 25ml of glacial acetic acid. Stirring was continued until a clear yellow solution appeared. The mixture was transferred to a clean, dark container and was stored at room temperature.
To prepare a working solution of ninhydrin-HFE-7100, 52ml of the concentrate solution was added in a 2L beaker and then 1000ml of HFE-7100 was added to the concentrate solution. The mixture then placed on a stirrer until a fully clear solution appeared. The working solution was transferred to a clean, dark container.

2.14.5 Ninhydrin Application Method

Office paper 30mm × 40mm was DNA decontaminated using a UV crosslinker for 15min each side of the paper as described in section 2.6. After hand washing, 60min prior to deposition, as described previously in section 2.4. CMRFs were deposited on the office paper by touching surface of the paper for 15sec followed by spraying the with the ninhydrin working solution either ninhydrin acetone base or ninhydrin HFE7100 base to completely saturate the paper and then it allowed to dry completely at room temperature. Once the latent impression developed the enhanced paper was subjected to DNA extraction.

2.14.6 Direct Mixing of Control DNA with Ninhydrin Acetone Base, Ninhydrin HFE-7100, Acetone and HFE-7100

In order to examine whether ninhydrin, acetone and HFE-7100 has an inhibitory effects on DNA amplification, control DNA was prepared in two different concentrations as indicated; 1.25ng/µl and 0.312ng/µl. 10µl aliquots of each concentration (12.5ng and 3.12ng) were mixed with equal volumes of acetone based ninhydrin, acetone, HFE based ninhydrin and HFE in separate experiments. The mixtures were uncapped and left in AirClean® Systems AC600 PCR Hood (AirClean®
Systems) at room temperature for 3-4h. Then 2µl of each mixture was taken for DNA quantification using qPCR.

2.14.7 Cyanoacrylate Ester (Superglue) Mark Development (CA) (Bowman 2004)

The loaded glass slides with fingerprint of middle and ring fingers were suspended within a 40×20×20cm plastic cabinet, the cabinet was placed inside a fume hood to remove fumes of cyanoacrylate vapour before the cabinet was opened and then slides were removed. Sufficient space between slides was maintained to ensure circulation of the vapours and exposure of all surfaces of glass slides. The cabinet was then humidified by placing a glass beaker of distilled water on a heater for 1h prior the addition of superglue. Then 3ml of superglue was evaporated from an aluminium foil pot on a heater at approximately 80ºC. The glass slides were left within the cabinet for one hour to allow cyanoacrylate fuming to take place, the development process of the samples was monitored to halt the development cycle if it looked as if overdevelopment of prints is beginning to occur. The developed slide was left at room temperature and immediately taken for DNA extraction.

2.15 Recovering DNA from Fingerprints Deposited On Glass Using Swabbing Technique

Palm and/or finger prints deposited on glass were swabbed using a double swab technique as described by Sweet et al. (1996). 50µl of filtered dH₂O water was loaded onto a crosslinked cotton swab so that it was completely wet. This swab was rolled over the CMRFs loaded surface of glass slide with firm pressure and using circular motions,
traversing over the target area multiple times. In case of palmprint, as the glass plate surface is larger than slides, the swab was rewetted during the swabbing process by dropping another 50µl of filtered dH₂O onto the swab. A second, dry cotton swab was then immediately rolled over the same surface using approximately similar pressure and movements as the first swab, ‘mopping’ up all residual moisture on the surface.

2.16 Recovering DNA from Fingerprints Deposited On Paper

2.16.1 Cutting Method

Office paper was cut into approximately 2mm² portions with decontaminated scissors and suspended in 800µl of filtered dH₂O containing Proteinase K (100µg/ml) and immediately subjected to DNA extraction.

2.16.2 Liquid Nitrogen

Two sets of samples were prepared; the first set was CMRFs deposited on office paper where three volunteers participated. The second set was 10µl aliquots of 1.25ng control DNA loaded onto paper. The paper was ground in liquid nitrogen into a powder using a mortar and pestle and re-suspended in 800µl of filtered dH₂O containing Proteinase K (100µg/ml) and immediately subjected to DNA extraction. Three replicates were prepared for each set of samples.
2.16.3 Collecting DNA/Cells from Office Paper Using Post it Tape Flags®

Volunteers were asked to deposit onto a paper section CMRFs as described in 2.7. Post it Tape Flags® were taken from their container and one end of the tape was held near to one side of the paper section, previously loaded with the fingerprints. The adhesive surface of the Post it Tape Flags® was rolled slowly over the prints. The flag tape was rubbed using the thumb at least 20 times before pulling up to ensure good adhesion to the latent prints and to maximise the DNA/cells transferred. The tape flag was then pulled away and was cut into approximately 2mm² portions with decontaminated scissors, placed in 2ml microfuge tube and then subjected to DNA extraction using E.Z.N.A kit as described in section 2.17.3. Office paper was cut into approximately 2mm² portions with decontaminated scissors and suspended in 800µl of filtered dH₂O containing Proteinase K (100µg/ml) and immediately subjected to DNA extraction.

2.17 DNA Extraction
2.17.1 Chelex® 100 Extraction

500µl of filtered dH₂O containing proteinase K (100µg/ml) was transferred into the 2ml microcentrifuge tube contained the swabs, vortex mixed for 30sec and incubated at 37°C for 60min, with occasional vortex mixing throughout the incubation period. The 2ml microcentrifuge tube was removed from the heat source, and the entire tube contents were transferred into a new DNA free spin basket (Promega) seated in a new 2ml microcentrifuge tube and then centrifuged at room temperature for 2min at 14000rpm to collect all liquid and cellular materials that might remain on the swab head. The spin basket was then removed from the 2ml microcentrifuge tube and
discarded. 200μl of resuspended 5% (w/v) Chelex® 100 (Bio-Rad, Hercules, CA, USA) stock suspension was added to the 2ml microcentrifuge tube and vortexed vigorously for 15s to re suspend the pellet before incubation at 56°C for 30min. The solution was then vortexed for 15sec and incubated at 100°C for 8min. The 2ml microcentrifuge tube was then centrifuged in a microcentrifuge for 1min at 14000rpm. Taking care not to transfer any Chelex® 100 beads, approximately 850μl of the DNA containing supernatant was then transferred by pipette, to a DNA free, labelled 1.5ml microcentrifuge tube for immediate use or storage at -20°C (Linacre, et al. 2010).

2.17.2 Amicon® Ultra-0.5 Centrifugal Filter Devices of Chelex® 100 extracted DNA

The Amicon® Ultra-0.5 device was inserted into one of the provided microcentrifuge tubes and approximately 450μl Chelex® 100 extracted DNA was transferred into the filter device by pipette, taking care to avoid touching the device membrane with the pipette tip. The device was then centrifuged in a microcentrifuge at 13000rpm for 20min to collect DNA in approximately 15-20μl. The filter device was then removed and the sample tube was discarded. The filter device was then inverted and placed into a new sample tube. The filter device and tube were then centrifuged in a microcentrifuge for 2min at 4000rpm, to collect the DNA in a reduced volume and concentrated. The DNA was then used immediately or stored at -20°C. Each sample was split into two parts and each part was concentrated with the Amicon® Ultra-0.5 device and then the two parts combined after concentration.
2.17.3 E.Z.N.A.® Blood DNA Mini Kit - Buccal Swabs Protocol

Prior to DNA extraction 60ml of 100% ethanol was added to dilute the DNA wash buffer and 10ml of propan-2-ol was added to dilute HBC buffer with both buffers being stored at room temperature.

The heads of both wet and dry swabs were cut off and placed into a sterile 2ml microcentrifuge tube. 500μL Phosphate Buffer Saline (PBS) followed by 25μL OB Protease Solution and 500μL BL buffer were added to the tube followed by vortexing at maximum speed for 30s. The tube was then incubated at 65°C for 10min. After this incubation, the swabs heads were removed from the 2ml microcentrifuge tube and were transferred to DNA-free Spin basket seated in a new 2ml microcentrifuge tube and centrifuged at 14000rpm for 2min at room temperature to collect all liquid and cell debris/free DNA remaining on the swab head. The collected liquid was then transferred by pipette into the original 2ml microcentrifuge tube and the swab head spin basket and 2.0ml microcentrifuge tubes were discarded. 500μl 100% ethanol was added and vortex mixed at maximum speed for 20s. The microcentrifuge tubes were then briefly centrifuged to collect any drops from inside the lid. A HiBind® DNA Mini Column was inserted into a 2ml collection microcentrifuge tube and 750μl of the sample was transferred onto a HiBind® DNA Mini Column and was centrifuged at 11000rpm for 1min. The HiBind® DNA Mini Column was transferred into a new 2ml collection tube, the remaining 750μl of the mixture was applied to the HiBind® DNA Mini Column and was centrifuged at 11000rpm for 1min. The HiBind® DNA Mini Column was then transferred into a new 2ml collection tube and the collection tube containing the filtrate was discarded. 500μl of HBC buffer was added to the HiBind® DNA Mini Column, followed by centrifugation at 11000rpm for 1min. The HiBind® DNA Mini Column was again transferred to a clean 2ml collection and the filtrate was discarded.
700μl DNA Wash Buffer was added to the HiBind® DNA Mini Column followed by centrifugation at 11000rpm for 1min. The HiBind® DNA Mini Column was again transferred to a clean 2ml collection tube and the filtrate discarded. These steps were repeated for a second DNA wash buffer-wash step. Then, the empty HiBind® DNA Mini Column was centrifuged for 2min at 13000rpm to dry the column matrix and remove all residual ethanol. The used collection tube containing the filtrate was discarded and the HiBind® DNA Mini Column was transferred into a labelled nuclease-free 1.5ml microcentrifuge tube. 50μl Elution Buffer, heated to 65°C, was transferred directly to the silica membrane of the HiBind® DNA Mini Column; this was incubated 65°C for 5min before elution of DNA by centrifugation at 13000rpm for 1min. The elution step was repeated using the eluate from the first elution. The recovered DNA was then processed immediately or was placed into storage at -20°C.

2.17.4 DNA IQ™ System- DNA Isolation from Stains and Buccal Swabs Protocol

Prior to DNA extraction reagents were prepared as follow:

15ml of 100% ethanol and 15ml of isopropyl alcohol were added to 2× Wash Buffer. The bottle caps were replaced, and mixed by inverting several times. The bottles were labelled to record the addition of alcohols then closed tightly to prevent any evaporation and the solution was stored at room temperature.

1M DTT was prepared by dissolving 5g of dithiothreitol (DTT) in 32.4ml of filtered dH₂O giving a final concentration of 1M. The DTT was dispensed into smaller aliquots that reflect usage, and frozen at -20°C.
Lysis buffer was prepared prior to use by adding 5μl of 1M DTT to 500μl of Lysis Buffer, mixed by inverting several times and the prepared solution was warmed up to 37°C before use.

The heads of both wet and dry swabs were cut off from the stem and were placed into a sterile 2ml microcentrifuge tube. 500μl of prepared Lysis Buffer was added to the tube followed by maximum speed vortex mixing for 20sec. The tube was then incubated at 70°C for 30min. The microcentrifuge tube was then removed from the heat source, the swabs heads were removed from the 2ml microcentrifuge tube and were transferred to a DNA free Spin basket seated into a new 2ml microcentrifuge tube and centrifuged at 14000rpm for 2min at room temperature. Spin basket and cotton swabs heads were discarded. The DNA IQ™ Resin was vortexed vigorously for 10sec (at this stage it was important to keep the stock resin resuspended while dispensing to obtain uniform results). 7μl of resuspended DNA IQ™ Resin was added to sample/Lysis Buffer mixture. The mixture of sample / Lysis Buffer / DNA IQ™ Resin was vortex mixed for 3sec at high speed. The mixture then incubated at room temperature for 5min. During the 5min incubation the mixture was vortexed for 3sec once every minute at high speed. The mixture was vortexed again for 2sec at high speed and quickly the mixture tube placed in the magnetic stand. The mixture tube was left for 10sec till separation occurred.

Carefully using a pipette, all of the solution was removed and discarded without disturbing the pellet on the side of the tube. 100μl of prepared Lysis Buffer was added; the 2ml tube was removed from the magnetic stand and was vortexed for 2sec at high speed. Then the tube was returned to the magnetic stand and the Lysis Buffer removed from the tube and discarded. 100μl of prepared 1× Wash Buffer was added to the tube
and the tube removed from the magnetic stand and was vortex mixed for 2 sec at high speed. The tube was returned to the magnetic stand and the Wash Buffer removed from the tube and discarded. Washing steps using the 1× Wash Buffer was repeated twice. All the Wash Buffer solution was removed and the tube was left in the magnetic stand, the lid was opened and was left to air dry at room temperature for 5 min. 50μl of Elution Buffer was added and the lid closed, then vortex mixed for 2 sec at high speed. Then the tube incubated at 65°C for 5 min. The tube was removed from the heat source and vortex mixed for 2 sec at high speed and immediately the tube was placed on the magnetic stand. Carefully, DNA-containing solution was then processed immediately or was placed into storage at -20°C.

2.18 DNA Quantification

The DNA Quantification was performed using Investigator® Quantiplex Kit supplied by QIAGEN (Crawley, UK) and quantification was carried out on the ABI® 7500 Real-Time PCR Systems (Applied Biosystems, USA).

Prior the DNA quantification process all reaction mixtures, Reaction Mix FQ and Primer Mix IC FQ (QIAGEN, Crawley, UK) were set up in an area separate from that used for DNA isolation and quantification standards as described in section 2.3. All solutions were mixed thoroughly before use to avoid localized concentrations of salt might be occurred. Fresh serial dilutions were prepared where the Control DNA Z1 was prepared according to table 2-1. Each dilution was vortexed for 5 s and centrifuged briefly before removing an aliquot for the next dilution. A new pipette tip was used for each dilution.
Table 2-1: Dilutions of DNA quantification standards. Serial dilutions of Control DNA Z1

<table>
<thead>
<tr>
<th>Serial dilution of Control DNA Z1 (ng/µl)</th>
<th>Undiluted Control DNA Z1 (µl)</th>
<th>QuantiTect Nucleic Acid Dilution Buffer (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>1.25</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>0.3125</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>0.078125</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>0.01953125</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>0.0048828125</td>
<td>10</td>
<td>30</td>
</tr>
</tbody>
</table>

The second stage was conducted by preparing the PCR master mix as below:

Master mix composition was calculated as below:

\[
\text{Master mix} = 11.5 \mu l \text{ of Reaction Mix FQ} + 11.5 \mu l \text{ Primer Mix IC FQ} \\
\times \{ \text{number of unknown samples} + \text{two No Template Controls (NTC)} \\
+ \text{two positive controls} + \text{3 samples for pipetting error} \}
\]

The master mix was vortexed thoroughly and then centrifuged briefly prior to dispensing into the 96 well PCR plate (MicroAmp® Fast Optical 96-Well Reaction Plate, Applied Biosystem). 23µl of the master mix was dispensed into the PCR plate. 2µl of QuantiTect Nucleic Acid Dilution Buffer was added to the NTC wells. 2µl of 1.25ng/µl control DNA was added to positive wells, as a positive control to monitor DNA quantification accuracy. 2µl of each DNA extract was added to the assigned wells as unknown (the total reaction volume per each well was 25µl). Then, the plate was sealed with the optical adhesive cover (MicroAmp® Optical Adhesive Film, Applied Biosystems) and centrifuged briefly.

The DNA quantity for each sample was calculated from two technical replicates. The results analyses were performed using SDS 1.9.1 analysis software (Applied Biosystems, USA). The assay platform used illustrated below.
• **Assay:** Absolute Quantification (Standard Curve)

• **Container:** 96-Well Clear Plate Template:

  Investigator_Quantiplex_Template_SDS1.4_Fast.SDT

• **Run Mode:** Fast 7500

  Two detectors were defined, FAM for the samples and VIC for the Internal PCR Control (IPC). The wells in use were selected and the box for both detectors was checked. Concentrations of the standards in the wells containing the control reactions for the FAM detector were entered. The task of each well was defined as one of standard, NTC or unknown sample. The VIC detector task for all wells was set as Unknown. After saving the file the PCR plate was loaded into the PCR machine for amplification. The cycling conditions illustrated in table 2-2.

### Table 2-2: Cycling conditions for 7500 Fast Real-Time PCR System

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp (°C)</th>
<th>Time (sec)</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial PCR activation step</td>
<td>95</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>Combined annealing/extension</td>
<td>60</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

The criteria of DNA standard curve interpretation were maintained during the processes was based on the manufacturer’s recommendations where the slope ranges of standard curve between −3.0 to −3.6, and the $R^2$ value ≥0.99. Any standard curve did not meet these criteria would result in discarding the quantification results. Also the Cycle Threshold ($C_T$) of the IPC was monitored for the potential inhibition of the PCR reaction. The total DNA was calculated from DNA concentration $\times$ total volume of the final DNA extract.
The following table 2-3 depicts the scoring of quantification results as recommended by Qiagen, the kit supplier.

**Table 2-3: Criteria were used in Interpreting Quantification Results.**

<table>
<thead>
<tr>
<th>Specific human target</th>
<th>IPC</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No amplification</td>
<td>Positive amplification</td>
<td>No human DNA detected</td>
</tr>
<tr>
<td>No amplification</td>
<td>No amplification</td>
<td>Invalid result</td>
</tr>
<tr>
<td>Positive amplification with low $C_T$ and high fluorescence signal</td>
<td>No amplification or $C_T$ higher than 32</td>
<td>IC result inconclusive</td>
</tr>
<tr>
<td>Positive amplification with high $C_T$ and low fluorescence signal</td>
<td>No amplification or $C_T$ higher than 32</td>
<td>PCR inhibition present</td>
</tr>
</tbody>
</table>

### 2.19 DNA Profiling

The DNA profiles of references samples of all of volunteers were previously determined; thus, the profiles generated from this study could be compared and verified.

#### 2.19.1 Direct Amplification

Three experimental settings were examined in this part of the research, the first one in which CMRFs were deposited on a UV-crosslinked piece of office paper (see section2.7). Approximately 2mm$^2$ of the loaded office paper was cut out and placed directly into a 0.2ml sterile thin walled PCR tube containing 50µl of AmpFLSTR® SGM Plus® PCR Amplification Kit (Applied Biosystems, USA) which contained 21µl AmpFLSTR® PCR reaction mix, 11µl AmpFLSTR® SGM Plus™ Primer Set, 1µl AmpliTaq Gold DNA polymerase and 20µl QuantiTect Nucleic Acid Dilution Buffer. A 34 cycle protocol was followed. The PCR was performed using a GeneAmp® PCR
System 9700 (Applied Biosystems, USA) with gold-plated silver 96 well blocks, following the 9600 emulation mode. Three replicates were performed on each occasion.

The second experimental setting of direct PCR was conducted by depositing CMRFs onto a UV-crosslinked piece of office paper, and then the paper was cut into portions then placed into 2ml centrifuge tube. 250μl of filtered dH2O containing proteinase K (100µg/ml) was added and incubated at 37°C overnight. 20μl of the 250μl was transferred into 30μl of PCR mix (PCR mix was prepared as described in first experimental setting) then amplified following the same conditions of the first direct PCR experimental setting.

The third setting was conducting in the same way of second experimental setting but the filtered dH2O-proteinase K was replaced with 1% Triton X100 containing proteinase K (100µg/ml).

2.19.2 Profiling of Extracted DNA

Low copy number (LCN) DNA analysis using 34 PCR cycles is the method used (Gill 2001) to generate DNA profiles. PCR was performed for 34 cycles on all samples using the AmpFLSTR® SGM Plus® PCR Amplification Kit (Applied Biosystems, USA). SGM Plus® PCR was conducted in a total reaction volume of 25μl. 11.5μl AmpFLSTR® PCR reaction mix, 5.5μl AmpFLSTR® SGM Plus™ Primer Set, 0.5μl AmpliTaq Gold DNA polymerase and 7.5μl DNA extract containing (100 – 200pg). Template DNA was mixed in a 0.2ml thin walled PCR tube. PCR was performed using the GeneAmp® PCR System 9700 with gold-plated silver 96well block, following the 9600 emulation mode according to the parameters listed in table 2-4. Amplification
positive control was maintained throughout the amplification process with the control DNA provided with AmpFLSTR® SGM Plus kit. 7.5μl of 007 control DNA (0.1ng/μl) was added to 17.5μl of PCR mix. Also, an amplification negative control was included in which DNA template replaced with PCR grade nuclease free water (Ambion® RT-PCR Grade Water, Life Technologies). PCR controls were included in every reaction. PCR products were then analysed on an ABI 310 Genetic Analyser (Applied Biosystems, USA) as described in 2.19.3.

Table 2-4: Cycling conditions for AmpFLSTR® SGM™ Plus PCR

<table>
<thead>
<tr>
<th>Stage</th>
<th>Initial</th>
<th>34 cycles</th>
<th>Final</th>
<th>Hold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation</td>
<td>Denaturation</td>
<td>Annealing</td>
<td>Extension</td>
</tr>
<tr>
<td>Temp</td>
<td>95</td>
<td>94</td>
<td>59</td>
<td>72</td>
</tr>
<tr>
<td>(°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>11 min</td>
<td>1 min</td>
<td>1 min</td>
<td>1 min</td>
</tr>
</tbody>
</table>

2.19.3 Capillary Electrophoresis SGM plus samples

The amplified DNA was analysed using the ABI Prism™ 310 DNA analyser using 310 Data Collection software version 3.0+ (Applied Biosystems).

All the samples were prepared for electrophoresis on the 310 instrument immediately before loading. A master mix of size standard and Hi-Di™ Formamide was needed to prepare the samples for electrophoresis. A master mix of 1μl of GeneScan – 500 ROX size standard size standard (Life Technologies) and 24μl of Hi-Di™ Formamide (Life Technologies) reflected calculated usage. The required volumes
of components were pipetted into 2ml polypropylene tube. Into 0.5ml sample tubes, 25μl of the formamide: size standard mixture and 1.5μl of PCR product or allelic ladder were added alternatively. The tubes were sealed with septa, and then briefly centrifuged to ensure that the contents of each tube are mixed and collected at the bottom. The tubes were heated in a heating block for 3min at 95°C, then immediately the tubes were placed on ice for 3min. The sample tray was placed on the autosampler to start the process. The electrophoresis was performed in a 36cm array filled with POP-4™ polymer heated to 60°C. The run parameters are indicated below:

**Dye Set:** F

**Internal Sizing Standard:** ROX

**Module Template:** Fragment Analysis 36-POP4

**Injection time (s):** 5

**Injection voltage:** (kV) 15

### 2.19.4 Criteria of DNA Profile Interpretation

All DNA profiles were generated using an ABI 310 Genetic Analyser (Applied Biosystems, USA). Analysis was performed using GeneMapper® ID v3.2 Software. The consistency of DNA profile interpretation was maintained during this study by following a number of criteria based on published interpretational guidelines and the requirements of the work undertaken in this thesis such that was proposed by Caragine et al. (2009) with slightly modification by reducing the rfu threshold to 50:
- Peak High Ratio (PHR) was calculated according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) interpretation guidelines for autosomal STR typing as follow: RFU (lower rfu peak) / Peak B rfu (higher RFU peak) \times 100 = PHR% 
- Peaks above 50 Relative Fluorescence Units (rfu) of the peak height is counted as true allele. 
- DNA profile should be reproducible between duplicates of the same DNA sample. 
- A homozygous allele is that with ≥150 rfu of the peak height and is approximately the summation of rfu of two heterozygous alleles. 
- A mixed profile is a profile that showing severe imbalance between heterozygous alleles at different loci with more than two peaks in the expected size range of particular locus and the stutter product was high at >20%. 
- The stutter peak is a peak that appears in n-4 stutter position and the peak height is less than 15% of the true allele and any peak in stutter positions above the 15% threshold is reported as potential allele. 

2.20 Histology Method-Haematoxylin

To examine whether latent CMRFs or palmprints contain nucleated cells, CMRFs and palmprints were applied onto Poly-L-lysine coated microscope glass slides (Polyscience Inc.). The palm mark was deposited onto eight slides aligned into two rows each of four slides abutted along the long edge. The fixation process was performed using the wet fixation technique with pre-cooled methanol (-20°C) followed by air-drying according to the protocol by Keebler and Facik (2008) with the slight
modification of dropping the fixative onto sample slides. Care was taken not to shake or move slides vigorously to prevent dislodging cells from the slide surface. Three drops of pre-cooled -20°C absolute methanol were applied onto deposited glass slides for 10min. These were left to air dry at room temperature. Then, slides were stained with iron Haematoxylin-anol solution for 10min to visualize the nucleus. The excess stain was removed, before being left to air dry at room temperature for 10min. Slides were mounted in xylene and coverslip prior to microscopic examination using ×100 and ×400 magnification under Axioscope-2 FS MOT white light microscope (Zeiss, Germany).

2.21 Statistical Analysis

Statistical analysis of data collected from the experimental work was performed to determine any statistically significant relationships or differences. To facilitate this, t-test analyses, one-way analysis of variance (ANOVA), and two-way ANOVA multiple comparisons were performed using GraphPad Prism version 6.00 for Windows, (GraphPad Software, La Jolla California, USA). The mean and standard deviation of each data set were performed using Microsoft Excel™ 2010.
3 Results: A comparison of Methods for DNA extraction from Fingerprints: E.Z.N.A.® Blood DNA kit, Chelex-100 and the IQ system

In view of the different choices of extraction methods that will affect the success rate of the subsequent DNA profiling step, the first component of establishing a baseline of DNA deposition from the volar surface was to determine most suitable DNA extraction technique to be used for DNA recovery from such challenging samples. Existing DNA extraction and purification procedures vary in reproducibility and cost efficiency. An ideal procedure should have a limited number of steps to reduce experimental error, a limited use of hazardous solvents, a reduced need for specialized equipment, and a method that is affordable (Lickfeldt et al. 2002). Based on these concerns, the objectives of this part of the study were to compare three DNA extraction methods for DNA yield quantitatively and qualitatively, presence of inhibitors, time efficiency, number of tubes and tips came into direct contact with DNA materials, and cost.

Three different DNA extractions methods were examined; Chelex (Linacre et al. 2010), E.Z.N.A. ® Blood DNA Kit (Omega Bio-Tek, Inc., US) and IQ system (Promega Corporation, US). The performance of each extraction method was assessed based on; 1) The efficiency of extraction method in terms of the total yield of DNA, presence of inhibitors which measured by monitoring co amplification of IPC of extracts, and the potential efficiency of each method on the quality of the full SGM plus DNA profiles obtained. 2) Time-efficient method to extract DNA from a single sample. This was calculated from the time needed to complete each method starting from placing swabs
into the extraction vessel ending by obtaining the final ready to use DNA extract. 3) The number of centrifuge tubes and tips used during extraction, this was calculated based on how many centrifuge tubes and tips came into direct contact with DNA containing materials starting from the first centrifuge tube in which swabs were placed ending with the last centrifuge tube in which DNA would be stored. The importance of scoring extraction methods on this comes from that minimizing the transfer between tubes would avoid a further loss that could be encountered during extraction for example, retention of DNA on vessel walls and/or in pipette tips (Van Oorschot et al. 2003). The costs of materials for each method were calculated from 2014 products catalogues. The equipment costs were not quantified, but all extraction methods required the use of microcentrifuge, vortex and heat block.

Twelve volunteers participated in this experiment. Samples were collected at four events on separate days for each extraction method. In total 288 samples were collected 96 each method. At each sampling event, CMRFs were deposited on glass microscope slides, DNA containing materials were collected using the double swab protocol (see 2.15) and extracted utilizing one of the three extraction methods (see 2.17). All samples were quantified for DNA using real time PCR (see 2.18.18). Samples with a total DNA above 200pg were used to generate DNA profiles after 34 cycles of PCR amplification.

The result of DNA recovery from CMRFs deposited onto glass slides showed that recovered amount of DNA was highly variable among different DNA extraction methods. However, the results of DNA recovery from CMRFs which illustrated in table 3-1 establish that the recovered amount of total DNA was higher in those samples extracted using the E.Z.N.A.® Blood DNA kit, with an average ranging from 0.0pg to
2714pg whereas samples extracted by DNA IQ system and modified Chelex yielded an average ranging between 0.0 – 1500pg and an average ranging from 0.0 – 1600pg, respectively. Two-way ANOVA multiple comparison analysis of DNA extraction methods versus average amount of recovered DNA indicated there was a significant difference in the average DNA yield resulting from E.Z.N.A.® Blood DNA kit compared to that of Chelex ($p=0.015$) and that of IQ system ($p=0.0001$) methods while the difference was not significant between Chelex and IQ system methods ($p=0.23$).

Figure 3-1 details the distribution of total DNA amount obtained from CMRFs among DNA extraction methods. Compared with E.Z.N.A® kit, both Chelex and IQ resulted in a lower percentage of extracts with total DNA amount above 1000pg. 10% of the total DNA extracted by E.Z.N.A® kit were above 1000pg compared to 4% of DNA samples obtained by Chelex and IQ. Up to 63% of the IQ extracts yielded DNA with less than 100pg, compared with 46%, 48% of E.Z.N.A® kit and Chelex, respectively.
Table 3-1: DNA Recovery from CMRFs Deposited onto Glass Slides. The total DNA calculated from two experimental replicates plus two technical ones.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Chelex</th>
<th>E.Z.N.A.® Blood DNA Kit</th>
<th>IQ System</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample Event- mean of total DNA yield (pg)</td>
<td>Sample Event- mean of total DNA yield (pg)</td>
<td>Sample Event- mean of total DNA yield (pg)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>1</td>
<td>252.20</td>
<td>0.00</td>
<td>365.60</td>
</tr>
<tr>
<td>2</td>
<td>90.52</td>
<td>20.70</td>
<td>78.65</td>
</tr>
<tr>
<td>3</td>
<td>97.63</td>
<td>10.00</td>
<td>86.91</td>
</tr>
<tr>
<td>4</td>
<td>118.45</td>
<td>71.83</td>
<td>143.24</td>
</tr>
<tr>
<td>5</td>
<td>159.58</td>
<td>56.57</td>
<td>52.22</td>
</tr>
<tr>
<td>6</td>
<td>1420.8</td>
<td>1415.7</td>
<td>649.00</td>
</tr>
<tr>
<td>7</td>
<td>62.12</td>
<td>50.09</td>
<td>159.44</td>
</tr>
<tr>
<td>8</td>
<td>113.38</td>
<td>160.51</td>
<td>203.81</td>
</tr>
<tr>
<td>9</td>
<td>22.5</td>
<td>85.86</td>
<td>125.77</td>
</tr>
<tr>
<td>10</td>
<td>34.12</td>
<td>31.38</td>
<td>67.73</td>
</tr>
<tr>
<td>11</td>
<td>498.32</td>
<td>34.97</td>
<td>322.78</td>
</tr>
<tr>
<td>12</td>
<td>660.96</td>
<td>377.19</td>
<td>242.51</td>
</tr>
</tbody>
</table>
Figure 3-1: Distribution of Total DNA Recovery from CMRFs Deposited onto Glass Slides. Three DNA extraction methods, E.Z.N.A.® Blood DNA kit, Chelex and IQ system were examined. n= 288, 96 each method.
Examining table 3-2 establishes that no inhibition of real time PCR amplification was encountered in any of the extraction methods used, the Ct values were all within the optimum range of 31±1. The average Ct value of IPC of IQ system was low (30.56) compared to that of E.Z.N.A. Blood DNA kit (31.17) and Chelex (31.56).

Table 3-2: Ct Values Obtained from DNA Samples Extracted with E.Z.N.A. Blood DNA kit, Chelex and IQ system. n= 144 (48 sample each extraction method)

<table>
<thead>
<tr>
<th>Sample</th>
<th>IPC</th>
<th>Sample</th>
<th>IPC</th>
<th>Sample</th>
<th>IPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>31.44</td>
<td>1.79</td>
<td>31.59</td>
<td>0.30</td>
<td>33.04</td>
</tr>
<tr>
<td>SD</td>
<td>0.95</td>
<td>31.77</td>
<td>0.09</td>
<td>33.64</td>
<td>0.56</td>
</tr>
<tr>
<td>M</td>
<td>34.34</td>
<td>1.56</td>
<td>31.67</td>
<td>0.30</td>
<td>34.07</td>
</tr>
<tr>
<td>SD</td>
<td>0.70</td>
<td>31.58</td>
<td>0.58</td>
<td>32.23</td>
<td>1.78</td>
</tr>
<tr>
<td>M</td>
<td>33.87</td>
<td>0.50</td>
<td>31.63</td>
<td>0.27</td>
<td>31.4</td>
</tr>
<tr>
<td>SD</td>
<td>1.26</td>
<td>31.39</td>
<td>0.42</td>
<td>32.67</td>
<td>1.20</td>
</tr>
<tr>
<td>M</td>
<td>33.05</td>
<td>1.07</td>
<td>31.94</td>
<td>0.41</td>
<td>33.13</td>
</tr>
<tr>
<td>SD</td>
<td>0.99</td>
<td>31.96</td>
<td>0.40</td>
<td>32.74</td>
<td>3.28</td>
</tr>
<tr>
<td>M</td>
<td>34.01</td>
<td>0.57</td>
<td>31.50</td>
<td>0.62</td>
<td>34.28</td>
</tr>
<tr>
<td>SD</td>
<td>1.28</td>
<td>31.35</td>
<td>0.71</td>
<td>33.29</td>
<td>0.61</td>
</tr>
<tr>
<td>M</td>
<td>31.82</td>
<td>1.11</td>
<td>31.16</td>
<td>0.44</td>
<td>32.08</td>
</tr>
<tr>
<td>SD</td>
<td>1.11</td>
<td>31.16</td>
<td>0.44</td>
<td>34.27</td>
<td>1.81</td>
</tr>
</tbody>
</table>

In order to assess the quality of the full profiles obtained by each extraction method, the average peak heights of SGM plus DNA profiles were evaluated. Table 3-3
shows the average peak heights per locus obtained from each extraction method in which 200pg DNA was added to PCR for each extraction method. E.Z.N.A. ® Blood DNA kit extracts resulted in the highest peak heights on average compared to extracts of Chelex and IQ system. Additionally, it can also be observed that the average peak heights varied across the loci tested, with the larger loci generally resulting in lower peak heights. All DNA extraction methods showed that full DNA profiles could be obtained. Figure 3-2 and table 3-3 compare representative electropherograms of full SGM plus profiles generated from DNA extracts obtained from the three extraction methods examined herein.
Table 3-3: Mean of Peak Highest of full SGM Profiles. Each profile represents an extraction method of E.Z.N.A. ® Blood DNA kit, Chelex and IQ system. PHR depicts peak high ratio, M: mean, and SD: standard deviation.

<table>
<thead>
<tr>
<th>Locus</th>
<th>E.Z.N.A. Blood DNA kit</th>
<th>Chelex</th>
<th>IQ system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PHR%</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>D3S1358</td>
<td>87</td>
<td>1250.5</td>
<td>153.4</td>
</tr>
<tr>
<td>vWA</td>
<td>88</td>
<td>867</td>
<td>83.44</td>
</tr>
<tr>
<td>D16S539</td>
<td>82</td>
<td>121.5</td>
<td>16.26</td>
</tr>
<tr>
<td>D2S1338</td>
<td>76</td>
<td>2855</td>
<td>404.5</td>
</tr>
<tr>
<td>AMEL</td>
<td>85</td>
<td>1851.5</td>
<td>351.43</td>
</tr>
<tr>
<td>D8S1179</td>
<td>74</td>
<td>1544</td>
<td>178.2</td>
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<tr>
<td>D21S11</td>
<td>60</td>
<td>384</td>
<td>80.61</td>
</tr>
<tr>
<td>D19S433</td>
<td>82</td>
<td>1324.5</td>
<td>463.15</td>
</tr>
<tr>
<td>TH01</td>
<td>70</td>
<td>697.5</td>
<td>99.7</td>
</tr>
<tr>
<td>FGA</td>
<td>70</td>
<td>623.5</td>
<td>154.9</td>
</tr>
</tbody>
</table>
E.Z.N.A.® Blood DNA kit

Chelex

IQ
Figure 3-2 Electropherograms (separated by dye colour) for CMRFs Deposited onto Glass Slide Extracted with E.Z.N.A.® Blood DNA kit (top plot), Chelex (middle plot) and the IQ (bottom plot). All rfu scales are normalized to the highest peak height seen per dye. 200pg DNA was added to PCR.
Furthermore, in order to give a better understanding about the quality of the DNA obtained from these methods, thirty DNA extracts (10 each method) were amplified with SGM® plus PCR amplification kit to reveal if there is any difference in the completeness of DNA profiles generated from these extraction methods.

In the current research, however, the success rate for obtaining a full SGM plus DNA profile from touched deposits was low where only 8 out of 30 samples underwent SGM plus profiling resulted in full profiles in which all 11 loci were called. Figure (3-3) illustrates the completeness of SGM plus DNA profiles among the three extraction methods at 34 cycles. All extracts of E.Z.N.A.® Blood DNA kit gave profiles, of the 10 samples four were full profiles as all 11 loci (corresponding to the references sample) have been called, and six were partial profiles. While of 10 samples recovered using modified Chelex, two showed no locus-specific products, six showed partial profiles, and the remaining two were full profiles. The samples recovered using the IQ system were 7 showed partial profiles, two were a full profile and the remaining one was with no locus-specific products. Examining figure 3-3 shows that there is a preference in loci amplification, conversely to small STR loci, majority of locus dropout incidents were observed in large STR loci such as D16S539, D2S1338, D18S51, and FGA. No drop in incidents was appeared in samples extracted with IQ system while that was observed in extracts samples of E.Z.N.A.® Blood DNA kit and Chelex.
The mean values of time needed to complete each method (n=5 each method) were recorded. Table (3-4) demonstrates that E.Z.N.A.® Blood DNA kit required less time than either other method (45min). The time needed for E.Z.N.A.® Blood DNA kit extraction and IQ system to extract a single sample was relatively close. On contrary Chelex method was required approximately 140min for extracting a single sample which is approximately three times more than the time needed by E.Z.N.A.® Blood DNA kit.

<table>
<thead>
<tr>
<th>Loci</th>
<th>DNA Kit</th>
<th>Chelex</th>
<th>IQ System</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>Green</td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td>vWA</td>
<td>Green</td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td>D16S539</td>
<td>Red</td>
<td>Yellow</td>
<td>Red</td>
</tr>
<tr>
<td>D2S1338</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td>AMEL</td>
<td>Red</td>
<td>Yellow</td>
<td>Red</td>
</tr>
<tr>
<td>D8S1179</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Red</td>
</tr>
<tr>
<td>D18S51</td>
<td>Yellow</td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td>D21S11</td>
<td>Red</td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td>D19S433</td>
<td>Yellow</td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td>TH01</td>
<td>Yellow</td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td>FGA</td>
<td>Yellow</td>
<td>Red</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

Figure 3-3: Completeness of SGM plus DNA Profiles. Number of loci of profiling results obtained from CMRFs DNA samples at 34 cycles (n = 30) by different extraction methods. 200pg DNA was added to PCR at each method. Green: full profile, red: locus dropout, yellow: Allele dropout and black: Alleles drop in.
Also table 3-4 depicts number of centrifuge tubes and tips were required to process a single sample. Fewer tubes and tips (5 tubes and 6 tips) were used with E.Z.N.A.® Blood DNA kit compared to 12 (6 tubes and 6 tips) and 20 (5 tubes and 15 tips) were needed for Chelex and IQ system respectively.

In addition table 3-4 illustrates material costs for the three DNA extraction methods. Cost per sample in sterling pound (£) was £1.84 for E.Z.N.A.® Blood DNA kit, £2.88 for the Chelex method, and £3.69 for the IQ system extraction.

Table 3-4: Comparison of Material Costs per Sample (£), Processing Time, and Numbers of Centrifuge Tubes and Pipet Tips of DNA Extraction Methods. All costs are based on recent catalog pricing (June. 2014).

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>E.Z.N.A.® Blood DNA kit</th>
<th>Chelex</th>
<th>IQ system</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Item</strong></td>
<td><strong>Cost £ (sample)</strong></td>
<td><strong>Cost £ (sample)</strong></td>
<td><strong>Cost £ (sample)</strong></td>
</tr>
<tr>
<td>Kit</td>
<td>1.32</td>
<td>0.021</td>
<td>Kit</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.04</td>
<td>Proteinase K</td>
<td>0.23</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>0.018</td>
<td>Amicon concentrator</td>
<td>2.3</td>
</tr>
<tr>
<td>PBS buffer</td>
<td>0.005</td>
<td>Centrifuge tube and spin baskets</td>
<td>0.21</td>
</tr>
<tr>
<td>Centrifuge tube and spin baskets</td>
<td>0.26</td>
<td>Pipet tip</td>
<td>0.12</td>
</tr>
<tr>
<td>Pipet tip</td>
<td>0.2</td>
<td>Total cost</td>
<td>2.88</td>
</tr>
<tr>
<td>Total cost</td>
<td>1.84</td>
<td></td>
<td>Pipet tip</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total cost</td>
</tr>
<tr>
<td><strong>Time required to process a single sample (min)</strong></td>
<td>45</td>
<td>150</td>
<td>60</td>
</tr>
<tr>
<td><strong>Number of centrifuge tubes and tips came into direct contact with DNA containing materials</strong></td>
<td>10 (4 tubes and 6 tips)</td>
<td>12 (6 tubes and 6 tips)</td>
<td>20 (5 tubes and 15 tips)</td>
</tr>
</tbody>
</table>
3.1 Discussion

These results of this part of the current research suggested that for samples of CMRFs deposited onto glass slides, the E.Z.N.A.® Blood DNA kit gave the highest average DNA quantities (394.53, SD=330.88), yet there was statistical significance between the data obtained from the E.Z.N.A.® Blood DNA kit and the other two methods. On the other hand there was no significant difference between the average of total DNA amount recovered by Chelex (210.5, SD=124.87) and IQ system (167.43, SD=134.5) methods. The large variation in DNA quantity within each method is most likely due to the variation seen between individuals. However generally low DNA recovery was noticed in samples extracted with IQ system, this might be explained by high number of tube and tips were came directly into contact with DNA containing materials during extraction (5 tubes and 15 tips), therefore some DNA amounts might be lost as a result of retaining DNA onto extraction vessels walls and lids as well as that retained by pipetting tips (Van Oorschot et al. 2003).

As Investigator Quantiplex quantification system has an IPC, which should indicate the presence of inhibition in a sample. This was not seen in this experiment; however, Investigator Quantiplex is a robust amplification system and may not suffer from lower amounts of inhibitory molecules, which could affect less efficient systems. However, $C_T$ value of IPC of IQ extracts was low (average 30.56) demonstrating less inhibitors were presented in theses samples which means IQ extracts were more purified than that of E.Z.N.A.® Blood DNA kit and Chelex method. This result supports the results of Pakdeenarong (2008) who suggested that 83.33% was the rate of PCR efficiency of DNA extracted from fingerprints applied
to three different electrical adhesive tapes using the DNA IQ™ System. The low DNA amount obtained by IQ system along with highly purified samples might be as a result of repeating washing step twice as recommended by the manufacturer protocol. In the same context Haak et al. (2008) established that magnetic bead extraction may reduce contamination, as it is semi-automated but as yet it has not been evaluated for samples with low levels of DNA and problems extracting mixed samples have already been recognised.

There were variances between the total DNA yield among the three extraction methods examined in relation to the current research. These results are consistent with those of other authors, (Phipps and Petricevic 2007; Castella et al. 2006) where the use of different methods for extracting DNA from fingerprints results in differences in yield of extracted DNA. Likewise the differences between laboratories using different methods of extraction have been noticed (Raymond et al. 2008).

The differences in DNA recovery among extraction methods were pronounced in the quality of SGM plus multiplex that generated by each method. Assessing whether the difference in quant value results would be the same with DNA profiling revealed that incomparable full SGM plus profiles in terms of peak highest were obtained from extraction methods, which is unexpected as the amount of DNA added into the PCR was comparable.

E.Z.N.A.® Blood DNA kit successfully gave full SGM plus profiles with high peak heights outperforming both Chelex and IQ system, possibly due to that of more amplifiable DNA was captured by the silica binding columns of the kit. Such results corporate other research results as it was established that DNA isolated with
silica columns is more readily amplifiable than DNA isolated using Chelex (Castella et al. 2006; Graham 2007; Jakubowska et al. 2010). Silica column based DNA extraction kits performed as well as or better than all of the other commercial and non-commercial methods evaluated for the extraction of amplifiable DNA (Fahle and Fischer 2000; Castella et al. 2006; Rechsteiner 2006). Moreover the silica column based method gives higher amplifiable DNA yields for challenging samples such as ancient samples than other commercially available kits (Rohland et al. 2010). Fewer tubes and tips that came into direct contact with DNA containing materials (4 tubes and 6 tips) plus keeping the sample into a single binding column during the extraction and purification process until reach the final elution step it might be helped in minimizing the loss of DNA in E.Z.N.A.® Blood DNA kit.

Sample concentration using filter centrifugation subsequent to Chelex extraction (Linacre et al. 2010) was intended to increase the DNA concentration to within a range that could be successfully profiled. Others have shown that the use of filtration devices to concentrate DNA samples showed an increase in the percentage of allele calls for each swab extract when compared to non-concentrated samples (Yang et al. 1998; Graham 2007). However low DNA amount was observed in Chelex extracts, also it was observed in the quality of SGM plus profiles which was less than that of E.Z.N.A.® Blood DNA kit. It has been established that samples extracted with Chelex resin might prone to inhibition as a result of transferring some resin accidently into the PCR tube, but this is not the case here as there was no indication of inhibitory effects on PCR where $C_T$ values of IPC were within the optimum threshold (average 31.56). The fact that Chelex yielded less DNA, also low peak heights might be due to the alkaline environment and high temperatures were used during extraction process (Phillips et al. 2012) and/or using Amicon
concentrator, which contributes towards losing DNA. This was demonstrated by Noren et al. (2013) research in which they aimed to evaluate the DNA recovery using Amicon Ultra 30K and Microsep 30K filtration devices, they reported that quality of filters, the membrane DNA binding properties, and the plastic ware of filter devices were found more likely contribute towards DNA loss. Moreover DNA resulting from Chelex extraction is a denatured single strand (Lincoln 1998) considering that in the current research DNA samples obtained from fingerprints, which are more likely, to be degraded (Fuchs 2007) that could degrade the DNA to a greater extent. Therefore it is more likely the DNA yield would be less quantitatively and qualitatively.

The high proportion of partial profiles obtained in this research, and the trend of losing signal strength along with the allelic drop out incidence of the large STR loci might be attributed to the DNA quality. It has been suggested that the DNA present in fingermarks are degraded as result of the action of deoxyribonuclease during keratinocyte migration toward the outer surface of epidermis (Fuchs 2007). A study by Kita et al. (2008) aimed to conclusively determine the source of DNA present in skin transfer, through the use of immunoelectron microscope analysis. They found single-stranded DNA was present in both the corneal layer of skin and in swabs from skin, which suggest that it is conceivable that the DNA from touched objects originates from the corneal layer sloughed from the surface and from sweat. The Kita et al. (2008) research indicates that DNA from the skin surface is likely to be already degraded as it leaves the surface, further compounding the difficulty in retrieving such DNA in a forensic sense.
The time required to process a single sample revealed that Chelex method required a much more amount of time for processing a single CMRFs sample compared to the other two methods. The fixed time required per sample for incubation, centrifugation, and drying ranged from 27min for E.Z.N.A. ® Blood DNA kit, 120min for the Chelex method, and 47min for the IQ system method. If it is assumed that the remainder of the total time listed in Table 3-4 was used for pipetting, and sample transfers, all methods E.Z.N.A. ® Blood DNA kit, Chelex and IQ required relatively less time for operations.

Compared to Chelex and IQ system methods, E.Z.N.A. ® Blood DNA kit was cost effective method, as the total cost required to process a single sample was quite low (£1.84).

The performance of extraction methods examined in relation to this research was different where E.Z.N.A. ® Blood DNA kit outperformed both Chelex and IQ system in obtaining a significant DNA amount from CMRFs deposited onto glass slides. E.Z.N.A. ® Blood DNA kit gave more amplifiable DNA yield, no inhibition was companied its extracts, requires less time to process a single sample, requires fewer number of tubes and tips came directly into contact with DNA containing materials, and E.Z.N.A. ® Blood DNA kit was cost effective where the cost was approximately half the cost of other two methods. The findings in this work propose an extraction approach as follows: 1) Casework samples shall be extracted with E.Z.N.A. ® Blood DNA kit due to their higher throughput, is preferable for the touch DNA samples, whereas IQ system is preferable for extracting samples when encountering of inhibitors, due to the highly purified samples obtained with this method; and 2) control known samples, e.g. buccal swabs, can be extracted with
Chelex, due to its lower reagent cost per sample when concentration step using Amicon is not needed.
4 Results: Variation in DNA Shedding by Different Parts of the Hand

The following chapter focuses on the issue of the potential of different parts of the volar surface, in particular the palm and the distal phalanx of the CMRFs, to shed DNA, when CMRFs and palm prints are deposited onto a glass surface. This work is intended to answer questions about crime scene practice with respect to evidence recovery, such as the issue raised by Ferraro (2012) concerning “whether to develop a scene mark for pattern analysis or rather sample it for DNA?” Furthermore, this work highlights anatomical location as an additional factor that has an influence on the DNA shedding process. It is important in defining best practice to improve the knowledge of the factors, which govern the deposition of contact DNA evidence.

4.1 Differences in Shedder Status

Before commencing examining the variation in propensity of shedding DNA by different parts of the hand, participated volunteers shedding statuses were defined based on the total DNA amount collected during four sampling events. Volunteers were classified as heavy, intermediate and light shedders following Allen et al. (2008). They designated shedder status as that heavy shedder deposits more than 300pg in a fingerprint, intermediate shedder would typically deposit somewhere between 50 and 300pg of total DNA in a fingerprint. Volunteer deposits less than 50pg of total DNA on glass slide designated as a light shedder. Considering that in the current research two fingerprints were examined so shedder
status of volunteers was redefined as two times more than that suggested by Allen et al. (2008). Heavy shedder would deposit total DNA more than 600pg in CMRFs. Intermediate shedder would deposit total DNA somewhere between 100 and 600pg, while the light shedder deposits total DNA less than 100pg in CMRFs. The DNA recovery was conducted using double swabs method, DNA extracted with E.Z.N.A. 

Blood DNA Kit which was better performed in obtaining DNA from CMRFs as detailed in section (3), DNA amount was estimated with real time PCR. Figure 4-1 shows recovered DNA levels in which, generally, there are wide inter- and even intra-individual differences. Volunteer 6 shows a consistent high level of DNA deposition, as represented by the mean of total DNA yield which range from 565.8pg to 2718.3pg, in contrast volunteer 3 consistently deposited poor levels of DNA so that total DNA yield ranged from 0.00pg to 39.51pg. Volunteer 11 produced relatively constant levels of DNA, varying between 191.77pg and 414.1pg. However, volunteer 6 can be classified as heavy shedders as their propensities in shedding DNA were high (2017.97pg, SD=296.12) which is above 600pg threshold. Seven volunteers were intermediate shedders as their shedding propensities within the range of 100-600pg. The rest, four volunteers were classified as light shedders as their shedding propensities were less than 100pg of total DNA.
Figure 4-1: Shedding Status of Volunteers. Recovery of DNA from glass slides touched by CMRFs of 12 volunteers. The dashed horizontal lines depict the boundaries of shedder groups. Each point represents mean of the total DNA recovery. N=48 (four sampling events of 12 volunteers). Error bars denote standard deviation.
4.2 Shedding DNA from Palm Vs CMRFs

Figure 4-2 shows the results of the DNA amount recovered from palm prints deposited on glass plate, recovered using double swabs method, DNA extraction conducted with E.Z.N.A.® Blood DNA Kit, and DNA total amount was estimated using real time PCR. The results are displayed as a mean of DNA amount of 12 volunteers at four sampling events (n=48). The mean of total DNA recovery that retrieved from palm prints revealed that four volunteers deposited DNA above was 100pg. in contrast the rest 8 their DNA deposition was below the threshold of low copy number DNA of 100pg.
In order to standardize experimental conditions, experiment was repeated again with the same volunteers as they asked to deposit both palm and CMRFs at the same time. Figure 4-3 shows the average and variation between palm and CMRFs for each volunteer. The average DNA amount reported in the current study was within a range of tens to a few hundreds of picograms in either palm or CMRFs, but the palm prints in most instances yielded less DNA when compared to CMRFs from the same donor. The total quantity of DNA recovered from palm prints ranged from 35.4pg to 785.2pg for volunteer 6, whilst for CMRFs of the same volunteer the total DNA range was 359.1 to 1998pg. That was not the case with volunteer 3 as DNA recovered from palm print was higher (range 43.31-89.89pg) from that of CMRFs (0.0-42pg). Two-way ANOVA multiple comparison analysis revealed that there was significant differences in DNA recovery of palm and CMRFs within seven volunteers.

Figure 4-2: Average of DNA Recovery from Palm Prints Deposited on Glass Plate. 12 volunteers were recruited and results are based on four sampling events (n=48). Error bars depict standard deviation. Dashed line denotes low template DNA threshold of 100pg.
4.3 Confirmatory Experiments

To further evaluate the previous finding in which DNA shedding propensity of palm and CMRFs was different and to establish whether the observed difference is not an artefact that relates to the surface areas and adherence of DNA to glass surfaces, therefore it was necessary to confirm that the differences in DNA relate to the anatomical location of the volar skin not the experimental protocol. Accordingly, confirmation experiments have been carried out by directly swabbing technique (see 2.13) either palms or the two fingertips, effect of applied forces during deposition, and microscopically, examine the DNA contained materials deposited in both palm and two fingers and force applied during deposition.
4.3.1 Direct Swabbing of Palm and Fingers

The amount of retrievable DNA from the palm or CMRFs was evaluated by directly swabbing different parts of the volar surface using the double swab technique. In each case, the entire friction ridge skin of the palm or CMRFs was swabbed. As shown in table 4-1, the amount of DNA retrieved directly from the palmar surface is less than from two fingers, even though the palm has a much larger surface area (for surface area calculations see section (2.8)). For example, volunteer 1 yielded an average of palm vs. CMRFs of 703pg (SD: 199.0) 1069.2pg (SD: 180.4) respectively. The results here show that the DNA recovered from print deposits of fingers and palm results from differences in the amount of DNA present on the respective volar surface.

Table 4-1: Comparison of DNA Recovery from CMRFs and Palm. DNA was recovered by directly swabbing the palm and two fingers. The results are the mean of four sampling events of two donors. M denotes mean and SD denotes standard deviation.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Surface area (mm²)</th>
<th>Mean of total DNA quantity (pg)</th>
<th>DNA yield per unit surface area (pg / mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Palm</td>
<td>8400</td>
<td>599.9</td>
<td>7575</td>
</tr>
<tr>
<td>CMRFs</td>
<td>899</td>
<td>63.7</td>
<td>689</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>M</th>
<th>SD</th>
<th>M</th>
<th>SD</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>0.08</td>
<td>1.19</td>
<td>3.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3.2 The Effect of Applied Force

This experiment investigated the influence of pressure applied during contact to the amount of DNA deposited in prints. Following the method described earlier in (2.9), these results depicted in figure 4-4, A and B demonstrate the highest amount of DNA was obtained with an estimated force of 2.5N (equivalent to 250g on balance reading) and 25N (2500g) with fingers and palm respectively, for example volunteer 11 deposited an average of 386.6pg at 2.5N in fingers and an average of 247.8pg at 25N in palm. The DNA amounts were observed to decline with increasing pressure in both fingers and palm. The lowest amount of DNA was recorded at the highest pressure.
DNA samples recovered from CMRFs were underwent SGM plus profiling. The results relatively mirrored that of total DNA quantity recovered at different forces during deposition. Figure 4-5 depicts electropherograms of DNA recovered...

Figure 4-4: Mean Deposit of DNA at Different Deposition Forces. A: total DNA amount recovered from CMRFs. B: Total DNA amount recovered from palm prints. DNA extracted using E.Z.N.A.® Blood DNA Kit. Two volunteers were participated at four sampling events. Error bars denote standard deviation. V12: Volunteers 12, V11: Volunteer 11.
from CMRFs deposited at different forces. It is apparent from figure 4-5 that more alleles were called by increasing force and then unexpectedly alleles start dropping out when force increases. The maximum alleles calling were observed within forces of 2.5 and 5N. Although DNA was recovered from CMRFs at force of 7.5N and above, but only allele 14 of D19S433 have been called (Figure 4-5) at 7.5N while no profiles were obtained from DNA recovered at 10N force. Profiling process was repeated for samples obtained at high force (7.5 and 10N) but same results of calling alleles were observed.
Figure 4-5: Electropherograms (separated by dye color) for CMRFs Deposited onto Glass Slide Extracted with E.Z.N.A kit. Plots depicted the force (N) that profile obtained at. All rfu scales are normalized to the highest peak height seen per dye. 200pg DNA was added to PCR.
Only one DNA sample that obtained from palm deposited at 25N showed alleles calling which depicted in Figure 4-6. On contrary, there were no alleles calling observed within other samples that obtained at 10, 50, 75 and 100N forces.
Figure 4-6: An Electropherogram of SGM plus DNA profile obtained from palm deposited at 25N. 200pg of DNA was added to PCR. Arrow indicates dropping out incidence.
4.3.3 Microscopic Examination:

To examine whether CMRFs or palmprints contain nucleated cells, iron Haematoxylin-anol solution staining was used for cell visualization (Quinones 2011). The results of light microscope analysis of palm and CMRFs, Table 4-2 shows that more nucleated cells and stripped nuclei were deposited from latent prints of CMRFs when compared to the palm. In general, microscopy revealed the number of both nucleated cells and stripped nuclei left in the print of two fingers was 14 in total. Both stripped nuclei and nucleated cells (Figure 4-7) were found together along with nucleus-free corneocytes in morphological analysis of cells from CMRFs or palmprints deposited directly onto glass and subsequently stained. Averages of eight nuclei per CMRF, compared to three nuclei per palm print were observed. On the whole the majority of epithelial cells from both palm and fingers prints were nuclei-free corneocytes.
Table 4-2: Difference in DNA-Containing Material Recovered from CMRFs and Palmprints Assessed by Light Microscopy. The number of nucleated cells and stripped nuclei were obtained from either CMRFs or the palm of three volunteers. The prints were deposited on Poly-L-lysine coated microscope glass slides, and stained iron Haematoxylin-anol solution. Results obtained from two replicates each volunteer.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Nucleated cells</th>
<th>Stripped nuclei</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Palm</td>
<td>CMRFs</td>
<td>Palm</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Mean</td>
<td>2.33</td>
<td>5.66</td>
<td>0.66</td>
</tr>
<tr>
<td>SD</td>
<td>2.30</td>
<td>4.04</td>
<td>1.15</td>
</tr>
</tbody>
</table>
Figure 4-7 Cellular Material Deposited with CMRFs. Direct light microscopic examination ($\times400=B2$, scales bar 50µm. $\times100=A$, B1 and C) of material retained on Poly-L-lysine coated microscope glass slides after CMRFs deposition and staining with iron Haematoxylin-anol solution: A: control epithelial cells obtained from the oral cavity; B1 & B2: nucleated cells; C: stripped nucleus (indicated by arrow) and nucleus- free cells. Pictures obtained from CMRFs.
4.4 Discussion

Expectedly, none of the 12 volunteers who participated in this research showed comparable shedding propensities. It is widely accepted that significant inter- and intra-individual differences exist in the amount of trace DNA deposited by various individuals (see figure 4-1). To date, the reason for these differences is unknown. In the current research, washing hands, substrate, force, time, collection, extraction, quantification and mode of deposition are all standardized, such variables have been deliberately included in this assay to reflect the wide range of interactions that take place during a violent crime or through a passive interaction. However, the reason for the difference in quantity of DNA left in print deposition is believed to be grounded in a wide variety of factors as has been thoroughly discussed earlier in section (1.4). For example, these factors include but not limited to, the propensity of the donor to deposit DNA (Lowe *et al.* 2002); the properties of the surface on which the DNA is being deposited (Raymond *et al.* 2004); the nature of the contact and the delay between sample deposition and collection (Phipps and Petricevic 2007; Allen *et al.* 2008).

The high standard deviation observed in the current research reflects both the natural variability in propensity to shed DNA, as well as the variation that results from operations of DNA analysis. As such this study gives us an indication of the whole range of shedding that may be anticipated or encountered from different individuals. Although there was high variation inter- and intra-volunteers, but there was, generally, a trend of DNA shedding propensity of each volunteer. For instance volunteers 6, 11, and 12 tend to deposit more DNA compared to that of other volunteers see figure 4-2. Conversely volunteer 3 was deposited low DNA amounts across all experiments. These volunteers
seem to underpin the notion of good shedders and bad shedders, so that when all other factors are maintained constant, inter individual differences in DNA deposition are one of the dominant forces determine the amount of DNA deposited.

The results in this chapter show that despite the large surface area of palm compared to that of CMRFs (see table 4-1) there is variation in DNA shedding propensities of palm and fingers of the same person (figure 4-3). Of 12 volunteers 11 the DNA deposited in CMRFs was higher than that of palm. The difference was statistically significant in 7 of the 11 volunteers. The direct swabbing of palm and CMRFs results confirmed that more DNA was recovered from CMRFs compared to that of palm. Although in both the DNA recovery process was the same. It seems possible that these results are due to the fact that there are regional differences in the thickness of the stratum corneum (SC) and also the number of desquamated cells of the SC was found to vary according to its location throughout the body, which reflecting the function of each anatomical location (Holbrook and Odland 1974; Yoshikawa et al. 1994). The SC of the glabrous skin found in palm is remarkably thick (0.5-2mm) and there is a firm attachment between each corneocyte (Fuchs 2007) when compared to that from fingertips (average 51µm) (Whitaker et al. 1995). Also there are large inter-individual differences in cornification thickness ranging from 130 to 795µm for the right index finger (Fruhstorfer et al. 2000). Reliance on that, it could be an indication of current DNA extraction, E.Z.N.A. ® Blood DNA Kit, may fail to break down the considerably thick cornified cell envelope obtained from palm print. Thus it may be possible that keratinocytes contain unequal amounts of residual, undigested DNA. The cell envelope is considered to be a composite biomaterial that is very resilient and a major contributor
to the protective function of the skin. Resilience is due to cross linking of proteins by ε-(
γ-glutamyl)-lysine isopeptide bonds, reinforced by disulphide bridges (Jarnik et al. 1998).

Another possible explanation of the variation in shedding DNA between palm and CMRFs, is that in this study the washing step was performed an hour prior to deposition allowing time for DNA to be shed and ensuring that the DNA quantified originates exclusively from the part of the hand sampled. Since the rinse of epidermal surface with mild detergent may remove some squames and how many are removed is correlated with the number of squames shed spontaneously over a long period of time, and the epidermal renewal time in healthy individuals will vary according to the SC thickness and location of sampled area of the body, which that it does not exceed maximum of 39 days in any case (Izuka 1994). Therefore the palm needs more time to renew its epidermal cells than that needed by fingers.

The above explanations may apply to the results with reference to number of stripped nuclei and nucleated cells, which were in general, limited to a few units in both palm and CMRFs. More stripped nuclei and nucleated cells were seen in CMRFs. The result was supportive and mirrors that of more DNA could retrieve from CMRFs compared to palm. For instance the volunteer 6 who was categorized as a heavy shedder, average of total DNA amount was 2017.97 pg, displayed a high incidence of DNA-containing components, 14 units (10 nucleated cells and 4 stripped nuclei). The significant thickness of SC might be prevented obtaining sufficient permeabilization (in this study -20 methanol was used) to provide an access into intracellular (Jamur and
Oliver 2010) or releasing stripped nuclei. Therefore the stain prevented from penetrating into cells of palm. Also it might be washing hands step reduced number of cells in palm, which needs more time for renewal epidermal cells as discussed above. However, the obtained units in this study were less when compared to those reported by Alessandrini et al. (2003). In their research, a single thumbprint was deposited immediately after vigorous hand washing, whereas in the current experiment CMRFs were deposited waiting after an hour since washing hands prior to mark deposition. The majority of epithelial cells obtained from CMRFs in this experiment were nucleus-free corneocytes, which was expected. These results correspond with an earlier study by Balogh et al. (2003) in which fingerprints were applied onto microscope slides, stained first with iron hematoxylin Weigert solution, and then with van Gieson solution. Also their report was scant in providing definitive figures of how much whether nucleated or nuclei free cells were obtained, but their results showed that that the majority of epithelial cells from latent fingerprints were nuclei free corneocytes, with a minimal incidence of nucleated cells, which were enough to obtain amplifiable DNA.

Examining the influence of using an equal force during CMRFs and palm deposition was supportive to the result of that less DNA is shed by palm compared to that of CMRFs. Additionally, increasing force in the current study was found to first increase and then decrease the amount of DNA deposited and these outcomes mirrored by SGM plus profiles obtained in relation to this experiment. Such a phenomenon may be explained by that DNA-containing materials might re-adhere again to the surface of either palm or fingers when higher pressure was involved or even be forced into the furrows that lie between the volar ridges. In general there is very limited research
concerning the influence of force amount applied during deposition of prints on DNA amount deposited. In contrast to the current results, Goray et al. (2010) demonstrated that factors such as pressure can relate to how much DNA is transferred onto a secondary touched object. They reported that an increase in the amount of force applied tends to lead to an increase in the amount of DNA transferred and the application of friction to the contact increases the amount of DNA transferred even further. Whatever, the case their study neglected that the amount of pressure as a factor might govern DNA deposition. This finding merits to be explored further in which different surfaces might be examined with wider population; such findings would help to improve awareness of force applied during touching or lifting objects as a factor impacts DNA deposition as well as manual strangulation cases.

A plan for fingermark evidence recovery will almost always need to be drawn up with reference to the recovery of other forensic evidence types. Also, it is important to broaden awareness of criminal scene investigator of all stages of the evidence recovery and analysis process in order to maximise success. However, based upon the findings of this part of the current study prioritize developing a palm mark in a crime scene for pattern analysis is preferable due to its low DNA contents, delaying the swabbing until the fingermark has been visualised may give the potential for both DNA and fingermark evidence. However, the impact of this on DNA must also be considered, hence the importance of an integrated forensic evidence recovery plan. Furthermore, this part of the current study highlights anatomical location as an additional factor that has an influence on the total DNA deposited. Also this would advance the knowledge of the factors that govern the deposition of traces DNA evidence. Although wide range of
factors governs DNA shedding propensity and the retrieved DNA amount is highly variable but individual shedding propensity is the key factor in determining the amount of DNA deposited.
5 Results: Retrieving DNA from Paper Deposited, Ninhydrin Enhanced CMRFs

5.1 Recovery of DNA from Office Paper

The establishment of a baseline for DNA recovery from office paper and an examination of whether office paper as a rough semi-porous surface retains more DNA follows intuitively that DNA will be deposited more readily on rough or porous substrates than on smooth surfaces (Harbison et al. 2008; Sewell et al. 2008; Leemans et al. 2006). The current experiment aimed to determine the DNA recovery level from office paper in comparison to that of the smooth non-porous substrate, glass. Six volunteers participated in this part of the work. Volunteers were asked to wash their hands an hour prior to deposition as previously described in section (2.4). Volunteers deposited CMRFs on separate days, either on glass slides or on a portion of office paper. The double swab method was used to recover DNA from glass and the cutting method was used in recovering DNA from office paper. E.Z.N.A.® Blood kit was used to extract DNA from both substrates. Then, DNA samples were quantified as described previously in section (2.18). Figure 5-1 illustrates averages of overall DNA recovered from CMRFs deposited onto glass compared to that of paper where the average recovery of DNA from CMRFs deposited on paper was 150.94pg (SD: 172.76) while 221.816pg (SD: 268.18) was recovered from CMRFs deposited on glass.
As detailed in Figure 5-2, in all instances and among all volunteers the recovery of DNA deposited in CMRFs onto paper was less than that from glass. For example volunteer 12 deposited an average of 670.81 pg (SD: 397.16) onto glass substrate whereas the average DNA deposited onto paper substrate was 462.05 pg (SD: 232.23). However, irrespective of the substrate, volunteers 11 and 12 show a consistent high level of DNA deposition, when 100 pg of DNA is defined as above low template DNA threshold; this quantity being obtained in 90% of samples recovered, whilst volunteer 7 consistently deposited lower levels of DNA so that a few to tens of picograms were observed in 90% of samples. However, no significant difference in the mean of DNA

Figure 5-1: Average of DNA Recovery from CMRFs Deposited on Glass VS Office Paper. Black bar represents glass. Orange bar represents office paper. DNA extraction was carried out using E.Z.N.A.® Blood kit. Each bar represents mean of total DNA obtained from 48 samples (n=96). Error bar depicts standard deviation.
levels recovered either from office paper and glass substrates was observed intra volunteers, as assessed using multiple comparison two-way ANOVA. A general (although not statistically significant $p=0.69$) trend existed in which higher levels of DNA were recovered from glass than from office paper.

Figure 5-2: Total DNA Recovery from Office Paper and Glass Slide among Six Volunteers. Dashed black line depicts 100pg threshold of low template DNA. Error bars show standard deviation. n=60 each substrate (10 replicates each volunteer).

DNA recovery results showed considerable variation in depositing total amounts of DNA, which skews the distribution; Figure 5-3 illustrates the range of DNA quantities recovered from paper and glass substrates. It was noted that 33% of all samples recovered from the six volunteers were over 100pg of DNA from paper compared to 63% of the total samples that recovered from glass which were over 100pg of DNA.
Figure 5-3: Cumulative Percentage Histogram of DNA Recovery Levels. Blue bar: frequency of DNA recovery level. Red line represents cumulative percentages expressing the frequency distribution of DNA recovery obtained from paper and glass. A: DNA recovery from paper substrate. B: DNA recovery from glass substrate. n=60 each substrate. Red dashed line depicts low template DNA threshold of 100pg.
5.2 Effects of Ninhydrin on DNA Recovery

This part of the research systematically examines the effects of ninhydrin on subsequent analysis of DNA recovered from CMRFs.

Five volunteers participated in the current experiment. Samples were prepared in an identical manner as described previously in section (2.7). Five sets of office paper portions were prepared according to the method described in (2.6) and loaded with CMRFs. The first set of paper was treated with ninhydrin acetone base solution, and called Ninhydrin Acetone Base-enhanced CMRFs (NAB). The second set was treated with ninhydrin HFE base solution, Ninhydrin HFE-7100 Based-CMRFs (NHFEB). The third set was treated with HFE-7100 alone, HFE-7100 treated paper (HFE), and the fourth set was treated with acetone only, Acetone Treated Paper (ACT). Finally, set of paper was loaded with CMRFs without any treatment was used as a control for comparison. The sample sets that were treated with either acetone or HFE-7100 were used as a control in order to observe if either acetone or HFE-7100 contribute towards any DNA reduction and interference with the DNA profiling process. Separately, acetone, HFE-7100 and ninhydrin solutions were applied by spraying the pieces of office paper with either solvent or ninhydrin working solutions to completely saturate the paper and then allowed for an hour to dry completely at room temperature. Following treatment, DNA was extracted from all samples using the E.Z.N.A.® Blood kit, quantified with real time PCR and profiled with the AmpFLSTR® SGM Plus® PCR Amplification Kit.

The results depicted in Figure 5-4 confirmed that the amount of DNA obtained
from CMRFs treated with NAB, NHFEB and HFE was reduced compared to that recovered from unenhanced paper and ACT. Among all treatments significant differences were found when one-way ANOVA analysis was conducted ($p=0.001$). Generally, more DNA was recovered from unenhanced paper where the mean was 47.82pg, while means of DNA recovery of ACT and HFE samples were 45.97pg and 33.10pg respectively. The NHFEB showed relatively less DNA amount (24.01pg) compared to ACT, NAB and HFE. Minimal differences were noticed in terms of DNA recovery between ACT and unenhanced paper.

**Figure 5-4: Effect of Different Treatments on DNA recovery from Paper.** Mean of DNA quantity obtained from CMRFs deposited on white office paper. DNA extracted using E.Z.N.A.® Blood kit method (n=20 each treatment). Error bars depict standard deviation
In order to provide an indication of the potential inhibition of the PCR reaction of samples that might have occurred on each occasion, $C_T$ values for ACT, NAB, NHFEB, HFE, and internal PCR control (IPC) were monitored. Figure 5-5 compares the results of $C_T$ values of both samples and the associated IPC obtained on each occasion. There was no indication of PCR inhibition; as in all instances there was a positive amplification of both sample and the associated IPC, this was along with $C_T$ values of IPC were within the range of optimum range of 31±1 (depicted in Figure 5-5 as the red line) as recommended by the kit manufacturer (Investigator® Quantiplex- QIAGEN, Crawley, UK). Also, DNA recovery of samples was low on all occasions by comparing with $C_T$ values.

![Figure 5-5: The Inhibitory Effects of Different Treatments on PCR.](image)

Means of $C_T$ values of DNA samples (black bars) and their associated IPC (orange bars) obtained from paper loaded with CMRFs and treated as indicated; NAB: Ninhydrin Acetone Base. ACT: Acetone. NHFEB: Ninhydrin HFE base. HFE: HFE7100 treated paper. Red line indicates the optimum $C_T$ value (31) for the IPC control.
5.3 Evaluation of the Effect of Ninhydrin on DNA Profiling

To further explore the low yield of DNA caused by ninhydrin treatment, a confirmation experiment was carried out by directly mixing known concentrations of control DNA (1.25ng and 0.312ng) with Ninhydrin Acetone Base (NAB), Acetone (ACT), Ninhydrin HFE Base (NHFEB) and HFE each separately as described earlier in section 2.14.6.

The results obtained here and presented in Figure 5-6 showed that in all instances concentration of control DNA mixed directly with ninhydrin prepared in acetone or HFE-7100 was less than that of non-mixed DNA. The acetone itself demonstrated an insignificant reduction in the DNA concentration in comparison to untreated samples. The greatest reductions of 1.25ng/µl control DNA was observed with samples that contained ninhydrin, whatever the solvent or amount of control DNA. In contrast, the highest DNA concentration was seen in samples treated directly with acetone. Apart from samples subjected to HFE-7100, the results of 0.312ng/µl control DNA were in accordance of that reported with 1.25ng/µl control, the lowest DNA concentrations were shown in samples mixed with NHFEB and NAB and the highest was experienced in acetone mixed samples. The obtained samples from mixing HFE were found to be the lowest (mean 0.0048ng/µl).
The results of $C_T$ values showed that there were no indications of PCR inhibition as in all occasions $C_T$ values of IPC were within optimum range of $31 \pm 1$ along with positive amplifications incidences within samples.

Figure 5-6: Evaluation of Ninhydrin Treatment on DNA Amplification. DNA quantity derived from control DNA samples mixed directly with: Ninhydrin acetone base (NAB), black bar. Acetone treated (ACT) blue bar. Ninhydrin HFE base (NHFEB) red bar. HFE treated samples (HFE) purple bar. Control DNA with no mixing orange bar. The results were obtained from five replicates for each treatment. Error bars represent standard deviation.
5.4 Maximizing the Yield of DNA Recovered From CMRFs on Paper

Whilst the results obtained in this investigation concluded that office paper impacts on the DNA recovery process, the aim of this part of the research was to optimise the recovery of DNA from office paper, as this received little attention in several prior research studies (Sewell et al. 2008). Several approaches were applied, which were based mainly on dislodging the cellular material from the substrate, as this is a critical step, releasing the DNA containing material into solution, allowing the normal extraction processes to be applied.

5.5 Incubation of Paper Substrate with Surfactants

The first attempt to maximize DNA recovery from office paper was aimed to incorporate three different surfactants (1% Triton™ X-100, 2% CTAB and 0.5% SDS) prior to the DNA extraction stage to facilitate the dislodging of DNA containing materials from paper surface. Samples were incubated with one of the three surfactants in the presence of proteinase K. Following the incubation step, DNA extraction was conducted using the E.Z.N.A.® Blood DNA kit as described previously in (2.17.3). A set of samples was incubated with proteinase K solution as a control group. Also glass was maintained throughout these maximizing experiments for comparison. Then the extracted samples underwent quantification using real time PCR. Four volunteers participated; five CRMFs of each volunteer were collected on paper or glass.

The results illustrated in Figure 5-7 show that the samples recovered with 1%
Triton™ X-100 outperformed in terms of yield of DNA recovery when compared to SDS, CTAB and water (control). The highest recovery was observed when 1% Triton™ X-100 was used. The maximum DNA recovered was observed in paper; the means were 366.19pg (paper) 297.57pg (glass). Virtually undetectable amounts of DNA were recovered from both samples of white office paper and glass substrates treated with 2% CTAB and 0.5% SDS incubation. More DNA was recovered from paper when compared to glass when 1% Triton™ X-100 was used per contra the DNA yield obtained from glass was higher compared to paper when a water incubation step was applied. One – way ANOVA analysis revealed that 1% Triton significantly outperformed other treatments ($p=0.001$)

![Figure 5-7: Effect of Using Different Surfactants on DNA Recovery from Paper VS Glass Substrate.](image)

**Figure 5-7: Effect of Using Different Surfactants on DNA Recovery from Paper VS Glass Substrate.** DNA quantity (pg) obtained CMRFs deposited on paper (P) and glass (G). DNA extraction was conducted using E.Z.N.A® Blood kit. n=20 each treatment (five CMRFs from each of four volunteers). Error bars show standard deviation.

$C_T$ values of the qPCR were monitored in order to include an objective measure of potential PCR inhibition of those samples recovered with different surfactants. Table 5-1 details the mean of $C_T$ values of CMRFs samples deposited on office paper plus their
relevant IPC. The results show the $C_T$ values obtained from samples treated with three different surfactants and water. The $C_T$ values were seen to be high in samples incubated with 0.5% SDS and 2% CTAB compared to samples of 1% Triton™ X-100 and water. The samples recovered with 1% Triton™ X-100 exhibited the lowest $C_T$ values for samples (32.26) and their associated IPC (30.77). Conversely, samples obtained with 2% CTAB showed the highest $C_T$ values where the mean 33.90 for sample and 32.58 for that of IPC.

Table 5-1: Inhibitory Effect of Different Surfactant. Means of $C_T$ values of DNA samples and its associated IPC obtained from CMRFs were loaded to portion of paper and treated with 1% Triton™ X-100, 0.5% SDS 2% CTAB and $H_2O$ (control). n=5 each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$C_T$ Value</th>
<th>Sample</th>
<th>IPC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>1% Triton</td>
<td></td>
<td>32.26</td>
<td>0.77</td>
</tr>
<tr>
<td>0.5% SDS</td>
<td></td>
<td>33.68</td>
<td>0.48</td>
</tr>
<tr>
<td>2% CTAB</td>
<td></td>
<td>33.90</td>
<td>1.00</td>
</tr>
<tr>
<td>$H_2O$</td>
<td></td>
<td>31.81</td>
<td>0.82</td>
</tr>
</tbody>
</table>

1% Triton™ X-100 was included in all further experiments carried out during the research, due to the promising results of maximizing DNA recovery that have been noticed. Since the results obtained from samples in the presence of 0.5% SDS and 2% CTAB showed PCR inhibition. An additional purification step was incorporated prior the final elution in order to remove remaining traces of surfactant that might inhibit PCR.
amplification. 100μl of filtered dH₂O was added to binding column that holds the DNA extract to wash off impurities and then centrifuged at 10000rpm for 1min. This washing step was repeated twice. The Cₜ values for samples and IPC were also monitored in order to evaluate the additional washing step results and the remaining levels of PCR inhibition.

Figure 5-8 illustrates the Cₜ values obtained prior and post introducing the washing step. The results showed a decrease in the Cₜ values of both samples and IPC post washing, along with detection of measurable amounts of DNA. The mean of Cₜ values (sample/IPC) of samples treated with 0.5% SDS or 2% CTAB dropped from 33.68/32.32 to 32.22/30.73 and from 33.91/32.58 to 32.29/30.67 respectively. The Cₜ value decline was seen to be higher in those samples extracted with 0.5% SDS in comparison to those of 2% CTAB.

![Figure 5-8](image-url)

**Figure 5-8: Effect of Incorporating an Additional Washing Step on PCR Inhibitory.** Means of Cₜ values of DNA samples and its associated IPC obtained from CMRFs loaded to portion of paper, treated with 0.5% SDS and 2% CTAB before and after addition of a distilled water washing step. Purple bars depict mean of Cₜ values obtained from samples. Green bar depict mean of Cₜ values obtained from IPC (n=5).
5.6 Maximizing DNA Recovery from Ninhydrin Treated CMRFs on Paper Using 1% Triton™ X-100

The current experiment seeks to examine the use of 1% Triton™ X-100 in improving DNA recovery from office paper following enhancement with ninhydrin. Four volunteers were recruited in this experiment. In general, on paper substrate, volunteers 7 and 8 tended to shed DNA below the 100pg while 11 and 12 shed DNA above the 100pg threshold (see Figure 5-2). After washing hands, and after waiting for an hour (as described previously), volunteers deposited CMRFs onto a decontaminated paper portion. The paper was treated with ninhydrin as described previously in (2.14.5). Then, the samples were left for an hour at room temperature. The papers were cut into small pieces and placed into 2ml microfuge tubes. 500µl of 1% Triton™ X-100 provided with proteinase K (100µg/ml) was added and the sample incubated for an hour at 37°C.

Examination of Figure 5-9 reveals that more DNA was recovered when 1% Triton™ X-100 was used in sampling ninhydrin enhanced paper when compared to water treated samples. Also, it can be seen that the average DNA recovered from CMRFs deposited onto paper treated with ninhydrin was lower than that of untreated paper. In all instances, the recovery of DNA was higher with unenhanced samples. The mean DNA quantity obtained from ninhydrin enhanced samples, treated with 1% Triton™ X-100 was 161.43pg (SD: 40.45) compared to 198.82pg (SD: 80.36) retrieved from unenhanced samples. Multiple comparisons Two-Way ANOVA analysis showed no significant differences among DNA recovered samples (p=0.07).
In order to evaluate the amplified DNA profiles obtained post 1% Triton™ X-100 treatment, a comparison of the rfu of the peak highest of the called alleles within the DNA profiles obtained from ninhydrin enhanced samples was carried out. DNA profiles generated from samples obtained from CMRFs treated with water were used as a control. 150pg of DNA was added to PCR in order to generate each profile. Three profiles were generated for each treatment.

In all instances, DNA profiles obtained with 1% Triton™ X-100 scored higher rfu values compared to that of profiles treated with water. The results showed (Figure 5-10) that DNA profiles were obtained from ninhydrin enhanced paper and treated with 1%

**Figure 5-9: Effect of Using 1% Triton on DNA Recovery from Ninhydrin Acetone Base Enhanced paper.** The results show the mean of DNA recovery (pg) from office paper with and without enhancement with ninhydrin acetone base. Samples were treated with 1% Triton X100 (purple bars). A separate group of samples were lysed with water used as a control (green bars). DNA extraction was conducted by E.Z.N.A. ® Blood kit. Each bar depicts DNA mean of five replicates. Error bars represent standard deviation. NIN: ninhydrin enhanced CMRFs.
Triton™ X-100 demonstrated higher rfu of the peak height among all loci. A full representative electropherogram of SGM plus obtained from ninhydrin enhanced CMRFs treated with 1% Triton™ X-100 and water depicted in Figure 5-11.

**Figure 5-10: Effect of 1% Triton™ X-100 Treatment on the Quality of SGM plus DNA Profile.** Each bar shows the mean of RFU per locus of SGM plus multiplex obtained from DNA samples recovered from CMRFs deposited on paper, with ninhydrin acetone base enhancement. Water treatment used for comparison. n= 3 each treatment.
Figure 5.11: Electropherograms (separated by dye colour) for CMRFs Ninhydrin Enhanced Treated with 1% Triton™ X-100 (top plot), Water (bottom plot). All rfu scales are normalized to the highest peak height seen per dye. 150pg DNA was added to PCR.
5.7 Direct PCR

A direct PCR approach was trialled for retrieving touch DNA from white office paper. In an effort to improve the success rates of DNA profiling results from paper, it was decided to assess the relative merits of carrying out direct PCR on touched office paper using SGM plus multiplex kit which not designed for direct PCR application. The approach of direct PCR may be beneficial in certain cases, such as paper. CMRFs were loaded to portions of paper and were prepared as previously described in sections (2.19.1). The first attempt was conducted by adding a portion of loaded paper to the PCR mix in the sample tube. The SGM plus profile results showed no alleles were called in those samples directly placed into the PCR master mix. A second attempt was conducted by lysing a portion of loaded paper with proteinase K in water, and then 20µl of the lysate was added into the PCR vessel. The results again demonstrated no SGM plus DNA profiles were generated. The third attempt incorporated Triton™ X-100 into direct PCR approach. Portions of loaded paper were lysed with 1% Triton™ X-100 for 24h at 37°C in the presence of proteinase K. In contrast to the first two attempts, complete and partial DNA profiles were obtained from CMRFs deposited on paper. On the other hand no full DNA profile was obtained from CMRFs enhanced with ninhydrin; results are detailed in Figure 5-12. An example of a DNA profile obtained from paper for one of the volunteers is shown in Figure 5-13.
Figure 5-12: Completeness of SGM Plus DNA Profiles Obtained with Direct PCR. Number of loci obtained from CMRFs deposited on a portion of office paper with and without ninhydrin enhancement at 34 cycles (n = 10, 5 each treatment). Samples either treated with 1% Triton™ X-100 or water. NIN show DNA profiles obtained from sample enhanced with ninhydrin acetone base. Green: full profile, red: locus dropout, yellow: allele dropout, and black: allele drop in.
Figure 5-13: An Electropherogram of a Representative SGM plus DNA Profile Obtained by Direct PCR. CMRFs were deposited on a portion of paper, and then incubated with 1% Triton in the presence of proteinase K for 24h at 37°C. PCR was conducted following low template DNA protocol of 34 cycles.
5.8 Recovery of DNA Using Liquid Nitrogen

In optimising approaches to overcome the interference of office paper substrate with DNA recovery, freezing the substrate with liquid nitrogen may help break up the paper sample. This experiment was carried out in order to facilitate the release of fingerprint DNA, cells and cell-debris from the substrate into the digestion solution. Conversion of the paper into a fine powder would increase the surface area of extraction. The samples were prepared following the method described in section (2.16.2). The results, shown in Figure 5-14, compare DNA recovery from CMRFs deposited upon office paper with and without using liquid nitrogen to break up the paper. Virtually no DNA was detectable throughout CMRF deposition and control DNA experiments that were carried out compared to untreated samples.

![Figure 5-14: Effect of Liquid Nitrogen on DNA Recovery from Paper.](image-url)

DNA average recovered with and without use of liquid nitrogen. Control DNA-NLN: control DNA loaded to office paper no liquid nitrogen was used (purple bar). Control DNA-LN: Control DNA loaded to office paper liquid nitrogen was used in sample preparation. CMRF-NLN: combined middle and ring fingerprints, no liquid nitrogen was used (green bar). CRMF-LN: combined middle and ring fingerprints, liquid nitrogen was used in sample preparation. Each bar represents a mean of DNA recovered from five replicates. Error bars show standard deviation.
5.9 Collecting DNA Containing Material from Office Paper Using Post It Tape Flags®

This part of the work seeks to utilize Post it Tape Flags® to investigate the potential of recovering DNA/cells from fingerprints deposited upon office paper with the intention to develop a practical method to be used in forensic science. In the current experiment, Frosted Lifting Tape (Sirchie® Fingerprint Laboratories, Youngsville, NC); Three-layer adhesive tape (Industrial Self Adhesives Limited [ISA], Nottingham, UK) and Fingerprint lifting tape 50mm x 20m (ISA, Nottingham, UK) were first tried but it found to be highly adhesive and would remove the paper surface as well as lifting any skin cells that are present and further residues of ninhydrin might also be transferred. Two volunteers participated in this experiment following the method described in section (2.16.3).

Results are summarized in Table 5-2 DNA levels recovered from paper and its associated Post It Tape Flag® were shown to vary between volunteers, between different sample times and between substrates (tape and paper). Volunteer 11 displayed the biggest DNA yield with tape, with average recovery 66.31pg, whereas lowest DNA average was shown by volunteer 12 (28.49pg). Conversely, volunteer 11 showed relatively low levels of DNA from paper compared to volunteer 12, with an average of 41.79 and 73.87pg, respectively. Also Table 5-2 illustrates the amount of DNA transferred by tape. On some occasions the DNA transferred to the tape was higher than the residual DNA that remained on the paper. For instance, the average obtained from volunteer 11 in sampling event 2 with tape was 90.02pg compared to 1.14pg was recovered from paper, whereas the amount transferred from paper following volunteer
12 depositions was low on most occasions as most of the DNA amount was recovered from paper.

**Table 5-2: DNA recovery from paper and its associated Post It Tape Flag®.** All points are averages of two technical replicates in qPCR-based quantification. DNA extraction was carried out using E.Z.N.A.® Blood kit. Two volunteers participated with four sampling events each.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Average DNA yield (pg)</th>
<th>Sampling event</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paper</td>
<td>Tape</td>
</tr>
<tr>
<td></td>
<td>1 2 3 4 M SD</td>
<td>1 2 3 4 M SD</td>
</tr>
<tr>
<td>11</td>
<td>81.34 1.14 13.4 71.29 41.79 40.39</td>
<td>107.7 90.02 49.76 17.76 66.31 40.44</td>
</tr>
<tr>
<td>12</td>
<td>87.69 121.2 21.39 65.21 73.87 41.87</td>
<td>15.746 1.56 93.01 3.65 28.49 43.46</td>
</tr>
</tbody>
</table>

### 5.10 Collecting DNA-containing Material from Ninhydrin Enhanced CMRFs Using Post It Tape Flags®

Two volunteers were asked to wash their hands an hour prior to deposition. They were instructed to deposit CMRFs on a portion of office paper. Then CMRFs were enhanced with ninhydrin acetone base. Samples were left to air dry at room temperature for an hour. Tape Flags® were applied and rolled over the CMRFs. Both paper and its associated Tape Flag® were cut and each placed in 2ml microfuge tube. DNA extraction was carried out using the E.Z.N.A.® Blood kit. Samples were quantified as described previously in 2.18 and profiled as mentioned in section 2.19.2.

The results depicted in Figure 5-15 demonstrated the means of DNA levels
recovered from both ninhydrin enhanced paper (NEP) and its associated post it Tape Flag®. The average DNA recovery was found to be high in NEP samples obtained from volunteer 12 (59pg). The lowest recovery was shown in NEP samples of volunteer 11 (24pg). The average of DNA samples recovered from tape was found to be higher in those obtained from volunteer 11 (36pg) compared to volunteer 12.

![Figure 5-15: Using Post it Tape Flags® in Recovering DNA from Ninhydrin Enhanced Paper (NEP).](image)

As can be seen in Figure 5-16 below, the fingerprints obtained after ninhydrin enhancement and tape lifting might still have adequate detail useful for the classical fingerprint comparison.
Table 5-3 details completeness of 20 SGM plus DNA profiles that were obtained from DNA samples recovered from ninhydrin enhanced office paper and its associated Tape Flag®. In all instances no full DNA profile was generated from DNA samples whether they were retrieved from paper or its associated Tape Flag®. Two of 5 profiles generated from DNA samples of paper were partial with alleles greater or equal in number to 12 have been called compared to none found in its associated Tape Flag®. Two DNA profiles with less than 12 alleles were observed in DNA paper samples and four were seen in DNA Tape Flag® samples. The rest of DNA samples retrieved from paper and its associated Tape Flag® resulted in no profiles.
Table 5-3: Completeness of SGM Plus DNA profiles Obtained from Ninhydrin enhanced Paper VS Associated Tape. Samples were treated with 1% Triton™ X-100 in the presence of proteinase K and DNA extraction was carried out using E.Z.N.A. Blood kit. Top table illustrates SGM plus of volunteer 12, bottom table illustrates SGM plus profile of volunteer 11. R denotes replicate. + =Drop in allele, - =Drop out allele, and /- locus dropout.

### Volunteer 12

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<th>R1 Tape</th>
<th>R2 Paper</th>
<th>R2 Tape</th>
<th>R3 Paper</th>
<th>R3 Tape</th>
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<th>R5 Tape</th>
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</table>
Figure 5-17 shows representative DNA profiles obtained from ninhydrin enhanced paper and their associated Tape Flag®. In all instances, the obtained profiles were partial. Some allele calls were shared between the DNA profiles that were generated from paper and its associated Tape Flag® DNA samples; whilst other alleles either appeared only in DNA tape samples or the alleles’ appearance was limited to DNA from paper samples. Also, the DNA profiling results showed some incidences of non-systematic drop in/out alleles in the obtained profiles.
Figure 5-17: Electropherogram of a representative SGM Pus DNA profiles of Ninhydrin enhanced paper and its associated Tape. A: profile obtained from Tape Flag®. B: profile obtained from office paper. Blue arrows indicate some incidences of drop in alleles. DNA extraction was carried out using E.Z.N.A.® Blood Kit. Blue arrow: allele dropout, red arrow: locus dropout, green arrow: allele drop in, and black arrow imbalance alleles.
In order to evaluate the collection efficiency of the taping method from office paper, a comparison between tape lifting and cutting methods in DNA recovery from CMRFs deposited on office paper was conducted. Figure 5-18 shows DNA recovery results of both tape lifting and cutting methods in which the DNA recovery was higher in samples collected using the cutting method. For instance, the average DNA recovery of volunteer 11 was 120.04pg (SD: 40.2) while DNA recovery of taping method was 24.4pg (15.76). Two-way ANOVA multiple comparison revealed that difference in DNA recovery was significant among both volunteers (volunteer 11, $p= 0.018$ and volunteer 12, $p=0.001$).

![Figure 5-18: DNA Recovery from CMRFs Deposited on Office Paper, Tape lifting VS Cutting. DNA extraction was conducted by E.Z.N.A. ® Blood kit. n=5 each method. Error bars denote standard deviation.](image-url)
5.11 Discussion

The results showed a reduction in DNA quantity retrieved from office paper compared to glass. These findings partially agreed with the results of Sewell et al. (2008) in which no DNA was detected in qPCR from samples recovered from touched office paper; however some partial profiles were obtained when 34 PCR cycles of low template DNA approach was applied. The results of the current study were found to be similar to those of Raymond et al. (2004) who reported the DNA amount recovered from five fingerprints rubbed over the face and deposited on white office paper was extremely low. The low DNA amount observed in the current study and other studies mentioned earlier (Raymond et al. 2004; Sewell et al. 2008) were not consistent with the intuitive concept that touch DNA would be more likely to be recovered from mildly abrasive and semi-porous surfaces, such as paper rather than smoother non-porous surfaces like glass, as rough substrate would better abrade skin cells and retain DNA during and after contact (Wickenheiser, 2002). Raymond et al. (2004) attributed the low amount of recovered DNA from fingerprints deposited on office paper to bleach traces available in white office paper as the manufacturing process of white office paper includes a bleach treatment step. The sodium hypochlorite (bleach) found to be DNA destructive through oxidative damage, such as base modification, and the production of chlorinated base products (Ohnishi et al. 2002; Whiteman et al. 2002). Exposure of DNA to increasingly higher concentrations of sodium hypochlorite causes cleavage of the strands, breaking the DNA into smaller and smaller pieces, and eventually to individual bases (Kemp and Smith 2005).

The observation made by Balogh et al. (2003b) that most DNA recovered from
office paper is capable of yielding a profile that is suitable for identification does not agree with the observations presented here, either due to the differences in the fingers utilised, as thumb and index were implemented in their research compared to middle and ring fingers in this here, or to the hand washing step that has been applied prior to deposition in the methodology here. It is possible that the results of Balogh et al. (2003b) demonstrate that nucleated cells and their constituent DNA originated from other parts of the body rather than from the finger due to their lack of inclusion of a hand washing step in their described methods.

Results obtained in the current study were found to be supportive of the results of Pesaresi et al. (2003) as they reported that a high level of DNA was recoverable from a smooth non-porous substrate like glass, with total DNA recovery ranging from 0-4ng compared to that from a porous substrate, like untreated wood, which ranged from 0 to 0.8ng. They hypothesized that DNA deposition on smooth non-porous substrates might be high, due to increased perspiration during the interaction. In addition, it may also be because smooth rather than textured surfaces allow improved DNA recovery (Wickenheiser 2002). On the other hand, high DNA recovery from glass was not found by Swaran and Welch (2012) where they suggested that the lowest amount of DNA recovered from different substrates was found with glass in either direct PCR (40%) or extracted DNA based method (20%). Also, they proposed the low yield of recovered DNA from glass related to the fact that glass has an affinity to adsorb DNA and it is possible that small amounts of DNA bind tightly to glass surfaces. As mentioned earlier, several studies showed variation in obtaining DNA from different substrates (see 1.4.4). Newman (2009) noticed no significant difference between materials (plastic, glass,
wood, metal), as all showed a propensity to generate profiles.

Figure 5-2 supports the observation of differences found in volunteers’ shedding propensities on different substrates. This may be because certain individuals are better epithelial cell donors than others, or a higher proportion of nucleated compared to non-nucleated cells (Lowe et al. 2002; Bright and Petricevic 2004). Moreover the volunteers’ potential of sweat might contribute to the variation in propensity of shedding DNA that observed in the present study (Quinones and Daniel 2011).

Most of the samples were found to be below the 100pg low template threshold. None of the samples collected were in the desirable nanogram range. This is to be expected from fingerprints on paper as previous authors have stated (Schulz et al. 2004; Bhoelai et al. 2011; Balogh et al. 2003a).

The direct mixing of standard DNA with Ninhydrin, acetone and HFE results demonstrated that acetone has no detrimental effect on DNA quantity, which matched observations of Fukatsu (1999) as DNA from various sources were found to be preserved in 100% acetone at room temperature in good condition for several years and was apparently more robust than when stored in ethanol. The preservative properties of acetone were attributed to its high penetrability and dehydrating activity. These results also were consistent with those of Fujii et al. (2013) where various ninhydrin solutions including the acetone and acetic acid solution tended not to reduce the DNA concentrations, and provided full STR profiles. To our knowledge, this is the first study to investigate how ninhydrin affects DNA profiling by directly mixing control DNA with ninhydrin and or the solvents and monitoring the inhibition of DNA amplification.
Despite the low yield of DNA that was observed in some occasions, the $C_T$ data did not detect any evidence of PCR inhibition (NAB, ACT, NHFEB and HFE). A possible explanation for these results may be that the direct mixing of DNA with the chemicals has rendered some template DNA to be amplifiable. In principle, it would be possible that free primary amines of DNA template at carbon atoms of the nitrogen bases react with free ninhydrin molecules, if ninhydrin is membrane permeable or free DNA is already available (control DNA), such a chemical reaction could disrupt the hydrogen bonds (Schulz et al. 2004). Furthermore, absence of PCR inhibition might result from room temperature drying being sufficient in inactivate ninhydrin traces as well as the solvents, thus no interference with DNA amplification would have occurred. Ninhydrin traces were proposed to possibly interfere with PCR by inhibiting Taq DNA polymerase (Schulz et al. 2004).

In contrast to the current study, two studies of Raymond et al. (2004) and Sewell et al. (2008) neglected that the DNA template could react with ninhydrin, emphasizing interference of chemical enhancement with the DNA profiling process by acting as inhibitors to the PCR reaction. In the present research, as the ninhydrin effect was systematically examined, it might explain such phenomena, as it demonstrates that ninhydrin acetone base impacts on the availability of amplifiable DNA template rather than interferes with the amplification process.

It appears that using a surfactant during DNA extraction facilitated DNA recovery from fingerprints. This study produced results that corroborate the findings of El-Hashemite and Delhanty (1997) who observed the effect of different lysis solutions in
DNA extraction on the subsequent PCR amplification success. They supposed that the type of lysis buffer used during DNA isolation has a positive effect on the allelic drop out.

Logically, Triton™ X-100 facilitates the dislodging of the fingermark components into an aqueous suspension from paper substrate, as well as aiding solubilisation of non-cellular DNA process during DNA extraction (Bienvenue et al. 2006; Opel et al. 2008; Norris et al. 2007). It also can be explained by that Triton™ X-100 might provide a better wetting of the surface or it competent DNA for recipient surface binding sites.

The results gathered here broadly agree with those of Thomasma and Foran (2013). They showed that Triton-based swabbing solutions outperformed water, producing significant increases in DNA yield. On the other hand Thomasma and Foran (2013) presented results that SDS-based swabbing solution maximizes the DNA recovery from marks, contrary to the findings obtained in the current research. This variation observed might be due to the different substrates in each study (glass or office paper) (Sewell et al. 2008) or it may be a result of the difference in the way of using detergent solutions (swabbing solution v. digestion solution).

SDS and CTAB have shown inhibitory effects on PCR amplification (Manterola et al. 1995). Manterola et al. also ruled out that SDS, in concentrations between 3.36 and 12.42 mg/ml inhibited the amplification of qPCR used to detect *Mycobacterium tuberculosis*. PCR inhibition as a result of using SDS and CTAB might reflect the extraction efficiency as impurities present can interfere with cell lysis during DNA extraction (Jacobsen and Rasmussen 1992; Subrungruang et al. 2004) or degrade or
capture nucleic acids (Lienert and Fowler 1992). Bearing in mind the extraction method plays a vital role in the success of recovering amplifiable DNA (van Oorschot et al. 2003). Ionic detergents such as SDS are reported to be PCR inhibitors than non-ionic detergents such as Tween 20 and Triton™ X-100 (Rossen et al. 1991). Additionally, 0.01% SDS was found to inhibit Taq DNA polymerase by 90%, while 0.1% SDS reported to inhibit Taq DNA polymerase by 99.9% (Konat et al. 1994) CTAB and SDS concentrations of more than 0.005% were reported to inhibit PCR amplification (Peist et al. 2001), probably by acting as chaotropic agents, denaturing enzymes and rendering them inactive.

The data presented in Figure 5-7 of the study here appear to suggest that PCR amplification was more efficient due to the presence of Triton™ X-100 traces in DNA samples; or it might be that treatment with Triton™ X-100 prior to extraction was allowed to recover more operative DNA template. Different organic compounds are added to PCR mixture, which makes DNA available for different enzymatic reactions from low quality DNA. A variety of chemical additives like Triton™ X-100, glycerol, formamide, dimethyl sulfoxide (DMSO), spermidine, bovine serum albumin (BSA), Tween-20, polyethylene glycol 6000 (PEG 6000), Nonidet P40 (NP40) and gelatin are known to enhance amplification and are utilized to optimize amplification (Weyant et al. 1990; Varadaraj and Skinner 1994; Sarkar et al. 1990; Lu and Negre 1993; Ahokas and Erkkila 1993). Nonionic detergents, such as Triton™ X-100, have overcome the inhibitory effects of trace amounts of strong ionic detergents, such as 0.01% SDS (Gelfand and White 1990).
The use of nonionic detergent such as mild surfactants has some advantages. In addition to improving the solubility of cellular macromolecules and the release of intracellular materials in a soluble form (JI et al. 2009), it increases DNA polymerase stability and reduces the loss of PCR reagents through adhering to the PCR tube walls (Gelfand and White 1990). Nonionic detergents might foster loosening of DNA-containing material off the paper by breaking protein-lipid, lipid-lipid associations of fingerprint components as such surfactants are uncharged besides containing hydrophilic head groups allow breaking the linkage between macromolecules of cells (Arnold and Linke 2007) therefore, releasing the DNA into cell lysate. Also, because it is non-ionic, Triton™ X-100 does not denature enzymes, meaning that if present in the PCR mixture, would not inhibit enzyme activity in the amplification reaction, compared to ionic detergents such as SDS or CTAB (Arnold and Linke 2007). Moreover, Templeton and Linacre (2014) successfully implemented the Triton™ X-100 as swabbing solution to be used with direct PCR and were able to generate interpretable profiles from 71% of 170 fingermarks. These properties might explain the higher yield of DNA in this part of the research as well as the success of generating DNA profiles directly without DNA extraction. The amplification failure of samples placed directly into the PCR mixture might be due to white office paper having undergone a bleach treatment step as discussed earlier.

The results obtained here support the notion that DNA will be deposited more readily on rough or porous substrates than on smooth surfaces (Harbison et al. 2008). Furthermore, the results suggested that DNA-containing material that are deposited on rough or porous substrates are more difficult to recover, which often leads to lower DNA
recovery yields being obtained from rough or porous substrates (Leemans et al. 2006).

The results obtained here were partially in accord with those of Sewell et al. (2008) in which ninhydrin and or DFO treatment was found to cause a 60% reduction in DNA recovery, but profiles were still recovered. Similarly, Schulz et al. (2004) found that while the DNA recovery may be lower after fingerprint enhancement, profiles can still be achieved. Given the negligible effect it has on the profiling of DNA, ninhydrin has been tested as a screening tool to determine if swabs of touch traces contain sufficient DNA for a profile (Anslinger et al. 2004). They reported that 120 of the 158 swabs that gave a reaction to ninhydrin also produced a DNA profile. On the other hand, there was an agreement with more recent research where Lovejoy (2012) reported that DNA recovery was reduced and no profiles were recovered after fingerprints after enhancement with ninhydrin were left for different periods of time.

Variations in DNA quantity were noticed in DNA retrieved from tape and paper substrates. The observed variations might be that tape-lifts, on some occasions, recovered cells contained DNA from the paper surface bound traces, leaving deeper imprinted DNA contained materials on the paper, which in turn might retain enough DNA to be of evidential value.

The results obtained in the current study were consistent with some previous studies into the use of tape lifting as a DNA recovery method, in which a variable success rate was reported. For example, Hall and Fairley (2004) mention that in more than 150 cases, their mini-tape lifting method reached a success rate of 80%. However, Li and Harris (2003) demonstrated a success rate varying between 0% and 100% when
sampling DNA from different parts of hairless areas on the body. The area examined were ankle, arm, behind the ear, between fingers and back of the neck. Furthermore, the Swedish National Laboratory of Forensic Science (SNLFS) performed a preliminary evaluation for tape lifting, after a few months of introducing it as a standard DNA collection method (Gunnarsson et al. 2010). The SNLFS mentioned that in 232 samples recovered with mini-tape and analysed, 78.0% yielded quantifiable levels of DNA. The general success rate over all samples was 38.8%, defined as the number of mini-tapes yielding interpretable DNA profiles. In the current study it has been sought to recruit Post It Tape Flags® instead of mini-tapes as the latter are highly adhesive and would remove the paper surface as well as lifting any skin cells that are present. Residues of ninhydrin would also be transferred. The current study results showed variation in the detection of alleles in DNA profiles depending on whether obtained from paper or from its associated Tape Flag®. These results might be explained by that DNA quantity that recovered from each item was different.

In the current research, the cutting method outperformed tape lifting in recovering DNA from paper substrate. Although, these results suggest the cutting method is superior to tape lifting, there are some drawbacks to the cutting method which is that the original evidence will be lost as a result of collection and analysis of DNA as well as the limitation of the amount of substrate that can be added into the extraction vessels, meaning that DNA can only be extracted from a small area of the paper. This is not the case with tape lifting method that has been presented in this study as DNA analysis process can be conducted without damaging the original paper substrate on which DNA has been deposited. Also with tape lifting method it is possible to examine a larger
surface area than that examined by cutting method through reapplying the same tape over a larger surface area. The simple handling, robust performance, examining of large area, along with keeping original substrate undamaged during the recovery of evidentiary DNA, all together with the recent appearance of improved chemistry of profiling kits, tolerance of inhibitors and degraded DNA, such as PowerPlex® ESI 17 Fast System (promega) and AmpFSTR® NGM™ Kit (Life Technologies) (Andrade et al. 2011) will lead to even more simplified and efficient future use of tape lifting approach in forensic practice to recover DNA from paper substrate. Consequently, further studies and developments of using adhesive materials similar to that used in Tape Flag® would be beneficial to design a new tape can be used to recover DNA from such challenging evidence of paper.
6 Results: Effect of Different Commonly used Fingerprints Development Methods on the DNA Profiling Process

As discussed previously in 1.5.4, the review of literature shows that there is no consistency regarding the effects of cyanoacrylate, aluminium and magnetic powders on subsequent DNA analysis. Consideration of cyanoacrylate fuming, aluminium powder and magnetic powder as agents, which do not destroy DNA, may be oversimplified. In all studies that have been reviewed in relation to this part of the research, the research investigated the effect of fingerprint enhancement techniques on DNA samples obtained from “macro quantities” of biological material. For instance cyanoacrylate was tested with biological stains (Stein et al. 1996; von Wurmb et al. 2001). Likewise, it was tested with several fingermarks loaded with DNA from other parts of the body (Wickenheiser and Challoner 1999; von Wurmb et al. 2001; Bever et al. 2002; Leemans et al. 2006; Shalhoub et al. 2008). On the other hand, powder dusting has received very limited attention, especially in relation to aluminium powder, using several fingerprints in which no prior hand washing step was applied (Schulz and Reichert 2002; Norlin et al. 2013; Raymond et al. 2004) or, if so, washing with deionized water (van Hoofstat et al. 1998; Raymond et al. 2004).

This part of the research aimed to perform a systematic study of the effect that Cyanoacrylate fuming (CA), Aluminium (Al) and magnetic (MG) powders have on traces of DNA recovered from just CMRFs that have been deposited on glass. Such findings will expand the scope of earlier work and improve the effectiveness of the
forensic response to crimes by increasing the awareness of the effect of using fingerprint enhancement on DNA recovery.

Six volunteers were recruited in the following experiments, three of which tended to deposit DNA above 100pg on glass (see 9.4). DNA extraction was conducted using the E.Z.N.A. kit. CMRFs were deposited on glass slides as described earlier in section 2.7 and developed following the methodology in sections 2.14.1, 2.14.2 and 2.14.7. Untreated CMRFs were set up as a control. In order to examine whether Al and MG powders were contaminated with DNA as a result of prior powdering of CMRFs, 10mg from three opened (previously used for dusting fingerprints of mock casework by the students) and two unopened (not used) containers of each powder was underwent to DNA analysis. All samples were evaluated by nuclear DNA quantification using qPCR and profiling using SGM plus. Quantification and profiling data were then used to infer which of the tested visualization techniques hinder the DNA profiling process and at which stage DNA loss occurs.

The powders’ DNA contamination results, illustrated in table 6-1, showed that DNA background was observed in opened, previously used Al and MG powders compared to unopened powders where no DNA was noticed. The average of DNA yield from Al powder was 471.67pg (SD: 185.64) while 438.01pg (SD: 109.28) was obtained from MG powder. The SGM$^+$ profiling of DNA contaminated powders reveals that incidence of reproducible mixed SGM plus profiles in Al and MG contaminated powders, Figure 6-1 depicts electropherograms of SGM plus profiles obtained from 10mg powders of Al and MG.
Table 6-1: DNA recovery of Al and MG powders. DNA extraction was conducted using E.Z.N.A. kit. C: depicts opened previously used powder container. n=6, two replicates each container.

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<tr>
<th>Powder</th>
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<td>C3</td>
<td>M</td>
</tr>
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<tr>
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<td>513.43</td>
<td>312.69</td>
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</table>
Figure 6-1: Mixed Profiles Obtained from Previously Used Al and MG Powders. Top electropherogram represents SGM plus profile obtained from Al powder and bottom represents SGM plus profile obtained from MG.
The results depicted in Figure 6-2 revealed that DNA amounts retrieved from CMRFs deposited on glass slides and enhanced with CA, and Al and MG powders were less than in case of samples recovered from glass slides without any treatments. The mean quantity of DNA recovered from unenhanced CMRFs was 490.54pg, whereas the means of CA and Al enhanced CMRFs were 210.47pg and 190.35pg respectively. The lowest mean DNA recovery was observed in MG enhanced samples, which was 159.92pg. Multiple comparisons between treatments using one-way ANOVA statistical analysis revealed that statistically significant differences at the 5% level were found between non-enhanced and enhanced samples with Al and MG powders, in contrast with CA treatment ($p = 0.046$). From these results, it can be concluded that DNA recovery was approximately halved whichever visualization treatment was used.

![Figure 6-2: Effect of fingerprint visualization Methods on DNA Recovery from CMRFs Deposited on Glass.](image)

Figure 6-3 compares the $C_T$ values of samples and associated internal PCR control (IPC) of CA, MG and Al enhanced samples compared to non-enhanced CMRFs. No indication of potential PCR inhibition was observed. The $C_T$ values obtained from IPC were within recommended range of 31±1 as is usual with positive amplification of DNA samples.

![Figure 6-3: $C_T$ Values of Samples and Their Associated IPC for DNA Obtained from CMRFs Visualized with CA, MG and Al. Purple bar: $C_T$ values of samples. Green bar: $C_T$ value of associated IPC.](image)

DNA profiles were generated from 48 CMRFs that had not been enhanced and CMRFs developed with one of CA, Al and MG powders (12 profiles for each treatment). All samples analysed by DNA profiling contained 150pg DNA. 43 of the DNA samples did not produce a complete profile, and only 5 samples gave a full profile (four of these profiles were obtained from unenhanced CMRFs samples and one profile only was obtained from a sample visualized with CA).
Figure 6-4 shows the level of completeness of the profiles achieved. The completeness of SGM$^+$ profiles was found to be varying among volunteers as well as visualization method. For example, the majority of alleles calling were observed in SGM$^+$ profiles obtained from V12 where 44 incidences of alleles calling of the corresponding profiles were recorded among all 6 profiles that generated from V12. In contrast, the lowest alleles calling (16) were recorded in SGM$^+$ profiles obtained from all 6 profiles that generated from DNA obtained from V10. Also, examination of Figure 6-4 shows 53 incidences of locus dropout seen in samples visualized with Al powder compared to 40 with CA treatment. Samples obtained from Al and MG developed CMRFs showed no full SGM$^+$ DNA profile was obtained. No SGM$^+$ DNA profiles were obtained in five samples, two in each of Al and MG developed CMRFs and one in CA.

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### Figure 6-4: SGM plus DNA Profile Completeness of DNA Recovered from CMRFs Visualized with Different Enhancements

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CA: Cyanoacrylate, MG: Magnetic powder and Al: Aluminum powder. Number of loci of profiling results obtained from CMRFs DNA samples at 34 cycles (n = 48). 150pg DNA was added to PCR each treatment. Blank: full profile, red: locus dropout, yellow: allele dropout, and Blue: allele drop in. R denotes replicates. Ref denotes references sample. V: volunteer.
6.1 Discussion

An initial investigation of Al and MG powders used in mock casework situations indicated that mixed SGM plus DNA profiles present in powder containers that have been regularly used. These results emphasises that DNA can be accumulated in powders used regularly for developing fingerprints. Although no brushes were used in the current study to develop CMRFs, the present of DNA in powders might be as a result of previous regular dipping of brushes and magnetic powder applicators into powder containers and then used among different fingerprints visualization mock casework scenarios. The brushes and applicators can pick up DNA from fingerprints and subsequently transfer it to powder containers (van Oorschot et al. 2005).

In the current experiment a reduction in DNA recovery was observed with all samples enhanced with CA, AL and MG compared to control samples. Although the differences were not statistically significant with CA, there was a general trend of DNA loss with fingerprint enhancement. On the other hand, no indication of inhibition was observed in enhanced or unenhanced samples.

In contrast to the results obtained here, Murray et al. (2001) reported that the majority of fingerprints enhanced with CA fuming yielded full SGM plus profiles when DNA processing was carried out within a week of treatment. These results were supportive of the findings of a previous study by Shipp et al. (1993) as they reported CA enhanced samples extracted with organic method and profiled with RFLP DNA were consistent with the DNA pattern from untreated bloodstains.
Given the results of variation between volunteers in the SGM plus profiling results prior and post enhancement, one may have expected to obtain comparable profiles from samples generated from different volunteers when using 150pg DNA template in PCR for each profile generated. The reason for this difference is unclear but may be due to the difference in the level of DNA degradation of each volunteer, the DNA shed through skin being prone to degradation due to the action of deoxyribonuclease (Fuchs 2007).

In accordance with the present results, some previous studies have demonstrated that any difference in DNA recovery from CA treated samples, as noted earlier, might be a result of differences in the cyanoacrylate fuming methods used, which may result in varying amounts of cyanoacrylate deposition, for example Pitilertpanya and Palmbach (2007) demonstrated that heavier deposition of cyanoacrylate decreased the subsequent DNA typing results. Furthermore, they reported better typing results were obtained from samples fumed for 20–30 min compared with those fumed for 40 min. They also mentioned that other factors, such as the cyanoacrylate heating temperature and the size of the fuming chamber, could significantly affect the amount of cyanoacrylate deposition.

The findings regarding CA fuming in the current study corroborated that of Bille et al. (2009) in which they reported that average DNA quantitation results demonstrate a reduction, as four of the six fumed samples did not yield any detectable DNA while several of the non-fumed samples demonstrated low concentrations. They hypothesized that CA fuming did not demonstrate any protection of DNA samples against scrubbing
off the hold substrate and from environmental conditions during three-month intervals that were examined.

In relation to the current findings, the reduction in DNA quantity might be because DNA-containing materials were entombed due to CA fuming and neither collection nor extraction methods were efficient in releasing all of the DNA from fuming residues. As the DNA extraction method used was found to lower the DNA recovery in the presence of CA, such treatment significantly hampers the amplification of DNA from small stains (von Wurmb et al. 2001). Although CA fuming was proposed to entomb DNA materials, the cyanoacrylate layer deposited on the surface can actually be porous. Therefore, water or other molecules in the environment might still have access to the biological material (Wargacki et al. 2007). Moreover, additional parameters can be of significance for DNA recovery such as the humidity and temperature accompanying the fuming process (Schulz and Reichert 2002). There was limited evidence that cyanoacrylate fuming had a detrimental effect on the success of DNA analysis as has been reported previously (Pitilertpanya and Palmbach 2007). Only 40% magnetic powder developed prints demonstrated detectable amounts of DNA (Norlin et al. 2013). Norlin et al. (2013) hypothesized that reduction was caused due to the content of iron in the magnetic powder possibly inhibiting PCR (Lennard 2001). These findings might be relevant to the current study results, as in both there was DNA loss.

The current results reported no PCR inhibition; IPC $C_T$ values were within optimal range of 31±1 cycles. The results of Roux et al. (1999) indicated that magnetic powder is not recommended for use in a sequence of analyses that involves DNA typing,
whereas aluminium fingerprint powder may be used successfully. The latter finding agreed with Murray et al. (2001) as samples processed with aluminium powder yielded full DNA profiles when DNA processing was carried out within a week of treatment and even after 100 days of preservation.

In the current study, the observed reduction of DNA in all treatments, along with no indication of PCR inhibition, might be explained by the presence of Al, MG and CA particulates in the extraction vessel having clogged the spin cartridge, thus low DNA yield was recovered (Life Technologies, 2014). Also, such presence might lead to prevent a complete lysis having occurred. Another possibility might explain that reduction is that DNA adhering to the Al, MG and CA particulate materials.

The current results suggest that use of a cheap disposable brush for Al powdering of each object to avoid the possibility of transfer, and for the MG powdering, rather than repeatedly dipping the applicator into a single container for an extended period, consider preparing and using separate small aliquots of MG powder for each object, discarding the powder after a single use. In addition it might be worthwhile to consider a bigger mesh size when designing spin cartridge for DNA extraction kits to avoid clogging the spin columns, bearing in mind that DNA molecules would be captured by the silica while all impurities would be washed off regardless of its size.
7 Discussion and Conclusion

This chapter is divided into sections that assess the findings against the aims of the project. Also, this chapter sees the results given a practical context. The limitations of the research and potential areas for further work are discussed in the final component of this section.

Previous research studies carried out investigating DNA deposition in fingermarks have been basic, lacking replicates and controls, producing conclusions that merit further investigation (Lowe et al. 2002; Phipps and Petricevic 2007). Also, it has already been emphasized that early intervention of all appropriate forensic practitioners in the strategic thinking for a case should benefit the success of forensic evidence recovery (Home Office 2014).

The first aim of the project was to determine the most suitable extraction method to be used in DNA recovery from CMRFs deposited on glass. Optimizing DNA yields is of prime importance to crime laboratories, particularly when dealing with touch samples where limited quantities of DNA may be left behind (van Oorschot and Jones 1997). The current data illustrate that different extraction methods can result in very different outcomes, highlighted by significant differences in the three extraction methods used for fingerprints on glass. So the right choice of the DNA extraction method is very important step in the analytical procedure to ensure optimal results therefore forensic laboratories be aware of the potential capacity and limitations of their techniques. In the present study, the E.Z.N.A kit was more effective, not only in cost and DNA yield but
also in the quality of SGM profiles. The use of this kit was sensitive enough to be applied in real casework.

The second aim of the project was to investigate the propensity of different areas of the volar surface to deposit DNA as a factor to be considered by forensic scientists. This can be added to the current factors affecting DNA deposition. The purpose of this experimental component was to provide data to further the current understanding of the nature and source of DNA deposited through a hand touch, and to investigate whether such data collation is practical and useful to criminal investigations. Therefore, this increases our confidence in assigning a weight to DNA evidence obtained in such circumstances.

The anatomical location of the skin has a measurable effect on the amount of DNA shed by it, as demonstrated in the current findings (see 4.2) in which the friction ridge skin of middle and ring fingers shed more DNA than the entire palm. In this study, a stringent protocol was developed to ensure that the DNA deposited originated from the donor surface. Given the rigor of the procedure (see 2.4 and 2.7), all or nearly all the DNA should originate from the donor surface. These findings have implications for scene examination practice. Ferraro (2012) asks, “Should I swab for DNA or process for fingerprints?”. The research conducted here gives some guidance in that respect (see 4.4). With the emergence of compulsory ISO 17025 accreditation for all UK providers of forensic services, a plan for fingermark evidence recovery will always need to be drawn up, and there should be a preliminary analysis of the undeveloped mark at the scene (Home Office 2014). On the basis of the evidence presented here, palmprints
should, where there is evidence of visible ridge detail, be processed for pattern analysis prior to swabbing for DNA, as the likelihood of their yielding donor DNA for profiling is limited (see 4.2). Furthermore, there is evidence that a high proportion of palm marks obtained from crime scenes can be matched to the donor using pattern matching (Sutton et al. 2013). The decision as to whether to swab fingermarks will depend on a variety of factors. These include the extent and level of friction ridge detail that can be observed by eye and the relative size of fingerprint, palmprint and DNA databases in the jurisdiction. Some may question the relevance of this type of research, seeing these results are based on experiments featuring a controlled DNA deposition process. In crime scenes a large number of factors may affect DNA deposition. These might include contact of the palms or fingers with other parts of the donor body resulting in intra-individual DNA transfer and potentially higher DNA levels or contact with a surface that contains DNA from someone other than the donor. Given the multitude of variables involved in obtaining DNA from fingermark evidence, it is unlikely that conclusive answers can ever or would ever be provided of the DNA amount deposited in a simple touch, but such current research can give an additional indication of the probability of obtaining a set of results taken into account information provided by forensic scientist. This in turn will be offered greater assistance to plan the forensic evidence recovery procedure. The Caddy report (2008) mentions the need for standardised processes and recovery techniques in relation to low template DNA as well as enhanced training for practitioners. Therefore, the recovery procedure should be cost effective (e.g. of chemicals and equipment), including reducing the time and human resource taken to conduct a DNA analysis, especially with such a small amount of DNA coming from the donor surface, and the
probability of getting difficult to interpret mixed profiles increases.

The results shown herein demonstrate that DNA deposition is highly variable, but can yield amounts of DNA, in some occasions, sufficient to generate a DNA profile through low copy number DNA typing techniques. This was found to be influenced by collection and extraction methods, but mainly by the individuals’ propensity to shed DNA. However, the concept of “good” and “poor” shedders was relevant to some extent in this study, although high inter- and intra-individual differences were observed in the deposited DNA amount, which is likely to be a result of a combination of behavioural as well as inheritable conditions as discussed previously in 1.4. By and large, volunteers with either high or low propensity to deposit DNA showed a degree of consistency in their capabilities to shed DNA throughout the different experiments that were conducted in this study. Volunteers, who tended to deposit a high amount of DNA on glass, showed the same ability with paper substrate, this observation being mirrored with other volunteers. In addition, the fingermark application force results suggest that a more forceful interaction does not necessarily increase the amount of DNA that is deposited, and that a number of factors will influence the amount of DNA deposited such as the substrate that fingermarks are deposited upon and the depositor characteristics.

The difficulty in predicting the amounts deposited, when combined with the complications associated with the location of DNA deposits, and the reported low success rates when processing this type of evidence, make DNA from fingerprints an unappealing form of forensic evidence in its current state. Conversely, the identification value of DNA evidence, its ubiquitous nature and the reduced awareness amongst
perpetrators of this kind of forensic evidence make it deserving of further investigations to improve the success rates.

The third aim focused on a systematic evaluation of the impact of different fingerprint development processes on DNA availability. The mechanism by which influence occurs was explored to ascertain whether the impact on the DNA analysis process can be predicted and this knowledge used to improve crime scene evidence recovery.

Previous publications have concluded that the impact of fingerprint visualization on DNA analysis from fingermarks is limited to the inhibition of the PCR (Van Hoofstat et al. 1998; Gino and Omedei 2011; Norlin et al. 2013). In contrast to the conclusions of these authors and although in some instances DNA recovery was low, the present study revealed that no PCR inhibition took place within samples obtained from touch DNA samples enhanced with ninhydrin, cyanoacrylate, magnetic and aluminium powders. These results suggest that such visualization materials interfere with the processes of sample collection and DNA extraction rather than PCR amplification. The results of experiments in which ninhydrin was directly mixed with control DNA suggest that ninhydrin affected the template DNA rather than inhibiting the PCR reaction (see 5.2). Moreover, solvents used to prepare ninhydrin have showed some indication of inhibiting DNA recovery (see 5.3). However, the reduction observed within DNA samples obtained from fingerprints enhanced with the other visualization materials is more likely attributable to the interference with DNA collection and extraction processes, by entombing DNA containing materials, clogging filters and columns used
during extraction process or DNA binding to powder particles or cyanoacrylate fuming layers. These results would partly address the issue raised in the Home Office Manual (2014) in which the manner of impact of fingerprint visualization processes on DNA analysis is unclear and there is a need for more research in that respect. These results also suggest that DNA extraction should be considered as the key to maximize the DNA recovery from fingerprints, considering that every enhancement material is different. This, in turn, would inform scene evidence recovery practice, emphasising the importance of an integrated forensic evidence recovery plan.

The fourth aim was to maximize DNA recovery from CMRFs deposited upon white office paper prior to as well as post ninhydrin development. The purpose of this experimental component was to overcome obstacles associated with DNA recovery from office paper and to investigate whether such findings may be of more assistance to criminal investigations in terms of increasing the evidential value of white office paper.

The current results demonstrated treatment of fingerprint loaded paper with 1% Triton™ X-100 in the presence of proteinase K in both ninhydrin enhanced and non-enhanced samples was successful in increasing DNA recovery from paper compared to that lysed in the presence of SDS, CTAB and water (see 5.5). Therefore, implementing use of improves extraction efficiency. The efficacy of this method in increasing DNA recovery from fingerprints deposited on paper was demonstrated by improved DNA recovery (see 5.4) compared to that recovered from glass substrate. This additional reagent is relatively cheap and can be used in forensic analysis to assess DNA recovery in challenging evidence types such office paper.
In response to the increased demand for DNA analysis, laboratories are continuously implementing improved methods that allow them to process database and casework samples more efficiently. These improvements include direct PCR. Since 2009, where the first report of using direct PCR in forensic analysis was published (Wang, et al, 2009), there has been a sudden explosion of commercial kits available in the market for direct PCR. For instance AmpFLSTR® Identifiler® Direct PCR Amplification Kit from Applied Biosystems®, FastGene™ Direct PCR kit (Nippon Genetics Co. Ltd.) and PowerPlex® Fusion Systems from Promega, showing that there has been a significant demand for rapid analysis of forensic samples. It also indicates that direct PCR as a technique has the potential for further application and development in the field of forensic science. One of the examples of that is examining direct PCR and the resulting DNA profile after chemical enhancement of fingermarks (Templeton and Linacre 2014).

The novelty of use of direct PCR to generate DNA profiles from fingerprints deposited upon office paper using a kit that has not been designed for direct PCR such as SGM plus was explored in this study. In this study direct PCR was achieved successfully when paper samples were pre-treated with 1% Triton™ X-100. In contrast, no DNA profiles were obtained when office paper was applied directly into the PCR vessel, or when the paper was incubated with water in the presence of proteinase K. However, success with direct PCR to generate DNA profiles from traces of DNA such as those in fingerprints on office paper indicated that this technique has the potential to be incorporated into routine forensic DNA testing. As a result of omitting extraction and quantification steps, the loss of DNA associated with extraction protocols, especially
with low target copy DNA samples, would be reduced, as would cost and processing time (Swaran 2014).

The last aim was to examine tape lifting for collecting DNA containing material from office paper. It has been emphasized that there is always a need to examine the best recovery techniques for different surface types that can be used at a crime scene (Newman, 2009). The cutting method is the most common technique used to recover DNA from porous surfaces such as office paper. The current study, for the first time, demonstrates that tape lifting is an approach which can be applied to recover DNA from office paper without ripping or delaminating the paper surface when post it Tape Flags® were used. This can also be integrated into the normal workflow of forensic evidence processing by establishing best recovery techniques for each surface type and office paper might need to be processed in a specialist DNA facility. This could be presented in the same way as the Fingerprint Development Handbook (Home Office 2014), which gives different flow charts of techniques for surface material types.
7.1 Future Work and Limitations

There are a number of limitations to this research that must be taken into consideration and it may be possible to highlight areas for further research. For example, the sample size or number of replicates used in an experimental component limits the conclusions that can be made as to the extent of their effect on DNA recovery. As discussed earlier, in a crime scene, a large number of factors may affect DNA deposition. These include contact of the palms or fingers with other parts of the donor body resulting in intra-individual DNA transfer and potentially higher DNA levels or contact with a surface that contains DNA from someone other than the donor in a secondary transfer. The latter is often proffered by the defence as the reason their client’s DNA came to be present at the crime scene. A large scale study across the general population into the nature and quantity of DNA recovered from hand marks deposited under ‘natural’ conditions (blind study), such as those found in a crime scene, could shed further light on scene practice. In such a study some other factors would be beneficial to consider such as age and skin type as additional factors govern DNA deposition.

Although this research evidenced conclusions based upon the experiments undertaken, the availability of participants who took part in our research study was one of the main limitations faced this research. As the current study was based on recruiting participants on a voluntary basis, due to the cost limitation, and because of the hour waiting after washing hands plus the number of replicates that were required either for the extraction methods used or the fingermarks enhancement were examined from each participants, it was difficult to achieve a greater number of volunteers.

Another issue with this project is that it was difficult to achieve the best results
whilst maintaining relevancy and consistency with the commercial kits currently used in the forensic investigation in the UK. The kit used for DNA profiling of samples was SGM\textsuperscript{+} rather than one of the new kits in which new chemistry was incorporated to accommodate degraded and low content DNA samples. This was due in part to the limited budget that was available; these kits were not available when the project commenced; SGM plus being retained in order to ensure comparability of data throughout the work. The new kits were launched to use in UK for caseworks and database as discussed early in 1.1. Examples of these kits include the PowerPlex\textsuperscript{®} ESI 17 Fast System and AmpF\textsuperscript{®} STR NGM\textsuperscript{™} Kit, which were accredited by the UKAS to be used for comparison across England and Wales. It may be that these kits will make up a significant contribution to the overall recovery of a useable DNA profile from such challenging samples of DNA deposited in fingerprints.

In the present study there was a drawback of tape lifting using post it Tape Flags\textsuperscript{®} as DNA recovery was low compared to that recovered by cutting method (see Figure 5-18). This is might a result of adhesive material on the post it Tape Flags\textsuperscript{®}, which may inhibit DNA recovery. Solvents such as xylene have been reported effective in releasing DNA from tape-lifts (TAPE-iT, LGC Forensics) (May and Thomson 2009). In this respect, further research might maximize DNA recovery using Tape Flags\textsuperscript{®}. Such research will broaden its application to casework items, particularly as this method has not only simplified sampling method but is non-destructive.

In the current research, DNA was obtained from fresh fingerprints developed with ninhydrin, Al, MG and CA. The effects of these enhancements were found related with availability of amplifiable DNA post development rather than inhibition of PCR. Further research is needed to examine aged fingerprints present on different surfaces for
some time against different environmental conditions (i.e. exposure to heat or humidity) would shed more light on the success rate of DNA profiling from fingerprints.

Also future work may be carried out to assess the limitations of direct PCR and to determine what effect chemical and physical enhancement methods will have on the DNA template and DNA profiling process. Tape lifting using post it Tape Flags® results presented herein are based on DNA recovery from just two fingertips post hand washing in ideal lab conditions, which is not the case in real casework as it is more likely that several fingermarks would present at the crime scene, this suggests more research exploring the applicability of this approach on large scale and among casework is merited. Likewise, developing a kit, in which materials similar to that of post it Tape Flags® are used to recover DNA containing material from paper substrate, may be of benefit for crime scene investigators.
8 References


Branch, L. (2011) *Generating Forensic DNA Profiles from Contact DNA on Cartridge Cases and Gun Grips*.


Spear, T., Clark, J., Giusto, M., Khoshkebadi, N., Murphy, M. and Rush, J. (2005) Fingerprints & cartridge cases: how often are fingerprints found on handled cartridge cases and can these fingerprints be successfully typed for DNA? *California Department of Justice, Bureau of Forensic Services*. 


## 9 Appendixes

### 9.1 DNA extractions, Chelex, volunteers 1-6

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237
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- **Experiment File Name**: Applied forces, CMRFs and Palm
- **Experiment Run End Time**: sds7500fast
- **Passive Reference**: ROX

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**9.12 Glass Vs Paper DNA recovery - Glass**

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Chemistry          TAQMAN
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Experiment File Name: Glass Vs Paper DNA recovery -Paper  
Experiment Run End Time  
Instrument Type: sds7500fast  
Passive Reference: ROX

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**9.18 1% Triton - Paper**

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- Experiment File Name: Triton
- Experiment Run End Time: Paper
- Instrument Type: sds7500fast
- Passive Reference: ROX
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| A2  | FAM-BHQ       | STANDARD  | FAM | None | 19.91891366 | 19.91014367 | 0.546593986 | 20  |
| A2  | VIC_BHQ       | UNKNOWN   | VIC | None | 30.28543168 | 32.50211984 | 0.840634675 |
| A3  | FAM-BHQ       | STANDARD  | FAM | None | 22.1092251  | 22.24696237 | 0.728981272 | 5   |
| A3  | VIC_BHQ       | UNKNOWN   | VIC | None | 31.48678666 | 32.46944505 | 0.558714745 |
| A4  | FAM-BHQ       | STANDARD  | FAM | None | 22.38470155 | 22.24696237 | 0.728981272 | 5   |
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| A6  | FAM-BHQ       | STANDARD  | FAM | None | 24.4629181  | 24.50222284 | 0.589773939 | 1.25 |
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364
### 9.19 1% Triton - Glass

**Block Type**: 96Fast  
**Chemistry**: TAQMAN  
**Experiment File Name**: Triton Glass  
**Experiment Run End Time**:  
**Instrument Type**: sds7500fast  
**Passive Reference**: ROX

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**Experiment File Name**: SDS Paper  
**Experiment Run End Time**:  
**Instrument Type**: sds7500fast  
**Passive Reference**: ROX  

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Experiment File Name: SDS Glass  
Experiment Run End Time:  
Instrument Type: sds7500fast  
Passive Reference: ROX

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**9.22 2% CTAB - Paper**

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Experiment File Name: CTAB Paper  
Instrument Type: sds7500fast  
Passive Reference: ROX
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| A3   | FAM-BHQ | STANDARD | FAM | None | 22.22655688 | 22.36429415 | 0.846313054 | 5                  |
| A3   | VIC_BHQ | UNKNOWN  | VIC | None | 31.60411845 | 32.58677683 | 0.676046527 |                    |
| A4   | FAM-BHQ | STANDARD | FAM | None | 22.50203333 | 22.36429415 | 0.846313054 | 5                  |
| A4   | VIC_BHQ | UNKNOWN  | VIC | None | 31.56943522 | 32.58677683 | 0.676046527 |                    |
| A5   | FAM-BHQ | STANDARD | FAM | None | 24.65885744 | 24.61955462 | 0.707105721 | 1.25               |
| A5   | VIC_BHQ | UNKNOWN  | VIC | None | 30.62998019 | 32.43100176 | 0.932918409 |                    |
| A6   | FAM-BHQ | STANDARD | FAM | None | 24.58024989 | 24.61955462 | 0.707105721 | 1.25               |
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| A7   | VIC_BHQ | UNKNOWN  | VIC | None | 30.44907961 | 32.45395861 | 0.658423076 |                    |
| A8   | FAM-BHQ | STANDARD | FAM | None | 26.53346072 | 26.56730471 | 0.699383068 | 0.3125             |
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### 9.26 CA – Treatment

- **Block Type**: 96fast
- **Chemistry**: TAQMAN
- **Experiment File Name**: CA effects
- **Experiment Run End Time**: 
- **Instrument Type**: sds7500fast
- **Passive Reference**: ROX

- **Well A1**
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  - **Target Name**: STANDARD
  - **Task**: FAM
  - **Reporter**: None
  - **Quencher**: None
  - **Ct**: 20.97084206
  - **Ct Mean**: 20.99829262
  - **Ct SD**: 0.031177756
  - **Quantity (ng/µl)**: 20

- **Well A1**
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  - **Task**: VIC
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  - **Quencher**: None
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  - **Ct SD**: 0.076624871
  - **Quantity (ng/µl)**:                

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- Sample: Identifier of the sample.
- V9: Position of the sample.
- Barcode: Barcode identifier.
- Cycles: Number of cycles.
- Status: Status of the sample.
- Peak Height: Peak height value.
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