A Critical Evaluation of Touch DNA Recovery Methods for Forensic Purposes.

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Copyright Statement

Abstract

Over the past decade there has been a significant increase in the number of submissions of touch DNA evidence to forensic laboratories. Previous research has indicated that analysis of these samples produces poor results, with only 5-6% of handled items generating a full profile (Quinones and Daniel, 2012). Published research, as well as case work review by forensic practitioners, has also indicated more consideration of how to improve the evidential value of touch DNA samples is needed. Therefore, this research aims to critically evaluate low-level DNA recovery and analysis methods in order to maximise efficiency for forensic identification purposes. Typical evidential items, such as plastic handled screwdrivers, aluminium cans, drinking glasses and wooden handles, were handled in a mock-operational trial. The deposited DNA was recovered from these items using a range of swabbing materials including cotton, polyester, nylon flocked, foam and rayon (also known as viscose). These samples were then quantified using human specific quantitative PCR and profiled using AmpFℓSTR™ NGM SElect™ and the RapidHIT™ 200 instrument. The DNA quantity and quality were compared and a statistically significant difference was found to be present between recovery methods from the different surfaces. The findings of this research allow for an optimal recovery strategy to be recommended based upon the surface type the DNA is being recovered from. Additionally, it was determined that it is possible to analyse touch DNA evidence using Rapid DNA technologies which may provide great benefits to criminal investigations. The way in which the DNA interacts with the surfaces and the swabbing materials was also preliminarily evaluated to determine the impact this has upon the recovery efficiency of each recovery method. This research will inform best practice for the recovery and

analysis of low-level DNA samples from forensic exhibits and can influence the ISO validation procedures for crime scene examination processes (ISO17020).

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Chapter 1: Introduction and Literature Review

1.1. Overall Introduction

Forensic Science can be defined as 'science that is used in the service of the justice system' (Jackson and Jackson, 2011). The analysis of Deoxyribonucleic Acid (DNA) for this purpose was developed in 1984 by Sir Alec Jeffreys when it was referred to as 'DNA Fingerprinting' (Jeffreys *et al.* 1985) and this was first used in an immigration case in 1985 (Jeffreys *et al.* 1985a). The use of repeat regions of DNA sequences has been utilised in various techniques including Short Tandem Repeat (STR) profiling, which is commonly referred to as DNA profiling, is regularly used in Forensic Science (Butler, 2005). Since the first uses by Sir Alec Jeffreys, DNA analysis and profiling has been, and continues to be, developed which has been revolutionary for the discipline of Forensic Science as this allows a strong link to be made between biological evidence and the individual who deposited it (Jackson and Jackson, 2011). As a result of these processes becoming more developed and more sensitive, it is now possible to analyse DNA samples with lower quantities of DNA present such as DNA collected from handled items which was discussed by Wickenheiser and Challoner (1999). When looking at these samples the term 'touch DNA' is often used which typically means DNA transfer that originates from contact between an individual and a surface also known as direct transfer, although this is not always the case. As the term 'touch DNA' can sometimes be misleading as it infers contact through touching which may not always be known, it is also commonly referred to as 'trace DNA' which encompasses all possible transfer mechanisms (Meakin and Jamieson, 2013). With these advancements, the analysis of samples commonly referred to as 'touch' or 'trace' DNA has become more commonplace which leads to the need for additional consideration to be given to alternative propositions, in regards to the activity that led to the occurrence of this evidence being present (Raymond *et al.* 2004). Due to this, DNA evidence can only definitively be used to give an indication of the source of the evidence i.e. who this biological material belongs to,

which means currently it cannot be used to suggest the activity which led to this evidence being present or whether or not an offence was committed as this relies upon other circumstantial information (Cook *et al*. 1998). Some of these considerations were previously thought be unlikely, however, with the complex nature of 'touch' or 'trace' DNA, propositions from the defence around how that DNA could have been inadvertently transferred to a crime scene need to be considered more heavily (Raymond *et al.* 2004). To aid with this, further research has been completed to assess the optimal analysis and interpretational methods which will be discussed in the remainder of this thesis with particular focus on 'touch' or 'trace' DNA. Please note, for the purpose of the research conducted in this thesis the term 'touch DNA' will be adopted as the transfer mechanism is known to be through direct transfer from the hands to the surface.

1.2. Touch DNA

1.2.1. Research into the Origins of Touch DNA

Touch DNA is genetic material that is deposited by individuals onto an item when they come into contact with its surface. This DNA typically exhibits low quality and quantity, often comprising of mixtures creating interpretational challenges (van Oorschot *et al*. 2003; Buckingham *et al*. 2016).

It has previously been suggested that touch DNA is generated by an individual's propensity to shed skin cells which occurs naturally through the course of their daily activities (Aditya *et al*. 2011) but this has been debated in published literature with no consensus being reached. This in itself, suggests that the origin of touch DNA is still unknown and may be more complex than simply the transfer of skin cells. However, many researchers have taken different approaches when trying to determine the origins of touch DNA which may contribute towards the differences of opinion.

The cells deposited within touch DNA are thought to be comprised of epithelial cells which are keratinized. It is also believed that the DNA originates from the epidermal cells that slough off the skin's surface but the relationship between the transfer of the DNA within cornified layers of the epidermis and the production of a genetic profile is not yet known (Kita *et al*. 2008). Additionally, when touch DNA deposits have been examined microscopically there has been no correlation reported between anucleate keratinocytes and the amount of DNA recovered suggesting this may not be the source of the DNA present (Stanciu *et al*. 2015).

A study conducted by Kita *et al*. (2008) found that there appeared to be nuclei present within the keratinized cells in the upper granular layer of the epidermis but these were flattened and had lost their shape. Additionally, they suggested that the DNA may originate from the fragmented DNA within the cornified layer of the epidermis which is sloughed onto a surface via sweat deposits.

Zoppis *et al*. (2014) also reported that fragmented DNA was found within the sebum secreted from sebaceous glands that are located over the dermis. However, these glands are not located on the palms of the hands or the fingers meaning that the presence of this within any touch DNA deposits suggests secondary transfer from other areas of the body to the hands prior to deposition. This adds weight to the suggestions previously made by Wickenheiser (2002) and van Oorschot and Jones (1997) that the DNA present within these touch DNA deposits is not comprised of only skin cells but may contain additional cells and DNA sources collected from other areas of the body or from other surfaces altogether. Burrill *et al.* (2019) also discussed the potential different sources of DNA present on the hands that may be deposited onto a surface and produced the image found in figure 1 which demonstrates the types of DNA that may be present and that will be discussed in this section.

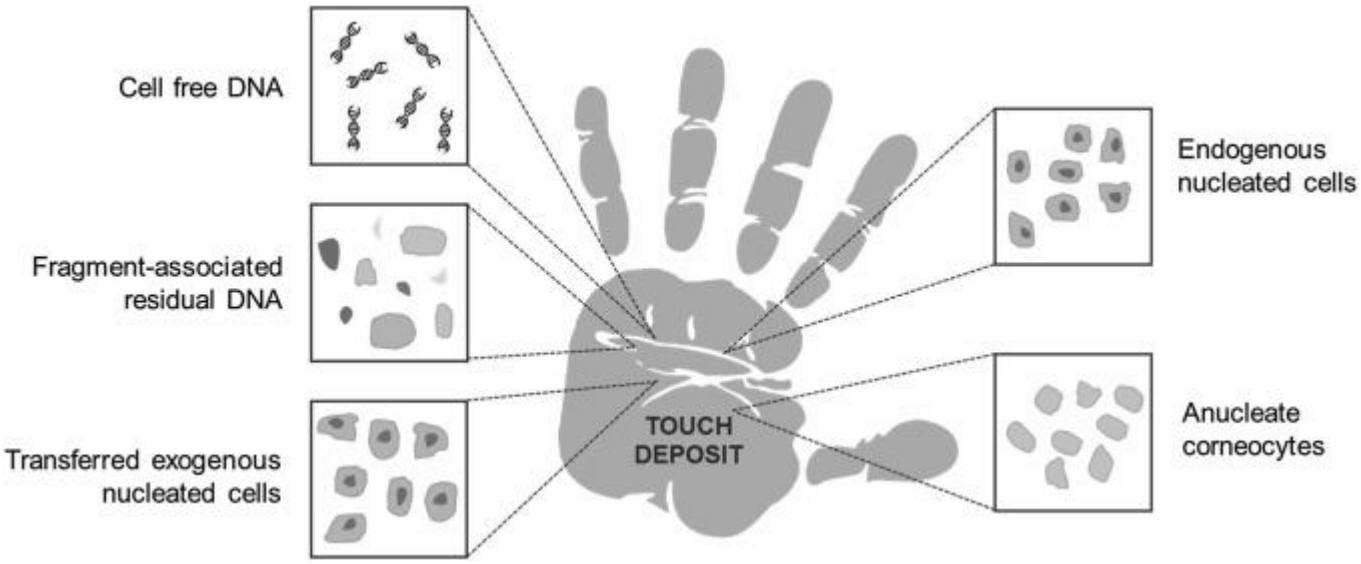


Figure 1. Infographic from Burrill et al. (2019) displaying the potential sources of DNA deposited from the hands.

Quinones and Daniel (2012) found that the levels of DNA present in cell free samples was variable both between different individuals and also intervariable depending on factors such as samples times with variation from one day to the next. Despite these variations it was still possible to generate a complete profile from 40% of the samples analysed. Additionally, Vandewoestyne *et al*. (2013) found that cell free DNA was present in 71.4% of contact trace samples from forensic casework which gave additional value to these samples in terms of the probability of this DNA profile being found.

Furthermore, extracellular DNA (exDNA) has been found by Wang *et al.* (2017*)* to be present upon the surface of epithelial cells. This exDNA originates within the cell nucleus and migrates to the cell membranes where they either remain attached or they can be unattached within fluids and secretions, such as sweat, as a result of apoptosis. The function of exDNA is not known but it has been suggested that this makes up a significant proportion of touch DNA samples (Wang *et al*. 2017). Such exDNA may be a biproduct of the waste production and excretion within the body that then collects on the surface of the hands in the

form of sweat. If this is the case, it suggests that such excretions may be invaluable in gaining a genetic profile from samples such as touch DNA but this factor needs to be further investigated.

Bouzga et al. (2020) investigated whether it was possible to identify the difference between skin epithelial cells and vaginal mucous membrane cells in relation to cases of sexual assault and/or rape. They found that the samples where vaginal mucous membrane cells were deposited had higher quantification values than those where skin epithelial cells were deposited which is to be expected. They also found that in the cases where the vaginal mucous membrane cells were deposited the main contributor to the DNA profile was the female whereas the samples where skin epithelial cells were deposited the main contributor was the male. However, despite investigating whether it was possible to differentiate the origins of these epithelial cells the research did not look into the cells themselves but rather the difference in the profile and quantification data which does not give indications on what is present in those contact traces but rather speculates that this contains skin epithelial cells.

Sauer et al. (2017) discuss how previously used immunological, histological and enzymatic techniques may not be appropriate for this as they do not have high sensitivity and specificity so may not be as useful for trace amount of DNA inferring that more appropriate analysis methods should be adopted. To further identify the components present within touch DNA, epigenetic approaches can be adopted to detect the presence of specific biomarkers.

Schultz et al. (2010) utilised a quantitative mRNA profiling assay-based method to evaluate the expression of cytokeratins to distinguish the presence of mucosal epithelial cells and epidermal epithelial cells. However, the specificity of some of the markers was not absolute with them also being found in a small number of vaginal samples. Further to this, the study conducted by Shultz et al. (2010) was not in relation to forensic samples so the application of this may not be appropriate for such samples.

Visser et al. (2011) conducted research focussed on the ability to identify skin from touch DNA evidence using mRNA profiling assays. Three skin specific gene transcripts were focussed upon, CDSN, LOR and KRT9 but lower expression levels of these genes were also detected in non-skin samples, however, these could be differentiated based on their ΔCt values. Lindenbergh et al. (2012) also investigated the use of CDSN and LOR markers for identifying the presence of skin within a sample. The CDSN marker was used as they encode the corneodesmosin that is involved in the desquamation process whereby the outer membrane layer of the skin is shed (Haftek et al. 1997; Jackson et al. 1993; Lindenbergh et al. 2012). The LOR marker was selected as this encodes loricin which is a component of the cornified cell envelope which is found in terminally differentiated cells (Candi et al. 1995; Lindenbergh et al. 2012). They also found that these markers were highly expressed in almost all skin samples and occasionally in vaginal mucosa and menstrual secretion samples which is in accordance to the findings of Visser et al. (2011). However, they also found that the mucosa markers, KRT4, KRT13 and SPRR2A, were also being detected within some of the skin samples despite these samples not representing a mucous membrane. This provides evidence to the suggestion that some of the DNA present in touch DNA samples may be due to secondary transfer from elsewhere on the body.

Lindenbergh et al. (2012) also completed NGM DNA profiling of the skin samples and found that there were some full profiles and some partial profiles. This may indicate that the skin samples which contained the additional mucosa markers as well as CDSN and LOR produced a full profile which would suggest that this secondary transfer was conducive to a fuller profile, however, the data around which samples produced the full profiles was not included in their report. In order to generate further conclusions around this, further research would be required.

The results of these reports demonstrate a need to identify specific biomarkers for skin samples to adequately identify the components of touch DNA deposits which is supported by Hanson et al. (2012).

Research conducted by Hanson et al. (2012) aimed to identify highly specific gene candidates for the positive identification of skin in touch DNA evidence. They identified five skin specific candidates which had a higher expression in skin than in other non-skin samples such as vaginal samples and had high specificity for skin. This included genes related to the late cornified envelope (LCE) which is part of the epidermal differentiation complex which is dominant in external epithelial skin samples. The most sensitive marker was LCE1C which was detected in 100% of mock touch DNA evidence samples with a significantly higher sensitivity and rate of detection than the other markers tested. However, further work is needed to assess the correlation of the identification of these biomarkers in comparison to the DNA profiles produced and to determine if this can be used to identify where these deposits originate from.

Haas et al. (2015) also conducted research to identify specific biomarkers for touch DNA samples by looking and markers indicative of skin cells. For the contact traces they analysed, they found that the presence of skin was difficult to identify with only a few of the laboratories used identifying skin when using the LCE1C and LOR markers which could suggest that the majority of a touch DNA deposit does not contain large amounts of skin cells. However, they also found that the housekeeping genes were rarely detected which indicates there was minimal biological material present. However, when looking at the quantification data it was found that this did not correlate well with the input amount of biological material with the high input stains not showing elevated RNA quantification results so it is not clear what quantity of biological material was present in these samples. This does suggest that LCE1C and LOR are more highly expressed in these contact traces than the other markers IL1F7, LCE1D, LCE2D, CCL27, KRT9 and CDSN but it is also worth noting that LCE1C and LOR were also detected in the salvia samples. This may indicate that there were some skin cells from the lips within the saliva sample or it could be hypothesized that these markers were not skin specific and that the samples where these markers were detected may have also contained saliva or buccal epithelial cells.

In order to assess the skin specificity of the markers, Haas et al. (2015) also completed a RNA dilution series from commercial skin RNA and found that seven of the eight skin markers and all 3 housekeeping genes were detected in these samples down to 0.8ng. KRT9 however, was not detected in the samples below 50ng which suggests that this marker requires a higher quantity of input DNA in order to detect the presence of skin so this would not be suitable for touch DNA samples which typically contain low amounts of DNA.

Akutsu et al. (2018) investigated skin and sweat characteristic mRNAs to determine whether these were appropriate to infer whether an object has been handled by evaluating their specificity in comparison to other body fluids and casework samples. They found that LOR was detected in skin, saliva, urine and vaginal fluid samples but with significantly different expression levels overall but there was some overlap between some samples although which samples was not specified. They found that CDSN was also detected in all skin samples but was also expressed in sweat and vaginal fluid samples but with significantly higher expression levels in skin and sweat samples. Additionally, they found that LCE1C was detected in under half of the skin samples and also in the sweat and vaginal fluid samples but again with significantly higher expression in skin and sweat with no overlap in expression levels. KRT10 was found in all of the blood, semen and vaginal fluid samples but in less than half of the skin samples. KLK5 expression was significantly higher in skin samples than in the other body fluids but vaginal fluid samples and urine samples displayed similar expression levels. They also looked at FLG which showed moderate dCt values in saliva, semen and vaginal fluid samples but had significantly higher expression levels in skin and sweat samples. The detection of DSC1 was the lowest of all the markers in skin samples but the DSC1-positive skin samples showed significantly higher expression levels in skin samples than in the blood and semen samples with good separation between skin and other body fluids. DCD was the only marker than was only detected in skin and sweat samples with higher expression in sweat than skin samples. This demonstrates that there is often crossover with these skin markers and other body fluids but the difference in

expression levels could be used to indicate if the detection of these markers are likely to originate from skin or other fluids. However, as these markers are detected in skin swabs as well as other fluids, it is not clear whether these other fluids are present within touch DNA deposits so further investigation is needed to determine if these markers are simply not specific or if these fluids are present within the touch DNA deposits.

Further to this, Akutsu et al. (2018) completed mRNA analysis on mock casework samples to assess the expression of these markers on simulated touch DNA deposits. They found that the housekeeping gene ACTB was detected in all samples bar one of the samples collected from a doorknob. Of the markers they selected they found that CDSN and LCE1C were expressed from the majority of the mock casework samples. FLG was the third most detected gene in these samples and DCD was detected in 50% of the swabbed samples with the highest dCt values but was not present in the samples collected from the collar of a shirt via a tape lifting method. DSC1 showed the lowest detectability in these samples which suggests that this is not an appropriate marker as there was low expression in simulated touch DNA deposits as well as skin swabs themselves. They did note that the dCt values for the mock casework samples were almost within the range of the skin swabs and sweat samples analysed previously with some samples exceeding these levels which suggests that this methodology is appropriate for use of touch DNA deposits.

Despite the promising results demonstrated by Akutsu et al. (2018) in relation to mock casework samples, they were only able to detect human genomic DNA in 2 of the 14 samples they analysed when quantifying the DNA present which may suggest that while these markers are present the DNA itself is not of a high quantity. They did not continue their analysis to look at the DNA profiles obtained from these samples so it is not possible to assess if these samples would generate a high-quality profile meaning it is unclear what contribution these genes have upon the overall profile from a touch DNA deposit.

Lacerenza et al. (2016) conducted a study where they aimed to assess the presence of skin and other body fluids present upon the palmar surface of the hands and fingers in an attempt to suggest what touch DNA deposits are comprised of. As part of this research, they collected DNA from the palmar surface of the hands and fingers using swabs and tape lifts and then subjected these to mRNA and DNA analysis. It was identified that 55% of the samples they analysed contained the markers for only skin or skin and mucosa followed by 15% of the samples containing markers for other body fluids as well as skin. When looking at the DNA yields it was found that a greater yield was present in samples which contained other tissues and fluids than samples which only contained skin and mucosa which may suggest why some touch DNA samples generate a better quality profile than others. This gives a strong indication that touch DNA deposits are comprised of skin related epithelial cells and general mucosa and that occasionally other body fluids may be present through secondary transfer, which they proposed could be identified by the presence of both skin and other body fluids rather than other body fluids alone such as in cases of primary transfer. However, as these samples were collected directly from the palmar surface of the hands rather than from a touched object or surface this data may not be representative of a touch DNA deposit as there is additional friction being applied during the collection process that is not present when this DNA is deposited naturally. Therefore, this needs to be further investigated to determine if these results are comparable when analysing an evidential touch DNA deposit.

Lindebergh et al. (2012) suggested that epigenetic processes such as micro-RNA analysis are promising for the future analysis of these low-level DNA samples but that at that time there was limited information available to adopt this approach. However, the use of both micro-RNA (miRNA) markers alongside messenger RNA (mRNA) markers could produce a more robust analysis method for the identification of touch DNA samples and may allow the identification of the genetic components of touch DNA deposits. The characteristics of miRNAs enable them to be well suited to the analysis of forensic samples such as touch

DNA as they are less prone to degradation due to their small size of 18 – 25nt (Kulstein et al. 2016; Sauer et al. 2017). Additionally, there is a reduced amount of sample loss as miRNA and DNA can be co-extracted from the same specimen (Li et al. 2014). It has been established in previous research by Courts and Madea (2011) and Sauer, Madea and Courts (2014) that the analysis of miRNAs is applicable to forensically relevant body fluids but they did not determine the validity of this approach in lower quantity samples such as touch DNA evidence.

In a study conducted by Sauer et al. (2017) they identified two miRNA markers for skin epithelial cells which displayed good separation properties with no overlapping with other organ tissues. However, these have also been identified as markers for saliva suggesting that these may not be specific to skin related epithelial cells but to epithelial cells in general including those obtained from a buccal scrape. This could pose potential complications when using these markers to identify the origins of touch DNA as any touch DNA deposits could also include saliva such as from the action of an individual wiping their face/mouth as discussed by Stanciu et al. (2015).

Sirker et al. (2017) attempted to identify 3 miRNA markers for skin which were specific and sensitive. They assessed the markers miR139, miR494 and miR3169 however, they found that miR139 and miR494 were not specific to skin and could not be used to separate this tissue type from other body fluids. Further to this, miR3169 had high expression levels in skin samples but also in saliva and moderate expression levels in menstrual blood and vaginal secretions. This suggests that these markers are being expressed in epithelial cells in general and not those skin specific cells which was also found in the study conducted by Sauer et al. (2017) when looking at the hsa-miR-203a-3p and hsa-miR-205-5p markers. However, Sirker et al. (2017) did comment that it was possible to separate the skin samples from the other body fluids by using specific markers for that body fluid. For instance, the marker miR451 is expressed in saliva but not in skin so when using this in conjunction with miR3169 it would be possible to identify if the sample included saliva. However, this marker

is also expressed in menstrual blood so an additional specific marker would be required to rule out the evidence originating from this body fluid.

Wang et al. (2017) investigated the nanoscale characteristics of forensically relevant epithelial cells and found that you could differentiate between the surfaces of cells from the skin and cells not exposed to the environment such as buccal cells based upon their visual characteristics as cells exposed directly to the environment are fully keratinized whereas those not exposed to the environment are either only partially keratinized or not keratinized at all. This suggests that while miRNA analysis may not be able to differentiate between skin related epithelial cells and buccal epithelial cells (Sauer et al. 2017), it is possible to do so using Atomic Force Microscopy (AFM) to visualize their nanoscale characteristics. Additionally, Wang et al. (2017) found that the cell morphology did not change even when exposed to the air for 2 to 3 weeks which indicates that these cells from the palm of the hand do not display visual degradation in this time. This suggests that it would be possible to visually identify these cells even after being exposed to the elements for a prolonged period of time although further research into this would be required to determine the limits of this.

There is a common assumption that touch DNA deposits contain shed epithelial cells which are comprised of corneocytes that are thought to lack DNA due to their lack of nuclei (Burrill et al. 2019). However, a later study by Burrill et al. (2021) looked to assess the DNA contribution of these corneocytes to touch DNA samples and investigate methodologies to maximise the amount of DNA extracted from these samples. When assessing different lysis methods for corneocytes it was determined that some traditional lysis buffers are not as effective as those that contain a reducing agent as a reduced DNA yield was obtained which suggests that this DNA may be being missed when utilising standard lysis protocols. Furthermore, when the DNA quantity was assessed using quantitative PCR this demonstrated an increase in DNA post-lysis of the corneocytes. However, when these samples were profiled the higher quantity did not necessarily reflect in the completeness of the profile which is likely due to this DNA being fragmented and degraded meaning this is

not being detected in the larger amplicons. This suggests that a large proportion of the DNA present in touch DNA samples is not being amplified successfully with current profiling methods and therefore, quantitative PCR may not be providing the quantity of the profilable DNA present within these samples.

In addition to assessing the DNA yield using quantification Burrill et al. (2021) also assessed the microscopic features of the corneocytes and observed that these cells adopt the appearance of anucleate cells however, the nucleic acid dyes still bound to some of the cellular contents suggesting there is a high DNA content in these cells despite the lack of nuclei which has been previously reported to suggest this is not the source of DNA within these samples (Stanciu et al. 2015).

Despite some substantial discoveries being made in the study by Burrill et al. (2021), it is important to note that this did not utilise mock evidential touch DNA deposits but instead collected rinses from the surface of the hands following hand washing to remove any cell free DNA meaning further investigation is required to determine if these results would be comparable in a traditional touch DNA deposits and also to determine the contribution that cell free DNA has to the overall DNA yield and profiles from these samples.

While Burrill et al. (2021) did not assess the impact of cell-free DNA (cfDNA) or extracellular DNA (eDNA) in touch DNA deposits, Wang et al. (2017) was able to detect eDNA in both buccal and palm cells. They were able to map the eDNA levels in order to quantitatively analyse the presence of these in both cell types by looking at the number of binding events between the lactoferrin AFM probe and eDNA. It was found that there was a higher quantity of eDNA in buccal cells than in palm cells which may indicate that this eDNA is a contributor to the profilable DNA within these DNA samples. However, the average DNA content did decrease in both cell types following 3 washes which suggests that a large amount of the DNA in touch DNA deposits may be collected onto the palm cells from other sources and not from the palms themselves which supports the theory by Zoppis et al. (2014) who proposed

that the DNA on the hands is secondary transfer from other areas of the body. Although this research by Wang et al. (2017) demonstrates that eDNA is present in both types of epithelial cells, they do not investigate whether this source of DNA is able to generate a DNA profile so it is not clear what contribution this has to the overall DNA profile obtained from touch DNA deposits.

Additionally, it was discussed by Quinones and Daniel (2012) that cfDNA can be found in a range of biological fluids including sweat with an average quantity of 11.5ng/mL and both partial and complete profiles were obtained from these samples indicating that this DNA is of sufficient quality to contribute to the DNA profile. This is also support by Stanciu et al. (2015) who reported that this cfDNA may contribute more to the DNA quantity than cellular DNA. In an additional study by Burrill et al. (2021a) they stated that further research is required to determine the relevance and contribution of cfDNA to forensic touch DNA evidence. In their study they did determine that the amount of cfDNA was significantly higher in hand rinses from unwashed hands when compared to washed hands which gives a strong indication that this cfDNA may be accumulated on the hands from secondary transfer, however, it is not clear whether this is secondary transfer from the donor or a third party.

A later study by Burrill et al. (2021b) aimed to assess where this DNA is accumulated from by separating the DNA built up from the hands themselves to that which accumulates from secondary transfer through the use of sterile gloves. They found that samples taken from the inside of the gloves had a higher cell count than the samples taken from the external surface of the gloves but both sets of cells were almost exclusively corneocytes with very few nucleated cells being present across all donors which gives a strong indication that the DNA within a touch DNA deposit does not originate from nucleated cells. However, the DNA levels were much higher from the external surface of the gloves than those obtained from the rinses collected from inside of the gloves and on the hands and this was found in the overall samples as well as the separated pellet DNA and cfDNA which suggests that more DNA is accumulated exogenously through activity than endogenously. They also noted that

the endogenous DNA comprised of higher levels of pellet DNA than the exogenous DNA which had higher levels of cfDNA which supports the hypothesis that cfDNA is accumulated via secondary transfer either from other areas of the body or from external sources. These endogenous samples had limited exposure to the environment yet still displayed profile quality indicative of degraded DNA which demonstrated that this degradation is likely to originate from DNA fragmentation rather than through external factors. Additionally, more complete profiles were obtained from the pellet DNA than the cfDNA in both the endogenous and exogenous samples which indicates that this has a higher contribution to the overall profile although cfDNA can contribute additional alleles not detected in the pellet DNA as reported by Burrill et al. (2021a). It was noted that 56% of the profiles obtained from the external surface of the gloves were mixtures whereas there were only 25% of profiles with sporadic non-donor alleles from the samples from the inside of the gloves which suggests that the high cfDNA levels in exogenous samples is a result of secondary transfer from other individuals rather than other areas of the donor body.

Burrill et al. (2021b) also assessed the degradation levels of the DNA obtained from exogenous and endogenous samples using a Degradation Index (DI). They found that samples from the outer surface of the gloves were significantly less degraded than samples from the insides of the gloves suggesting that the intact DNA contributions to touch DNA samples are more likely obtained from exogenous sources than due to them being naturally occurring on the surface of the hands.

1.2.2. Transfer of Touch DNA

DNA is deposited onto a surface through the transfer of genetic material from an individual to a surface. Such transfer is in accordance with Locard's Principle of Exchange, whereby every contact leaves a trace (Byard *et al*. 2016).

When DNA is found upon a surface, this can be deposited either through direct or indirect transfer. Indirect transfer of DNA occurs when an individual's DNA is transferred to a surface through an intermediary such as via a second person. This is often referred to as secondary transfer, but there can be multiple intermediaries transferring this DNA (Meakin and Jamieson, 2013). Such indirect transfer can cause difficulties to arise when investigating how DNA came to be present on a surface which is discussed by van Oorschot *et al.* (2019) as the number of indirect transfers of the DNA is often unknown and there are a vast number of possibilities that need to be considered. To demonstrate some of the possibilities of transfer, van Oorschot *et al.* (2019) used the below figure (figure 2) visually represents the wide range of scenarios through which DNA can be transferred all of which need to be considered when interpreting this evidence.

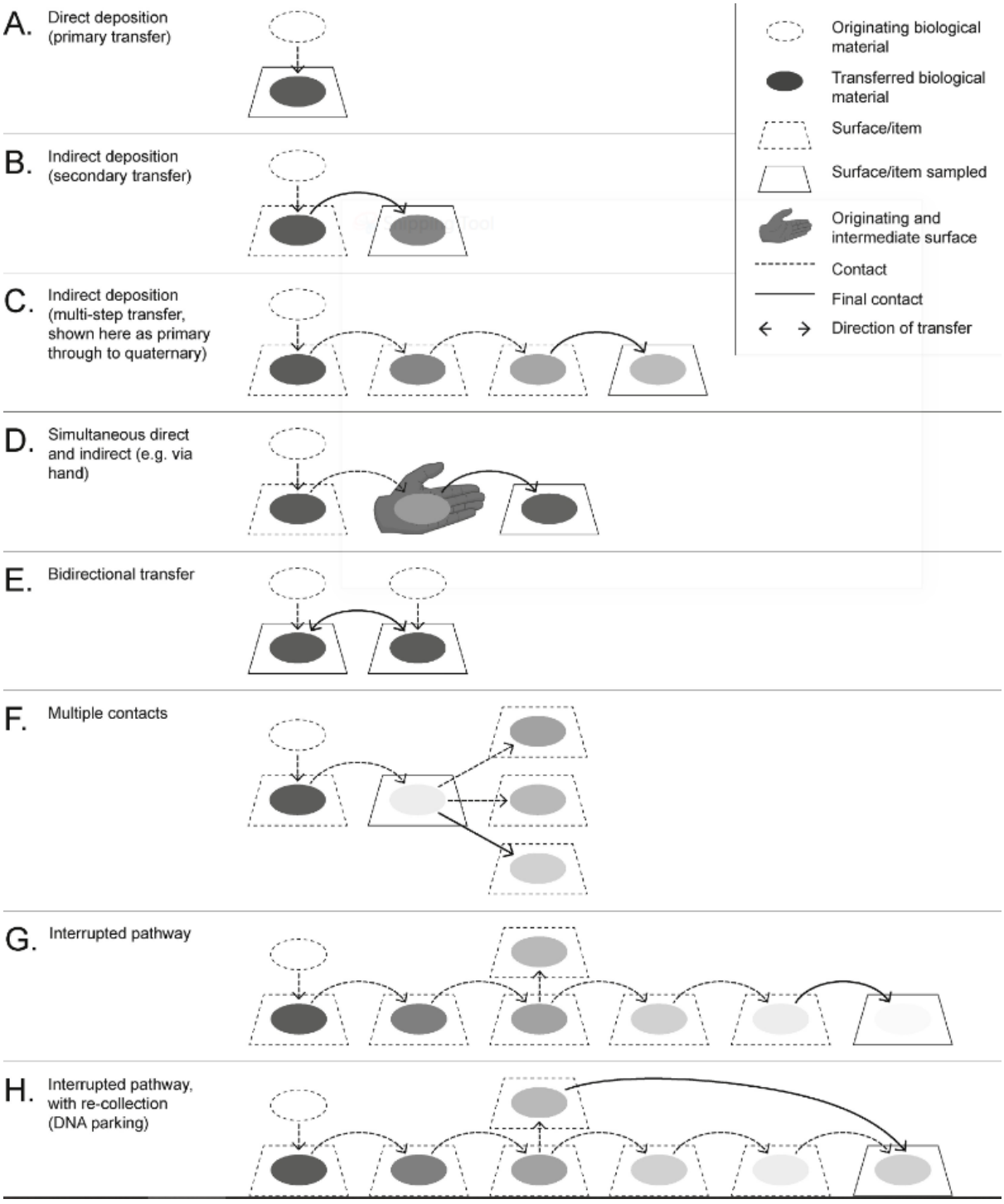


Figure 2. Infographic from van Oorschot et al. (2019) demonstrating the various modes of DNA transfer.

The first report of such indirect transfer was published by van Oorschot and Jones (1997) who indicated the need to exercise caution when interpreting profiles from touched items

due to the presence of non-donor alleles on items due to indirect transfer. Further to this, van Oorschot and Jones (1997) found that when items were handled by more than one donor, the DNA of the first donor to handle an item was found on the hands of the second donor after handling the same item, which demonstrates how indirect transfer can pose interpretational difficulties for such evidence types. Since this report, DNA analysis techniques have become more sensitive with lower quantities of DNA being detected which increases the need for caution when interpreting touch DNA samples (van Oorschot *et al.* 2019).

It was found by Port *et al*. (2005) that full DNA profiles could be found on surfaces when in the vicinity of a static speaking individual. This was found up to 115cm from the item when the individual is in the standing position. Full profiles were also generated from individuals in the kneeling and sitting positions when they are approximately half a metre away from the surface. Such results were also found by Finnebraaten *et al*. (2008) in relation to contamination within the laboratory environment when an individual was speaking without protection. However, in contrast to Port *et al*. (2005), they found that only standing individuals produced a full DNA profile. It was hypothesised that this difference may arise due to the height of the individuals, as the tallest individuals in the study conducted by Finnebraaten *et al*. (2008) produced the complete profiles. However, more published data is needed to fully assess the impact that height has upon the prevalence of DNA deposited via indirect transfer when speaking.

Goray *et al*. (2016) found that secondary transfer can occur under a number of circumstances with some donor profiles being completely overridden by the secondary profile. It is also discussed that this transfer can be inadvertent in nature and be from indirect transfer from an object rather than from an individual as also discussed by van Oorschot and Jones (1997). This poses potential interpretation difficulties when analysing touch DNA samples from an unknown donor as the profile may have been inadvertently transferred without any direct contact. However, it is of note that the items used in the study by Goray *et*

al. (2016) were precleaned so their findings may not be applicable when looking at items that are regularly used by an individual. In contrast to this, Meakin *et al*. (2017) looked at regularly used items and found that the regular user of the object such as a knife persisted longer, and was more prominent, than that of indirectly transferred DNA. Additionally, Meakin *et al.* (2017) found that the indirect transfer of DNA was minor within the profiles with the regular user being most prominent which poses complications for interpretation of such samples when looking at the considerations for activity level reporting (van Oorschot *et al.* 2019).

While the possibility of such indirect transfer has been discussed in published research, it is also important to assess how probable it is that this will occur at a crime scene and how this will impact the profiles produced (van Oorschot *et al.* 2019). One way that this has been assessed is in relation to the amount of DNA recovered from a surface. Meakin and Jamieson (2013) reviewed the available published data at the time and found that the amount of DNA recovered from an item which has been touched once varied substantially between 0-150ng. In addition to this, it was observed that items that have been regularly used also recovered DNA in the range of 0-75ng which indicates it is not possible to distinguish between a single contact and regular use based solely on the quantity of DNA present. In addition to this, Oldoni, Castella and Hall (2016) found that the quantity of DNA deposited by different pairs of individuals varied significantly from 0.90ng to 86.41ng. This, in conjunction with Meakin and Jamieson (2013) suggests that, due to the variability in individuals propensity to deposit DNA, it is not possible to determine whether an item has been handled by single or multiple donors solely based on the quantity of DNA recovered which means that further detail is needed when analysing these samples. Further to this, Oldoni, Castella and Hall (2016) found that whether a regular user or single secondary user was the major contributor to a profile varied depending upon the surface this was recovered from which suggests that we cannot always determine the regular user from the secondary user based on their contribution level to the DNA profile as the length of time the secondary

user handled the item can influence whether they are a major contributor in the profile. Additionally, Oldoni, Castella and Hall (2016) found that in some cases, unknown DNA deposited via indirect transfer exceeded the contributions of the regular and second user. This is in concordance with the complications discussed by van Oorschot *et al.* (2019) which should be considered when interpreting such 'touch' DNA evidence.

Rolo *et al.* (2019) found when assessing the shedder status of individuals that indirect transfer occurred in up to 57.14% of the samples with non-self DNA being deposited onto the items even when washing their hands 15 minutes before the sample deposition. The amount of non-self DNA detected varied depending upon the surface and also was found to be higher in samples where a handprint was deposited rather than a thumbprint which is to be expected. This further demonstrates the propensity for individuals to have their DNA transferred to a surface without direct contact occurring as even with a short time since handwashing this was still prevalent in the samples. Champion, van Oorschot and Linacre (2019) also found that the propensity for secondary DNA transfer varied depending upon the surface with DNA readily transferring from aluminium to cotton, polyester and aluminium but transferring in lower quantities from aluminium to plastic.

Further to this, Rolo *et al.* (2019) assessed the amount of background DNA present on samples which are regularly used by a single donor and found that non-self DNA was present in 61.11% of the samples analysed which shows that this is commonly present on regularly used items and may be being accrued by the regular user indirectly transferring the non-self DNA to the items although Rolo *et al.* (2019) did discuss there was potential for these items to have been used by another individual which would also contribute to this non-self DNA being present. Aside from the amount of non-self DNA present on regularly used items, Rolo *et al.* (2019) found that these regularly used items produced less partial profiles indicating that regularly used items are more likely to generate a full DNA profile although caution is needed to assess the presence of indirect transfer. However, one factor that may help with this interpretation process is the mechanism of the transfer, if known, as

Champion, van Oorschot and Linacre (2019) found that secondary transfer occurred in the greatest quantities when friction and pressure were present in the transfer rather than when friction or pressure alone were present in the transfer mechanism.

While a range of research has been conducted into the transfer of DNA via different mechanisms, there has not been a definitive interpretational approach published which allows the differentiation between direct and indirect transfer, as discussed by Gosch and Courts (2019) in their review of the research published in this area, as there are many differing approaches and outcomes with a lot of the data varying between studies.

Another factor influencing the transfer of DNA is the amount of DNA deposited which varies between individuals and can be influenced by many factors (Gosch and Courts, 2019). Research has been conducted, with varying results, into the ability to categorise an individual's shedder status along with the factors that influence this. Research conducted by Lowe *et al*. (2002) suggested that it was possible to categorise an individual as a 'good' or 'bad' shedder. In contrast, Phipps and Petricevic (2007) found that categorising an individual's shedder status is much more complex than simply if they produced a full profile. Both papers consider environmental factors such as the influence of hand washing and the dominant vs non-dominant hand being used. There was much variation within the profiles produced under all circumstances with no single factor conclusively linked to poor or good shedding, suggesting that it was not possible to define an individual's shedder status in relation to DNA deposition. Further to this, Rolo *et al.* (2019) attempted to assign shedder status to the participants used in their study by using whether a complete profile was obtained regularly regardless of contact area and substrate however, they were only able to assign a good shedder in 1 out of 10 participants with most of these being classified as an intermediate shedder as the amount of DNA deposited and therefore producing a profile varied considerably. Additionally, Kanokwongnuwut *et al.* (2018) were able to categorise individuals used in their study as heavy, intermediate or light shedders in repeat studies. However, they did find that when looking at the profiles produced from each of these

categories, there was a clear distinction between light and intermediate shedders but not between intermediate and heavy shedders which they suggested was more continual. This supports the statements previously made by Phipps and Petricevic (2007) that categorising an individual's shedder status may be more complex.

Despite the difficulties and contrasting results when looking at the categorisation of an individual's propensity to shed skin cells, Goray *et al*. (2016) suggest that this determination is still extremely useful in criminal investigations. This is also supported by Lim *et al*. (2016), who states that this is one of the many factors that impacts the quantity and quality of DNA collected from handled items.

Burrill *et al.* (2019) summarised that from the published data, there is no clear biological reason for the differences observed in shedder status yet this can impact how much DNA is transferred which was also discussed by Gosch and Courts (2019). Burrill *et al.* (2019) further suggests that, having a greater understanding of what is included in a touch DNA deposit may aid in understanding what influences an individual's shedder status. Additionally, consideration should be given to other factors influencing DNA transfer such as those discussed by Gosch and Courts (2019) and investigated by Kanokwongnuwut *et al.* (2018) including, but not limited to, the time since hand washing, the manner of contact, surface type and duration of contact.

1.2.3. Touch DNA Analysis

Research conducted by Farash, Hanson and Ballantyne (2017) suggested that the method used to analyse such low-level DNA has an impact upon the quality of the resulting profile with enhanced methods being required for samples that have quantities below 0.1ng. This is further discussed by Dong *et al*. (2017) along with the influence that the processing, recovery and storage has upon the results produced from touch DNA samples.

The sensitivity of the laboratory processes could have been a factor that could cause variation, due to the difference in extraction processes between the research conducted by Lowe *et al*. (2002) and Phipps and Petricevic (2007). However, similar variations have been found in later research such as that conducted by Kanokwongnuwut *et al.* (2018) and Rolo *et al.* (2019) which demonstrates that these variations are still present when using the more sensitive processes available.

1.2.4. The Impact for Forensic Investigations

The number of cases where low-level DNA has the potential to be evidentially valuable, and therefore is being analysed, has greatly increased (Dong *et al*. 2017). In particular, 'touch' or 'contact' DNA from individuals and surfaces has become one of the most analysed sample types in forensic cases (Oldoni, Castella and Hall, 2016). If this material is deposited in sufficient quantity on an item or surface at a crime scene, there is potential for this to be recovered and profiled (Aditya, *et al*. 2011).

Burrill *et al.* (2019) suggest that knowing the origins of touch DNA evidence can inform on the best practice for the recovery and analysis of this evidence type. With this evidence type typically producing reduced quantities of DNA, it is vital that the recovery and analysis of this evidence is optimal to increase the success rates of this evidence (Hess and Haas, 2017). Therefore, an increased understanding of the genetic components within touch DNA deposits will inform investigative and analytical approaches (Quinines and Daniel, 2012).

1.3. A Review of Different Methods of DNA Recovery

1.3.1. The Impact Recovery Method has on the Success Rates of Touch DNA Evidence

The effectiveness of DNA recovery methods has been the topic of discussion for many years with conflicting results being published and no consensus around the most effective method being gained. However, despite these conflicting results, it is vital that the correct recovery method is used to maximise the amount of DNA recovered from a surface (Mulligan *et al.* 2011; Hess and Haas, 2017; Aditya *et al*. 2011; May and Thomson, 2009). To date, a large proportion of this research has been focussed upon higher quantity samples such as blood, semen and saliva, yet as discussed by Kirgiz and Calloway (2017) the recovery methods that are effective for these samples may not be as effective when recovering lower quantities of DNA such as touch DNA. Verdon *et al.* (2014) also found that recovery methods that were deemed as successful with higher quantity samples were not as effective at recovering trace amounts of DNA which supports the need for further research in this area.

Mulligan *et al*. (2011) state that while many factors of LCN (Low Copy Number) DNA recovery have been investigated, the importance of the swabbing material has not been extensively reported suggesting that further work is needed in this area in order to optimise the recovery of touch DNA. Furthermore, Hansson *et al*. (2009) suggested that due to the success of touch DNA recovery being influenced by multiple factors, it is important to continually assess available methods to ensure the optimal method is being used. Additionally, Verdon *et al*. (2014) state that a comprehensive evaluation of which recovery methods are most efficient for trace DNA collection is required.

Mulligan *et al*. (2011) also suggested that the inefficient recovery of low-level DNA can exacerbate the issues with the profiling of these samples with more allelic dropout, stochastic fluctuation and insufficient profile data being present. Verdon *et al*. (2014) also state that if the most efficient swabbing material is not utilised, in relation to both the surface

type and sample type, then the vital biological evidence may not be sufficiently retrieved indicating the importance of comparing a wide range of recovery methods. This further supports the need to adopt an optimal recovery strategy to ensure that the potential of gaining a useful profile of high quality is maximised which will aid in increasing the success rates of this evidence type.

1.3.2. Swabbing Materials

There are many factors that contribute towards the effectiveness of a swabbing material including its chemical and physical characteristics. In order for a swabbing material to be efficient, it must possess the capacity to both absorb and release any genetic material well. It was suggested by Marshall *et al*. (2014) that swabbing materials that are proficient at collecting cellular material often do not release this material well, and that swabbing materials which release any collected DNA well do not absorb the DNA as well.

Mulligan *et al*. (2011) suggested that factors such as whether the material is natural, semi-synthetic or completely synthetic can affect the efficiency of the swab for recovering touch DNA. In addition to this, Mulligan *et al.* (2011) suggested that the structure of the swab, such as whether the material is woven, non-woven or knit in construction around the shaft of the swab, may influence the recovery efficiency of the swab and may impact on the material's absorbent qualities. Verdon *et al*. (2014) also suggested that factors such as the swabbing material, thickness and structure may influence the efficiency of the recovery method along with the design of the swab and the shaft material. However, they also argue that the swab tip is the most important factor to consider as this is the area onto which any genetic material is collected.

Brownlow *et al.* (2012) reported that routinely, in Police Services such as the Metropolitan Police Services (MPS), sterile cotton swabs, which have a mattress design, are used for the recovery of genetic material at crime scenes. This was also stated in the 2015 DNA

Recovery Validation Report by Staffordshire Police (Miller and Beckwith, 2015) that they routinely use cotton swabs for DNA evidence recovery. These swabs have a structure where the cotton is tightly wound around the shaft of the swab however, this may not be an effective recovery method as this structure could hinder the collection and release of the DNA (Brownlow *et al*. 2012). Bonsu, Higgins and Austin (2020) also reported that the use of cotton swabs can be problematic due to the biomaterial becoming trapped within the cotton matrix of the swab which results in reduced efficiency of these swab types.

While Brownlow *et al.* (2012) hypothesised that these factors may influence the effectiveness of the swab material, no statistical differences were found between swab materials or woven and non-woven fabrics. However, in their study Brownlow *et al.* (2012) found that knit fabrics recovered significantly lower quantities of DNA which does suggest that this may impact upon the recovery of touch DNA but the extent of this is unknown. This is further supported by Verdon *et al*. (2014) who also suggested that the way in which the material is wound around the shaft may influence the efficiency of the swab as cotton swabs that were wound performed better than those that were layered. When looking at a flocked design such as the nylon flocked swab, Verdon *et al.* (2014) suggested the structure allowed for rapid absorption and the release of DNA from the swab was maximised. However, Brownlow *et al*. (2012) found that the nylon flocked swabs did not have an increased potential to collect and release DNA when compared to the cotton swab, which suggests that this structure does not facilitate increased DNA recovery. This suggests that more than just the material itself should be considered and that the optimal structure has not yet been determined.

The thread count of the fabrics was also assessed by Mulligan *et al*. (2011) to determine the impact that this has upon the recovery efficiency of a swabbing material. They found that fabrics such as cotton that were woven with a low thread count recovered more cellular material from glass surfaces when water was used as a moistening agent. It was suggested that this was due to the space available between threads allowing cellular material to

penetrate further into the swab material which may also allow for more efficient release of this DNA as this will not become trapped within the swab interior. This factor should be further analysed in relation to other swab materials to determine if this is also applicable.

Brownlow *et al*. (2012) found that cotton swabs produced a higher quality profile than nylon flocked swabs, therefore suggesting that cotton swabs are more effective at recovering evidential DNA when recovering saliva samples and diluted saliva samples. They suggested that the reason for this may be due to the nature of the samples being in a dried form which they reported was common at crime scenes rather than being an abundant moist sample as has been used in other fields such as medicine and microbiology where good success rates were achieved with the nylon flocked swabs. This is also supported by Hansson *et al*. (2009) who found that cotton swabs generated more full profiles than nylon flocked swabs. They also found that nylon flocked swabs generated the lowest yield of DNA. This suggests that nylon flocked swabs may not be suitable for forensic samples.

In contrast to this, Benschop *et al*. (2010) found that nylon flocked swabs performed better than cotton swabs with a higher yield of male DNA being obtained. However, these were from vaginal samples so the reason for the difference may be due to the nature of the sample collected as this has been suggested to directly impact the optimal recovery method (Kirgiz and Calloway, 2017). Additionally, as vaginal samples are wet samples rather than dried out, this may also be a factor in the success rates of evidence collection using nylon flocked swabs as discussed by Brownlow *et al*. (2012). This suggests that further research is required to determine the optimal recovery method based upon the sample type. Pamela *et al*. (2015) found that nylon flocked swabs produced a partial profile for 76.3 % of samples tested, whereas cotton swabs only produce this in 55% of the samples analysed. However, they also found that cotton swabs produced more complete profiles than nylon flocked swabs, with 32.5% of the samples analysed being a full genetic profile. This suggests that overall, more full profiles are gained using cotton swabs which may suggest that this is a more useful swabbing material.

Marshall *et al*. (2014) compared nylon flocked swabs to a novel swabbing material known as the X-Swab which has a dissolvable material, which holds the potential to increase DNA yield during the extraction process as the material itself dissolves reducing the loss of DNA during this process. However, when this was reviewed, it was found that there was no significant difference between the materials in regards to the yield of DNA from low-level samples. Despite the difference not being significant, it was noted that with samples of 5ng of input DNA the X-Swab recovered twice as much DNA than the nylon flocked swab suggesting that there is potential for this material to be effective as a recovery method.

Seah *et al*. (2004) reported that DNA recovered from materials such as cotton, nylon and rayon produced higher quality profiles than those recovered from polyester and acrylic. When assessing the reasons for this, they found that fabrics such as cotton, nylon and rayon have a high capability to produce strong hydrogen bonds with the DNA present due to having strong dipole-dipole interactions which facilitate this. However, polyester and acrylic fabrics have an abundance of polar carbonyl and cyano groups which produce weaker dipole-dipole bonding. This suggests that the chemical properties of the swab materials may directly impact their ability to collect and release DNA effectively.

Comte *et al.* (2019) conducted a study comparing the efficiency of four different swabs to determine if there were differences in the recovery efficiency in relation to the ability to release the DNA collected from three different surfaces. They found that nylon flocked swabs released a higher quantity of DNA from samples collected from clothing and steering wheels than two varieties of cotton swabs and a viscose swab. However, there was no difference between these recovery methods when looking at samples collected from a screwdriver. Despite a difference being observed in this study, the data was obtained purely from quantification data which while this provides a strong indicator for the success of a profile it is not definitive as discussed by Haas *et al.* (2015). Therefore, this data is not necessarily indicative of whether this swab type would generate a good quality profile from these samples. Additionally, Haase *et al.* (2019) found that cotton swabs were more effective

at touch DNA recovery than nylon flocked swabs in relation to samples collected from gun shell casings and glass slides which contrasts with the findings of Comte *et al.* (2019). However, this study again, did not include data regarding the success of the DNA profiles generated from these samples so it is unclear whether a difference in the overall success of this evidence was observed.

When looking at the recovery of touch DNA, Verdon *et al*. (2014) found that the swabs which performed well for the recovery of other biological samples were not as effective for the recovery of touch DNA. For instance, when looking at the recovery of saliva samples, both neat and dilute, the foam swab was ranked the highest of the swabs utilised, but when looking at touch samples, this swab was ranked seventh. This suggests that more research needs to be conducted to determine the optimal recovery method per sample type. Verdon *et al*. (2014) also suggested that a selection of swabs should be adopted based upon the surface type and sample type rather than just a single swab to be used for all samples. Overall, they found that the Puritan FABSwab which had a cotton tip was most efficient but when looking at differing surface types, they found that the cotton, foam, rayon and polyester swabbing materials outperformed the nylon flocked swabbing materials on all surfaces. Bonsu, Higgins and Austin (2020) discuss how the interactions between metal surfaces and the deposited DNA has not been extensively researched and that the formation of ionic bonds between the surface and the deposited DNA may impede the release of this DNA resulting in poor DNA profiling outcomes. Comte *et al.* (2019) also found that nylon flocked swabs produced a higher quantity of DNA from porous surfaces such as clothing whereas there were no significant differences found between the quantities of DNA produced from nylon flocked, cotton and viscose swabs taken from screwdrivers which suggests that an optimal recovery strategy is required in order to obtain the maximum amount of genetic material from different objects found at a crime scene. This is further supported by Hedman *et al.* (2021) who found that foam swabs generated higher yields from absorbent wooden surfaces than cotton and nylon flocked swabs, albeit in relation to saliva samples, which

further supports the need to investigate the optimal recovery method for differing surface types. However, when looking at touch DNA collected from the skin, Kallupurackal *et al.* (2021) reviewed cotton and nylon flocked swabs along with different processes for recovering this DNA such as the double swabbing method versus a single wet swab and found that there was no statistically significant difference found between any of the methods trialled in terms of the resulting STR profiles produced which suggests that further research may be needed to determine the optimal approach for the collection of touch DNA from skin samples.

1.3.3. Recovery Techniques

In addition to the swabbing material used, the technique utilised for collection DNA from a surface may also impact the success rate of this evidence type. In a study conducted by de Bruin *et al*. (2012), they stated that only a minimal amount of pressure was used when recovering DNA from the skin using a swab which may reduce the amount of DNA being recovered. This suggests that when recovering DNA from a surface using a swabbing mechanism, it is important to adopt a good level of pressure and force to ensure that there is a high level of interaction between the swabbing material and the surface increasing the potential for evidence recovery which is supported by Champion, van Oorschot and Linacre (2019) who found that using pressure and friction resulted in a greater amount of cellular material being transferred between substrates. Additionally, the design of the swab may impact upon the ability to recover DNA as this may influence the level of pressure that can be applied. Hansson *et al*. (2009) found that when using the nylon flocked swabs, the flexible nature of the shaft made sampling difficult. Due to the flexible nature, less pressure is able to be applied during the recovery process which then reduces the amount of DNA recovered from a surface. This was also found by Brownlow *et al*. (2012) who stated that when recovering low-level DNA, a firm pressure was applied using the cotton swabs but this was

made difficult when using the nylon flocked swab due to the plastic shaft being flexible in nature. This suggests that the method used to recover DNA from a surface impacts upon the success rates of this evidence type, suggesting that it is important to ensure the swab design allows for a good level of pressure to be applied alongside using an appropriate material. Verdon *et al*. (2014) also discussed the design of the swab in relation to the method of sampling and stated that the flexible nature of the foam matrix allowed for greater penetration into the substrate pores. This suggests that the design of the swab can influence the recovery potential based upon how this interacts with the surface the DNA is being recovered from.

Additionally, de Bruin *et al*. (2012) suggested that the double swabbing method performs better at collecting epithelial material than methods such as stubbing or tape lifting. This method involves applying a single moistened swab over the area followed by a single dry swab. This allows for the loosening of any dried genetic material through the moistened swab which can then be secured on the subsequent dry swab. However, the moistening agent utilised in the double swabbing method may also affect the recovery of low-level DNA (May and Thomson, 2009). Routinely a cotton swab is used with distilled water as the moistening agent (Templeton *et al*. 2013). Despite a cotton swab moistened with distilled water being the routinely used collection method for touch DNA further methods may be more effective.

In a study conducted by Lenz *et al*. (2006) it was suggested that the swabbing technique influences the success rates of touch DNA from common crime scenes such as cars. They also found that when looking at the moistening agent that this did not significantly impact upon the recovery of this evidence type. However, a study conducted by May and Thomson (2009) found that a swab with xylene solvent was most effective when recovering DNA from a large area and that this method of recovery should be applied to recover DNA from a tape lift. It was found that the xylene solvent in conjunction with the chelex extraction method effectively concentrated the DNA onto the swab and dissolved the adhesive present

suggesting that such a method would be effective when recovering DNA from a surface with an adhesive nature. Furthermore, this suggests that the solvent or moistening agent used can have an impact upon the optimal recovery of touch DNA.

Mulligan *et al*. (2011) hypothesised that polar solvents would facilitate the bonding of the cell to the fabric of the swab by forming hydrogen bonds with carbohydrates present on the membranes of epithelial cells, and that dipole-dipole interactions are likely to play a significant role in the collection and release of cellular material. However, they found that this was not the case and suggested this may be due to the solvent bonding to the swab reducing the potential for these bonds to be made with the cellular material. They also found that, when using water as the moistening agent a higher quantity of DNA was recovered than when using isopropanol and it was suggested that this was due to the difference in hydroxyl groups present for bonding. In contrast to this, Phetpeng *et al*. (2013) found that a higher yield of DNA was obtained for touch DNA when using an EO swab with isopropanol as the moistening agent. Furthermore, Thomasma and Foran (2013) found that moistening agents which included a detergent significantly increased the DNA yields recovered when using a cotton swab. They also found that detergents such as SDS and Triton x-100 produced the best results however, this was only in relation to fingerprints recovered from glass slides with cotton swabs, so this may not be applicable to other surface types or swabbing materials. While Phetpeng *et al*. (2013) and Thomasma and Foran (2013) found significant differences within the DNA yields produced from different swab and moistening agent combinations and differing moistening agents respectively, further factors such as the swabbing material and design of the swab itself should be considered.

1.3.4. Additional Factors Influencing Touch DNA Recovery

Factors other than the swabbing material may influence the recovery of touch DNA such as the interactions of the DNA with both the swab and the surface type. Verdon *et al*. (2014)

suggested that a comprehensive study comparing a range of recovery methods for the collection of a series of samples from differing surface types was needed.

Daly, Murphy and McDermott (2012) suggested that porous surfaces have a greater ability to adhere epithelial cells than non-porous surfaces indicating that this could impact upon the recovery of the DNA from such surfaces both in respect to how readily that DNA can be collected from the surface and also in relation to the retention of DNA on the surface over a prolonged period of time. They also found that samples recovered from wooden surfaces yielded more useful profiles than the samples recovered from fabric and glass, suggesting that the surface itself can play a major part in the success of touch DNA evidence.

In a study conducted by Hansson *et al*. (2009), it was found that when DNA was recovered from an absorbent surface, minitapes consistently produced higher quantities of DNA than the swab materials used. However, when DNA was recovered from a hard plastic surface, a self-saturating swab was more effective than the cotton swab and nylon flocked swab, but performed equally as well as minitapes. This suggests that the optimal recovery method may vary depending upon the surface that DNA is being recovered from. Verdon *et al*. (2014) investigated the optimal recovery method dependent upon surface type and found that foam swabs consistently recovered more DNA from wood than other swabbing materials. They also found that this was not always the case on other surface types which suggests that there may be other factors that influence the recovery of low-level DNA. Hansson *et al*. (2009) found in a similar study that foam swabs were more efficient on non-porous surfaces than cotton and nylon flocked swabs but that there was no difference in efficiency on porous surfaces such as fabrics. This may be contrasting with the research conducted by Verdon *et al*. (2014) as wood can be considered a porous surface depending upon if this has been treated. Therefore, further research may need to be conducted into this.

The success of touch DNA recovery may also be influenced by the extraction method being utilised, as if the recovered DNA is not released well during the extraction process then this

will hinder the success of this evidence type. Hansson *et al*. (2009) found that the chelex extraction method recovered more DNA than the automated EZ1 method, however the quality of the profiles produced was better with the EZ1 method. This suggests that the quantification data may not be indicative of the quality of the DNA present and therefore may not be an appropriate indicator for whether a good quality profile will be produced. Similarly, Brownlow *et al*. (2012) found that when an automated extraction method was utilised the cotton swab performed significantly better than the nylon flocked swabs, whereas when using a manual extraction process, such as the QIAGEN DNA Investigator Kit, the nylon flocked swabs retrieved significantly more DNA than the cotton swabs. This suggests that the optimal combination of swab material and extraction method should be considered when analysing touch DNA samples to maximise the amount of DNA available for analysis.

1.4. A Review of Rapid DNA Analysis

1.4.1. What is Rapid DNA Analysis?

Short Tandem Repeat (STR) profiling is routinely used in forensic investigations for linking DNA evidence present at a crime scene to an individual, however standard STR profiling methods can be time consuming thus leading to a delay in investigative leads from this evidence type (LaRue *et al.* 2014). Due to this, instruments that allow for the rapid analysis of DNA have been developed (Gangano *et al.* 2013). One such instrument is the RapidHIT™ 200 from IntegenX, which is a self-contained system which allows for the STR analysis of samples in an automated benchtop unit. This system allows for the extraction, amplification, separation and analysis of biological samples within a single mobile unit which can be set up in a non-laboratory setting such as a custody suite (Holland and Wendt, 2015).

Rapid DNA analysis holds the potential to be extremely advantageous in forensic investigations due to its ability to analyse a profile within approximately 90 minutes, depending upon the sample (Gangano *et al*. 2013). These technologies can provide great

value due to the investigative leads that can develop through this rapid analysis of DNA evidence (Verheij *et al*. 2013 and Hennessy *et al*. (2013).

1.4.2. Rapid DNA Analytical Techniques

The RapidHIT™ instrument was launched in 2012 for the purpose of analysing reference samples, such as buccal samples, with the potential for this to be used in casework (Gangano *et al*. 2013). To date, Rapid DNA technologies, such as the IntegenX RapidHIT™ instruments, have been used to analyse reference samples and some evidential samples, such as blood and saliva, with this being used for casework samples by the DNA Profiling Laboratory, Health Sciences Authority, Singapore in 2015, but it has been suggested these technologies are not appropriate for the analysis of trace DNA (La Rue *et al*. 2014; Thong *et al*. 2015a). Furthermore, Hennessy *et al*. (2013) discuss the potential for this to be utilised both inside the laboratory environment as well as outside of this environment such as in police custody suites or at the crime scene itself. Additionally, Moreno, Brown and Callaghan (2017) state that such Rapid DNA technologies enable analysis of reference samples at the point of collection without further human interaction due to the integrated nature of the instruments.

While these Rapid DNA analysis technologies aim to provide an automated DNA analysis that could be used outside of the laboratory, research conducted by Date-Chong, Hudlow and Buoncristiani (2015) concludes that these systems were not yet suitable at that time for a “hands-off” approach, as even with high quantity samples, such as buccal samples, some alleles are flagged for expert review leading to a manual review of profiles being required prior to searching the National DNA Database. This suggests that while technologies such as the RapidHIT™ may be useful for both reference and crime scene samples, expert review of the profiles produced is still required. However, as the analysis time is reduced using

these technologies (Gangano *et al.* 2013), even with a manual review process the overall time between sample collection and the results being obtained is greatly reduced.

1.4.3. The Impact Rapid DNA has Upon Forensic Investigations

Rapid DNA analysis holds the potential to be extremely advantageous in forensic investigations due to its ability to analyse a profile within approximately 90 minutes, depending upon the sample which allows for rapid intelligence to be gained around the individual that DNA evidence is linked to (Gangano *et al*. 2013; Hennessy *et al.* 2013). The potential for this to be utilised both inside the laboratory environment as well as outside of this environment, such as in police custody suites or at the crime scene itself, has also been discussed by Hennessy *et al.* (2013). This provides even greater benefits to the investigative process as the portable nature of these technologies could be very advantageous in cases such as disaster victim identification and other rapid intelligence cases as they allow for the rapid analysis of the DNA evidence to gain information on the potential identity of suspects and victims (Mogensen *et al*. 2013). Additionally, Moreno, Brown and Callaghan (2017) state that such Rapid DNA technologies enable analysis of reference samples at the point of collection without further human interaction due to the integrated nature of the instruments reducing the need for expert review and analysis of these samples.

1.4.4. Success Rates for Low-Level Samples

Gangano *et al*. (2013) found that samples with a higher quantity of DNA, such as blood and saliva samples, produced profiles that are suitable for discrimination or for searching against a database. However, Thong *et al*. (2015) also suggest that urgent samples that involve

touch or contact DNA should not be analysed with RapidHIT™ and that only samples containing high amounts of DNA should be analysed with these technologies.

Mapes *et al*. (2016) state that it is unclear as to what quantity and quality of DNA is required in order to obtain a genetic profile when using Rapid DNA technologies. However, they do suggest that higher quantities of DNA are required than with the standard laboratory processes. This is in agreement with research conducted by Moreno, Brown and Callaghan (2017), who found that Rapid DNA analysis of samples with 50ng or less of total DNA inputted into the instrument resulted in consistently poor yield, with partial or no profile being produced. Additionally, they found that samples with 250ng or more of DNA produced full profiles that were concordant with conventional analysis, methods suggesting that a higher quantity of DNA is required to generate a full profile with these technologies. Further to this, Manna *et al*. (2016) found that samples containing 1.0µg of DNA or greater generate a complete STR profile with Rapid DNA analysis, such as DNAscan™. However, samples containing less DNA produce variable results with only partial profiles being generated at 0.5µg of DNA.

In an earlier study, Thong *et al*. (2015) found that a good success rate was generated using the RapidHIT™ system for high quantity samples with more than 80% of the alleles being called for samples down to 0.5µl of blood. However, this was considerably lower when analysing lower quantity samples. In agreement with these findings, Wiley *et al*. (2017) also found that lower quantity samples, such as serial dilutions of saliva, produced poorer results with a substantial increase in allelic dropout. This suggests that the sensitivity of the Rapid DNA systems are not suitable for the analysis of low-level samples such as touch DNA.

However, Wiley *et al*. (2017) suggest that by utilising the automated Rapid DNA technologies to analyse routine samples such as reference buccal samples, this can be taken out of the forensic laboratories, and can therefore relieve some of the demand to allow them to focus on more challenging samples, such as the lower quantity samples including

mixtures and touch DNA. In addition to this, Dawnay *et al*. (2014) propose that Rapid DNA analysis can be used as a screening process for selective sampling using standard laboratory processes.

One of the main limitations of Rapid DNA analysis in comparison to standard benchtop protocols is that there is a reduced sensitivity which limits the application of this analysis to reference samples or higher quantity evidential samples (Thong *et al.* 2015). This is further supported by Shackleton *et al.* (2019) who found that samples containing 3.125K cells or fewer produced profiles with allelic drop out whereas samples such as buccal scrapes with around 38K cells produced comparable results with benchtop methods demonstrating that with higher quantity samples, good success rates can be obtained that are in line with manual processing. Some Rapid DNA analysis instruments have been optimised for analysis of low content DNA samples which means it may be appropriate to analyse touch DNA with these systems as discussed by Turingan *et al.* (2016). However, the adaptations made to this instrument for this kind of analysis has the potential for sample loss in the form of the smaller exDNA fragments due to the elution process which are suggested to make up a large portion of touch DNA deposits as discussed in section 1.2.1. This suggests that while these adaptions to the Rapid DNA analysis process may allow for the analysis of lower quantity samples, this may not yet be ready for analysing touch DNA until further understanding of what touch DNA is comprised of is obtained.

1.5. A Review of the Uses of Diamond™ Nucleic Acid Dye

1.5.1 What is Diamond™ Nucleic Acid Dye?

Nucleic Acid binding dyes, such as SYBR® Green, Ethidium Bromide and Diamond™ Nucleic Acid Dye, have previously been used in forensic DNA analysis for gel electrophoresis, fluorescent quantification and within real-time PCR (Haines *et al.* 2015). Diamond™ Nucleic Acid Dye is a molecule that binds to the phosphate backbone in DNA via

external-groove binding, and can be visualised using fluorescent microscopy as it has excitation of 494nm and an emission of 558nm (Kanokwongnuwut *et al.* 2018, Hughes *et al.* 2022). Furthermore, Diamond™ Nucleic Acid Dye does not bind effectively to bacterial DNA, RNA and prokaryote supercoiled DNA, which aids in the visualisation of touch DNA, as this limits the amount of background fluorescence observed (Kanokwongnuwut, Kirkbride and Linacre, 2018a). Additionally, it has been determined to have a negligible impact upon the subsequent DNA profile of stained cellular material (Kanokwongnuwut, Kirkbride and Linacre, 2018a; Hughes *et al.* 2022).

1.5.2. Applications for Diamond™ Nucleic Acid Dye

Haines *et al.* (2015) discuss how being able to locate latent DNA at crime scenes will aid in the recovery of this evidence to maximise the success of this evidence type through a targeted approach. When reviewing different Nucleic Acid dyes Haines *et al.* (2015) found that Diamond™ Nucleic Acid Dye and SYBR® Green were able to detect latent DNA down to 0.5ng. Kanokwongnuwut, Kirkbride and Linacre (2018a) conducted a study to determine if Diamond™ Nucleic Acid Dye could be used to visualise latent DNA, deposited by holding an item for 5 seconds, on a range of surfaces typically of forensic interest at a crime scene. They found that deposited DNA could be identified from staining with Diamond™ Nucleic Acid Dye on all surfaces reviewed, these were; glass slides, credit cards, mobile phones, SIM cards, zip-lock bags, nickel cartridge cases and aluminium cartridge cases. Kanokwongnuwut, Kirkbride and Linacre (2018a) did note that the background colour of some of these items made it more difficult to visualise the green fluorescence of the stained material, however increased magnification typically resolved this allowing for the cells to be counted. Champion *et al.* (2020) further assessed the ability to visualise touch DNA on various substrates but as well as reviewing non-porous substrates, such as glass, they reviewed the use of Diamond™ Nucleic Acid Dye for visualising touch DNA on porous

surfaces, these were; a black cotton t-shirt, a wooden matchstick and three paper-based substrates. The results from the study conducted by Champion *et al.* (2020) demonstrated that, when Diamond™ Nucleic Acid Dye is diluted with water, cellular material could be visualised on absorbent surfaces, whereas a dilution with ethanol was more appropriate for non-absorbent surfaces. While visualisation was possible on all substrates reviewed by Champion *et al.* (2020) they did conclude that more research was needed into application methods when looking at items with a larger surface area.

Young and Linacre (2020) reviewed the use of a spray device for the application of Diamond™ Nucleic Acid Dye onto larger surface areas to locate touch DNA deposits. They found that it was possible to detect touch DNA over a large surface area using a spray device, with a pressurised continuous-spray being favourable due to observing higher intensities without large droplet sizes which may influence the fluorescence intensity (Young and Linacre, 2020). Cook, Mitchell and Henry (2021) applied this technique to casework samples using a mini air compressor to apply the Diamond™ Nucleic Acid Dye to the surface of these samples. They found that visualisation of touch DNA with Diamond™ Nucleic Acid Dye was possible with the majority of exhibits, however they noted that this was not suitable for all exhibits as background fluorescence and absorption of excitation was present in some samples, particularly those with a dark or black background. Despite their findings, Cook, Mitchell and Henry (2021) did suggest that the use of Diamond™ Nucleic Acid Dye may be appropriate with some samples but that further investigation was required.

This was further utilised by Kanokwongnuwut *et al.* (2018), when assessing the shedder status of 11 individuals, whereby they stained touch DNA deposits made by the individuals, and through the cell count visible and subsequent profiling of the DNA collected they were able to determine whether these donors were heavy, intermediate or light shedders. Similarly, Champion, van Oorschot and Linacre (2019) utilised Diamond™ Nucleic Acid Dye to visualise and assess the transfer of latent DNA between surfaces and were able to determine that, DNA transfers in greater quantities when friction and pressure are present in

the transfer mechanism. Additionally, through the use of this staining method, Champion, van Oorschot and Linacre (2019) were able to demonstrate that DNA transferred readily from aluminium to cotton, polyester and aluminium but did not transfer from aluminium to plastic in as high quantities.

A further study by Kanokwongnuwut, Kirkbride and Linacre (2018b), assessed the ability to visualise latent DNA on differing swab materials using Diamond™ Nucleic Acid Dye. Their findings suggest that not all swab materials are suitable for staining with Diamond™ Nucleic Acid Dye due to them auto-fluorescing without any DNA or Diamond™ Nucleic Acid Dye being present. In particular, Kanokwongnuwut, Kirkbride and Linacre (2018b) found that foam swabs, white cotton swabs, FLOQSwab™ Regular and FLOQSwab™, were not suitable due to their lack of contrast between DNA and the natural fluorescence of the materials. However, Cylinder and Ultrafine swabs exhibited a clear difference in fluorescence levels between negative samples and samples with DNA present and black cotton swabs demonstrated minor differences in fluorescence levels with extracted DNA with the cellular material being clearly present under 220x magnification. Therefore, these swabbing materials are suggested to be suitable for staining with Diamond™ Nucleic Acid Dye. Similar findings were also reported by Cook, Mitchell and Henry (2021) who found that cellulose based swab materials exhibited background fluorescence, which made it difficult to differentiate between swabs with solely Diamond™ Nucleic Acid Dye present versus those with DNA and Diamond™ Nucleic Acid Dye present. However, Kanokwongnuwut, Kirkbride and Linacre (2018b) found that the black cotton swab still had clearly detectable levels of cellular material after 4 weeks of storage at room temperature demonstrating that DNA can still be stained after being stored at room temperature for a prolonged period of time, as is often the case at a crime scene where the time since deposition is unknown, which demonstrates the potential benefits for integration with casework samples (Kanokwongnuwut, Kirkbride and Linacre, 2018b).

Similarly, Haase *et al.* (2019) found that they were able to visualise deposited cells on shell casings and glass slides and utilised this to determine how much DNA was recovered with cotton and nylon flocked swabs by staining the DNA prior to collection and reviewing the number of cells remaining following DNA recovery with these swabs. However, in contrast to Kanokwongnuwut, Kirkbride and Linacre (2018b), who found that staining the material prior to collection reduced the recovery efficiency, Haase *et al.* (2019) suggested that the recovery of touch DNA could be optimised by staining the cellular material in situ prior to recovery. Kanokwongnuwut *et al.* (2019) also found that it was possible, when using aluminium powder or white powder, to enhance a fingermark and then subsequently visualise the cellular material using Diamond™ Nucleic Acid Dye with the accurate recording of the number of stained cells being possible, which will aid in the dual analysis of fingermarks and DNA.

1.6. Aims and Objectives

1.6.1. Aims

This research aimed to critically evaluate the recovery methods of low-level DNA to maximise efficiency for identification purposes. This was achieved by using multiple swab materials to collect low-level DNA for profiling using the latest profiling technologies (NGM SElect™ and RapidHIT™). There is a lack of advanced level research in this field which has a significant impact on the success rates for this evidence type. Furthermore, without an understanding of the interactions between the materials and source DNA, there is a lack of opportunity to optimise recovery methods in the future.

1.6.2. Objectives

The objectives for this research are as follows:

- To assess the impact that the surface type has upon the recovery of touch DNA;
- To compare the quantitative and qualitative data produced from the analysis of touch DNA samples to determine the impact that the recovery method has upon the resulting data;
- To compare the RapidHIT™ and NGM SElect™ results from touch DNA samples to determine if the profile quality is impacted;
- To evaluate the interactions between the recovery method, DNA and surface type to design an optimal recovery strategy.

Chapter 2: Materials and Methods

2.1. Surface Preparation Method

Mock evidential items were purchased new at the beginning of this research where possible for the purpose of acting as surfaces onto which touch DNA could be deposited. In order to eradicate any DNA from the surfaces prior to use, a robust cleaning protocol was designed and adapted per surface type. The surface types used in this research were plastic handled screwdrivers, wooden handles, drinking glasses and aluminium drinks cans, these can be viewed in figure 3. These items were chosen due to their similarity to commonly experienced evidential items from which touch DNA is recovered. Additionally, in the 2015 DNA Recovery Validation Report by Staffordshire Police (Miller and Beckwith, 2015) similar items were used to replicate their common exhibits and therefore these items were selected to ensure relevant surfaces were being assessed. Similar items were also used in research conducted by Daly, Murphy and McDermott (2012) and Cook, Mitchell and Henry (2021).

Figure 3 Images of the surfaces used within this research.

Due to the nature of the surfaces, the plastic handled screwdrivers, wooden handles and drinking glasses were all subjected to the same cleaning regiment. However, this was found to not be suitable for the aluminium drinks cans so this was adapted for this surface type.

When cleaning the plastic handled screwdrivers, wooden handles and drinking glasses, the items were initially placed in 2% Virkon and left to soak for a 24-hour period. Following this, the items were removed from the 2% Virkon and allowed to dry. They were then placed into

pre-cut and sealed autoclave sterilisation roll to be autoclaved. These were then autoclaved at 121ºC at 15 psi for 15 minutes and allowed to dry in a dryer to ensure thoroughly dry.

The cans were cleaned twice with 2% Virkon by wiping the surface of the cans thoroughly with Medical Tissues soaked with 2% Virkon. The outer gloves were changed and cleaned between each can, to avoid cross contamination. Following the cleaning with 2% Virkon, the cans were wiped over with sterile distilled water to ensure that they will be safe to drink from.

Following cleaning, the items were then placed into brown paper evidence bags until needed for sample deposition. Paper evidence bags were selected due to the material being more breathable than a plastic bag and additionally, Goray, van Oorschot and Mitchell (2012) found that DNA transferred more readily from evidence items such as gloves to plastic evidence bags than to paper envelopes. However, there is some evidence transfer from the evidential items to the inside surfaces of the packaging is still expected along with the potential for this evidence to be redistributed over the surface of the items as found by Goray, van Oorschot and Mitchell (2012) and Stella, Meakin and van Oorschot (2022).

To ensure that this cleaning protocol was effective, one of each item was swabbed prior to handling and these were extracted and profiled. All of these negative controls taken from the cleaned items returned a profile with no detected alleles present indicating that this cleaning protocol is appropriate for eradicating any background DNA from the items.

2.2. Participants and Ethics

2.2.1. Ethical Approval

Prior to completing any practical work, ethical approval was sought from Staffordshire University's Research Ethics Committee via a Proportionate Review form. This was accepted and the research was then completed with all donor's being required to sign a consent form prior to being involved in the research.

2.2.2. Participants

Throughout the different experiments in this research the same donors were used (unless otherwise stated). Due to Staffordshire Police partially funding the RapidHIT™ section of this research, consideration of their practices was including during some of the method design, namely the selection of the evidential items to the donors that were, along with aiming to adopt an approach that is realistic in relation to commonly encountered exhibits which is supported by Gosch and Courts (2019). The donors were selected at random from the staff at Staffordshire Police Headquarters, as their DNA profiles had already been recorded by Staffordshire Police, with the expectation that they would be available throughout the duration of the study. Despite this expectation, there was some unforeseen unavailability of the donors. Due to this the donors did change between some of the studies conducted, however the donors were used for the entirety of each study for continuity. Staffordshire Police had previously conducted research into the potential shedder statuses of a selection of their staff members which was reported in their 2015 DNA Recovery Validation Report (Miller and Beckwith, 2015). However, as the shedder status of the donors is unknown in a real-life scenario, the proposed shedder status of the donors selected was not taken into consideration neither was the sex with a range of male and female donors being used.

There was a total of seven donors used throughout all studies which was made up of two males and five females, one of which is the researcher who was only used in the preliminary/proof of concept studies to ensure that in the studies where DNA profiling results were assessed, in regards to the success rates, contamination from the researcher could be easily identified. However, as not all donors were used in all studies please see table 1 for a breakdown of which donors were used in which studies from this research.

Donor	Preliminary Extraction Study	RapidHIT™ Study	RapidHIT™ Reanalysis Study	Phase 1 of Recovery Methods Study	Phase 2 of Recovery Methods Study	DNA Interactions Study
1 (Male)		X	X	X	X	
2 (Male)		X	X	X	X	
3 (Female)		X	X	X	X	
4 (Female)		X	X	X		
5 (Female)		X	X			
6 (Female)					X	
7 (Female) - Researcher	X					X

Table 1 Table indicating donor participation in various studies.

2.3. Sample Deposition Method

2.3.1. Sample Deposition Design

For the research conducted there were two sets of instructions, one in which some elements were controlled, such as handwashing and the handling process, to assess the results in a more controlled environment where sufficient DNA was allowed to accumulate (Kanokwongnuwut *et al.* 2018), and the second, in which only the process of handling was controlled, to provide more of a mock-operational trial, as in a real-life scenario the time since handwashing is unknown. There have been many studies published with varying times since handwashing being included, however one hour was decided for this study as this allows sufficient DNA to accumulate on the surface of the hands which is supported by Kanokwongnuwut *et al.* (2018) who found that the accumulation of cellular material appears to plateau after one hour.

The instructions outlined in the below sections 2.3.2. and 2.3.3. were provided to participants with the aim of controlling only a few factors. While the reproducibility of the data produced may be reduced by not controlling further variables, such as dominant vs non-dominant hands, the soap used for handwashing or the time taken for a can to be emptied, this research aims to be as applicable to real-world scenarios as possible which is also suggested by Gosch and Courts (2018) who discussed the need to address this with a large number of repeats. Additionally, by taking this approach, it balances the need for some reproducibility with the desire to ensure the results from this research could be implemented by practitioners, such as Staffordshire Police, as Cook, Mitchell and Henry (2021) discussed how this is not always possible from research conducted in a heavily controlled environment.

In an attempt to combat the potential reduction of reproducibility of the results obtained from the main studies described in this thesis, a good number of repeats was included as suggested by Gosch and Courts (2018) and a breakdown of the repeats is displayed in table

2. As can be seen in table 2, the share of repeats by the participants was not controlled for the RapidHIT™ and RapidHIT™ Reanalysis studies due to the donor impact not being assessed. However, this was adapted for the later studies to ensure there was an even range of donors across the samples to account for the potential impact their shedder status may have upon the results with this being unknown to the researcher, if for example a light or heavy shedder was to handle more items. Additionally, a fewer number of repeats was included for the DNA Interactions study due to this being a proof of concept study.

Study	**Repeats per Swab Material**	**Repeats per Surface Type**	**Repeats per Swab-Surface Combination**	**Repeats per Donor**
RapidHIT™ Study	36	36	9	N/A
RapidHIT™ Reanalysis Study	36	36	9	N/A
Phase 1 of Recovery Methods Study	32	32	8	32
Phase 2 of Recovery Methods Study	32	32	8	32
DNA Interactions Study	4	4	1	N/A

Table 2 Breakdown of repeats per study.

2.3.2. Phase 1 – Controlled Study Instructions for Participants

One hour before sample deposition, participants were required to wash their hands thoroughly. Following this, they were to continue with their normal activities. At the time of deposition, the test exhibit to be handled was collected in its evidence bag and removed by the participant then handled according to the below instructions.

Aluminium drinks can:

- Remove the item from the evidence bag.
- Open the can as normal.
- Drink from the can as normal while ensuring contact between the skin on their hand/palm and the side of the can.
- Once the can is empty, return item to the evidence bag and seal the bag.

Plastic handled screwdriver (to be completed once a day for 7 days):

- Remove the item from the evidence bag.
- Hold the screwdriver in their hand using the plastic handle for 5 minutes.
- Rotate the screwdriver in their hand every 1 minute to replicate normal use.
- Once the 5 minutes has been completed, return item to the evidence bag and seal the bag.

Wooden handles (to be completed once a day for 7 days):

- Remove the item from the evidence bag.
- Hold the handle in their hand for 5 minutes.
- Rotate the handle in their hand every 1 minute to replicate normal use.

- Once the 5 minutes has been completed, return item to the evidence bag and seal the bag.

Glass Tumbler:

- Remove the item from the evidence bag.
- Hold the glass in their hand as with normal use for 5 minutes.
- Rotate the glass in their hand every 1 minute to replicate normal handling.
- Once the 5 minutes has been completed, return item to the evidence bag and seal the bag.

Once the handling of the items had been completed, the samples were stored within the freezer ready for collection where possible.

2.3.3. Phase 2 – Mock-Operational Trial Instructions for Participants

For this phase no hand washing was required by the participants prior to handling the items as described in section 2.3.1. but the participants were advised to continue with their normal daily activities until the time of deposition. The test exhibit to be handled was then collected in its evidence bag by the participant, removed then handled according following the instruction detailed in section 2.3.2.

Due to some lab access restrictions during the COVID 19 pandemic these samples were unable to be stored in the freezer for 6 months post deposition, so they were stored securely at room temperature during this time.

2.4. DNA Extraction

Following DNA recovery using the double swabbing method and sterile water as a moistening agent (de Bruin *et al.* 2012), the swab heads were placed directly into a 1.5ml

Eppendorf tube, ready for extraction, to help reduce the potential for sample loss to the swab tube as described by Goray, van Oorschot and Mitchell (2012). The collected DNA was then extracted using the QIAamp DNA Micro Kit (QIAGEN) following the Forensic Casework Protocol.

2.5. DNA Quantification

Following DNA extraction, the extracted DNA was quantified using the human specific Genomic DNA quantification assay (Primer Design) in a half reaction mixture following the manufacturer's protocol with bright white plates being used and DNase free water being added rather than the internal extraction control, due to this not being available during DNA extraction as the samples were extracted prior to the purchase of the quantification reagents for the majority of samples.

2.6. DNA Profiling

The extracted DNA was then profiled using the AmpFℓSTR® NGM SElect™ PCR Amplification kit (Applied Biosystems) using a half reaction mixture following the manufacturer's protocol, with no dilutions of samples being made. MicroAmp® Optical 96-well Reaction Plates sealed MicroAmp® Clear Adhesive Film were used following amplification in a GeneAmp® PCR System 9700 with the silver 96-well block. Capillary electrophoresis was completed directly following amplification and was completed using the 3500 Genetic Analyser (Applied Biosystems) in MicroAmp® Optical 96-well Reaction Plates.

2.7. Data Analysis

Where applicable statistical comparisons were performed with SPSS v27.0 (SPSS Inc.)

using the Kolmogorov Smirnov (KS) test for normal distribution followed by significance testing with a 95% confidence interval, the details of the significance testing utilised will be discussed in the respective results chapters.

2.8. DNA Extraction Preliminary Study

A series of 15 treated aluminium drinks cans were used for this study following the preparation and deposition methods outlined in sections 2.1 and 2.3.3. respectively. The deposited DNA was then recovered using a cotton swab (Deltalab) following the double swabbing methodology with sterile water as the moistening agent (de Bruin *et al*. 2012).

The DNA was then extracted from the swabs using different extraction methods, four spin column methods and one magnetic bead method, to determine the impact this has upon the success rates of this evidence type. Three samples were extracted with each of the following extraction methods; blackPREP Swab DNA Kit (Analytic Jena) following the DNA isolation from buccal swab protocol, PuriSpin Fire Monkey Trial Kit (Revolugen) following the DNA/ Total Nucleic Acid extraction protocol, QIAamp DNA Investigator Kit (QIAGEN) following the Forensic Casework protocol, QIAamp DNA Micro Kit (QIAGEN) following the Forensic Casework protocol, genesig Easy DNA/RNA Extraction Kit (Primer Design) following the swab samples protocol. The extracted samples were then analysed with quantitative PCR as described in section 2.5. and DNA profiling was completed as described in section 2.6.

The purpose of this study was to determine if there was an optimal manual extraction method that should be adopted when analysing touch DNA deposits. The technologies used were selected based upon advice from the suppliers regarding their effectiveness for such samples.

The quantification data produced in this study, observed in table 3, was found to be non-normally distributed with a Kolmogorov-Smirnov test and produced a statistically significant difference between the different extraction kits used with an independent-samples Kruskal-

Wallis test (p=0.007), with the QIAamp DNA Micro Kit generating higher quantities of DNA than the PuriSpin Fire Monkey Trial Kit (p=0.001) and blackPREP Swab DNA Kit (p=0.009). However, no further significant differences were found between the remaining extraction methods. Using a Cohen's d formula the effect size was calculated as 0.39 for the comparison of QIAmp DNA Micro Kit with PuriSpin Fire Monkey Trial Kit and 0.32 for the comparison of QIAmp DNA Micro Kit with blackPREP Swab DNA Kit which indicates a small effect size.

Sample	Extraction Method	Quantification (ng/µl)
AJ1	blackPREP Swab DNA Kit	0
AJ2	blackPREP Swab DNA Kit	0
AJ3	blackPREP Swab DNA Kit	0.001
FM1	PuriSpin Fire Monkey Trial Kit	0
FM2	PuriSpin Fire Monkey Trial Kit	0
FM3	PuriSpin Fire Monkey Trial Kit	0
IK1	QIAamp DNA Investigator Kit	0
IK2	QIAamp DNA Investigator Kit	0.001
IK3	QIAamp DNA Investigator Kit	0.002
PD1	genesig Easy DNA/RNA Extraction Kit	0.002

PD2	genesig Easy DNA/RNA Extraction Kit	0.001
PD3	genesig Easy DNA/RNA Extraction Kit	0
QM1	QIAamp DNA Micro Kit	0.003
QM2	QIAamp DNA Micro Kit	0.001
QM3	QIAamp DNA Micro Kit	0.003

Table 3 Quantification data for the DNA extraction preliminary study.

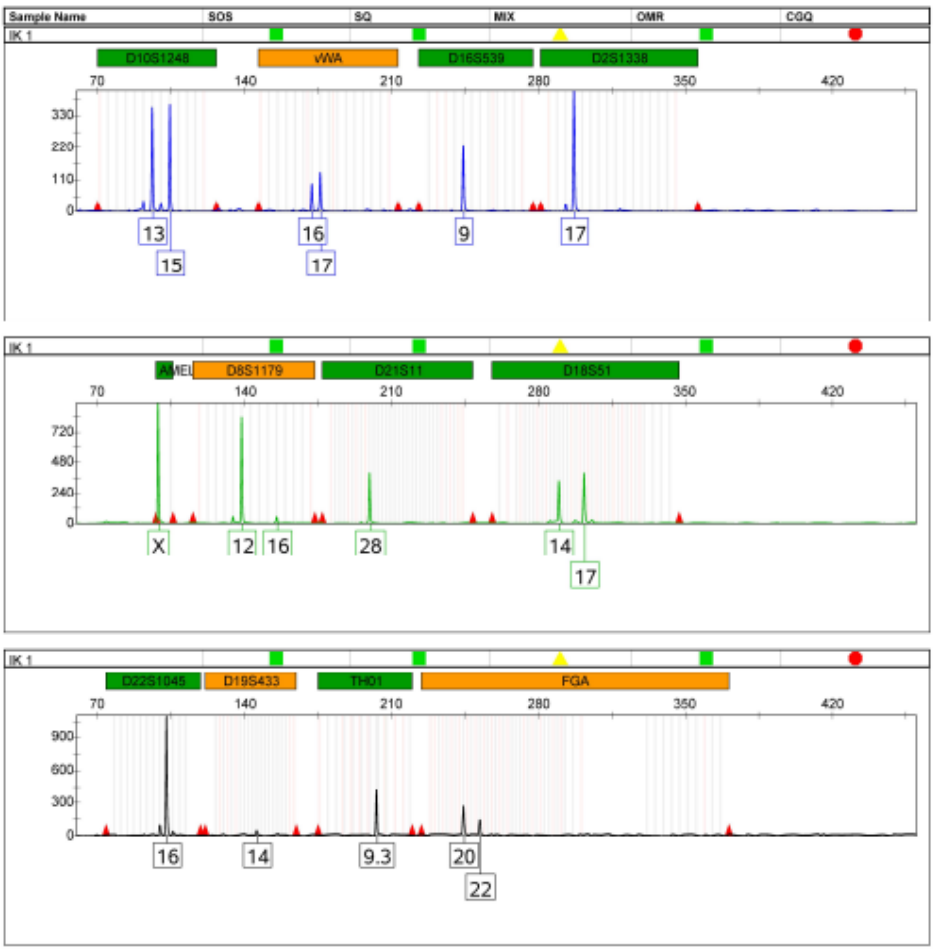
Following this, the profile data produced in this study was evaluated, which can be observed in table 4, to determine if there was a difference in the quality of profiles produced from these kits as indicated by the number of alleles present, the peak heights and the presence of any stutter peaks.

Sample	**Extraction Method**	**Total Number of Alleles**	**Number of Donor Alleles**
AJ1	blackPREP Swab DNA Kit	23	12
AJ2	blackPREP Swab DNA Kit	4	0
AJ3	blackPREP Swab DNA Kit	9	3
FM1	PuriSpin Fire Monkey Trial Kit	2	0
FM2	PuriSpin Fire Monkey Trial Kit	24	16

FM3	PuriSpin Fire Monkey Trial Kit	8	3
IK1	QIAamp DNA Investigator Kit	37	31
IK2	QIAamp DNA Investigator Kit	34	26
IK3	QIAamp DNA Investigator Kit	27	16
PD1	genesig Easy DNA/RNA Extraction Kit	22	15
PD2	genesig Easy DNA/RNA Extraction Kit	29	22
PD3	genesig Easy DNA/RNA Extraction Kit	12	4
QM1	QIAamp DNA Micro Kit	30	20
QM2	QIAamp DNA Micro Kit	32	16
QM3	QIAamp DNA Micro Kit	58	34

Table 4 Profile data from the DNA extraction preliminary study demonstrating the number of alleles and donor alleles produced with each extraction kit.

This data was found to be non-normally distributed with a Kolmogorov-Smirnov test and it was found that there was no statistically significant difference (p=0.061) between the number of donor alleles produced from the samples extracted with the different kits using an independent-samples Kruskal-Wallis test. However, when looking at the profiles themselves there was varying quality between the kits with the QIAamp DNA Investigator Kit and the QIAamp DNA Micro Kit producing more donor alleles than the remaining extraction kits with greater peak heights, an example of this can be seen in figure 4. As can be seen in table 3, all samples generated spurious alleles which are likely as a result of indirect transfer with non-donor DNA being collected on the hands in the time since handwashing, which was also observed by Goray *et al.* (2016) when looking at precleaned items. Similar results have also been found when comparing QIAGEN extraction kits to alternative manufacturers in published research (Phillips *et al*. 2012), however, this includes comparisons to automated methods which were not adopted for this research.



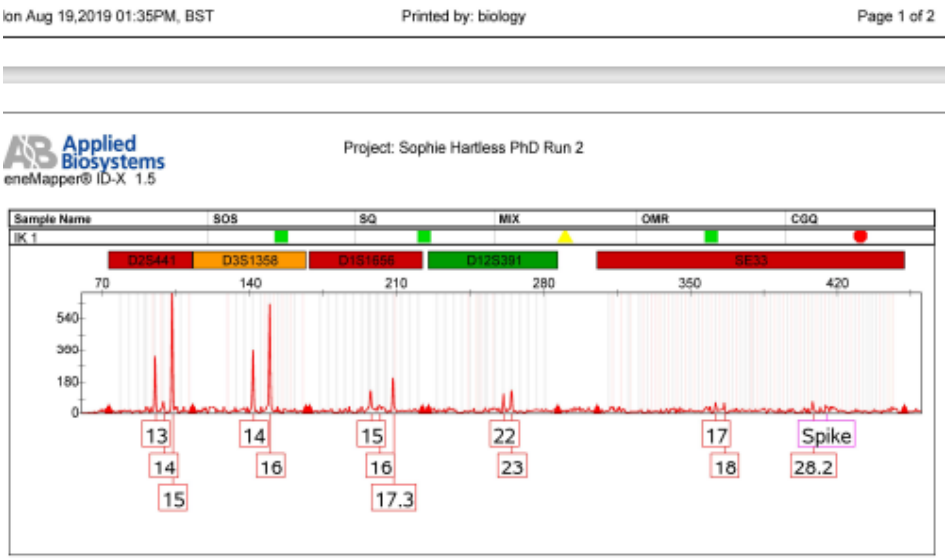


Figure 4. An example profile with good peak heights from the DNA extraction preliminary study.

Due to the data produced suggesting that the QIAamp DNA Investigator Kit and the QIAamp DNA Micro Kit produce higher quality profiles than the other extraction kits, a cost analysis was conducted to determine which kit to use. Based on the lower cost, the quantification values and profile data, the QIAamp DNA Micro Kit was selected for use within the remainder of this research.

Chapter 3: Analysis of Touch DNA Using RapidHIT™ 200.

3.1. Introduction

The benefits and limitations of Rapid DNA analysis have been discussed in section 1.4. of this thesis which form the basis of the rationale behind this study alongside the Home Office Strategy (2015-2020) which suggested a strong focus should be placed on real-time investigations. The limitations of Rapid DNA Analysis, namely the suggested inability for this technique to be used for touch DNA analysis (Thong *et al.* 2015a), led to Staffordshire Police looking to expand the research currently conducted to determine if this would be possible when utilising different recovery methods which was also suggested by Wiley *et al.* (2017). This was discussed with Staffordshire University and the researcher conducting this study, through the Staffordshire Forensic Partnership, to determine a research design that addressed this with the aim of determining if successful analysis of touch DNA with Rapid DNA Analysis was possible if an optimal recovery method was utilised.

Research conducted by Mapes *et al*. (2016) looked at common items found at a crime scene that had low-level DNA present and whether they generated successful profiles using Rapid DNA Analysis. Through this study they found that items with a porous surface, such as a ball cap, generated higher success rates than those with a smoother surface, such as a screwdriver. From this study, they conclude that samples that generate a high success rate with standard laboratory procedures are most likely to be successful with Rapid DNA analysis although the surface type will impact upon this.

In addition to the surface type, other factors such as the swab substrate may impact upon the success rates of low-level DNA analysis with Rapid DNA technologies (Wiley *et al.* 2017). Wiley *et al*. (2017) evaluated the impact that the swabbing substrate had upon the resulting profiles. They found that nylon flocked swabs (4N6-FLOQSwab™) and rayon swabs (MacroPur™) performed better with the RapidHIT™ system than the other materials tested including Whatman FTA cards and the EasiCollect swab. The nylon flocked swabs

and rayon swabs produced full profiles compared to the partial or no profiles produced with Whatman FTA cards and the EasiCollect swab. This suggests that the swab substrate material does impact upon the resulting profile quality and Wiley *et al*. (2017) suggest that this factor should be investigated further.

Bruijns, Tiggelaar and Gardeniers (2018) discuss the various factors that impact the success of DNA recovery and analysis. The researchers discuss factors such as swab morphology, absorption capacity along with the extraction and recovery efficiency. They found that the absorption capacity of the swab does not show a clear relation to the swab morphology as factors such as the size and porosity of the swab also impacts the absorption along with the material itself. As discussed by Bruijns, Tiggelaar and Gardeniers (2018) and Verdon *et al*. (2014) the way in which the material is wound around the shaft of the swab impacts upon the recovery efficiency. Verdon *et al*. (2014) suggest that swabs that are wound around the shaft perform better than those which are layered.

As low-level DNA is becoming increasingly common at crime scenes, the successful recovery and analysis of this evidence is vital in forensic investigations (Hess and Haas, 2017; Aditya *et al*. 2011). The number of cases where low-level DNA is potentially evidentially valuable has increased and as a result of this, the analysis of this evidence type has also increased (Dong *et al*. 2017).

Previous research into optimising DNA recovery has yielded conflicting results as discussed in section 1.3.2. and the optimal recovery method may vary depending upon the source of the DNA which is supported by Kirgiz and Calloway (2017) who state that the recovery methods utilised for higher quantity samples, such as sperm and saliva, may not be appropriate for lower quantity samples such as touch DNA. They also suggest that this should be further investigated to determine the optimal recovery method for touch DNA.

This chapter aims to address whether touch DNA can be successfully analysed using Rapid DNA Analysis when an appropriate swab material is utilised. This chapter will also begin to

suggest an optimal recovery strategy for this evidence type from aluminium and plastic surfaces. This will help to inform whether the benefits of this technique can be expanded which will enable for forces to implement this when they have a high throughput of low quantity samples, such as is the case for Staffordshire Police.

3.2. Materials and Methods

3.2.1 DNA Recovery

Touch DNA samples were deposited upon aluminium drinks cans (n=36) and plastic handled screwdrivers (n=36) as outlined in section 2.3.2. and recovered from the surfaces using mini-pointed cotton swabs, nylon flocked swabs, foam swabs and viscose swabs (a breakdown of the participants can be found in section 2.2.2. and a breakdown of the number of repeats can be found in section 2.3.1.). The items were swabbed in their entirety using a single swab moistened with distilled water rather than the double swab method discussed by de Bruin *et al.* (2012), which is due to the size of the cartridges for the RapidHIT™ 200 instrument not being able to facilitate two swab heads in each chamber. The swabs were stored at room temperature prior to use and were kept sterilised to reduce the potential for contamination.

To ensure a good level of repeats was possible that would remain statistically valid, due to the limited controls in place as discussed in section 2.3.1., only 2 commonly encountered surface types, in Staffordshire Police exhibits as per their 2015 DNA Recovery Validation Report (Miller and Beckwith, 2015), were used in this study.

3.2.2. Rapid DNA Analysis

DNA analysis with the RapidHIT™ 200 instrument was conducted using the Crime Stain 2 protocol which has been designed specifically for lower quantity samples and has an extended run time of 2.5 hours (Pagram, 2018). The extraction of DNA with this protocol

uses guadinium thiocynate (Teknova, Hollister, CA, USA) chemical lysis and a solid phase DNA separation and purification with paramagnetic beads (Micro-mod GmBH, Germany). This follows the same steps as the other protocols for other samples such as buccal samples with a standard concentration of the beads being utilised and these were suspended within 500µl of lysis buffer for 6 minutes (Shackleton *et al*. 2018). However, an enhanced bead capture time is included with this being 5 x 200 seconds (Pagram, 2018). Following extraction, there is no quantification step as the DNA IQ™ technique limits the quantity of isolated DNA sent to the PCR chamber to within a range of 4-20ng (Holland and Wendt, 2015). The extracted DNA is then moved to individual chamber where it is amplified using the PowerPlex® 16HS reagents from Promega and is further transferred to a 8-channel capillary electrophoresis plate where fragment separation occurs (Holland and Wendt, 2015; Shackleton *et al.* 2018).

The resulting electropherograms were interpreted using the GeneMarker® software using a threshold of 50 RFU (relative fluorescence units) due to this being the optimal RFU for this instrument.

3.3. Results and Discussion

3.3.1. Assessment of the Impact of Surface Type on the Success Rates of Touch DNA Analysis with RapidHIT™ 200.

Surface Type	**N (∑=72)**	**Median number of alleles per profile**	**Median number of donor alleles per profile**	**Median number of non-donor alleles**
Aluminium Drinks Can	36	10.50	10.00	0.00

Plastic Handled Screwdriver	36	20.00	18.00	3.00

Table 5. Median number of alleles, donor alleles and non-donor alleles recovered from the two surface types.

Touch DNA samples were deposited upon two surfaces throughout this study and these were then recovered as described in section 3.2.1. Table 5 displays the median number of alleles, donor alleles and non-donor alleles recovered from each surface. Overall, this data was found to be non-normally distributed (p=0.000, p=0.000) using a Kolmogorov-Smirnov test for normality demonstrating the variance in the profiles produced. A Mann-Whitney U test was conducted at a 0.05 significance level which determined that a statistically significant difference was present between the two surface types (p=0.041) with more donor alleles being produced from the plastic handles screwdrivers than from the aluminium drinks cans. A cohen's d test for effect size indicated a small effect size (0.38).

Oldoni, Castella and Hall (2016) suggested that the propensity to recover donor alleles from metal and plastic items varies considerably based upon a number of factors such as the time the item was handled for. These findings provide support for the data produced in this study, as the sample deposition method differed for the plastic handled screwdrivers and the aluminium drinks cans. The plastic handled screwdrivers were handled periodically for a short amount of time over 7 days, whereas the aluminium drinks cans were handled on a single day but likely for a longer period of time as these were handled simulating regular use with the donor drinking from the can until this was empty as described in section 2.3.2.

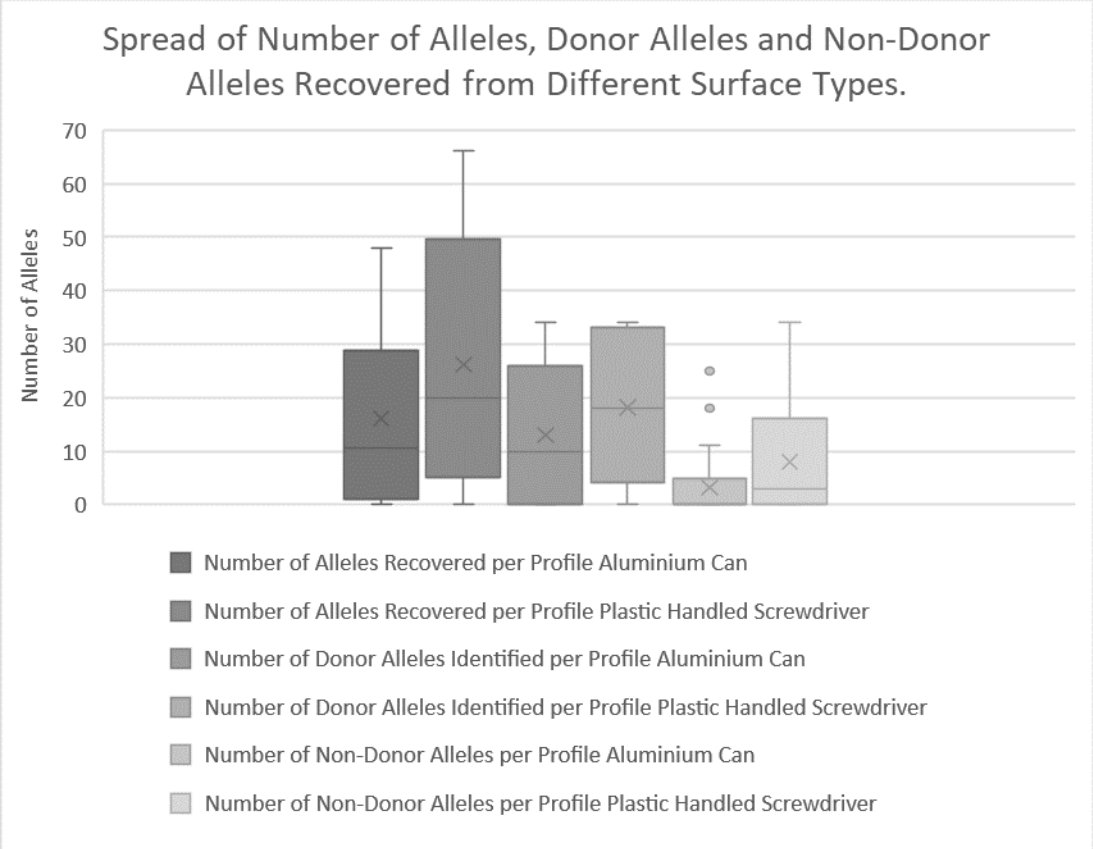


Figure 5. A box and whisker plot displaying the spread of the number of alleles, donor alleles and non-donor alleles recovered from aluminium drinks cans and plastic handled screwdrivers when analysed with RapidHIT™ 200.

Additionally, the nature of the objects were different with the plastic handled screwdriver being more textured allowing the transferred DNA to be retained upon the surface. Similar results were found by Daly, Murphy and McDermott (2012) who found that textured surfaces such as wood and fabric produced a higher yield of DNA than a smooth glass surface. Whereas, the aluminium drinks cans were handled with a prolonged period of contact while drinking from these but only upon a single day resulting in a shorter handling time. This is also supported by Oldoni, Castella and Hall (2016) who outlined that factors such as the nature of the object can influence the recovery of the donor alleles.

However, in contrast to this, Pamela *et al*. (2015) found that samples recovered from metal and glass surfaces produced a higher number of complete profiles suggesting that their characteristics were more facilitating of DNA recovery. However, this was in relation to the recovery of DNA from enhanced fingerprints which may alter the propensity of DNA retention on the surface due to the powder used to enhance the fingerprints themselves. The effect of other substances such as fingerprint powders could further impact the success of this evidence type and its recovery and should therefore be considered when approaching such evidential samples as although there may not be an inhibitory effect such materials may cause partial degradation of the DNA sample itself which can directly impact the success of this evidence type (Pamela *et al*. 2015). This is also discussed by Hess and Haas (2017) who suggest that some surface types such as clothing can act as an inhibitor when a swatch is taken and extracted due to the dyes within the fabrics.

Further to this, Verdon *et al*. (2014) also found that the surface type directly impacts the success of this evidence type with different recovery methods being required for the differing surfaces. For instance, it was presented that foam swabs were more effective when recovering touch DNA from wooden surfaces than with other surfaces. Whereas, cotton swabbing substrates were more effective when recovering touch DNA from pitted plastic surfaces. This suggests that an optimal recovery strategy is required depending upon the nature of the surface that the evidence is being recovered from.

3.3.2. Assessment of the Impact of Swab Material on the Success Rates of Touch DNA Analysis with RapidHIT™ 200

		Percentage of Donor Alleles Present per Profile					
Swab Substrate	**N (∑=72)**	**0%**	**1-20%**	**21-40%**	**41-60%**	**61-80%**	**81-100%**

Cotton	18	2	1	2	3	4	6
Foam	18	1	2	6	1	2	6
Nylon Flocked	18	10	4	1	0	0	3
Viscose	18	2	6	1	1	4	4

Table 6. Percentage of donor alleles present per profile generated with each swab material.

The touch DNA samples recovered in section 3.3.1. were recovered using four swab materials, to assess the impact that swab material has upon the success rates of touch DNA analysis with RapidHIT™ 200 the percentage of donor alleles present per profile, from both surface types, is displayed in table 6. The profiles produced in this study varied in quality and completeness both between the swab materials and within a single material causing the data produced to overall, be non-normally distributed using a Kolmogorov-Smirnov test for normality ($p=0.156$, $p=0.076$, $p=0.010$, $p=0.000$). However, the donor alleles recovered from cotton and foam swabs were normally distributed ($p=0.0156$ and $p=0.076$ respectively).

Despite this, a difference was observed between the percentage of donor alleles recovered using the different swab substrates. Further to this, a Kruskal-Wallis test with a 0.05 significance level was carried out to determine if this difference was significant. It was found that a statistically significant difference was observed between the profiles produced from the different swab substrates ($p=0.004$). This suggests that more donor alleles can be recovered when using different swab substrates.

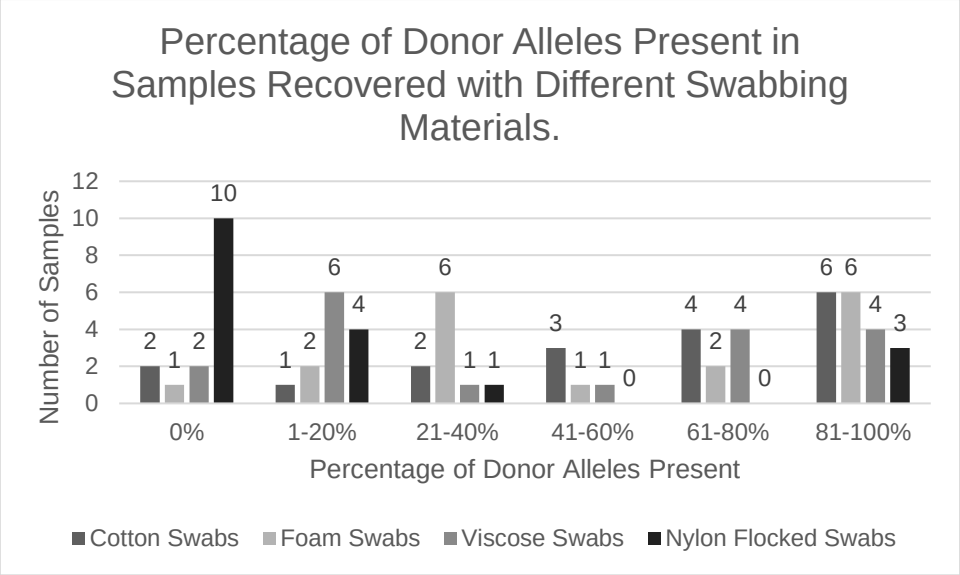


Figure 6. A chart of the percentage of donor alleles present in samples recovered using cotton, foam, viscose and nylon flocked swabs and analysed with RapidHIT™ 200.

In order to determine where this difference originates from post-hoc testing was conducted with a Bonferroni adjustment to the significance level ($p < 0.0083$). This demonstrated that there was a difference between the number of donor alleles recovered using cotton and foam swabs when compared to nylon flocked swabs ($p=0.003$, $p=0.002$ respectively). When looking at the data produced along with the statistical analysis, it can be observed that nylon flocked swabs recovered fewer donor alleles than cotton and foam swabs and these differences were determined to have a large effect size using the cohen's d test for effect size (1.04 and 0.92 respectively). The remaining post-hoc testing found no statistical difference between the remaining swab substrates.

This data shows that nylon flocked swabs are not as effective as other swabbing substrates for recovering touch DNA samples. Previous research conducted by Brownlow *et al*. (2012) also supports this conclusion as they reported that cotton swabs were more efficient than nylon flocked swabs for DNA recovery from crime scenes due to the nature of the design of the swab itself. This could account for some of the differences seen within this research as

the nylon flocked swab has a flexible plastic shaft which can cause some difficulty in the sampling process and retrieval of the touch DNA evidence. Such complications were reported by Brownlow *et al*. (2012) in relation to both high quantity DNA samples and decreased quantity samples.

Further to this, Pamela *et al*. (2015) found that in relation to generating a DNA profile from enhanced fingerprints, cotton swabs produced a higher number of complete genetic profiles (32.5%) than the samples recovered using nylon flocked swabs (30%). However, they also found that of the samples recovered with nylon flocked swabs more partial profiles were generated (76.3%) than with cotton swabs (55%). The findings present by Pamela *et al*. (2015) directly reflect those produced in this study as more complete profiles were generated with cotton swabs but less partial profiles when compared to the samples recovered with nylon flocked swabs. This in turn, suggests that a nylon flocked swabbing substrate is not as effective at recovering low-level DNA as other swabbing substrates.

In contrast to this, Benschop *et al*. (2010) found in an earlier study that nylon flocked swabs produce a higher yield of DNA than cotton swabs when analysing post-coital samples. They found in particular that a higher yield of male DNA was recovered using nylon flocked swabs than when recovered with cotton swabs. This suggests that when looking at a higher yield, wet sample the nylon flocked swabbing substrate may be more effective than other swabbing substrates. Such a suggestion is also supported by Kirgiz and Calloway (2017) who state that techniques that are used and have been found to be effective for higher quantity samples, such as semen and saliva, may not be as effective for touch DNA samples due to the differing quantities and nature of the samples.

Kirgiz and Calloway (2017) also suggest that methods of scraping to recover touch DNA from steering wheels may be more effective than the conventional double swabbing methods with cotton swabs. They stated that the yields of DNA produced were more highly dispersed when the double swabbing method with cotton swabs was used than with other methods

such as tape lifting and scraping with FTA cards. This may lead to less efficient DNA recovery and therefore a lower success rate for this evidence type. However, this research did not consider further swabbing substrates which may impact the effectiveness of touch DNA recovery.

Verdon *et al*. (2014) found throughout their research that the optimal swab varies based upon both the nature of the sample and also the surface type. Overall, it was found that foam swab were most effective for both neat and diluted saliva but they were not as effective when recovering touch DNA with a wound cotton swab being the most effective. This is in line with the data produced in this study as it was also found that while the number of complete profiles was equal between the cotton and foam swabbing substrates, the cotton swabs did produce a greater number of higher quality partial profiles than the foam swabs.

However, in contrast with the research conducted by Verdon *et al*. (2014) it was found that the foam swabbing substrate was more effective than the nylon flocked and viscose substrates whereas Verdon *et al*. (2014) found that nylon flocked swabs and rayon swabs performed better than foam swabs. Rayon swabs are very similar in nature to the viscose swabs used in this study (Bruijns, Tiggelaar and Gardeniers, 2018) and therefore suggests that these should perform better than the foam swabs but this was not found in this study as there were a higher number of complete profiles produced using the foam swabbing substrate than with a viscose swabbing substrate. In addition to this, the partial profiles produced using the foam swabs were of a higher quality with more donor alleles being present than from the samples recovered using the viscose swabs. This suggests that further research is required in this area to determine the optimal recovery strategy for touch DNA from such surfaces as conflicting results have been produced from this study and previous published research (Verdon *et al*. 2014).

In addition to this, this study assessed the effectiveness of each swabbing substrate in relation to the surface type in order to determine whether any swabbing substrate performed

better on the different surface types used. From this, it was discovered that the foam and viscose substrates recovered a higher number of donor alleles from the aluminium drinks cans than from the plastic handled screwdrivers. Also, cotton and nylon flocked swabs recovered more donor alleles from the plastic handled screwdrivers. This suggests, in accordance with previous research, that the surface type that the DNA is recovered from can determine what the optimal swabbing substrate is to gain a higher quality profile from touch DNA (Verdon *et al*, 2014; Oldoni, Castella and Hall, 2016; Daly, Murphy and McDermott 2012; Pamela *et al*. 2015; Hess and Haas, 2017).

Further to this, the collection method used when recovering the samples may also impact the success rate generated. de Bruin *et al*. (2012) suggested that the double swabbing method performs better at collecting epithelial material than other methods such as stubbing. This method involves applying a single moistened swab over the area followed by a single dry swab. This allows for the loosening of any dried genetic material through the moistened swab which can then be secured on the subsequent dry swab. However, the moistening agent utilised in the double swabbing method may also affect the recovery of low-level DNA. Routinely a cotton swab is used with distilled water as the moistening agent (Templeton *et al*. 2013). Despite this being the routinely used collection method for touch DNA further method may be more effective. Thomasma and Foran (2013) found that moistening a cotton swab with a detergent produced a higher yield of DNA from low-level samples such as touch DNA. Phetpeng *et al*. (2013) found in a similar study that the quantity of DNA recovered depended upon both the brand of the swab and the moistening agent used. In this study a single swab moistened with distilled water was utilised, this may have influenced the profiles generated as with only a single moistened swab there may be less genetic material recovered than with the double swabbing method as suggested by de Bruin *et al*. (2012).

3.3.3. Overall Assessment of the Success Rate of RapidHIT™ 200 for Analysis of Touch DNA.

	Percentage of Donor Alleles (%)				
	0-19	20-39	40-59	60-79	80-100
Number of Profiles	28	8	6	7	23
Percentage of Profiles (%)	38.89	11.11	8.33	9.72	31.94

Table 7. A comparison of the percentage of donor alleles present within the profiles produced.

This study combined all of the profiles produced in sections 3.3.1. and 3.3.2. to determine the overall success rates for touch DNA analysis with RapidHIT™ 200 and the percentage of donor alleles present in the profiles produced is presented in table 7, which also this illustrates the varying quality of the profiles generated from the touch DNA samples in this study. Despite this variability, it was found that 31.94% of the samples produced a profile with 80% or more of the donor alleles present and of these, 11.11% were full genetic profiles showing that 31.94 % of the samples produced a loadable DNA profile. Quinones and Daniel (2011) previously reported that the analysis of low-level samples such as touch DNA from handled items produces poor results with only 5-6% of the samples analysed producing a full genetic profile when analysed with AmpFℓSTR® NGM.

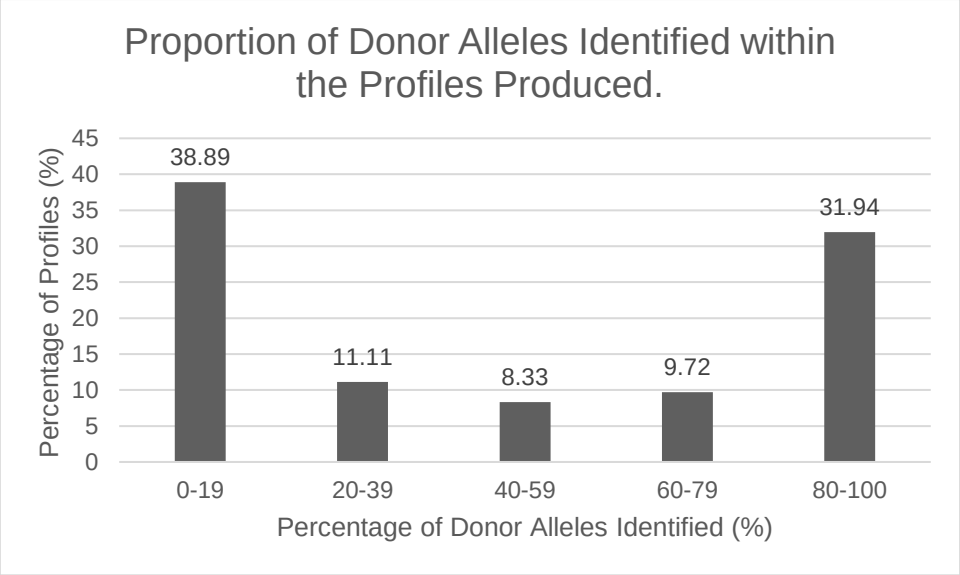


Figure 7. A chart displaying the proportion of donor alleles identified within the profiles produced from touch DNA samples analysed with RapidHIT™ 200.

Further to this, 31.94% of the samples analysed produced a profile of a quality appropriate for loading onto the National DNA Database (NDNAD). However, further to this, Gangano *et al*. (2013) suggest that samples with as few as 7 loci can be loaded onto a database for investigative leads which shows that a further 18.05% of the samples can be loaded onto the database for investigative leads. Overall, 50% of the profiles produced in this study could be loaded onto the NDNAD to aid investigations which is a substantial increase compared to the 5-6% of profiles that was previously reported by Quinones and Daniel (2012).

Further to this, the data produced in this study is further supported by Gangano *et al*. (2013) who suggested that similar low-level case samples could be analysed successfully with this instrument. In contrast to this Thong *et al*. (2015a) found that lower quantity samples produced poor results with only samples with a higher quantity of DNA such as blood and saliva producing good success rates with over 80% of the profiles being present. In a later study by Thong *et al*. (2015), it was suggested that a higher sensitivity is needed in order to

analyse low-level samples including touch DNA. They also advise that any urgent samples involving touch DNA should not be analysed with the RapidHIT™ systems.

While this study did produce some high-quality profiles, there was still the need for further interpretation using GeneMarker® HID v2.7.2 software. This analysis identified any stutter peaks present within the profiles produced along with the presence of any pull-up artefacts, which were identified through investigation of the peak heights and positioning of the artefacts to determine if they were true peaks or indeed artefacts. This is also discussed by Date-Chong, Hudlow and Buoncristiani (2015) who found that expert review is still required for the profiles produced with approximately 50% of the reference profiles they produced requiring further review by an expert. This suggests that while this system can be used for the analysis of touch DNA samples, further review by an expert would be required before these samples could be loaded onto a database and used to aid investigations.

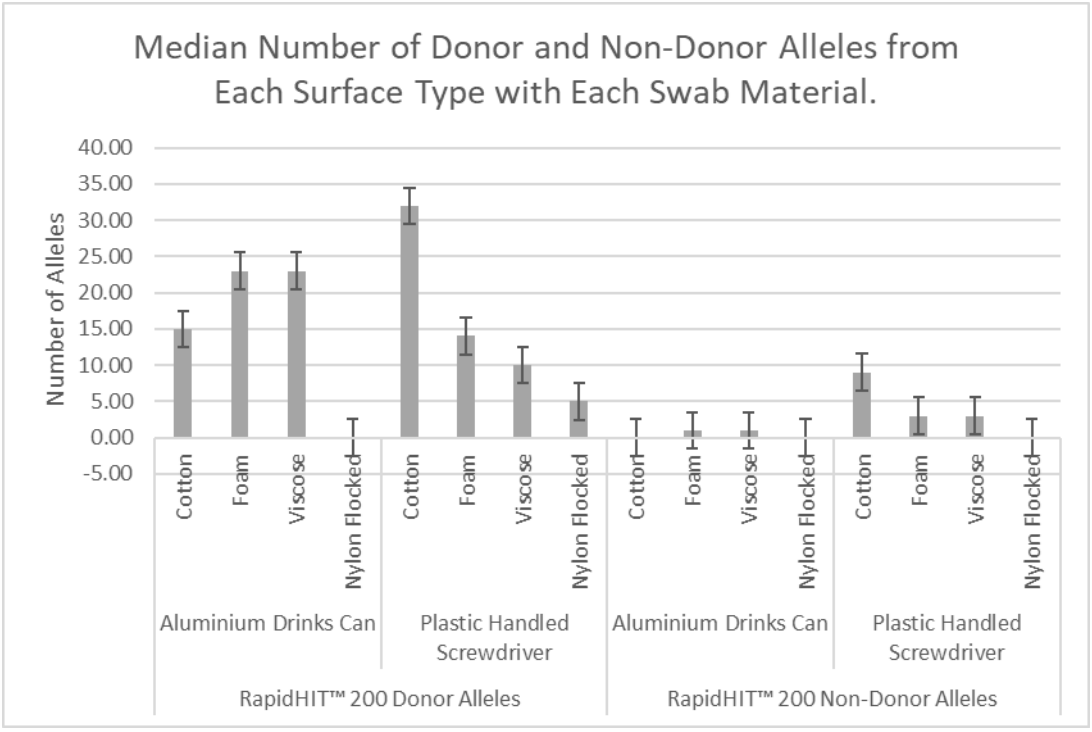


Figure 8. A chart displaying the median number of donor and non-donor alleles produced from each swab type on each surface when analysed with RapidHIT™ 200.

Additionally, a reduced amount of contamination and additional alleles was observed within the samples analysed using the RapidHIT™ 200 system, as only 1 sample contained alleles attributed to the researcher. In this study approximately 26.5% of the total alleles present were attributed to non-donor DNA when compared to reference samples from the donors as indicated in figure 8. This was also found in a study conducted by La Rue *et al*. (2014) who additionally found that any instances of contamination produced alleles with a lower intensity than is generally found in profiles which allowed this to be identified. This reduction in contamination, may be due to the minimal handling time required for analysis as described by Moreno, Brown and Callaghan (2017) as once the sample cartridges have been loaded into the instrument, no further human interaction is required with the samples unless these are being retrieved following analysis.

The completeness of the profiles varies greatly between the swabbing substrate used, surface type and the donors used. There are many factors that can influence the quality of the profile produced from handled items, as discussed in section 1.2.2 of this thesis. Another factor that can have an adverse effect upon the quality of the profile is the impact of the donor and the DNA that they shed upon the surface. This factor has been discussed in research conducted by Lowe *et al*. (2002) who concluded that individuals differ in their tendency to deposit DNA upon surfaces. However, they do identify that the reason for this difference is unknown but there may be a correlation between a donor's shedder status and the chemicals present within the skin secretions. Further to this, Kanokwongnuwut *et al.* (2018) also found that assigning a shedder status is more continual which is in line with the findings of Phipps and Petricevic (2007) who suggested that assigning shedder status was more complex than simply a good and poor shedder. This may have influenced the variation observed in this data however, consideration to shedder status was not included in an attempt to keep this study more mock-operational.

3.4. Conclusion and Further Work

The analysis of touch DNA with the RapidHIT™ 200 instrument was evaluated throughout this study to determine its appropriateness. This study found that more donor alleles were recovered from the plastic handled screwdrivers than from the aluminium drinks cans which suggests that the nature and texture of the surface may impact the propensity to recover touch DNA from the surface which is supported by Oldoni, Castella and Hall (2016). However, it was also found that the number of donor alleles recovered from each surface varied depending upon the swabbing material that was used, with more donor alleles being recovered from the aluminium drinks cans with the foam and viscose swabs. Additionally, more donor alleles were recovered from the plastic handled screwdrivers when using the cotton and nylon flocked swabs. This suggests that the interaction of the swabbing substrate with the surface type may directly impact the efficiency of the recovery of touch DNA. This was previously reported by a number of researchers however, the reason for this is yet unknown with conflicting results being published (Verdon *et al*, 2014; Oldoni, Castella and Hall, 2016; Daly, Murphy and McDermott 2012; Pamela *et al*. 2015; Hess and Haas, 2017).

It can also be observed from figure 8 that there were more non-donor alleles recovered from the plastic handles screwdrivers than from the aluminium drinks cans. However, overall the majority of samples were found to either be single source or with the donor being the major contributor to the profile. The difference in the sample deposition method between the aluminium drinks cans and plastic handled screwdrivers is likely to be a contributing factor to more non-donor alleles being found on the plastic handles screwdrivers as these were regularly used by the donor over a 7 day period. Similar findings were reported by Rolo *et al.* (2019) who found that background or non-donor DNA was found on regularly used items when donors shared an office space which was also the case for the data produced in this chapter. This further emphasises the need for caution when interpreting the profiles from touch DNA evidence (van Oorschot *et al.* 2019) as this indicates that indirect transfer can

occur readily on regularly used items particularly when the donor is within close quarters with other individuals such as in a work environment.

Additionally, this study found that cotton and foam swabbing materials perform statistically better when recovering touch DNA than nylon flocked swabs. Whereas other swabbing materials such as cotton, foam and viscose show no statistical difference which is in contrast with research published by Verdon *et al*. (2014) whereby they found that nylon flocked swabs were more effective. This suggests that further research is needed in this area to determine if a difference can be discerned between swabbing substrates and why this difference may be observed. There has been some suggestion as to this by a number of researchers but a consensus has not been reached (Verdon *et al*. 2014; Brownlow *et al*. 2012; Bruijns, Tiggelaar and Gardeniers, 2018).

Gangano *et al.* (2013) reported that a profile with a minimum of 7-10 loci present were above the threshold for uploading to the NDNAD for investigative leads. Based upon this threshold, throughout this study it was determined that approximately 50% of the samples analysed produced profiles that would be eligible to be loaded onto the NDNAD in order to aid in forensic investigations (Gangano *et al*. 2013). This is a significant increase when compared to previously reported success rates of 5-6% of samples analysed producing a full genetic profiles (Quinones and Daniel, 2011). Further to this, 11% of the samples analysed in throughout this study produced a full genetic profile which again is a substantial increase compared to the rates published by Quinones and Daniel (2011) and comparable to success rates published by Mapes, *et al.* (2016). This overall suggests that touch DNA may be analysed using the RapidHIT™ 200 instrument which in turn may lead to the potential of analysing this evidence type with other rapid DNA profiling technologies.

The results of this study will be used to inform the optimal recovery strategy for touch DNA from different surface types. In order to do so, this research will be expanded further to evaluate the interactions that occur between the swabbing substrate, surface type and the

DNA present in order to better understand the way in which this evidence type should be recovered to maximise efficiency. However, as nylon flocked swabs performed poorly on both surface types in comparison to the other swab substrates, this swabbing material will not be taken forwards for the remainder of this research into effective touch DNA recovery. This study succeeded in demonstrating that comparable success rates can be obtained for the analysis of touch DNA with Rapid DNA Analysis as similar success rates were obtained to those published by Mapes *et al.* (2017). This has the potential to greatly aid in forensic investigations by allowing more real-time results and investigative leads to be obtained and should be considered for use on evidential items moving forwards.

Chapter 4: Re-Analysis of Touch DNA with NGM SElect™ Following Analysis with RapidHIT™ 200

4.1. Introduction

The benefits of Rapid DNA analysis have been discussed in section 1.4. of this thesis, but there are additional benefits that are yet to be addressed which have the potential to greater aid the investigative process. One such benefit is the ability to retrieve a swab once analysed to be reanalysed with another instrument. This could be done in cases of experimental failure as described by Wiley *et al.* (2017) or to reanalyse samples using standard processes if further detailed analysis is needed. Wiley *et al.* (2017) describe how only a small portion of the sample DNA is removed from the swab for analysis via Rapid DNA methods which leaves a large proportion of this genetic material behind which can be used for re-analysis. They also found that good success rates and concordance was found when these samples were reanalysed with another Rapid DNA instrument which further demonstrates that this reanalysis is possible with minimal impacts to the overall quality of the DNA profiles produced. However, not all cartridges used in Rapid DNA analysis allow this sample retrieval and reanalysis. For instance, the EXT cartridges described by Amick and Swiger (2019) require small volumes of extracted DNA to be inputted rather than a swab. In these cases, to allow analysis using more than one instrument or method some extracted DNA would need to be retained prior to loading the cartridges as this would not be retrievable post analysis. Mapes *et al.* (2016) discuss that while the early indicative results from Rapid DNA analysis can have great benefits to an investigation, as the sample is partly consumed there is also potential for sample loss so it is important to select the correct samples to analyse using these methods. Should the sample be of sufficient quantity, it would be possible to reanalyse this sample using standard laboratory-based methods as discussed by Wiley *et al.* (2017) in relation to the ability to retrieve a swab to be reanalysed.

It was reported by Mapes *et al.* (2016) that there have been conflicting reports on what sample input is needed for Rapid DNA analysis to deliver a high-quality profile so it is currently unclear if these instruments can deliver comparable sensitivity to traditional benchtop methods. Additionally, Mapes *et al.* (2016) did propose that as with traditional methods, the success rates are related to the concentration of the DNA in the sample, those samples that have low quantities and poor success rates with the more sensitive benchtop methods would not be appropriate for analysis with these mobile Rapid DNA instruments. However, in their study they only hypothesised what results could be obtained using Rapid DNA analysis by using sensitivity values of 100pg/µL and 25pg/µL rather than analysing the samples using a Rapid DNA instrument so it is unclear whether this hypothesis would be accurate if the analysis was conducted.

To aid in determining what samples should be taken forward for full laboratory analysis, Dawnay *et al.* (2014) discussed using Rapid DNA analysis as a screening tool as only a small portion of the available DNA is used and these methods and typically more cost effective and can give a better visualisation on what DNA is present within a sample. They found that when using such a screening system there was no statistically significant difference between the quantification data of a sample that had been screened using Rapid DNA analysis and a sample that had no screening process. This supports the suggestion by Wiley *et al.* (2017) that this Rapid DNA analysis does not have to be the end of the sample analysis and that if required these samples could be analysed again. This would also negate the concerns raised by Mapes *et al.* (2016) around sample loss should the incorrect analysis method be selected based on the difference in sensitivity between the Rapid DNA analysis and standard benchtop protocols as there is no significant sample loss when using these instruments as a screening tool. This allows quicker investigative leads to be obtained using the Rapid DNA analysis instruments while also allowing for the possibility for further analysis. However, Dawnay *et al.* (2014) did find that 16.87% of the touch DNA samples produced a false negative when analysed using the Rapid DNA screening method in

comparison to the standard laboratory practice which suggests this may not be applicable for samples of a lower quantity. Since this study by Dawnay *et al.* (2014) was conducted the sensitivity of these instruments has been improved as discussed by Turingan *et al.* (2016) so these conclusions likely need to be re-evaluated using the more sensitive instruments available to determine if these samples are more concordant between rapid DNA analysis and more sensitive laboratory processes.

The remainder of this chapter aims to compare the sensitivity of Rapid DNA analysis methods with the standard laboratory processes in relation to the analysis of touch DNA samples to determine if the results are concordant and if it is possible to utilise Rapid DNA analysis for such low-level samples.

4.2. Materials and Method

Following analysis with the RapidHIT™ 200 instrument described in section 3.2.2. of this thesis, the samples were retrieved from the RapidHIT™ 200 cartridges by opening the seal using a sterile scalpel using a cross incision and the swabs were then retrieved using sterile tweezers and placed into a 1.5ml Eppendorf tube to be taken for reanalysis with AmpFℓSTR® NGM SElect™. These samples were then reanalysed following the DNA Extraction, Quantification and Profiling steps outlined in chapter 2. The profiles were then interpreted using the GeneMapper™ software using a threshold of 150 RFU which is the optimal RFU for this analysis.

4.3. Results and Discussion

4.3.1. Comparison of Impact of Surface Types on Success Rates

Surface Type	N (∑=72)	Quantification ng/µL
Aluminium Drinks Can	36	0.011
Plastic Handled Screwdriver	36	0.000

Table 8. Mean quantification data of samples collected from aluminium drinks cans and plastic handled screwdrivers.

Following the retrieval of the samples analysed with the RapidHIT™ 200 instrument these were re-extracted and quantified. Upon the analysis of this quantification data, it was found that the data was non-normally distributed using a Kolmogorov-Smirnov test for normality (p=0.000, p=0.000) and a statistically significant difference (p=0.000) was found between the quantity of DNA collected from the two surface types using a Wilcoxon-Signed Rank test, with a greater quantity of DNA being recovered from the aluminium drinks cans than on the plastic handled screwdrivers, which had a small effect size (0.37) using a Cohen's d test for effect size. However, as this quantification data was obtained post-analysis using the RapidHIT™ 200 where some of the sample recovered on the swab is consumed (Mapes *et al.* 2016) it is unclear what quantity of DNA may have been present on these samples at the time of deposition and initial analysis.

Additionally, it can be seen that despite having an average quantification of 0.00 ng/µL from plastic handled screwdrivers (table 8), profiles were still able to be obtained using both RapidHIT™ 200 and NGM SElect™ with an average of 26.14 and 24.08 alleles being called respectively from samples collected from these surfaces. Similar findings were obtained by Haas *et al.* (2015) who found that the quantification data for their samples did not appear to correlate input DNA for those samples as it suggested minimal biological material being present despite a higher input of DNA for the sample in question. This suggests that the quantification data may not be accurately capturing all of the profilable DNA within a sample

which is likely why a high number of alleles can be detected from samples with a small DNA quantification value.

Surface Type	N (∑=72)	Median Alleles per Profile with RapidHIT™ 200	Median Donor Alleles per Profile with RapidHIT™ 200	Median Alleles per Profile with NGM SElect™	Median Donor Alleles per Profile with NGM SElect™
Aluminium Drinks Can	36	10.50	10.00	27.00	9.00
Plastic Handled Screwdriver	36	20.00	18.00	19.00	9.00

Table 9. Median number of alleles and donor alleles per profile called using RapidHIT™ 200 at 50 RFU and using NGM SElect™ at 150 RFU for each surface type.

The samples retrieved from the RapidHIT™ 200 instrument were profiled using NGM SElect™ with a threshold of 150RFU to determine if there is a difference in terms of the profile completeness between these methods and if reanalysis is possible. There is a difference in the threshold frequencies used for RapidHIT™ 200 and NGM SElect™ which may influence this data however, it is important to compare this data using each method's optimal analytical threshold so as not to compromise the integrity of the data. Upon statistical analysis, it was determined that for both surface types the data was non-normally distributed across both the total number of alleles produced (p=0.000, p=0.012) and the number of donor alleles produced (p=0.000, p=0.014) using the Kolmogorov-Smirnov test for normality. Due to this a Wilcoxon Signed Rank test was completed to assess if any statistically

significant differences were present between the initial analysis and the reanalysis of these samples.

For samples collected from the aluminium drinks cans, it was found that there was a statistically significant difference between the total number of alleles called between the two analyses with more alleles being detected in the profiles produced from the reanalysis of these samples with NGM SElect™ ($p=0.042$), which was determined to be a low effect size (0.39) using a Cohen's d test for effect size. However, when looking specifically at the donor alleles detected there was no statistically significant difference between the profiles produced using the RapidHIT™ 200 instrument or the laboratory-based reanalysis with NGM SElect™ ($p=0.064$). This suggests that while the reanalysis of these samples produced more data due to the increased sensitivity of these traditional laboratory-based methods in comparison to Rapid DNA analysis (Thong *et al.* 2015), these additional non-donor alleles were as a result of contamination or background DNA rather than the DNA deposited by the donor which suggests that this additional sensitivity may not be providing further intelligence around the sample in relation to who handled the item in question. This could be attributed to the difference in laboratory environment, as the DNA recovery and loading of the RapidHIT™ 200 cartridges took place within Staffordshire Police Headquarters clean room, whereas the subsequent analysis of the sampled with NGM SElect™ was conducted within a fume cupboard which had been deep-cleaned to reduce any potential for contamination, however this was housed within a functional teaching lab so background DNA may have been gathered such as described by Port *et al.* (2005) and Finnebraaten *et al.* (2008).

When looking at samples collected from plastic handled screwdrivers, there was no statistically significant difference found between the total alleles or donor alleles produced in the initial analysis and reanalysis of the samples ($p=0.831$ and $p=0.086$ respectively). This shows that for samples collected from this surface type, reanalysis of the samples produced similar data demonstrating that sufficient DNA is retained on the swab post-analysis with the

RapidHIT™ 200 instrument to produce comparable data with the more sensitive NGM SElect™ method.

Previously Wiley *et al.* (2017) found that the reanalysis of samples with another Rapid DNA analysis instrument produced concordant results which suggested that this reanalysis was possible without loss of data from these samples. However, Dawnay *et al.* (2014) found that there was a 16.87% rate of false negatives when samples were analysed with both Rapid DNA instruments and standard laboratory methods. The data produced here is aligned with that of Wiley *et al.* (2017) as despite more total alleles being detected from the aluminium drinks cans when analysed with NGM SElect™ there was no statistically significant difference found in the remainder of the data when looking specifically at the surface types used. However, Dawnay *et al.* (2014) did not reanalyse the same samples with the two methods but used repeat samples in each method which may attribute to some of these differences as there can be variation with the results produced from a similar sample type.

Furthermore, Mapes *et al.* (2016) suggested that samples collected from drinking items have a greater potential for successful Rapid DNA analysis than samples such as a screwdriver. This is in contrast to the data presented in this study as despite more alleles being detected in the reanalysis of the samples collected from the aluminium drinks cans, more alleles and donor alleles were produced from the samples collected from the plastic handled screwdrivers when analysed using Rapid DNA analysis. This suggests that the data we have for standard profiling methods may not be applicable to Rapid DNA analysis and therefore should be used with caution when determining if these samples should be processed using Rapid DNA analysis methods. Additionally, as there were statistically no fewer alleles detected when samples from either surface type were reanalysed with NGM SElect™, this demonstrates that it is possible to reanalyse samples from aluminium drinks cans and plastic handled screwdrivers after analysis with Rapid DNA instruments without a loss of data which poses great investigative benefits by reducing the initial analysis time to provide profile information which may aid the development of a criminal investigation. Murakami *et al.*

(2020) discuss how being able to analyse evidential samples using Rapid DNA technologies has the potential to not only reduce the analysis times but also reduce the pressure faced by laboratories with labour shortages as the process of this analysis requires less physical interaction. The data produced in this study and in chapter 3 of this thesis supports the analysis of evidential samples using Rapid DNA analysis as it is not only possible to generate good success rates as detailed in chapter 3 but also to reanalyse these samples with standard profiling methods following their initial analysis and achieve comparable results.

4.3.2. Comparison of Impact of Swab Materials on Success Rates

Further to reviewing the differences with the data produced from each surface type when analysed with the RapidHIT™ 200 instrument and laboratory based NGM SElect™, differing swab materials were also assessed to determine if this influences the ability to reanalyse these samples.

Swab Material	**N (∑=72)**	**Quantification ng/µL**
Cotton	18	0.013
Foam	18	0.000
Nylon Flocked	18	0.002
Viscose	18	0.007

Table 10. Mean quantification data of samples collected using cotton, foam, nylon flocked and viscose swabbing materials.

The quantification data produced per swab type was found to be non-normally distributed with no statistically significant difference being present between the amount of DNA collected using each of these swabbing materials when using a Kruskal-Wallis test (p=0.256). However, when looking at the average quantification data per swab displayed in table 10, it can be observed that more DNA was present post-analysis with the RapidHIT™ 200 instrument on the cotton swabs which suggests that these swabs retain more DNA during the analysis process than the other swab types examined in this study.

Analysis Method	Total Number of Alleles	Total Number of Donor Alleles	Total Number of Non-Donor Alleles
RapidHIT™ 200	1523	1120	403
NGM SElect™	1760	830	930

Table 11. Total number of alleles, donor alleles and non-donor alleles produced using RapidHIT™ 200 (50 RFU) and NGM SElect™ (150 RFU) profiling methods.

Further to this, the profile data produced from each of these swabbing materials was assessed and compared between the two profiling methods which can be observed in table 12. Initially the overall total alleles and donor alleles were assessed to determine if this data is concordant between the two analysis methods. This data was non-normally distributed using a Kolmogorov-Smirnov test for normality (total alleles: p=0.000, p=0.003; donor alleles: p=0.000, p=0.014) and using a Wilcoxon Signed Rank test, it was found that there was no statistically significant difference between the total alleles or donor alleles produced with the RapidHIT™ 200 instrument or the laboratory-based NGM SElect™ method (p=0.234 and p=0.069 respectively). This suggests that when using the optimal analytical thresholds, the results produced with Rapid DNA technologies are comparable with those produced using

standard NGM SElect™ analysis. This provides further support that it is possible to analyse touch DNA samples with Rapid DNA technologies and that the success rates produced are comparable with those produced using NGM SElect™ analysis. Additionally, as the samples can be reanalysed with no statistically significant difference this suggests that further analysis can still be conducted following analysis with Rapid DNA technologies where needed. Mapes *et al.* (2016) expressed concerns over the use of Rapid DNA analysis for low-level samples due to the potential for sample loss during this analysis. However, the data produced in this study demonstrates that even with an element of sample loss, it is still possible to reanalyse these samples using standard laboratory processes and produce results with no statistically significant difference.

Swab Material	**N (∑=72)**	**Median Alleles per Profile with RapidHIT™ 200**	**Median Donor Alleles per Profile with RapidHIT™ 200**	**Median Alleles per Profile with NGM SElect™**	**Median Donor Alleles per Profile with NGM SElect™**
Cotton	18	25.5	24.5	5.0	2.5
Foam	18	25.5	17.5	21.5	11.5
Nylon Flocked	18	1.5	0.0	19.0	8.0
Viscose	18	19.0	15.0	38.5	19.5

Table 12. Median number of alleles and donor alleles per profile called using RapidHIT™ 200 at 50 RFU and using NGM SElect™ at 150 RFU for each recovery method.

As the data shows a large range of variability (table 12), this data was further broken down to compare the difference between the total and donor alleles detected per swab type using the different analysis methods.

The samples collected using cotton swabs were non-normally distributed, using a Kolmogorov-Smirnov test for normality (p=0.156, p=0.018), when looking at the total number of alleles detected however, no statistically significant difference was found using a Wilcoxon Signed Rank test between the number of alleles detected using the RapidHIT™ 200 instrument or the NGM SElect™ method (p=0.088). However, the number of donor alleles called with each profiling method was normally distributed (p=0.156, p=0.059) and showed a statistically significant difference using a Paired t-test (p=0.000) with more donor alleles being detected with the initial analysis with the RapidHIT™ 200 instrument than the reanalysis with NGM SElect™, with a large effect size (1.18) using a Cohen's d test for effect size. This demonstrates that despite the cotton swabs having a higher quantification value than other swab materials, a significant amount of those donor alleles are lost when the sample is reanalysed. This may be due to this part of the sample being consumed with during the Rapid DNA analysis as discussed by Mapes *et al.* (2016) or alternatively, it may be as a result of that additional DNA remaining trapped within the fibres of the swab so these are not fully released with that second analysis which is also discussed by Marshall *et al.* (2014).

When looking at the samples collected using foam swabs, the total and donor alleles displayed normal distribution, using a Kolmogorov-Smirnov test for normality (total alleles: p=0.124, p=0.200; donor alleles: p=0.076, p=0.200). Similarly to the samples collected with cotton swabs, the samples collected with foam swabs had no statistically significant difference between the number of total alleles detected with both profiling methods when using a Paired t-test (p=0.852). However, a statistically significant difference was found when looking at the number of donor alleles detected using a Paired t-test (p=0.025). It was determined that more donor alleles were also produced when the initial analysis with the

RapidHIT™ 200 instrument was conducted in comparison to the reanalysis using NGM SElect™, however this had a low effect size (0.16) using a Cohen's d test. In contrast with the samples collected with cotton swabs, the samples collected using the foam swab had an average quantification value of 0.00 ng/µL when this was obtained post-analysis with RapidHIT™ 200. This suggests that for this swab material, a large amount of the DNA on the swab is released during that initial analysis rather than being retained in the swab which suggests this swab material has a good potential to release the cellular material collected rather than this being trapped within the fibres of the swab head which is one of the factors discussed by Mulligan *et al.* (2011) in relation to the success of a recovery method.

Samples collected using nylon flocked swabs were also analysed and displayed non-normal distribution across both the total number of alleles collected and the donor alleles collected using a Kolmogorov-Smirnov test (total alleles: p=0.000, p=0.0128; donor alleles: p=0.000, p=0.189). A Wilcoxon Signed Rank test was completed on both the number of total alleles detected and also the number of donor alleles detected and it was found that there was a statistically significant difference between the total number of alleles called with each profiling method with more alleles being detected with the reanalysis of these samples with NGM SElect™ (p=0.025), which had a medium effect size (0.61) using a Cohen's d test. However, there was no statistically significant difference found between these analysis methods when looking at the number of donor alleles collected (p=0.054). This suggests that while more information is gained from that reanalysis of the samples using the more sensitive NGM SElect™ this information does not necessarily provide insight into the handler of the object but instead provides more information on the background DNA present on the item or contamination obtained during the analysis process. As there are more non-donor alleles being identified with this reanalysis, it may suggest that the minimal handling time needed for Rapid DNA analysis is reducing the level of contamination and background DNA within the samples as discussed by Moreno, Brown and Callaghan (2017). Furthermore, this background DNA or contamination may then be being reintroduced when these samples are

being analysed again within a standard laboratory environment with more hands-on steps being involved providing a greater opportunity for the samples to be exposed to contamination. In contrast with the data produced in this study, when looking at buccal samples collected using different swabbing mediums and analysed using Rapid DNA instruments Moreno, Brown and Callaghan (2017) found that nylon flocked swabs had higher success rates than cotton swabs. This suggests that while nylon flocked swabs may perform well with Rapid DNA analysis instruments in relation to higher quantity samples, this does not correlate with lower quantity samples as more alleles were detected when these samples were reanalysed with more sensitive traditional analysis methods than with Rapid DNA analysis.

When looking at the samples collected using a viscose swabbing material, the total number of alleles detected with both profiling methods was found to be normally distributed ($p=0.052$, $p=0.200$) with no statistically significant difference being observed with a Paired t-test between the number of alleles called with either method ($p=0.054$). However, the data around the number of donor alleles produced was non-normally distributed ($p=0.010$, $p=0.200$), yet a Wilcoxon Signed Rank test demonstrated there was also no significant difference between the number of donor alleles called with either the RapidHIT™ 200 instrument or the more sensitive NGM SElect™ method ($p=0.0427$). The viscose swabbing material also displayed an average quantification value of 0.007 ng/µL which suggests similarly to the cotton swab that some of the biological material collected with this swab type is retained within the fibres of the swab head allowing for sufficient DNA to be extracted during the reanalysis process.

Despite there being no statistically significant difference found between the overall total number of alleles and the number of donor alleles produced either with the initial Rapid DNA analysis or the subsequent reanalysis with NGM SElect™, it was found that there were some differences based upon the recovery method used. Based on this information, it may not be appropriate for the reanalysis of samples from all recovery methods. For instance,

cotton and foam swabs had statistically fewer donor alleles when the samples were reanalysed than with the initial Rapid DNA analysis. This suggests that while a concordant number of total alleles was detected for these recovery methods, these may consist more of contamination or background DNA than that of the individual who handled the items. The study conducted by Wiley *et al.* (2017) which determined it was possible to reanalyse samples using another Rapid DNA instrument was in relation to buccal samples collected using a cotton swab. As a larger amount of DNA is retained in a cotton swab, as demonstrated from the quantification data in table 10, it may be that with a higher quantity sample like a buccal sample this sample loss during the initial analysis does not have a negative impact on the reanalysis of the same sample. In contrast to this, nylon flocked swabs were suggested by Moreno, Brown and Callaghan (2017) to perform better with Rapid DNA analysis than cotton swabs however, the data produced in this study contradicts this as there were more total alleles detected in the reanalysis of these samples using NGM SElect™. The only recovery method used in this study which did not have a statistically significant difference between analysis methods was the viscose swab which suggests that this swab has a good balance between its absorption properties and ability to release DNA from within its fibres which Mulligan *et al.* (2011) discussed was often unbalanced in many recovery methods. This suggests that the optimal recovery method for the reanalysis of touch DNA samples with NGM SElect™ following Rapid DNA analysis would be a viscose swab. However, in chapter 3 it was found that this swabbing material was not as efficient as recovering touch DNA from a more textured surface such as the plastic handled screwdriver.

4.4. Conclusion and Further Work

The aim of this study was to compare the sensitivity of Rapid DNA analysis methods with the standard laboratory processes in relation to the analysis of touch DNA samples to determine

if the results are concordant and if it is possible to utilise Rapid DNA analysis for such low-level samples. In order to assess this, touch DNA samples that were previously analysed with the RapidHIT™ 200 instrument in chapter 3 of this thesis were reanalysed using NGM SElect™ and the results were reviewed to determine if a difference was observed between the methods.

Overall, there was no statistically significant difference found between the number of donor alleles or total number of alleles produced from the analysis of touch DNA samples with the RapidHIT™ 200 instrument and reanalysis with NGM SElect™ which demonstrates that it is possible to reanalyse these samples without loss of data. Despite this, it was also found that more total alleles were detected from aluminium drinks cans with NGM SElect™ than with Rapid DNA analysis. Additionally, it was found that there were some statistically significant differences between the different recovery methods utilised in this study with cotton and foam swabs producing fewer donor alleles and nylon flocked swabs producing more total alleles with NGM SElect™ than with the RapidHIT™ 200 instrument. No other statistically significant differences were found with the remaining comparisons.

While the overall data is in accordance with Wiley *et al.* (2017) and suggests that samples can be reanalysed without any significant data loss, the data varies depending upon the surface type and recovery method used. For all surface types, there were statistically no fewer alleles with NGM SElect™ than with RapidHIT™ 200 which demonstrates that despite there being some differences with this data, there is no significant data loss as there is no fewer alleles being produced at reanalysis and there is no statistically significant difference between the donor alleles detected with each analysis method which indicates that the additional data gained is in relation to background DNA or contamination from the more hands-on approach utilised within the laboratory. However, there were statistically fewer donor alleles detected when samples collected with cotton and foam swabs were reanalysed with NGM SElect™ after initial analysis with the RapidHIT™ 200 instrument. This demonstrates that some of the data can be lost during the Rapid DNA analysis which was

proposed by Mapes *et al.* (2016). However, as this data is initially detected using the Rapid DNA analysis, the profile information is not lost and therefore the touch DNA samples obtained from these swab types could still be analysed using Rapid DNA analysis but further reanalysis may not provide additional information.

In contrast to Shackleton *et al.* (2019) who suggested that higher quantity samples would produce good quality profiles that are comparable to manual processing, in this study, profiles were obtained from samples which produced a 0 pg/µL quantification value which suggests that the quantification data should not be used as a clear indicator for the success of samples analysed with Rapid DNA instruments. While it is unclear what quantity of DNA was present in these samples prior to analysis with the RapidHIT™ 200 instrument, it can be seen that the quantity of DNA post-analysis was very varied between samples with an overall average of 0.0000001 ng/µL. However, as discussed by Haas *et al.* (2015) the quantification data does not necessarily correlate with the quality of the profile produced or the input amount of DNA. This further supports the suggestion that quantification data should not be used as an indicator for the success of the profiling data for a sample.

Overall, the data produced in this study provides strong support that touch DNA samples can not only be analysed using Rapid DNA instruments but also reanalysed with standard laboratory processes with limited loss of profile data between analysis methods demonstrating that Rapid DNA analysis could be used as a primary source of analysis, a screening method or a first step in the DNA analysis to gain information in a timelier manner to aid in the investigative process. However, as there were some differences observed with the samples collected using cotton and foam swabs with fewer donor alleles being detected when these samples were reanalysed using NGM SElect™ it may be prudent to assess further recovery methods to determine the optimal methods for reanalysis following Rapid DNA analysis.

Chapter 5. Analysis of Touch DNA Using NGM SElect™

5.1. Introduction

The impact that the recovery method and technique has upon the analysis of touch DNA has been discussed in section 1.3. of this thesis. While it is important to use the correct recovery method for the evidence type to maximise the amount of DNA recovered for analysis (Mulligan *et al.* 2011; Hess and Haas, 2017; Aditya *et al.* 2011; May and Thomson, 2009), it is also important to take into consideration the surface that this evidence is being recovered from as this also has the potential to impact the success of any subsequent DNA analysis.

Overall, the published data produced to date has begun to suggest optimal recovery methods based upon surface type or evidence type but extensive research into an optimal recovery strategy for touch DNA has not yet been published. Due to this, the remainder of this chapter aims to compare the quantitative and qualitative data produced from the analysis of touch DNA samples to determine the impact that the recovery method and surface type has upon the resulting data. This data will be used to inform an optimal recovery strategy for touch DNA evidence from a range of surface types.

5.2. Materials and Method

5.2.1. DNA Recovery

Touch DNA samples were deposited upon aluminium drinks cans (n=32), plastic handled screwdrivers (n=32), drinking glasses (n=32) and wooden handles (n=32) as outlined in section 2.3.2. and duplicated following section 2.3.3. with a touch DNA being deposited on a further 32 of each surface type. Information regarding the participants utilised in these studies can be observed in section 2.2.2. and a breakdown of the replicates can be found in section 2.3.1.

These samples were recovered from the surfaces using cotton swabs, foam swabs, polyester swabs and rayon swabs which were selected due as they are reportedly the same as viscose swabs (Bruijns, Tiggelaar and Gardeniers, 2018) but are more cost effective. Unlike in the studies in chapter 3, there were no restrictions related to the number of swabs that could be used so the items were swabbed in their entirety using a single swab moistened with 2 drops of sterile distilled water followed by a single dry swab using moderate pressure with a rotating motion. The swabs were stored at room temperature prior to use and were kept sterilised to reduce the potential contamination. Once the DNA had been recovered from the surface these were transferred to a sterile 1.5ml Eppendorf tube for processing.

5.2.2. DNA Analysis

The recovered DNA from section 5.2.1. was then processed following the DNA Extraction, DNA Quantification and DNA Profiling steps outlined in chapter 2, however, no dilutions of the samples were made in order to assess the concordance between the quantification and profile data. The profiles were then interpreted using the GeneMapper™ software using the optimal analytical threshold of 150 RFU.

5.3. Results and Discussion

5.3.1. Assessment of the Impact of Surface Type on the Success Rates of Touch DNA Analysis with NGM SElect™

5.3.1.1. Phase 1 – Controlled Study

Touch DNA samples were deposited upon and recovered from four different surface types and analysed as described in section 5.2. The amount of DNA recovered from these surfaces varied as displayed in table 13.

Surface Type	**N (∑=128)**	**Quantification ng/µL**
Aluminium Drinks Can	32	0.008
Drinking Glass	32	0.006
Plastic Handled Screwdriver	32	0.001
Wooden Handles	32	0.010

Table 13. Median quantification data of samples collected from aluminium drinks cans, drinking glasses, plastic handled screwdrivers and wooden handles.

Statistical analysis using a one-sample Kolmogorov-Smirnov test demonstrated that the data was non-normally distributed (p=0.000, p=0.000, p=0.000, p=0.000). Further analysis using a Kruskal-Wallis test found that a statistically significant difference was present between the amount of DNA recovered from each surface (p=0.005) with a higher amount of DNA being collected from the plastic handled screwdriver than the wooden handles (p=0.007), with a small effect size (0.16) using a Cohen's d test. No further significant differences were displayed between the remaining surface types. As the wooden handles were an absorbent wood due to being untreated, this may be a factor in why less DNA was recovered from these samples than from the plastic handled screwdrivers as some of the DNA may be

absorbed into the surface of the wood during the deposition or recovery process. Similar findings were discussed by Hedman *et al.* (2021) who found that a greater DNA yield was obtained from ridged plastic than from absorbent wood which suggests that absorbent wood may not retain touch DNA on its surface as well as other substrates. However, this data is contrasting with the data published by Daly, Murphy and McDermott (2012) who found that a higher quantity of DNA was obtained from wooden surfaces in comparison to glass and fabric surfaces. The reason for this difference may be due to the differing deposition protocols used as Daly, Murphy and McDermott (2012) had only a 60 second handling time for all samples whereas this study was designed to replicate regular use of these items and therefore had a prolonged handling time in comparison which is supported by Oldoni, Castella and Hall (2016) who found that the proportion of DNA on an object increased with handling time.

As quantification data does not always correlate with the data obtained during DNA profiling (Haas *et al.* 2015) it is important to also assess the success of the DNA profiling from these surfaces to determine if more valuable profile information can be obtained from a certain surface type. Therefore, the profile data was also assessed in relation to the total alleles produced and also the number of donor alleles produced, displayed in table 14. It was found that both sets of data were non-normally distributed (total alleles: p=0.200, p=0.200, p=0.200, p=0.007; donor alleles: p=0.184, p=0.006, p=0.120, p=0.032) and no statistically significant differences were found between the total number of alleles or number of donor alleles recovered from the different surfaces (p=0.487 and p=0.199 respectively). This suggests that despite the difference found in the quantification data with a lower quantity of DNA being obtained from the wooden handles than the plastic handled screwdrivers, comparable DNA profiles were produced from all surfaces reviewed.

Surface Type	**N (∑=128)**	**Median Number of**	**Median Number of**	**Median Number of**

		Total Alleles per Profile	Donor Alleles per Profile	Non-Donor Alleles per Profile
Aluminium Drinks Can	32	29.50	17.50	8.00
Drinking Glass	32	21.50	9.50	7.50
Plastic Handled Screwdriver	32	22.00	11.00	7.50
Wooden Handles	32	34.00	24.00	9.00

Table 14. Median number of alleles, donor alleles and non-donor alleles detected per profile obtained for each surface type following the phase 1 sample deposition protocol.

While no statistically significant differences were found between the profiles obtained from each of the surface types used in this study. It can be seen that, the least donor alleles were obtained from the plastic handled screwdrivers which demonstrates that despite this mock evidential item generating a higher quantity of DNA than the wooden handles, this did not translate into the profiling data with the wooden handles generating the most alleles and donor alleles. As it remains unclear which part of touch DNA deposits contribute towards the DNA profiles as discussed in section 1.2.1. of this thesis, it could be hypothesised that the DNA being quantified during the quantitative PCR process is not the element of the touch DNA deposit that generates the profile information which would explain why the quantification and DNA profiling data produces conflicting results. Haas *et al.* (2015) also found when looking at quantification data that the samples with a high input amount did not always provide high quality results which further supports this.

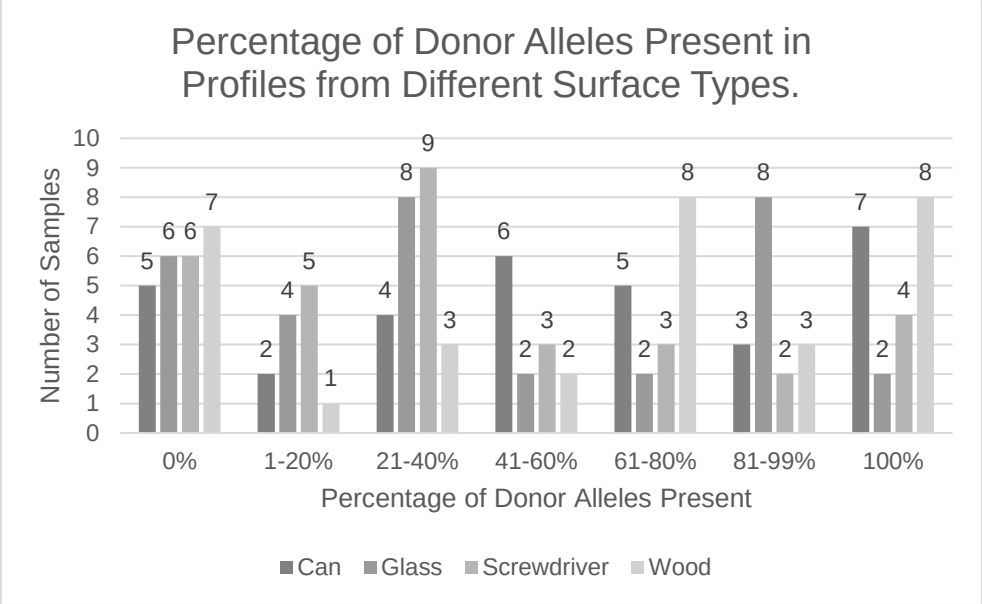


Figure 9. A graph displaying the percentage of donor alleles present within the profiles produced from touch DNA samples deposited on different surface types following the phase 1 deposition method.

Furthermore, it was reviewed to determine the percentages of the donor profile present from each surface which is displayed in figure 9. From this it can be seen that the largest amount of complete donor profiles were obtained from the can and wooden surfaces which conflicts with the findings of Hedman *et al.* (2021) who found that ridged plastic produced greater yields of DNA. In this study, we can see that surfaces such as the plastic handled screwdriver produced a larger number of partial profiles with only 21-40% of the donor alleles being present. This suggests that while this surface may retain a large amount of the deposited DNA due to it's more textured nature, this DNA may not be of value to a criminal investigation as it is not that of the donor in this instance. Similar findings were obtained by Daly, Murphy and McDermott (2012) who found that wooden surfaces produced more useful profiles than glass surfaces. While there are more complete donor profiles obtained using the wooden surfaces than the glass surfaces, it can also be seen that there are more partial profiles with 81-99% of donor alleles from the glass surface than from the wooden surface which suggests that these surfaces may be comparable in terms of generating a useful DNA

profile. Furthermore, it can be seen from table 14 that the wooden handles produced the greatest median number of non-donor alleles which suggests that the absorbent nature of this surface allows for greater retention of non-donor DNA which may have been indirectly transferred to the surface. Cook, Mitchell and Henry (2021) mention in their study that Diamond™ Nucleic Acid Dye appears to be absorbed by an untreated wood surface. As Diamond™ Nucleic Acid Dye binds to the phosphate backbone of DNA (Kanokwongnuwut *et al.* 2018, Hughes *et al.* 2022), it could be inferred that DNA would also be absorbed by an untreated wood therefore increasing it's potential to retain any indirect transfer that occurs.

This difference could be observed due to the difference in handling time, as discussed in chapter 3 in relation to the difference observed between the plastic handled screwdrivers and aluminium drinks cans, with the wooden handles being handled daily over a 7 day period as this would allow for a large amount of indirect transfer or background to build up on the surface during the participants' daily activities. Additionally, as discussed by Goray and van Oorschot (2015) social interactions, such as those that occur in an office environment, can lead to detectable DNA being transferred to a surface. However, it is noted that the plastic handles screwdrivers had the same handling time as the wooden handles, yet they had a reduced amount of non-donor DNA detected in comparison which indicates that the surface morphology itself is contributing to this non-donor DNA being accumulated.

5.3.1.2. Phase 2 – Mock Operational Study

To provide a comparison of samples obtained in a less controlled environment, touch DNA samples were deposited upon the same four surface types but without any hand washing requirements being stipulated. The average quantities of DNA recovered from these surfaces when no hand washing requirements were provided is displayed in table 15.

Surface Type	N (∑=128)	Quantification ng/µL

Aluminium Drinks Can	32	0.00
Drinking Glass	32	0.00
Plastic Handled Screwdriver	32	0.00
Wooden Handles	32	0.00

Table 15. Median quantities of touch DNA recovered from aluminium drinks cans, drinking glasses, plastic handled screwdrivers and wooden handles when no hand washing requirements were in place.

The quantification data produced from these samples was found to be non-normally distributed (p=0.000, p=0.000, p=0.000, p=0.000) with no statistically significant difference being present when using a Kruskal-Wallis test (p=0.335). Despite this, it can be seen in the raw data that, a larger amount of DNA was obtained from the drinking glasses than the other surfaces. This contrasts with data published by Daly, Murphy and McDermott (2012) who found that greater amounts of DNA were obtained from porous surfaces such as wood. However, this is in line with the findings of Hedman *et al.* (2021) who obtained greater yields of DNA from glass surfaces than plastic or wooden surfaces albeit in relation to saliva stains.

Additionally, this data varies greatly from that produced from phase 1 of this study where hand washing was controlled as is displayed with this data being non-normally distributed (p=0.000, p=0.000). From the samples where hand washing was not controlled there is a lower quantity of DNA obtained from all surfaces apart from the drinking glasses. However, overall, there is no statistically significant difference present between the quantities of DNA obtained in either phase 1 or phase 2 of this study using a Mann-Whitney U test (p=0.270) which indicates that the level of hand washing does not significantly impact upon the resulting quantities of DNA obtained from a touch DNA deposit despite the variation observed. Such factors were also discussed by Lowe *et al.* (2002) and Phipps and Petricevic

(2007) in relation to assessing an individual's shedder status where they found that there was a lot of variation but hand washing was not a significant factor in how much DNA was deposited by an individual. This directly supports the data produced here as while it can be seen that, while there was a reduction in deposited DNA when there was no hand washing requirements stipulated, this difference was not significant.

To further assess the impact that the surface type has upon the success of touch DNA recovery from mock evidential items, the total number of alleles and donor alleles obtained from each surface type was investigated and the data is displayed in table 16.

Surface Type	**N (∑=128)**	**Median Number of Alleles per Profile**	**Median Number of Donor Alleles per Profile**	**Median Number of Non-Donor Alleles per Profile**
Aluminium Drinks Can	32	4.00	2.00	0.00
Drinking Glass	32	2.00	1.00	0.00
Plastic Handled Screwdriver	32	12.00	11.00	0.50
Wooden Handles	32	4.00	2.50	0.00

Table 16. Median number of alleles donor and non-donor alleles detected per profile obtained for each surface type following the phase 2 sample deposition protocol.

The profile data was found to be non-normally distributed for both the total number of alleles per profile (p=0.000, p=0.000, p=0.200, p=0.000) and the donor alleles per profile (p=0.000,

p=0.000, p=0.120, p=0.000) and no statistically significant differences were found using a Kruskal-Wallis test (p=0.203 and p=0.138 respectively). This is in line with the data produced from phase 1 where hand washing was controlled which suggests that while the surface type may be influencing the success rates of this evidence type due to the variability, this impact is not significant. This is in contrast with Daly, Murphy and McDermott (2012) who found statistically significant differences between samples recovered from glass, fabric and wooden surfaces. It has been discussed by Hansson *et al.* (2009) and Verdon *et al.* (2014) that the success of touch DNA recovery is influenced by the substrate it is being recovered from however, this was in relation to requiring the optimal recovery method per surface type which suggests that the surface type alone is not the contributing factor to the success rate of touch DNA evidence. This is supported by the data produced in this study as while there were no statistically significant differences found between the profiles obtained from each surface type, there were some differences and this should be further reviewed in relation to the recovery methods used on these surfaces. This will be discussed further in section 5.3.2.

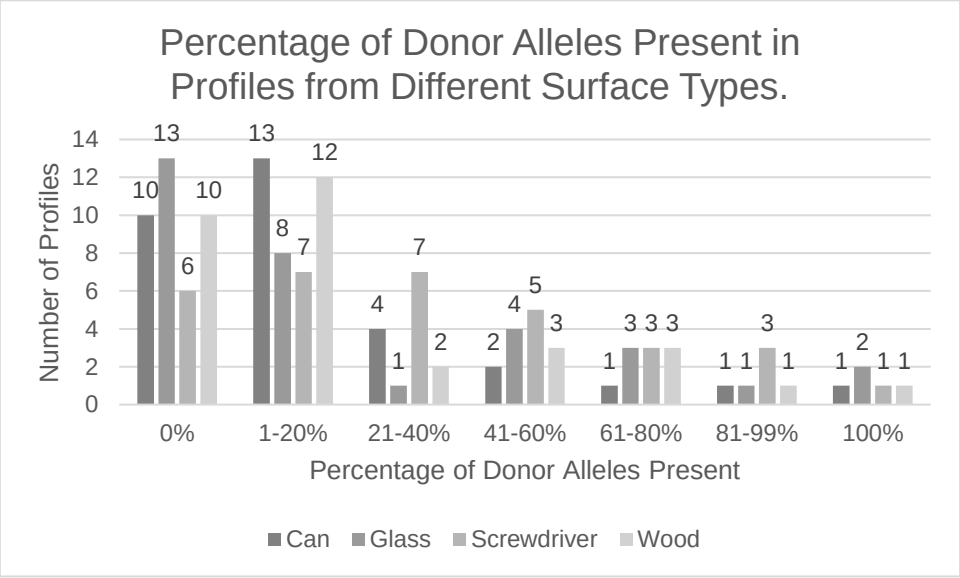


Figure 10. A graph displaying the percentage of donor alleles present within the profiles produced from touch DNA samples deposited on different surface types following the phase 2 deposition method.

In relation to the completeness of the donor profiles produced from each surface type, it can be seen from figure 10 that in comparison to the samples analysed in phase 1, a larger proportion of this data had no donor alleles present or a small percentage of donor alleles present within the sample. This suggests that while the impact of hand washing may not be significant, it can be seen to reduce the number of complete donor profiles obtained when there is no regulation around hand washing. As the actions of the donor are unknown in a criminal investigation, it is important to take into consideration the impact this may have upon the resulting data as this may reduce the potential of providing a discriminative profile. Despite this, Daly, Murphy and McDermott (2012) indicated that a profile is considered as discriminative with as few as 6 alleles meaning any samples with 20% or more of the alleles would be considered as discriminative and would therefore be useful within a criminal investigation although this may be more for exclusionary purposes than identification. Gangano *et al.* (2013) also suggested that samples with as few as 7 alleles could be loaded onto a database to gain investigative leads which suggests despite the low number of donor alleles present within the samples obtained from mock evidential items, a large number of these can still be utilised within a criminal investigation.

Furthermore, as can be seen from table 16, there are very few non-donor alleles being detected which suggests that when these samples are stored at room temperature, while also reducing the overall number of alleles detected, this also reduces the amount of non-donor DNA persisting on the item which suggests that the DNA of regular/primary user of the item will persist on the surface for longer in sub-optimal conditions than any indirectly transferred cellular material.

5.3.2. Assessment of the Impact of Swab Material on the Success Rates of Touch DNA Analysis with NGM SElect™

5.3.2.1. Phase 1 – Controlled Study

As discussed by Mulligan *et al.* (2011), Hansson *et al.* (2009) and Verdon *et al.* (2014) the success of a touch DNA sample can be directly impacted by the recovery method used to collect this evidence. Due to that, a range of recovery methods were assessed to determine the impact of these upon the resulting quality and quantity of the DNA evidence and the data produced was interpreted in relation to the impact of the recovery method. The quantification data for the recovery methods utilised in this study can be seen in table 17.

Swab Type	**N (∑=128)**	**Quantification ng/µL**
Cotton	32	0.009
Foam	32	0.008
Polyester	32	0.002
Rayon	32	0.000

Table 17. Median quantification data obtained from touch DNA samples deposited following the phase 1 deposition method and recovered using cotton, rayon, foam and polyester swabs.

The quantities of DNA obtained from these different swab materials can be seen to be varied and a statistical analysis of this data demonstrated that this data was non-normally distributed (p=0.000, p=0.000, p=0.000, p=0.000). Despite the varied quantities of DNA from each of these recovery methods, there was no statistically significant difference found between the amount of DNA obtained from these swab materials using a Kruskal-Wallis test (p=0.186). However, it can be observed that cotton and foam swab materials had higher median quantities of DNA than the rayon and polyester swabs which suggests that these

materials are more proficient at recovering touch DNA samples. This data is ins concordance with that obtained by Haase *et al.* (2019) who found that a cotton swabbing material obtained greater DNA yields than the other swabs they reviewed. However, as the amount of DNA deposited by an individual is extremely variable, it may be that the contributing factor to these differences is due to the donor and not the swab material itself which has not been reviewed in this study. The impact of a donor's ability to deposit touch DNA has been extensively reviewed with no defining factors being determined as discussed in section 1.2.2. of this thesis.

As the quantification data produced in such studies is not indicative of the success of the STR profiling of these samples, the recovered DNA was subjected to DNA profiling and the resulting number of alleles and donor alleles can be found in table 18.

Swab Type	**N (∑=128)**	**Median Number of Alleles per Profile**	**Median Number of Donor Alleles per Profile**	**Median Number of Non-Donor Alleles per Profile**
Cotton	32	35.00	25.00	10.00
Foam	32	38.00	25.00	12.50
Polyester	32	27.00	17.00	9.50
Rayon	32	6.00	2.00	0.00

Table 18. Median number of alleles, donor alleles and non-donor alleles collected using four different swab types from touch DNA deposits made following the phase 1 deposition protocol.

A large variation in the profile data can be seen in regard to the success of rayon swabs in comparison to the other swabs utilised in this study. This further demonstrates that the quantification data obtained from touch DNA samples may not be representative of the components of the deposits that generate a DNA profile as rayon swabs were found to generate a large DNA yield yet provided the lowest amount of alleles from STR profiling.

The data produced from the DNA profiling of these samples was found to be non-normally distributed for both the total alleles produced (p=0.005, p=0.001, p=0.200, p=0.200) and the donor alleles (p=0.046, p=0.000, p=0.002, p=0.058). Further statistical analysis using a Kruskal-Wallis test demonstrated that there was a significant difference in the number of total alleles produced using these different swab materials (p=0.000) with fewer alleles being produced from rayon swabs than cotton, foam or polyester swabs when pairwise comparisons were conducted (p=0.000, p=0.000 and p=0.002 respectively). A Cohen's d test for effect size determined that these differences had a large effect size (1.4, 1.48 and 0.98 respectively). However, no statistically significant differences were observed between the remaining swab materials. A Kruskal-Wallis test was also conducted on the donor alleles produced using the different swab materials and a statistically significant different was found (p=0.000) again, with fewer donor alleles being obtained from rayon swabs than cotton, foam or polyester swabs with a pairwise comparison (p=0.000, p=0.000 and p=0.026 respectively) which were determined to have a large (1.29), large (1.21) and medium (0.74) effect size respectively using a Cohen's d test. No further significant differences were found between the remaining swab types. This data is supported by Verdon *et al.* (2014) who found that cotton swab materials produced more complete profiles than rayon swabs despite rayon swabs outperforming the nylon flocked swabs. However, they found that foam and polyester swabs recovered less alleles than rayon swabs which is contrasting with the data produced in this study with foam and polyester obtaining significantly more alleles than the rayon swabs. Additionally, Seah *et al.* (2004) found that DNA samples recovered using a rayon material produced a higher yield than those recovered with a polyester material and

they proposed this was due to the chemical bonding that takes place between the DNA and the material. However, this is contrasting with the data produced in this study whereby more alleles were obtained using a polyester swab material than a rayon swab material. While there is some variability within the data produced from this study and the previously published data, it has been observed that rayon swabs consistently underperform in comparison to the commonly used cotton swabs (Brownlow *et al.* 2012) so may not be appropriate for touch DNA recovery. This is further supported by the data displayed in figure 6 where it can be seen that no complete profiles were obtained using the rayon swabs yet they produced the highest number of samples with no donor alleles being detected.

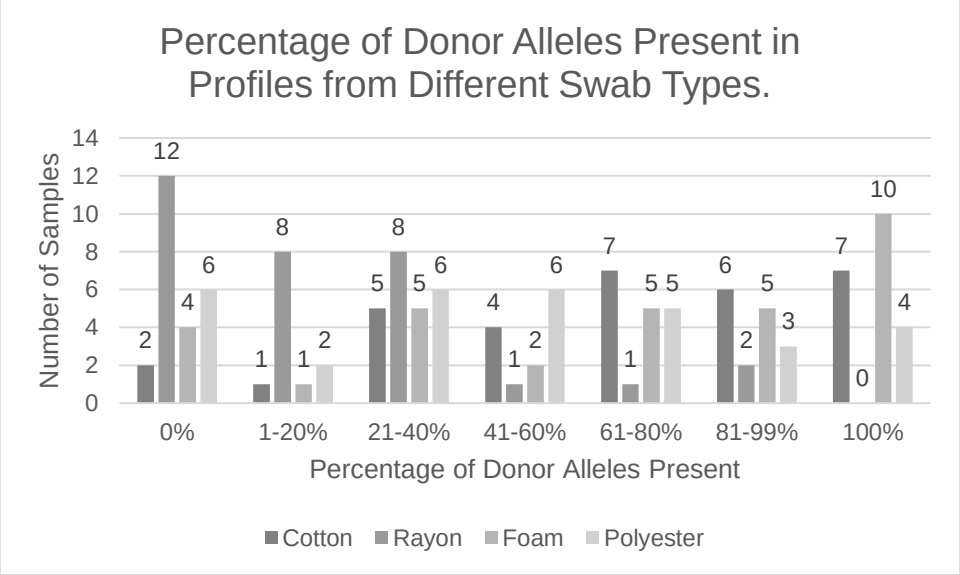


Figure 11. A graph displaying the percentage of donor alleles present within the profiles produced from touch DNA samples recovered using different swab materials following the phase 1 deposition method.

Despite no statistically significant differences being found between the cotton, foam and polyester swabs, the data displayed in figure 11 shows that foam swabs produced the highest number of complete donor profiles suggesting that this swab material may be better suited to the recovery of touch DNA samples. The cotton, foam and polyester swabs also

consistently produce profiles with more than 20% of donor alleles being detected (85.29%, 82.35% and 70.59% of samples respectively) which suggests these swabs regularly produce profiles that are of value for investigative leads as they have 7 or more alleles present (Daly, Murphy and McDermott, 2012 and Gangano *et al.* 2013).

As the optimal recovery method has been suggested to vary based upon the surface the DNA is being recovered from (Hansson *et al.* 2009; Daly, Murphy and McDermott, 2012 and Verdon *et al.* 2014) this was assessed to determine if any of the swab materials utilised in this study perform differently on the different surface types utilised in section 5.3.1. This data is displayed in table 19.

	Aluminium Drinks Can				Drinking Glass				Plastic Handled Screwdriver				Wooden Handles			
	Cotton	**Foam**	**Polyester**	**Rayon**	**Cotton**	**Foam**	**Polyester**	**Rayon**	**Cotton**	**Foam**	**Polyester**	**Rayon**	**Cotton**	**Foam**	**Polyester**	**Rayon**
Total Alleles	37.5	32.5	29	8	34.5	30	16.5	8	35.5	39.5	22	1	34.5	44.5	38	7
Donor Alleles	30.5	24.5	17.5	1.5	26	23	7	7	15.5	21.5	8	0	24.5	34	27.5	5
Non-Donor Alleles	8.5	9	11	0.5	8.5	13.5	8	0	12.5	16	6	0	10.5	13	10.5	1

Table 19. Median alleles, donor alleles and non-donor alleles produced from touch DNA deposited using the phase 1 deposition protocol onto aluminium drinks cans, drinking glasses, plastic handled screwdrivers and wooden handles and then recovered using various swabbing materials.

When looking at the total alleles recovered from the aluminium drinks cans, the data was found to be normally distributed (p=0.200, p=0.200, p=0.200, p=0.200) however, a one-way independent-measures ANOVA determined that there was a statistically significant difference between the number of alleles recovered using the different swab materials (p=0.002). Post-hoc testing demonstrated that this difference was between the number of alleles recovered with rayon swabs in comparison to cotton, foam and polyester swabs with fewer alleles being recovered using the rayon swabs (p=0.003, p=0.017 and p=0.022 respectively). An eta squared test for effect size determined that the observed differences had a large effect size (0.4). This suggests that rayon swabs are not as effective at recovering touch DNA from a metal surface. Bonsu, Higgins and Austin (2020) suggested that due to the nature of the metal surface this is likely to readily bond with the structure of DNA so it is likely that rayon swabs do not produce a strong enough interaction with the DNA on the surface to break the bonds that this forms to the metal. However, it can be seen that the other swab materials were able to recover further alleles suggesting they may have a stronger chemical interaction with the DNA on this surface.

The amount of donor alleles recovered from the aluminium drinks cans was found to be non-normally distributed (p=0.051, p=0.013, p=0.200, p=0.025) and a Kruskal-Wallis test identified that a statistically significant difference was present between the swab materials used on this surface (p=0.003). A pairwise comparison of the swab materials found that cotton swabs produced significantly more donor alleles than rayon swabs (p=0.016) with a large effect size (1.59 with a Cohen's d test) however, no further statistically significant differences were found between the remaining swab types. This along with the data displayed in figure 12 suggests that cotton swabs are the most effective swab at recovering touch DNA from a metal surface such as these drinking cans despite Bonsu, Higgins and Austin (2020) suggesting that these swabs may not be effective at DNA recovery from a metal surface due to the potential for the cellular material to become entrapped within the swab matrix. There has been limited published research around the

recovery of touch DNA from metal surfaces so it is unclear at this stage whether this data is comparable to the findings of other researchers.

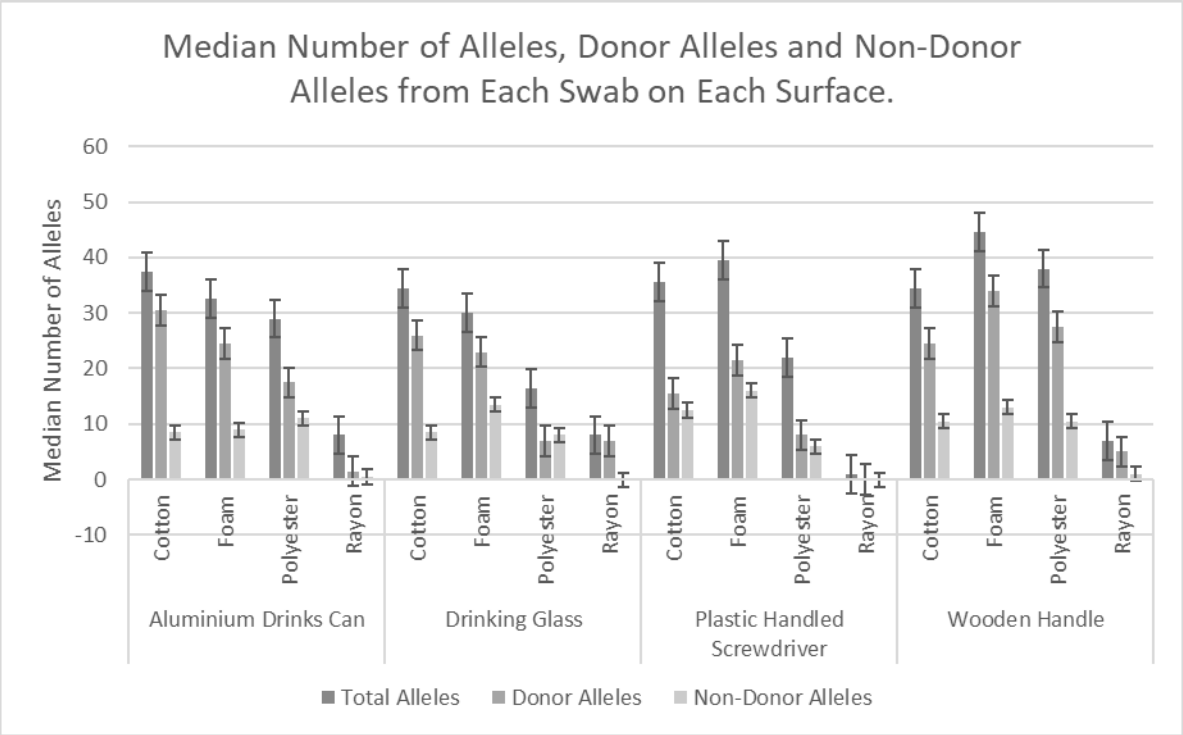


Figure 12. A chart displaying the median number of alleles, donor alleles and non-donor alleles per profile recovered from touch DNA deposits on aluminium drinks cans, drinking glasses, plastic handled screwdrivers and wooden handles following the phase 1 deposition protocol using a variety of swab materials.

The total number of alleles and the number of donor alleles recovered from drinking glasses were found to be non-normally distributed (total alleles: p=0.200, p=0.036, p=0.200, p=0.200; donor alleles: p=0.168, p=0.007, p=0.200, p=0.024) and a Kruskal-Wallis test indicated that a statistically significant difference was present in the number of total alleles between the different recovery methods used (p=0.045). A pairwise comparison found that this difference originated between rayon swabs and cotton and foam swabs with cotton and foam swabs recovering more alleles than the rayon swabs (p=0.029 and p=0.021 respectively), which a Cohen's d test confirmed was a large effect

size (1.15 and 1.24 respectively). No further significant differences were present. However, there was no statistically significant difference present using a Kruskal-Wallis test when comparing the number of donor alleles recovered with the different swab materials (p=0.129). When looking at the donor allele data displayed in figure 12 it can be seen that, more donor alleles were obtained using the cotton and foam swabs with cotton swabs producing the most donor alleles despite this difference not being significant. Data produced by Verdon *et al.* (2014) contrasts with the data produced in this study as they found that polyester swabs were ranked higher than all other swab types for the recovery of DNA from a glass surface. However, this was in relation to the quantity of DNA recovered rather than the profiles produced from this swab type which may account for the differences as the quantification data does not necessarily indicate a successful DNA profile as was also discussed by Verdon *et al.* (2014) in relation to touch DNA samples producing a high quantification value but a substandard profile. Contrastingly, the data produced in this study is supported by research conducted by Haase *et al.* (2019) who found that cotton swabs recovered more DNA from glass in comparison to nylon flocked swabs. Similar results were also obtained by Mulligan *et al.* (2011) where cotton swabs were found to recover more cellular material from glass surfaces when water was used as the moistening agent albeit in relation to saliva samples but no statistically significant difference was found between the cotton and polyester swabs. This suggests that while there is a visible difference in the amount of DNA obtained using cotton swabs in comparison to alternative recovery methods, this difference is not significant and therefore further testing may be required to determine if cotton swabs would continue to outperform other closely ranked swabs such as the foam swabs over a greater number of samples.

The total number of alleles recovered from plastic handled screwdrivers utilising different swab materials was assessed and the data was found to be normally distributed (p=0.200, p=0.073, p=0.200, p=0.200) with a statistically significant difference displayed between the recovery methods using a one-way independent-measures ANOVA (p=0.000). Post-

hoc testing demonstrated that this difference occurred between the number of alleles recovered with rayon swabs in comparison to cotton, foam and polyester swabs (p=0.000, p=0.000 and p=0.026 respectively) with rayon swabs recovering fewer alleles than the other swab materials. Further to this, foam swabs were also found to recover a significantly greater number of alleles than polyester swabs (p=0.027). These differences were observed to have a large effect size using an eta squared test (0.62). No further significant differences were found between the remaining swab materials.

When looking at the number of donor alleles recovered from the plastic handled screwdrivers, it was found that this data was non-normally distributed (p=0.200, p=0.00, p=0.200, p=0.200) but there was again a statistically significant difference (p=0.000) found between the recovery methods using a Kruskal-Wallis test. Pairwise comparisons with a Bonferroni adjustment to the significance level identified that this difference originated from rayon swabs recovering statistically fewer donor alleles than cotton and foam swabs (p=0.004 and p=0.001 respectively) which had a large effect size using a Cohen's d test (1.54 and 1.78 respectively). This data is supported by Hansson *et al.* (2009) who found that when recovering trace DNA from a hard plastic surface a self-saturating foam swab performed better than cotton and nylon flocked swabs. However, this data is contrasting with that of Verdon *et al.* (2014) who found that cotton swabs were more effective at recovering low-level DNA from plastic surfaces. While there is no statistically significant difference between the cotton and foam swabs produced in this study, it can be seen from table 19 that foam swabs generated more alleles and donor alleles than cotton swabs which suggests that these swabs may be more efficient at recovering DNA from a plastic surface although cotton swabs do still recover a high number of alleles and donor alleles.

Wooden handles produced non-normally distributed data (p=0.060, p=0.200, p=0.031, p=0.065) when looking at the number of total alleles recovered from this surface using different swab types. A Kruskal-Wallis test on this data found that a statistically significant difference was present (p=0.027) with pairwise comparisons using a Bonferroni

adjustment to the significance level identifying that more alleles were recovered with the foam swabs than the rayon swabs (p=0.031), with a large effect size (Cohen's d value of 1.3), but no further differences were observed. The donor alleles recovered from the wooden handles were also non-normally distributed (p=0.200, p=0.200, p=0.003, p=0.200) with a statistically significant difference being found using a Kruskal-Wallis test (p=0.041). When conducting pairwise comparisons on this data with a Bonferroni adjustment to the significance level it was found that foam and polyester swabs recovered significantly more donor alleles than rayon swabs (p=0.010 and p=0.018 respectively), which was found to be a large effect size (1.26 and 1.3 respectively) using a Cohen's d test. This data is supported by that of Hedman *et al.* (2021) who found that foam swabs recovered a greater yield of DNA from absorbent wooden surfaces than cotton swabs. Additionally, Verdon *et al.* (2014) found that foam swabs consistently produced a higher DNA yield from wooden surfaces than other swab types reviewed. However, this also included polyester swabs which is contrasting with the data produced in this study with polyester swabs performing very similarly to foam swabs with slightly more donor alleles being recovered than with the foam swabs.

The data produced from this study suggests that when touch DNA is deposited upon a smooth, non-porous surface such as metal or glass a cotton swab may be more efficient at recovering the deposited DNA which is contrasting with the suggestions made by Hansson *et al.* (2009) who suggested that foam swabs were more efficient at DNA recovery from non-porous surfaces. While foam swabs did display good success rates from non-porous surfaces in this study with no statistically significant differences being observed between the cotton and foam swabs, the cotton swabs produced a greater number of donor alleles from both surfaces. However, in line with Hansson *et al.* (2009) foam swabs were slightly more efficient at touch DNA recovery from plastic handled screwdrivers than cotton swabs suggesting that from a more textured/ridged plastic foam swabs may be more proficient. Verdon *et al.* (2014) also suggested that cotton swabs

were most effective from a pitted plastic surface which while the foam swabs did recover more alleles and donor alleles that cotton swabs, the difference was not significant suggesting that good success rates can be obtained using either of these swabbing materials on a plastic surface. Additionally, in line with published research by Verdon *et al.* (2014) and Hedman *et al.* (2021) foam swabs were found to be highly efficient at touch DNA recovery from an absorbent wooden surface. However, in contrast with this published data, polyester swabs were equally as efficient at recovering touch DNA from this surface which is in accordance with the data produced by Mulligan *et al.* (2011) where polyester swabs were as efficient at DNA recovery from porous surfaces as cotton swabs albeit in relation to saliva stains.

5.3.2.2. Phase 2 – Mock Operational Study

To determine if the data produced from phase 1 was replicable using mock evidential items, the study was repeated however, participants were given no requirements regarding the time between hand washing and handling the items. The items were still handled for the same lengths of time to replicate regular use of these commonly encountered evidence items to provide a more mock-operational trial. In addition to this difference in methodology, the storage of the samples differed from those produced in phase 1 as due to the COVID 19 pandemic access to the laboratory was not possible for a 6-month period post deposition of the touch DNA upon these samples meaning they were instead stored at room temperature prior to the recovery and analysis of the touch DNA. Similar storage was detailed in a study by Shackleton *et al.* (2019) for blood and saliva samples.

When looking at the quantification data obtained from the samples collected using cotton, rayon, foam and polyester swabs it can be observed that this varies greatly between swab materials as displayed in table 20.

Swab Type	N (∑=128)	Quantification ng/µL
Cotton	32	0.00
Foam	32	0.00
Polyester	32	0.00
Rayon	32	0.00

Table 20. Median quantification data obtained from touch DNA samples deposited following the phase 2 deposition method and recovered using cotton, rayon, foam and polyester swabs.

Statistical analysis upon this data determined that this was non-normally distributed (p=0.000, p=0.000, p=0.000, p=0.000). However, despite the variations in the amounts of DNA recovered from each swab material, no statistically significant difference was found using a Kruskal-Wallis test (p=0.719). When looking at the quantification data produced from the phase 1 samples per swab type, it can be seen that there was an overall decrease in the amount of DNA collected from the samples where hand washing was not controlled yet, cotton swabs displayed an increase in the amount of DNA recovered. While this difference in quantification was not significant, as outlined in section 5.3.1.2., it suggests that more DNA is recovered from cotton swabs when the samples are stored at room temperature for a prolonged period than with the other swab materials utilised in this study. This is in accordance with the data produced by Haase *et al.* (2019) who found that cotton swabs generated higher yields of DNA than other swab materials. However, as discussed by Haas *et al.* (2015) the DNA yield does not always correlate with the success of this evidence type which was also experienced by Verdon *et al.* (2014) who found that in some instances, samples with a high DNA yield did not produce a high-quality profile.

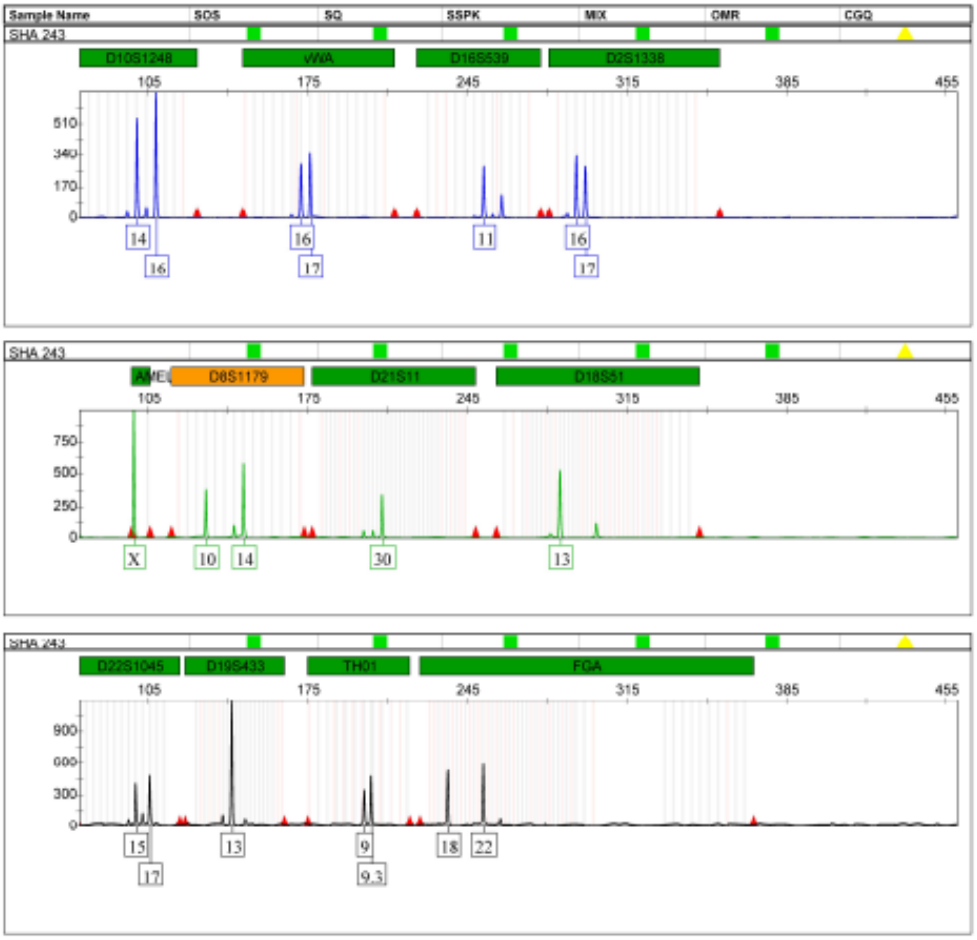
To further assess this in relation to the swab materials used to recover the touch DNA samples in this study, all samples were analysed using the NGM SElect™ kit as detailed in chapter 2 with no dilution to assess the concordance between quantification values and DNA profile quality. The mean number of alleles and donor alleles produced from these samples can be found in table 21.

Swab Type	N (∑=128)	Median Number of Alleles per Profile	Median Number of Donor Alleles per Profile	Median Number of Non-Donor Alleles per Profile
Cotton	32	3.00	2.00	1.00
Foam	32	3.00	1.00	0.50
Polyester	32	7.00	4.50	0.00
Rayon	32	10.00	9.00	0.00

Table 21. Median number of alleles, donor alleles and non-donor alleles collected using four different swab types from touch DNA deposits made following the phase 2 deposition protocol.

Both the data in relation to the total alleles per profile (p=0.000, p=0.018, p=0.000, p=0.000) and donor alleles per profile (p=0.000, p=0.032, p=0.000, p=0.004) were found to be non-normally distributed and no statistically significant differences were obtained from either set of data using a Kruskal-Wallis test (p=0.203 and p=0.138 respectively). However, it can be seen that, despite recovering the greatest yield of DNA, cotton swabs produced the least alleles upon DNA profiling which was also suggested by Haas *et al.* (2015) who found that the higher input samples did not produce the best results at further

analysis. This further suggests that the genetic components of touch DNA deposits that generate the DNA profiles are not being quantified using current quantitative PCR methodologies. In contrast with Verdon *et al.* (2014) and Haas *et al.* (2015) it was also found in this study that samples that gave a 0ng/µL quantification value, generated a profile with over 85% of the alleles being called which all belonged to the donor as can be observed in figure 13. Burrill *et al.* (2021) discussed how many quantitative PCR methods have a shortest amplicon of 80 base pairs meaning that the smaller fragments of DNA thought to be found in touch DNA deposits may not be being detected from these methods. This would explain the instances of low DNA yields producing a higher quality DNA profile as these smaller fragments may still be contributing to the overall DNA profile despite not being detected during quantitative PCR analysis.



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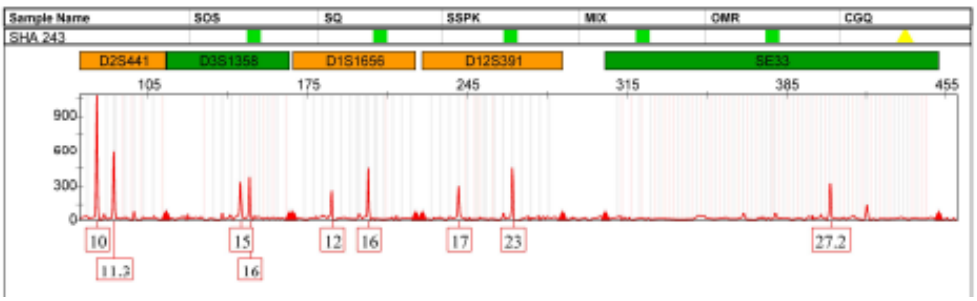


Figure 13. DNA profile obtained from a 0ng/µL sample using a polyester swab from a plastic handled screwdriver.

In order to further assess the success rates of touch DNA evidence recovered using different swab materials, the percentage of donor alleles was reviewed and can be seen in figure 14. This shows that in comparison to the samples recovered in phase 1, there are much fewer complete donor profiles being obtained with rayon swabs producing most profiles with 61% or more alleles. In contrast to the profiles produced during phase 1 of this study, there are a larger proportion of samples containing 20% or fewer alleles which suggests that these are will not provide investigative leads in line with the requirements outlined by Gangano *et al.* (2013) and Daly, Murphy and McDermott (2012).

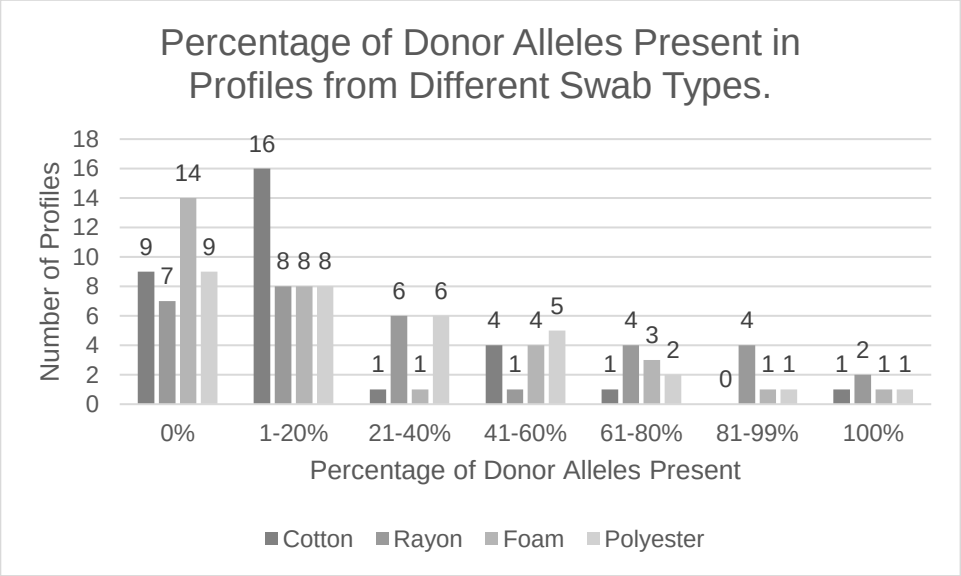


Figure 14. A graph displaying the percentage of donor alleles present within the profiles produced from touch DNA samples recovered using different swab materials following the phase 2 deposition method.

Furthermore, when comparing the efficiency of the different recovery methods on each of the different surfaces, no statistically significant differences were found for the data

produced from the phase 2 deposition protocol which is contrasting with the data produced from phase 1. However, some differences were observed when looking at the raw data despite these not being significant as can be seen in figure 15. It can be observed that more total and donor alleles were recovered from the aluminium drinks cans using foam swabs which supports the findings of Hansson *et al.* (2009) who suggested that foam swabs were more efficient at DNA recovery from non-porous surfaces. In contrast with this, it can be seen that from drinking glasses foam swabs recovered the least alleles. However, polyester swabs recovered the most total alleles but only a small number of these were attributed to the donor suggesting that a large portion of this is background DNA collected upon the donor's hands prior to deposition and transferred to the item via an indirect transfer mechanism such as those discussed in section 1.2.2. The researcher hypothesises that this may have been gathered from the social setting within the office environment in which the participants share, as has been reported by Goray and van Oorschot (2015). However, due to the lack of controls in this study and the varying recovery methods used, it is not possible to infer a likely transfer mechanism as explained by Gosch and Courts (2019). In contrast to the data from phase 1 of this study, rayon swabs were found to recover the highest number of donor alleles from drinking glasses and plastic handled screwdrivers which suggests that in a less controlled environment these swabs may perform well on non-porous surfaces which supports the findings of Verdon *et al.* (2014) who found that rayon swabs outperformed the foam swabs other than on a wooden surface which is further supported by the data produced in this study as foam swabs produced a greater number of alleles from wooden surfaces than the other swab materials.

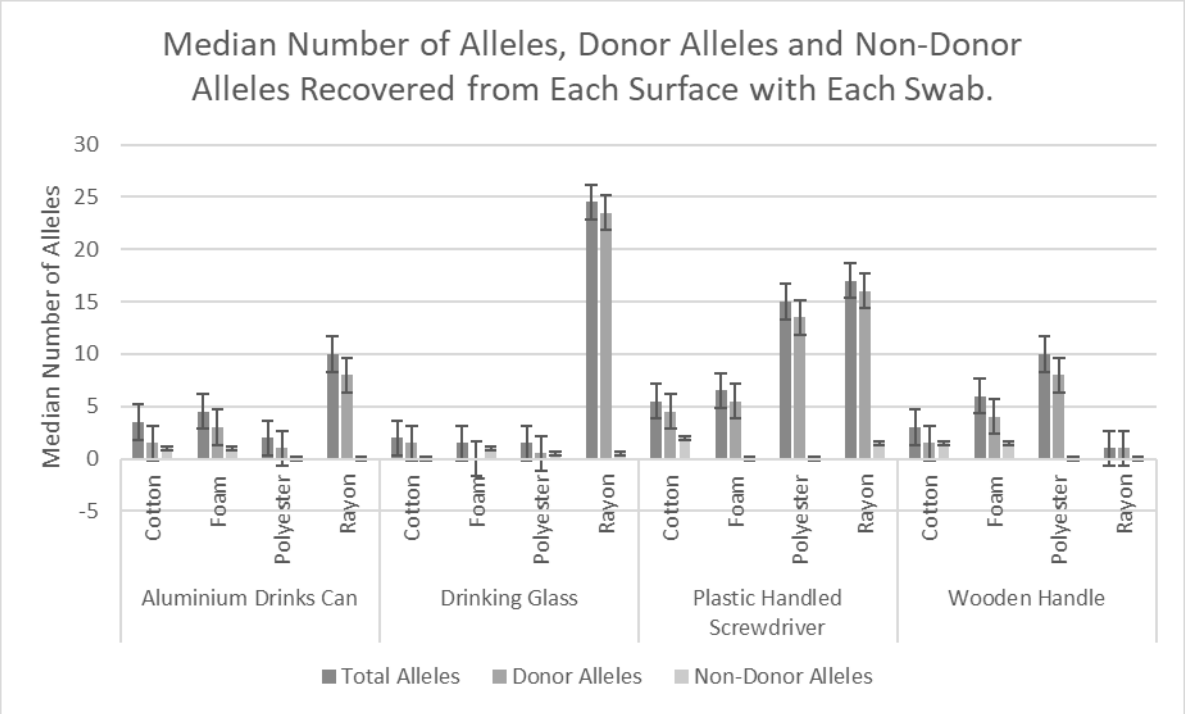


Figure 15. A chart displaying the median number of alleles, donor alleles and non-donor alleles recovered from aluminium drinks cans, drinking glasses, plastic handled screwdrivers and wooden handles using different swab materials from touch DNA samples deposited following the phase 2 deposition protocol.

The decrease in profile quality in comparison to phase 1, was found to be non-normally distributed (total alleles p=0.002, p=0.000; donor alleles p=0.000, p=0.000) and statistically significant in relation to both the total number of alleles produced and the number of donor alleles produced when using an independent-measures t-test (p=0.000 and p=0.000 respectively) and this difference was found to have a medium effect size (0.7) with a Cohen's d test. The reason for this decrease in profile quality could be attributed to the lack of hand washing requirements meaning participants may have washed their hands immediately prior to depositing their DNA upon the surface which may be reducing the amount of cfDNA upon the hands as described by Burrill *et al.* (2021). However, Lowe *et al.* (2002) and Phipps and Petricevic (2007) discovered that despite a large amount of variation being found in relation to hand washing, the differences were not

significant and therefore is unlikely to be the main contributor to poor DNA deposition. Another factor that may have contributed to the reduced profile quality obtained from phase 2 of this study is the difference in the sample storage conditions as the samples produced in phase 1 were sampled as soon as collected and the swabs were stored within a freezer to reduce sample degradation before the analysis took place. Shackleton *et al.* (2019) discussed how samples stored at room temperature for up to 120 days demonstrated no obvious effect upon the DNA profiles produced. However, they found that samples stored for more than 250 days began to show signs of degradation with reduced peak heights and an increase in the number of profiles with 0% of the donor alleles present although this was not found to be significant. This supports the observations made in relation to the samples produced from phase 2 of this study as there is an increase in profiles with no donor alleles present and a general reduction in the profile completeness from all swab materials.

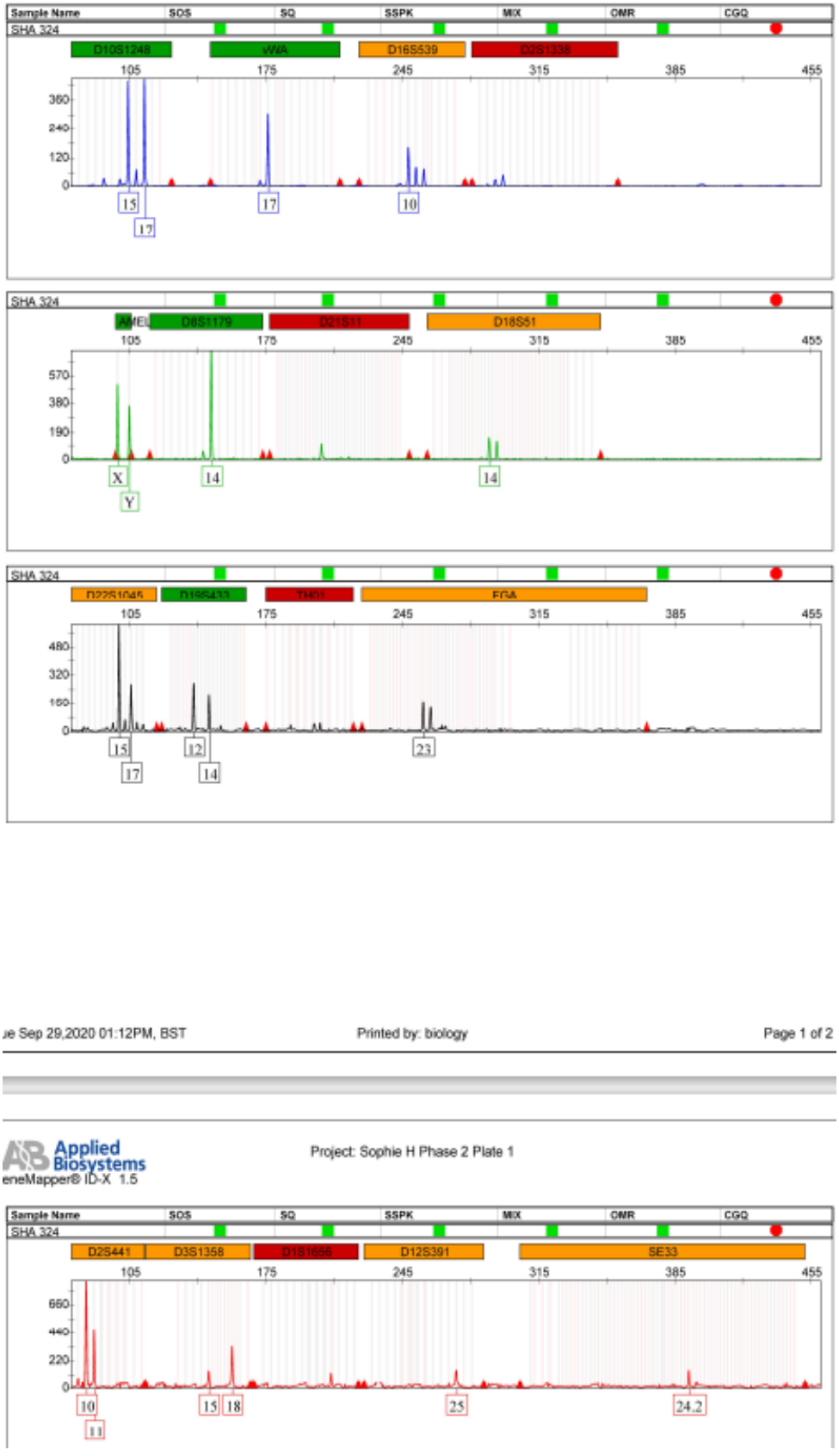
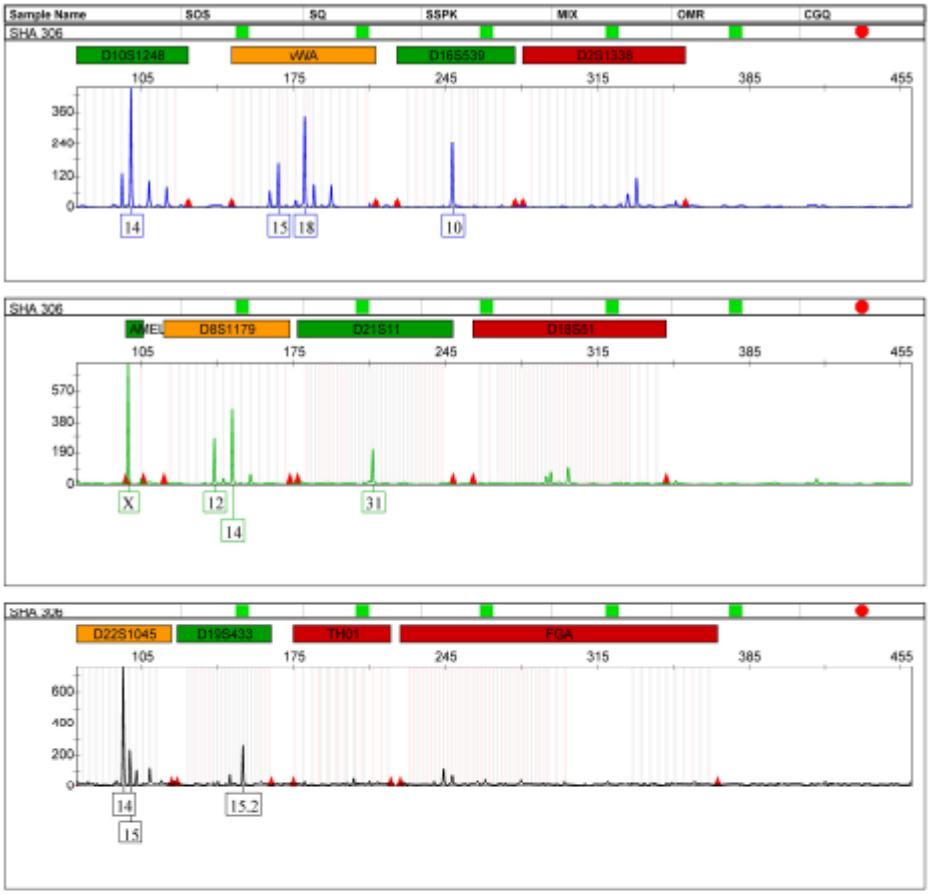


Figure 16. An example DNA profile displaying samples degradation with reduced peak heights being observed over the larger amplicons.

In addition to the reduced number of alleles observed in the profiles from phase 2 of this study, an increased amount of sample degradation can be seen through lower peak heights being found in the larger amplicons than the shorter amplicons which was also discussed by Burrill *et al.* (2021) and Burrill *et al.* (2021b) in relation to touch DNA deposits. This is observed in the profile displayed in figure 16 where the “ski-slope” pattern can be seen with those larger amplicons such as FGA and SE33 producing alleles with a much lower peak height than the shorter amplicons. In addition to the reduced peak heights observed in some of the profiles from this study, complete loci dropout was also observed further indicating the degradation of the samples from phase 2 of this study. An example of this can be seen in figure 17 where no allele data was obtained from the larger loci demonstrating that loci dropout. This suggests that the prolonged storage of these samples at room temperature before DNA recovery has impacted the overall quality of the DNA sample which is a significant factor in the results obtained from this section of the study. However, as the time since deposition and environmental factors are often unknown with evidential items collected from a crime scene, this data is still relevant as it provides insight into the potential to recover touch DNA from items that have been exposed to sub-optimal conditions for a prolonged period of time.



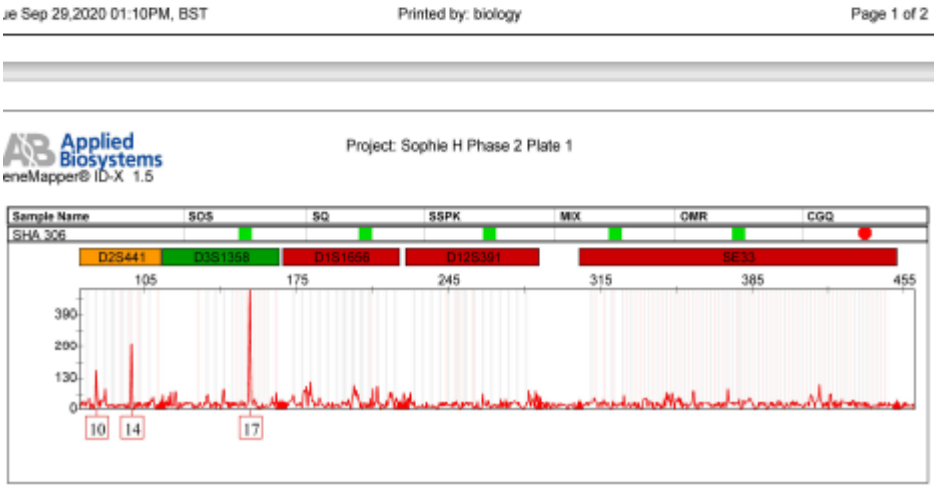


Figure 17. An example DNA profile produced using the phase 2 deposition protocol displaying sample degradation with loci dropout at the larger amplicons.

5.4. Conclusions and Further Work

This chapter aimed to compare the quantitative and qualitative data produced from the analysis of touch DNA samples to determine the impact that the recovery method and surface type has upon the resulting data. The quantitative data obtained throughout this chapter has displayed a high level of variability between surfaces and recovery methods however, this data was not concordant with the success of the relevant DNA profiles produced. Similarly to Verdon *et al.* (2014) and Haas *et al.* (2015) it was found that samples that produced a high quantity of DNA with quantitative PCR analysis did not necessarily produce a high quality DNA profile with samples containing over 1.2ng/µL of DNA producing profiles with less than 10 alleles in some cases suggesting that the DNA being detected during this analysis is not contributing to the overall DNA profile. In contrast to this, there were also samples that had a 0ng/µL that produced profiles with 80% or more alleles. This supports the suggestion made by Burrill *et al.* (2021) that quantitative PCR analysis may not be detecting the smaller fragments of DNA found within touch DNA deposits which suggests that the quantitative data produced may not be useful in determining whether a sample is likely to produce a high-quality DNA profile.

When looking into the impact that surface type alone has upon the resulting DNA profiles, the differences observed were found to not be statistically significant suggesting that the surface itself is not having an overall impact upon the success of this evidence type. However, there were some differences observed between the swab types with rayon swabs consistently producing less alleles than cotton, foam and polyester swabs when hand washing was controlled. This suggests that this swab material may not be appropriate for use in touch DNA recovery despite having higher success rates with other biological samples (Seah *et al.* 2004 and Verdon *et al.* 2014).

Despite the surface type alone not significantly impacting the success of touch DNA analysis, the use of different swab materials on these surfaces did produce some significant differences, with large effect sizes demonstrating the impact of these findings, which further supports the need to determine an optimal recovery strategy for this evidence type. Rayon swabs were consistently found to produce significantly fewer alleles than the other swabbing materials on all surface types suggesting that these swabs are not efficient at touch DNA recovery from metal, glass, plastic or wooden surfaces. While the remainder of the swab comparisons did not produce a statistically significant difference, except from screwdrivers where foam swabs recovered significantly more alleles than polyester swabs, there were still some differences observed.

The differences produced from the data from phase 1 samples demonstrates that when touch DNA is deposited upon a smooth, non-porous surface such as metal or glass a cotton swab can be more efficient at recovering the deposited DNA. Foam swabs did display good success rates from non-porous surfaces in this study with no statistically significant differences being observed between the cotton and foam swabs, however the cotton swabs produced a greater number of donor alleles from both surfaces. Additionally, foam swabs were more efficient at touch DNA recovery from plastic handled screwdrivers than cotton swabs suggesting that from a more textured/ridged plastic foam swabs are more proficient. However, the difference was not significant suggesting that good success rates can be obtained using cotton or foam swabbing materials on a plastic surface. Additionally, foam swabs were found to be highly efficient at touch DNA recovery from an absorbent wooden surface. Additionally, polyester swabs were equally as efficient at recovering touch DNA from this surface so either of these swabbing materials would be recommended for the recovery of touch DNA from an absorbent wooden surface.

In contrast to this data, the samples produced from the phase 2 deposition and storage displayed no statistically significant differences between the swab materials used on each surface type. However, differences were observed in the raw data produced in this study

with more total and donor alleles being recovered from the aluminium drinks cans using foam swabs. It can also be seen that from drinking glasses foam swabs recovered the least alleles. However, polyester swabs recovered the most total alleles but only a small number of these were attributed to the donor suggesting that a large portion of this is background DNA collected upon the donor's hands prior to deposition and transferred to the item via indirect transfer (Meakin and Jamieson, 2013; Gosch and Courts, 2019; Rolo *et al.* 2019; Goray and van Oorschot, 2015) which may be due to the lack of hand washing requirements in this study. Additionally, it was found from reviewing the non-donor alleles throughout the studies in this chapter, that alleles from other participants could be found in some samples. As they are known to work in a relatively small office space, it is likely that indirect transfer is prevalent as described by Goray and van Oorschot (2015) in relation to indirect transfer in social settings. While this can be inferred due to a knowledge of the participants environment, this is not typically known in relation to a evidential item and furthermore, detailed inferences around mechanisms of transfer are less reliable in these studies due to other factors potentially influencing this such as the variances with the swab material and surfaces being utilised. As explained by Gosch and Courts (2019), a standardised methodology, in relation to these factors, is more desirable when assessing DNA transfer events. In contrast to the data produced from phase 1 samples, rayon swabs were found to recover the highest number of donor alleles from drinking glasses and plastic handled screwdrivers which suggests that in a less controlled environment these swabs may perform well on non-porous surfaces which supports the findings of Verdon *et al.* (2014) who found that rayon swabs outperformed the foam swabs other than on a wooden surface which is further supported by the data produced in this study as foam swabs produced a greater number of alleles from wooden surfaces than the other swab materials.

Overall, this research suggests that foam swabs consistently produce high quality DNA profiles from wooden surfaces and perform comparably across all surfaces with cotton

swabs. Despite the rayon swabs producing more alleles from drinking glasses and plastic handles screwdrivers when samples were deposited using the phase 2 deposition protocol, these swabs would not be recommended for the recovery of touch DNA samples from the surfaces used in this research as they produced significantly fewer alleles from samples in phase 1 than all other swabbing materials. Based upon this data, the researcher proposes that the following recovery strategy should be adopted to increase the DNA recovery potential for touch DNA evidence: foam swabs would be recommended for touch DNA recovery from wooden surfaces and textured or ridged plastic surfaces and cotton swabs would be recommended for touch DNA recovery from metal or glass surfaces.

Finally, it was observed that prolonged storage of samples at room temperature significantly impacted the quality of the DNA profiles produced from touch DNA samples with greater degradation being observed in these samples. This provides valuable insight into the potential to obtain successful DNA profiles from these samples collected from a crime scene as the environmental factors and time since deposition is unknown. However, when the time since deposition is thought to be short, there is a greater potential of obtaining a successful DNA profile when the sample is recovered and stored within a freezer until analysis than when samples have been left at room temperature for a prolonged period of time prior to recovery.

Chapter 6. A Review of DNA Interactions Using Diamond™ Nucleic Acid Dye

6.1. Introduction

While the efficiency of recovery methods has been assessed, there has been limited research into the interactions that take place between the deposited DNA, the surface and the recovery method. Bonsu, Higgins and Austin (2020) discussed how the chemical interactions taking place between the DNA and metal surfaces it is deposited on can have an impact upon the successful recovery from such surfaces but did not look into the reactions taking place between the recovery method and the DNA when this is used on a metal surface. Bruijns, Tiggelaar and Gardeniers (2018) also discussed the swab morphology, absorption capacity along with the extraction and recovery efficiency of different swab materials in relation to saliva samples but this was not evaluated in relation to touch DNA which may alter these factors due to this DNA typically comprising of smaller, more fragmented DNA (Kita *et al.* 2008 and Zoppis *et al.* 2014) which may be less likely to remain trapped within the swab morphology.

Some studies have begun to utilise cell staining techniques to visualise the DNA present upon a surface to indicate the presence of touch DNA and provide inferences around the interactions which take place such as in the study conducted by Haase *et al.* (2019) and these have been further discussed in section 1.4. of this thesis. Due to the increasing success at staining touch DNA using reagents such as Diamond™ Nucleic Dye (Kanokwongnuwut *et al.* 2018; Haase *et al.* 2019; Champion *et al.* 2020 and Young and Linacre, 2020), this was utilised to track the interactions taking place between the deposited touch DNA, surfaces and swab types to provide insight into why different recovery methods perform variably upon different surfaces.

The remainder of this chapter aims to address the DNA interactions taking place between the DNA, swab and surface along with aiming to assess the collection and release efficiencies of the swabs utilised in chapter 5 of this thesis. Such an application of Diamond™ Nucleic Acid Dye to this extent has not, to the researchers knowledge, been conducted, therefore this study acts as a proof of concept for the use of this Nucleic Acid Dye in this way.

6.2. Materials and Methods

Touch DNA samples were deposited by the researcher upon aluminium drinks cans (n=4), plastic handled screwdrivers (n=4), drinking glasses (n=4) and wooden handles (n=4) as outlined in section 2.3.2.

To visualise the DNA deposited on these items, Diamond™ Nucleic Acid Dye (DD, Promega, USA) was diluted 100x in 75% ethanol. Due to the nature of the mock evidential items being larger than those utilised in published data, the Diamond™ Nucleic Dye was diluted 100x in 75% ethanol to ensure this was of a viscosity and volume that would cover the entirety of the mock evidential items which differs from the dilution factor used in published data (Kanokwongnuwut *et al.* 2018; Haase *et al.* 2019; Champion *et al.* 2020 and Young and Linacre, 2020), however staining of the touch DNA was still clearly visible upon the surfaces of these items.

The mock evidential items were then rinsed in the diluted Diamond™ Nucleic Acid Dye so the entirety of the item was exposed and then left to dry. The items were then viewed using the DCS®5 instrument with a 494nm to 558nm filter and images were taken. Following this, the samples were recovered from the surfaces using cotton swabs, foam swabs, polyester swabs and rayon swabs using the method described in section 5.2.1. The touch DNA that was previously stained on the items was transferred, during this recovery process, to the swabs and the swabs were then visualised and images were

taken using the DCS®5 as above. The recovered DNA was then extracted following the DNA Extraction protocol explained in chapter 2 but the swabs were retained and re-imaged using the DCS®5 to determine the release rate of the swabs.

The images of the visualised cells were then viewed and the number of cells fluorescing was manually counted in each set of images to determine the collection and release rate of each recovery method. As there was potential for human error with this approach with manually counting the cells, this was completed three times and a mean was taken of the values to reduce any errors within the data.

The number of cells present upon each of the surfaces were manually counted before and after DNA recovery to determine the number of cells recovered by the different swab materials to account for any cells not observed upon the images due to them being present within the interior fibres of the swabs. Following DNA extraction, the cells remaining upon the swabs were counted to determine the release rate of each swab material for each of the surfaces.

To calculate the recovery rates for each swab material the number cells left upon the surface following recovery was divided by the number of cells present on the surface prior to recovery and this was multiplied by 100 to generate a percentage. Similarly to calculate the release rate for each swab material the number of cells left upon the swabs post-extraction was divided by the number of cells present on the swabs prior to extraction and this was again multiplied by 100 to generate a percentage.

6.3. Results and Discussion

The cell counts per surface are displayed in table 22 and these can be seen to vary considerably between repeats indicating that the number of cells deposited by a donor is inconsistent which has been discussed by Phipps and Petricevic (2007) who found that the amount of DNA deposited by an individual can vary considerably based upon

environmental factors. Due to this variability, the collection and release rates calculated from this data may not be as accurate and further repeats are required to obtain more accurate data. To illustrate some of this variability, figures 18-21 display the images taken from the plastic handled screwdrivers following sample deposition.

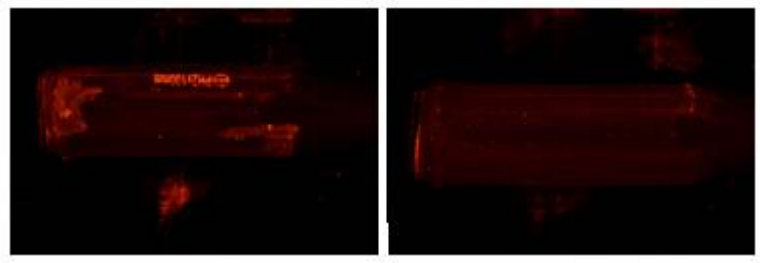


Figure 18. Touch DNA Deposits on Screwdriver 1

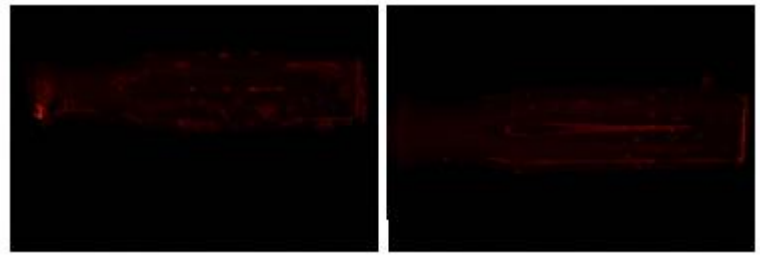


Figure 19. Touch DNA Deposits on Screwdriver 2

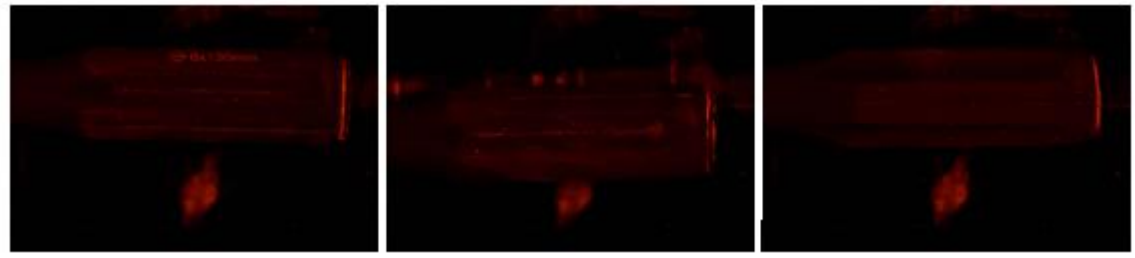


Figure 20. Touch DNA Deposits on Screwdriver 3

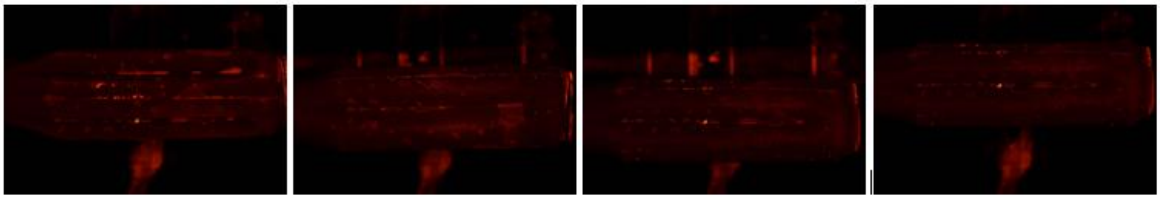


Figure 21. Touch DNA Deposits on Screwdriver 4

Surface Type	Swab Material	Initial Cell Count	Post-Recovery Cell Count	Recovered Cells	Post-Extraction Cell Count
Aluminium Drinks Can	Polyester	1980	634	1346	18
Aluminium Drinks Can	Cotton	1546	276	1270	23
Aluminium Drinks Can	Foam	1924	164	1757	22
Aluminium Drinks Can	Rayon	2358	179	2179	9
Drinking Glass	Polyester	784	103	681	16
Drinking Glass	Cotton	183	21	162	5
Drinking Glass	Foam	296	21	271	6
Drinking Glass	Rayon	321	6	315	4
Plastic Handled Screwdriver	Polyester	828	51	777	38

Plastic Handled Screwdriver	Cotton	212	70	142	8
Plastic Handled Screwdriver	Foam	337	69	268	10
Plastic Handled Screwdriver	Rayon	286	92	194	2
Wooden Handle	Polyester	373	28	345	4
Wooden Handle	Cotton	68	13	55	3
Wooden Handle	Foam	222	58	164	15
Wooden Handle	Rayon	232	78	154	4

Table 22. Cell counts obtained from touch DNA deposits upon varying surface types and recovered using cotton, rayon, foam and polyester swab materials.

From these cell counts, collection and release rates were calculated for each swab type upon each surface which are displayed in figure 22. This data shows that polyester swabs had the lowest collection rate from aluminium drinks cans and drinking glasses which supports a lower number of alleles and donor alleles being recovered from these surfaces

in phase 1 and 2 of the recovery method study. Cotton swabs were observed to have the lowest recovery rate from plastic handled screwdrivers which was also found in phase 2 of the recovery method study in chapter 5 with cotton swabs producing the lowest number of alleles from this surface type. When looking at the recovery rates for wooden handles, it can be seen that rayon swabs produce the lowest recovery rate which aligns with the data produced in relation to the number of alleles recovered from this surface in phase 1 of the recovery method study in chapter 5.

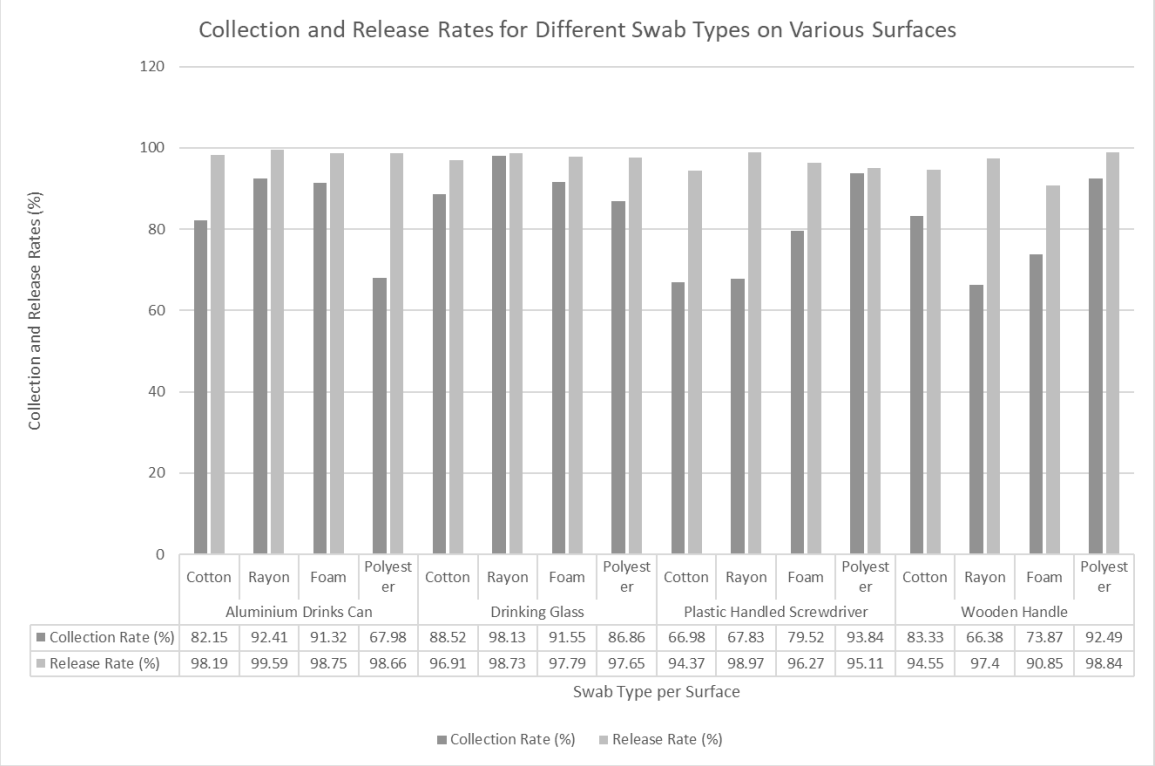


Figure 22. A chart displaying the collection and release rates for different swabs from aluminium drinks cans, drinking glasses, plastic handled screwdrivers and wooden handles.

When looking at the release rates for the different swab materials from the mock evidential items used in this study, it can be seen that all swabs have a release rate of 90% or higher. While this suggests that the majority of the touch DNA recovered is being released from the swabs and not being retained within the swab, this data may not be accurate. This is due to the images of the swabs post-extraction being taken with no

magnification unlike the images taken in published research (Kanokwongnuwut *et al.* 2018; Haase *et al.* 2019; Champion *et al.* 2020 and Young and Linacre, 2020). Due to this, there may be cells that are trapped within the inner fibres of the swabs that are not visible upon the images. Additionally, while limited autofluorescence was observed when images were taken of the swabs prior to DNA extraction, this did increase following DNA extraction which is likely due to some of the extraction reagents being retained within the swabs, an example of this can be seen in figure 23. Similar findings were discussed by Kanokwongnuwut, Kirkbride and Linacre (2018b) and Cook, Mitchell and Henry (2021), however their findings were in relation to swabs prior to DNA extraction taking place.

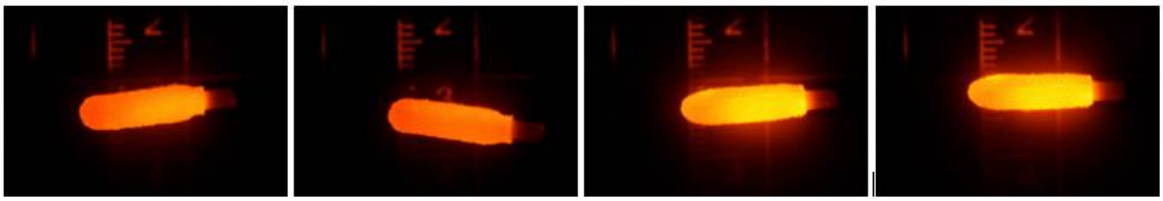


Figure 23. Remaining DNA from Can 3 on Foam Swabs Post-Extraction demonstrating the auto-fluorescence observed.

Bruijns, Tiggelaar and Gardeniers (2018) also conducted a study to assess the extraction efficiency (termed release rate in this study) of various swab materials in relation to saliva samples and found that foam swabs had a higher extraction efficiency than polyester, cotton and rayon swabs. However, the data displayed in table 23 demonstrates that for touch DNA samples, rayon swabs have the highest collection and release rate. The reason for this difference may be due to different biological samples being used as saliva stains are of a higher quantity than touch DNA samples and may interact with the swab materials differently as suggested by Kirgiz and Calloway (2017). Alternatively, these differences may be observed as an element of human error may be in occurrence with the data produced in this study due to the number of cells present on the surface types and swabs were manually counted, however control measures were taken to reduce this possibility, so this theory is less likely. Similar methodologies have been used by

Kanokwongnuwut *et al.* (2018) and Champion *et al.* (2020) however, the area observed was much smaller with cells being counted over a 1mm^2 surface area rather than the entirety of an evidential item as was conducted in this study with no magnification being applied to the samples.

Swab Type	**Collection Rate (%)**	**Release Rate (%)**
Cotton	81.09	97.61
Rayon	88.9	99.33
Foam	88.52	97.85
Polyester	79.42	97.59

Table 23. Collection and release rates for cotton, rayon, foam and polyester swabs.

While the data produced in this study is considerably varied, it provides some insight into the number of cells being deposited when an item is handled and the interactions taking place during the recovery and analysis process. To provide further insight into the release rates of these swab materials, the same study should be repeated using a higher magnification in an attempt to visualise any cells trapped within the interior of the swabs and provide a more accurate indication of the release rates of these swab types.

6.4. Conclusion and Further Work

This chapter aimed to visualise the interactions that take place between the DNA, swab and surface to give some insight into the recovery and release rates of the swabs and to indicate whether Diamond™ Nucleic Acid Dye could be used for this purpose.

From the data produced in the chapter, some initial insight was provided around the interactions that take place between the DNA, surface and swab materials throughout the recovery and analysis process. This data was based upon the number of cells counted on the surfaces of the mock evidential items before and after recovery and the number of cells detected on the surface of the swabs post-extraction. This data was found to be highly variable due to the small number of samples analysed and the variability in the donor's propensity to deposit touch DNA upon the mock evidential items.

Based on this data and the quantification data produced in chapter 5, further investigation is required to determine which genetic components of a touch DNA deposit contribute towards the DNA profiles produced as the cell counts and quantification data produced do not appear to correlate with the quality of the profiles produced in chapter 5.

Additionally, further data is required to assess the overall recovery and release rates of each swab type upon the surfaces reviewed in these studies. However, it does appear that Diamond™ Nucleic Acid Dye can be used to indicate the recovery and release rates of swab materials, which has yet to be used in published research. To make this application more robust and less time consuming, it is the recommendation of the researcher that a cell counting software could be utilised/adapted rather than a manual counting process. Furthermore, to make the application of the Diamond™ Nucleic Acid Dye easier and more evenly spread, it is suggested that a pressurised spray device is used as described by Young and Linacre (2020).

Chapter 7. Overall Discussions, Conclusions and Recommendations

7.1. Overall Discussion

While understanding the origins of touch DNA evidence will greatly improve the investigative process for this evidence type, including how to optimise the recovery and analysis of these samples, it is still possible to obtain good success rates from touch DNA deposits with currently available analysis methods despite the previously reported 5-6% success rates published by Quinones and Daniel (2011). However, selecting the correct recovery and analysis methods are vital to the success of this evidence type despite not yet having that greater understanding, informed decisions regarding this can still be made to optimise this process (May and Thomson, 2009; Aditya *et al.* 2011; Mulligan *et al.* 2011; Verdon *et al.* 2014 and Hess and Haas, 2017). For instance, the first step of the analysis process is the successful recovery of the touch DNA deposit from the evidential item. As discussed by Verdon *et al.* (2014) it is important to develop an optimal recovery strategy that takes into consideration the nature of the sample being recovered, the surface this has been deposited upon and the swab material used to recover the DNA present. Limited research has been conducted into this since Verdon *et al.* (2014) which utilised older, less sensitive DNA profiling techniques which are no longer used in practice. The data produced in chapters 3 and 5 of this thesis can be used to suggest an optimal recovery strategy for touch DNA that was previously only based upon success rate with other biological sources such as blood and saliva. It was found that more donor alleles were recovered from plastic handled screwdrivers than aluminium drinks cans when touch DNA samples were analysed using Rapid DNA technologies which suggests that the nature and texture of the surface may impact the propensity to recover touch DNA from the surface which is supported by Oldoni, Castella and Hall (2016). When utilising standard

laboratory practices, it was found that the surface type alone did not have a significant impact upon the quality of the DNA profiles produced from touch DNA samples. However, in both the analysis with RapidHIT™ 200 and NGM SElect™, it was found that the number of alleles and donor alleles varied per surface depending upon the recovery method used on that surface, further demonstrating the need to adopt an optimal recovery method for this evidence type. When utilising the RapidHIT™ 200 instrument to analyse touch DNA samples it was found that more donor alleles were recovered from the aluminium drinks cans using foam and viscose swab materials. Whereas, more donor alleles were recovered from the plastic handled screwdrivers when using the cotton and nylon flocked swab materials. In contrast to this, the data from phase 1 samples analysed using NGM SElect™ in chapter 5 suggests that when touch DNA is deposited upon a smooth, non-porous surface such as metal or glass a cotton swab may be more efficient at recovering the deposited DNA. While foam swabs did display good success rates from non-porous surfaces in this study, as well as the study conducted using the RapidHIT™ 200 instrument, no statistically significant differences were observed between the cotton and foam swabs, and the cotton swabs produced a greater number of donor alleles from both surfaces. However, foam swabs were slightly more efficient at touch DNA recovery from plastic handled screwdrivers than cotton swabs suggesting that from a more textured/ridged plastic foam swabs may be more proficient. However, the difference was not significant suggesting that good success rates can be obtained using cotton or foam swabbing materials on a plastic surface. Additionally, foam swabs were found to be highly efficient at touch DNA recovery from an absorbent wooden surface and polyester swabs were found to be equally efficient at recovering touch DNA from this surface so either of these swabbing materials would be recommended for the recovery of touch DNA from an absorbent wooden surface.

It was also found through the analysis of touch DNA samples with the RapidHIT™ 200 instrument that nylon flocked swabs produced significantly less alleles than the other

swab materials demonstrating that despite their success with higher quantity samples (Benschop *et al.* 2010), they are not efficient at touch DNA recovery and should therefore not be utilised for this type of evidence. This shows clear support for the suggestion made by Kirgiz and Calloway (2017) that recovery methods that have been proven to be effective at recovering high quantity samples may not be as effective at recovering trace DNA. Furthermore, when looking at the swab types used in chapter 5 that were analysed with NGM SElect™ it was found that rayon swabs also consistently produced significantly fewer alleles than other swab materials suggesting that this material is also not effective at recovering touch DNA samples. This data directly contradicts that produced by Wiley *et al.* (2017) who found that nylon flocked and rayon swabs produced a greater number of full and partial profiles than the cotton swab when using Rapid DNA technologies. However, this was in relation to blood samples so the reason for this difference is likely due to the differing nature of the samples analysed, further emphasising the need to adopt an optimal recovery strategy for touch DNA evidence that takes into account the recovery method and surface type combinations, such as the strategy suggested in this research in section 5.4.

To further optimise the analysis of touch DNA samples, the use of Rapid DNA technologies to analyse this evidence type was investigated. It has previously been reported that Rapid DNA analysis should not be utilised to analyse evidential items and that these technologies should only be used for higher quantity samples and reference samples and should not be utilised for the analysis of trace DNA (Thong *et al*. 2015). Additionally, Mapes *et al.* (2016) and Moreno, Brown and Callaghan (2017) suggested that a higher DNA quantity is required for a successful DNA profile to be obtained using Rapid DNA analysis than with standard laboratory analysis suggesting that as poor success rates were published for touch DNA samples using standard laboratory analysis (Quinones and Daniel, 2012) these samples would not generate high success rates using Rapid DNA analysis. However, in chapter 3 of this thesis, touch DNA samples were

recovered from aluminium drinks cans and plastic handled screwdrivers and analysed using the RapidHIT™ 200 instrument to determine if it was possible to obtain high quality DNA profiles using this instrument. The data produced from this study demonstrated that it was possible to obtain a full DNA profile from touch DNA samples using Rapid DNA analysis with 11.11% of the samples analysed producing a full STR profile. Furthermore, it was found that 31.94% of the samples analysed produced a profile of a quality appropriate for loading onto the National DNA Database (NDNAD) with 80% or more of the donor alleles being present which is substantially greater than the previously reported 5-6% success rates (Quinones and Daniel, 2012). This data was supported by Gangano *et al.* (2013) who were able to produce full profiles from other low quantity samples suggesting that while the Rapid DNA technologies are reported to have a reduced sensitivity (Thong *et al.* 2015 and Shackleton *et al.* 2019) it is possible to analyse low-level samples with these instruments. To provide further support to the ability to analyse these samples it can be observed from the analysis of touch DNA samples with NGM SElect™ in chapter 5 that full DNA profiles were obtained from 16.40% of the samples analysed during phase 1 of the study and 3.90% of the samples analysed during phase 2 of this study which demonstrates that the results produced using the RapidHIT™ 200 instrument is comparable to those produced using standard laboratory processes.

While this is a great development, in regards to the uses of Rapid DNA analysis, concerns were raised by Mapes *et al.* (2016) regarding the use of Rapid DNA technologies for lower level samples due to some of the sample being consumed during this analysis meaning it may no longer be possible to analyse this sample using standard laboratory processes should the analysis be unsuccessful. Wiley *et al.* (2017) reported that despite some sample consumption during Rapid DNA analysis, it is possible to reanalyse these samples in the event of a run failure and generate good quality results. This was further assessed in relation to touch DNA samples in chapter 4 of this thesis and it was determined that the samples that were analysed with RapidHIT™ 200 and then reanalysed with NGM

SElect™ demonstrated no significant differences in regards to the number of alleles produced which provides further reassurance that should the sample be analysed with Rapid DNA technologies unsuccessfully this sample can be reanalysed using standard processes with concordant results being produced. Despite there being no statistically significant differences between the data produced using the RapidHIT™ 200 instrument in comparison to the reanalysis with NGM SElect™ it was observed that there was fewer alleles produced from the reanalysis in some instances which demonstrates that some sample consumption is experienced as suggested by Mapes *et al.* (2016). However, in these cases, the alleles that were not present in the reanalysis were available in the initial analysis so the data itself was not lost and could still be used for investigative leads. To provide further support for the use of Rapid DNA technologies for low-level samples such as touch DNA, it was also observed that fewer non-donor alleles were produced when the samples were analysed using the RapidHIT™ 200 instrument in comparison to the reanalysis of these samples with NGM SElect™ which demonstrates that the reduced handling time of these samples provides a reduced opportunity for contamination to occur (Moreno, Brown and Callaghan, 2017).

This suggests that while there are still further advancements that can be made in relation to the understanding and optimisation of touch DNA analysis, the optimisation options currently available can aid the investigative process by allowing for quicker analysis of these samples (Gangano *et al.* 2013; Hennessy *et al.* 2013 and Verheij *et al.* 2013) and a more optimised recovery process taking into consideration the surface type the touch DNA is deposited upon as suggested by Verdon *et al.* (2014).

7.2. Conclusions and Recommendations

This aim of this research was to critically evaluate the recovery methods of low-level DNA to maximise efficiency for identification purposes. This was proposed to be achieved by

using multiple recovery methods to collect low-level DNA for profiling using the latest profiling technologies (NGM SElect™ and RapidHIT™).

Despite there being a lack of understanding of what is present within a touch DNA sample or how this DNA is transferred from the hands to an object, it was still possible to optimise the analysis of these samples by investigating differing recovery methods and analysis methods. This led to the discovery that, despite published researching suggesting it is not possible to analyse low-level samples such as touch DNA with Rapid DNA technologies (Thong *et al.* 2015 and Shackleton *et al.* 2019), this is not the case, as demonstrated in chapter 3 of this thesis, it is possible to analyse touch DNA samples with the RapidHIT™ 200 instrument. Touch DNA samples analysed with this method generated complete DNA profiles from 11.11% of the samples analysed. Additionally, 31.94% of the samples were deemed to be of sufficient quality for loading onto the NDNAD based upon requirements reported by Gangano *et al.* (2013). This means that investigative leads can be gained from touch DNA exhibits in as little as 2.5 hours, which is of even greater benefit for forces, such as Staffordshire Police, who have a high throughput of this evident type. It was also discovered in accordance with Wiley *et al.* (2017) that it is possible to reanalyse the DNA samples following Rapid DNA analysis and obtain results with NGM SElect™ analysis that are not statistically significant in comparison to those obtained from Rapid DNA analysis. This greatly widens the possibilities for the analysis of this evidence type as these samples can now be analysed within a much shorter timescale to provide investigate leads earlier within a criminal investigation while also having the possibility to reanalyse these samples using standard processes if required.

Further optimisation was also possible through the analysis of the efficiency of different recovery methods in relation to recovering touch DNA from a select set of surfaces. From this analysis it was determined that nylon flocked and rayon (also known as viscose) swabs produced significantly poorer results than cotton, foam and polyester swabs when recovering touch DNA from a variety of surfaces. This demonstrates that while these swab

materials were effective at recovering higher quantity samples (Wiley *et al.* 2017), they are not effective at touch DNA recovery and should therefore not be utilised for this evidence type. While no statistically significant differences were obtained from the remainder of the swab comparisons in relation to touch DNA recovered from aluminium drinks cans, drinking glasses, plastic handled screwdrivers and wooden handles, there were still observable differences which allow recommendations to be made in relation to the optimal recovery strategy. For instance, when touch DNA is deposited upon a smooth, non-porous surface such as metal or glass a cotton swab can be seen to be more efficient at recovering the deposited DNA. However, foam swabs were found to be slightly more efficient at touch DNA recovery from plastic handled screwdrivers than cotton swabs suggesting that from a more textured/ridged plastic foam swabs may be more proficient at recovering touch DNA. However, the difference was not significant suggesting that good success rates can also be obtained using a cotton swabbing material on a plastic surface. However, foam swabs were found to be highly efficient at touch DNA recovery from an absorbent wooden surface and polyester swabs were found to be equally efficient at recovering touch DNA from this surface so either of these swabbing materials would be recommended for the recovery of touch DNA from an absorbent wooden surface.

In addition to suggesting an optimal recovery strategy based upon the profile data produced, a preliminary study was also completed to visualise the DNA interactions taking place between the deposited DNA, surface and swab material used for recovery. While it was possible to visualise the cells deposited upon the surfaces and track these throughout the analysis process, the data obtained was considerably varied and did not fully correlate with the profile data produced from these samples. This was also found to be the case when looking at the quantification data produced from the touch DNA samples suggesting that the cells deposited and quantified may not be the main contributor to the overall DNA profiles produced which was also discussed by Burrill *et al.* (2021), in regards to the suitability of current quantification methods for touch DNA evidence. However, it was

determined that the interactions taking place can be visualised, from the deposition through to the extraction of the DNA, which to the researcher's knowledge has not been demonstrated in published research to date. It is therefore recommended by the researcher, that this be further reviewed with a wider range of participants and a larger number of repeats, in order to make these conclusions more robust. This has the potential to greatly aid the process of DNA recovery but will also allow for the optimal recovery strategy suggested here to be developed further which will lead to increased success rates for touch DNA evidence.

Finally, the samples deposited following the phase 2 protocol with no hand washing requirements and with prolonged storage at room temperature due to laboratory restrictions were found to produce significantly less alleles than samples the samples produced during phase 1 of the study where hand washing was controlled and the samples were recovered and stored within a freezer soon after deposition. This demonstrated that samples that have been exposed to the environment or were recovered after a prolonged period since deposition demonstrated a high level of degradation. However, as the time since deposition and environmental factors are often unknown with evidential items collected from a crime scene, this data is still relevant as it provides insight into the potential to recover touch DNA from items that have been exposed to sub-optimal conditions for a prolonged period of time.

Overall, it is recommended that touch DNA samples can be analysed using Rapid DNA technologies with comparative success rates being achieved to standard laboratory analysis which will greatly aid the investigative process through gaining these investigative leads in a timelier manner. It is also recommended that nylon flocked and rayon swabs should not be utilised for touch DNA recovery from metal, glass, plastic or wooden surfaces. However, an optimal recovery strategy for touch DNA from these surfaces is proposed with cotton swabs being most effective at touch DNA recovery from metal and glass surfaces and foam swabs being most effective at touch DNA recovery from textured

or ridged plastic and absorbent wooden surfaces. Despite these recommendations to provide the optimal success rates for touch DNA from these surfaces, as no statistically significant differences were observed between cotton, foam and polyester swabs when recovering DNA from metal, glass and wooden surfaces these swabs could be utilised on any of these surfaces with good success rates.

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Appendices

Appendix 1. Images of Touch DNA Deposits Staining upon Aluminium Drinks Cans with Diamond™ Nucleic Dye.

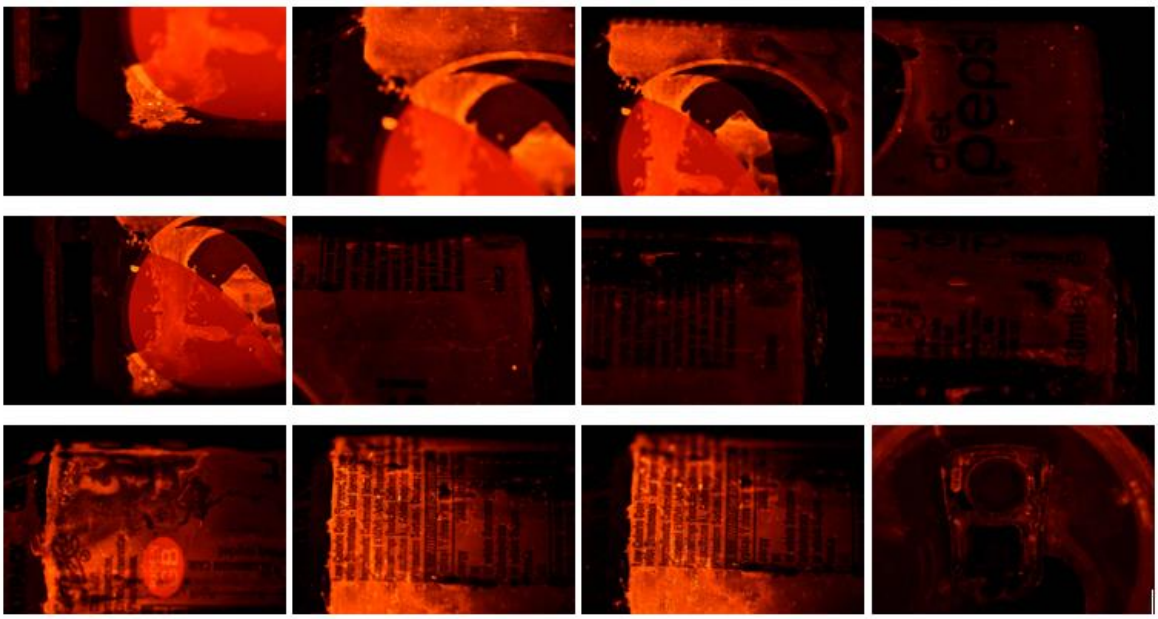

Figure 24. Touch DNA Deposits on Can 1

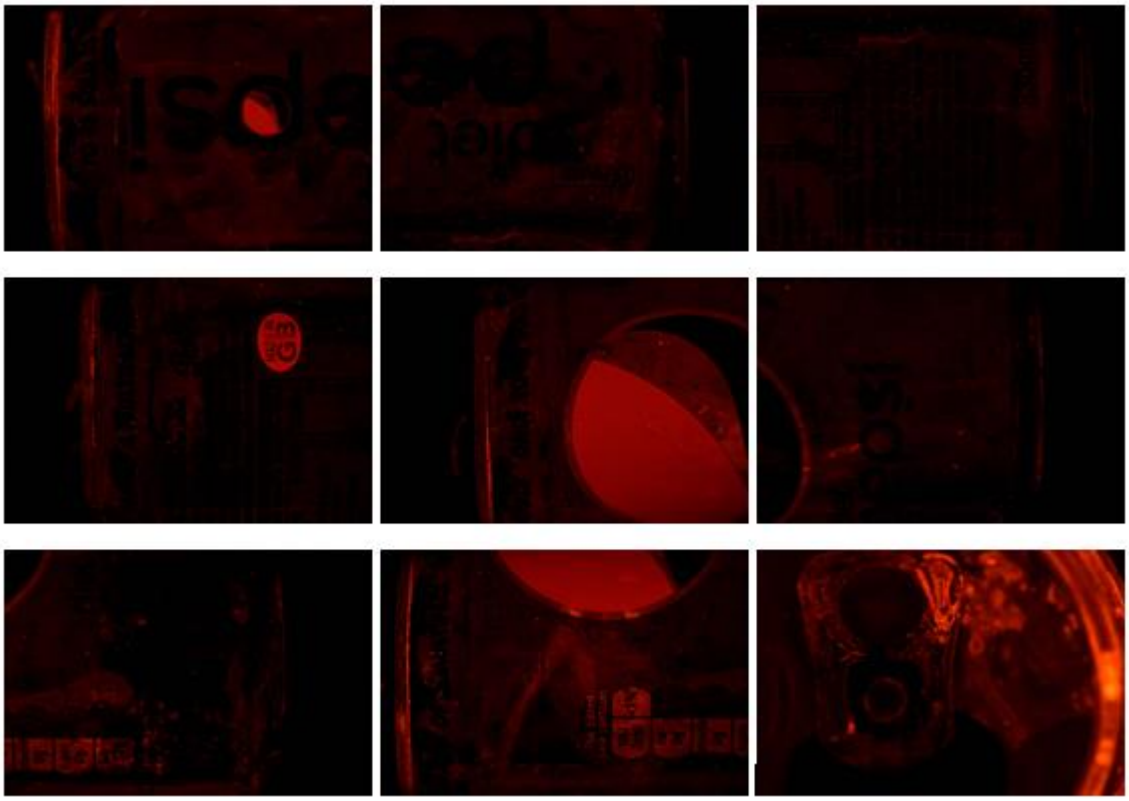

Figure 25. Touch DNA Deposits on Can 2

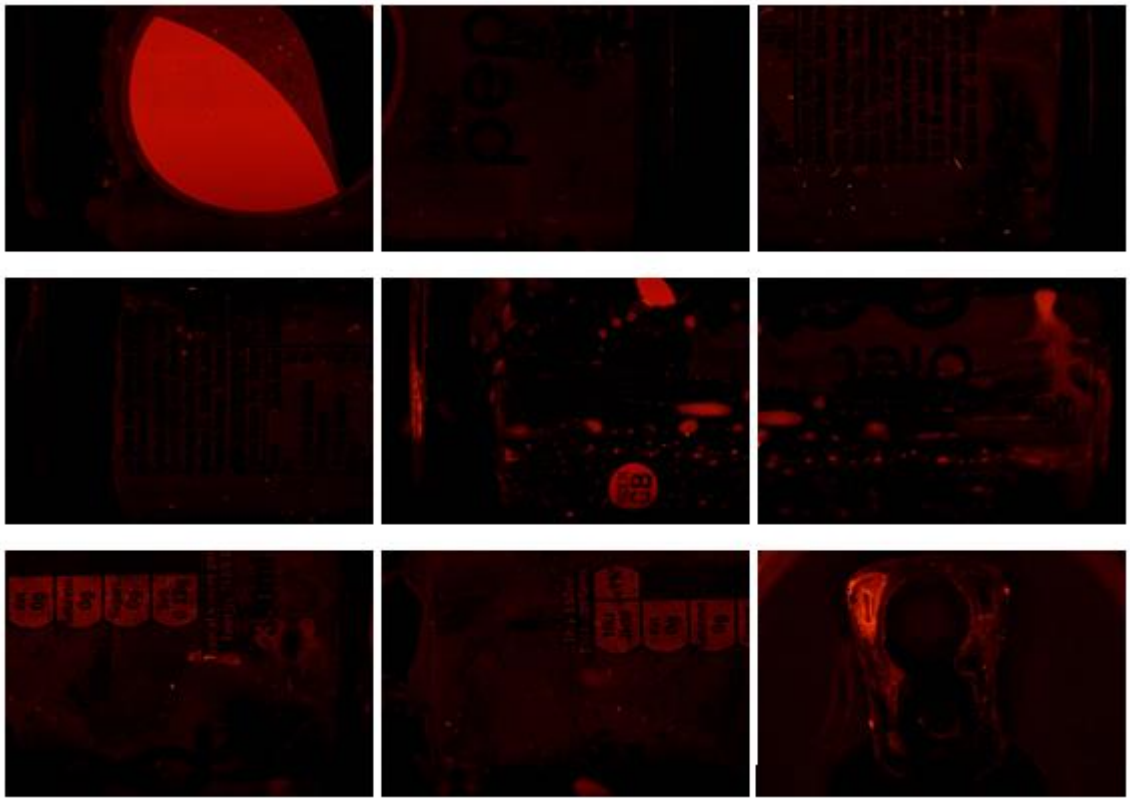

Figure 26. Touch DNA Deposits on Can 3

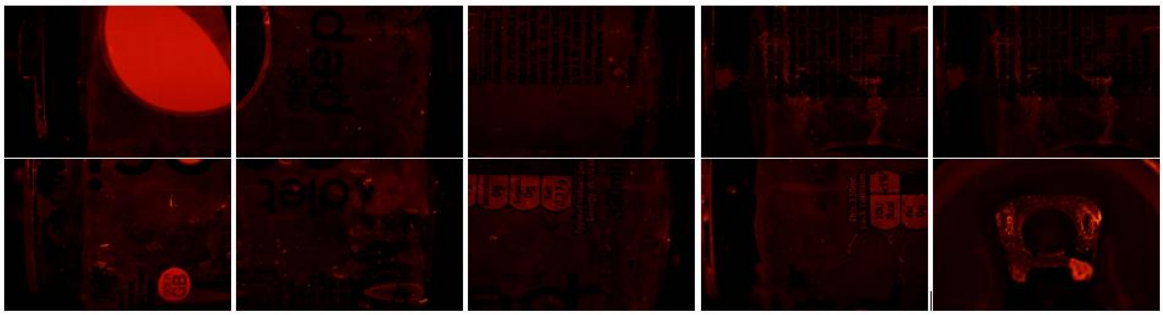

Figure 27. Touch DNA Deposits on Can 4

Appendix 2. Images of Remaining Stained Touch DNA on Aluminium Drinks Cans Post-Recovery.

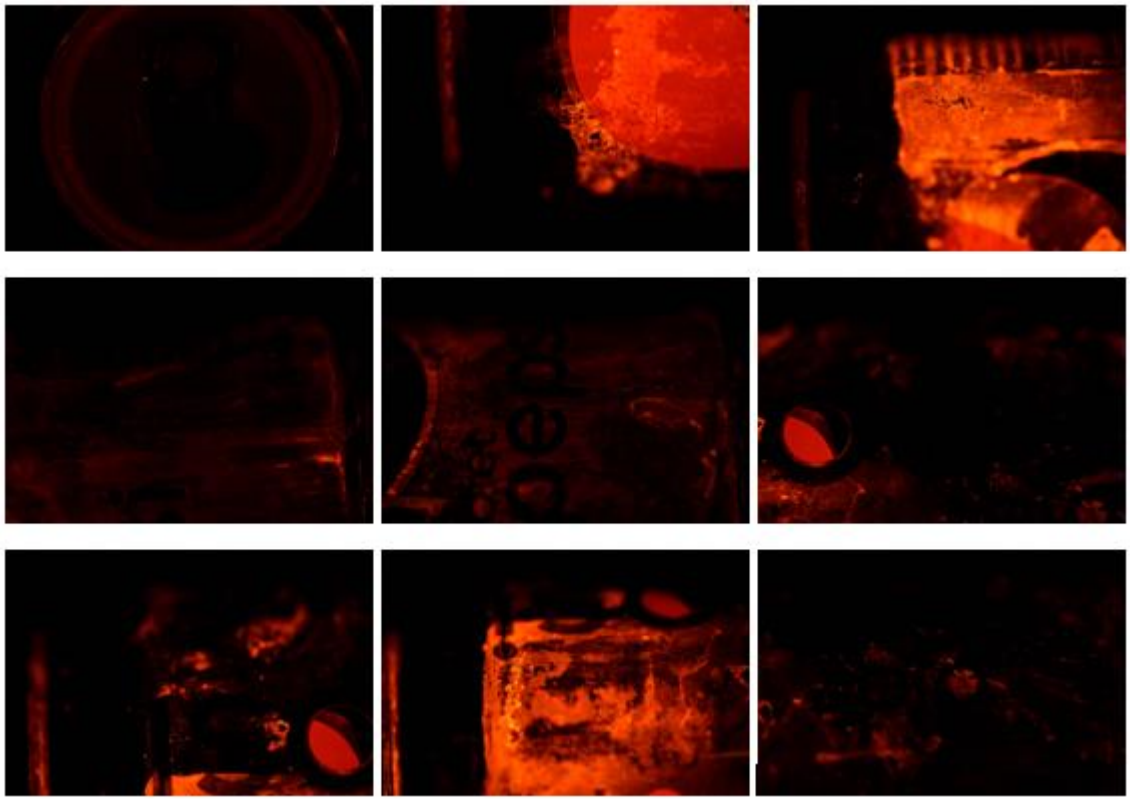

Figure 28. Touch DNA on Can 1 After Swabbing

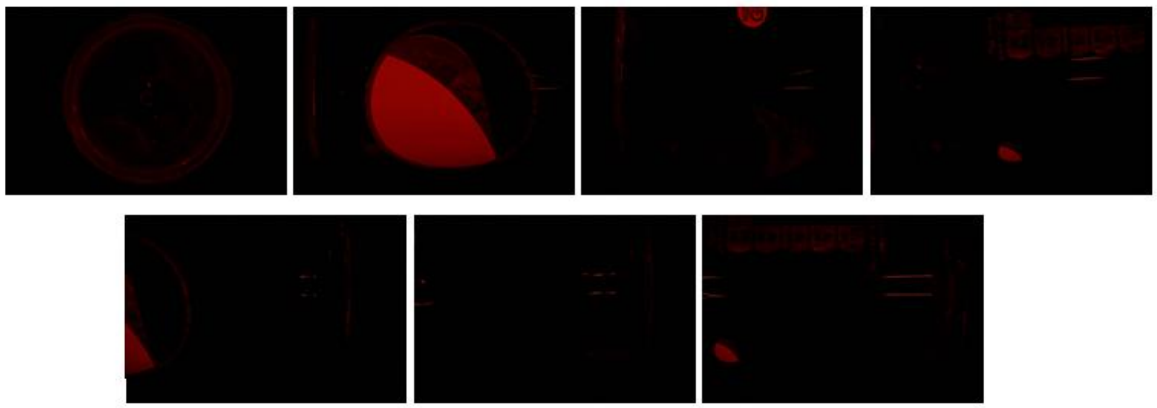

Figure 29. Touch DNA on Can 2 After Swabbing

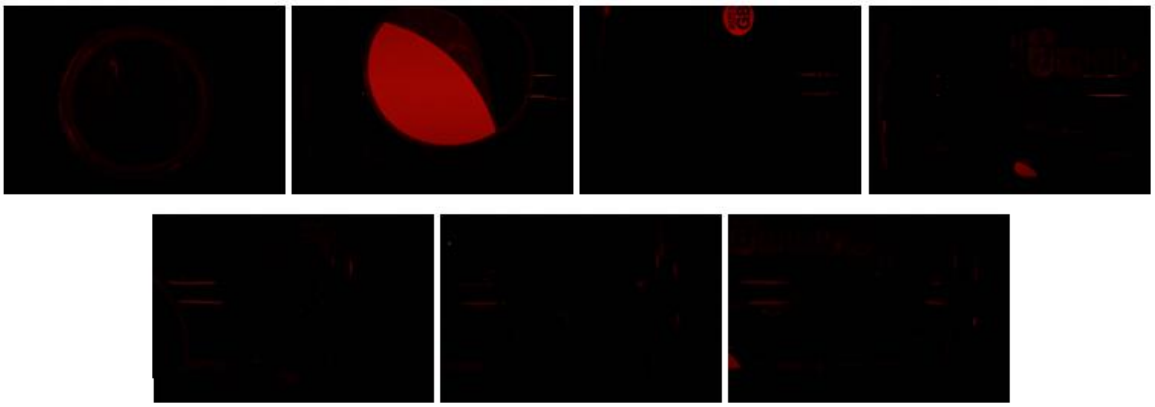

Figure 30. Touch DNA on Can 3 After Swabbing

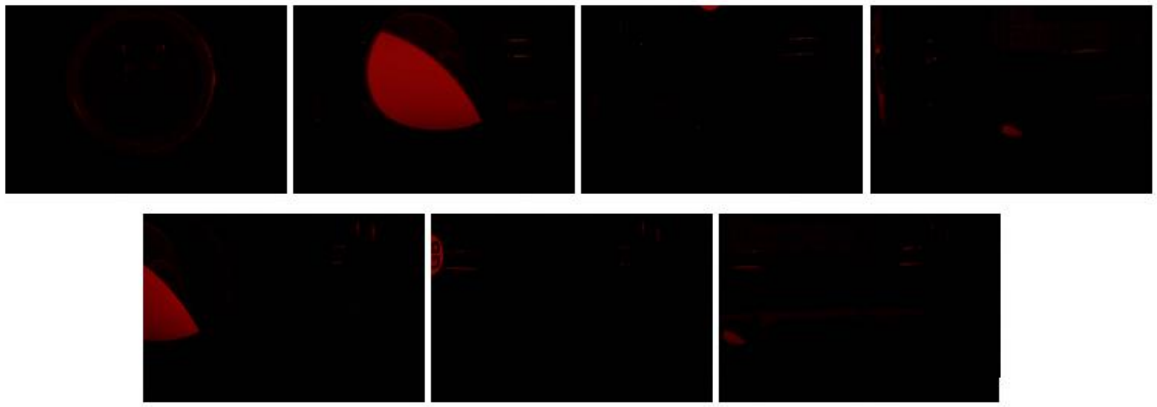

Figure 31. Touch DNA on Can 4 After Swabbing

Appendix 3. Images of Recovered Touch DNA on Swabs from Aluminium Drinks Cans Stained with Diamond™ Nucleic Dye

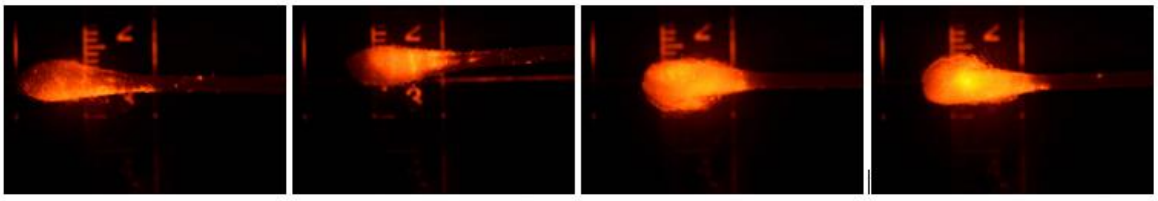

Figure 32. Recovered Touch DNA from Can 1 on Polyester Swabs

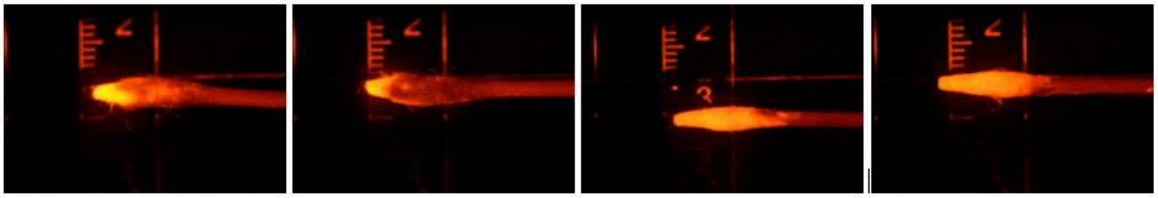

Figure 33. Recovered Touch DNA from Can 2 on Cotton Swabs

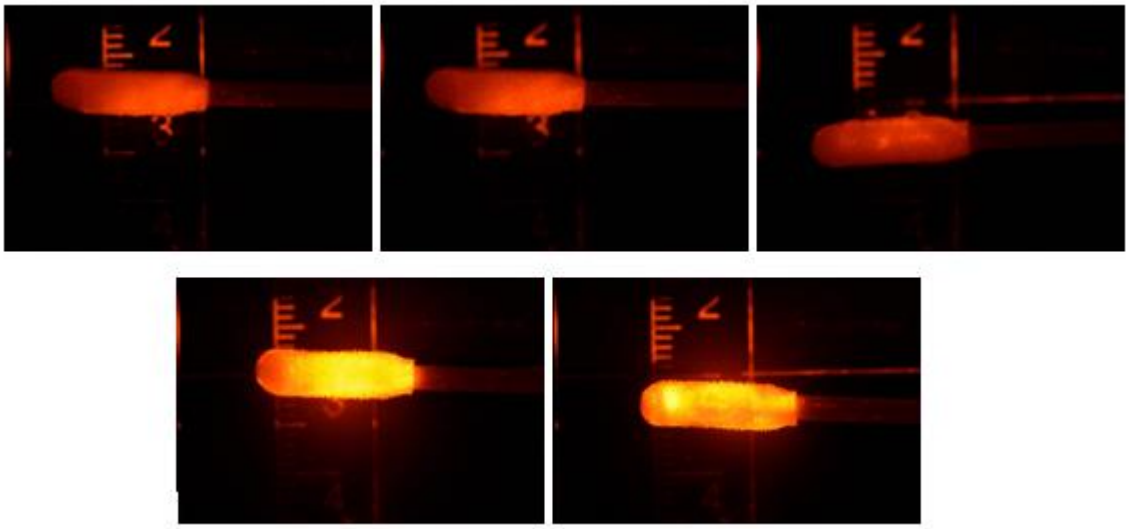

Figure 34. Recovered Touch DNA from Can 3 on Foam Swabs

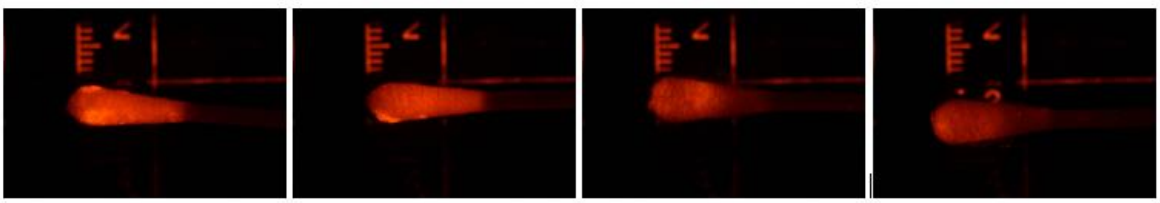

Figure 35. Recovered Touch DNA from Can 4 on Rayon Swabs

Appendix 4. Images of Remaining DNA on Swab Materials Used on Aluminium Drinks Cans Post-Extraction

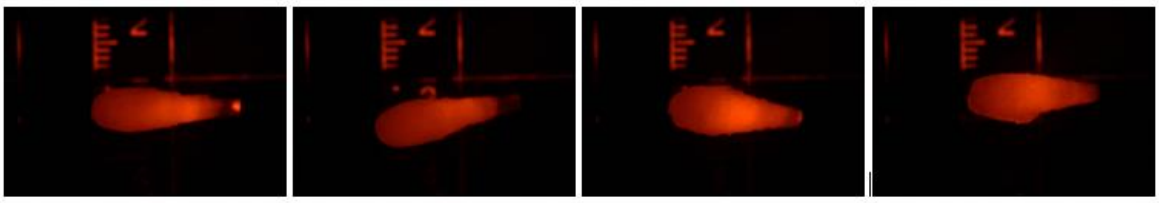

Figure 36. Remaining DNA from Can 1 on Polyester Swabs Post-Extraction

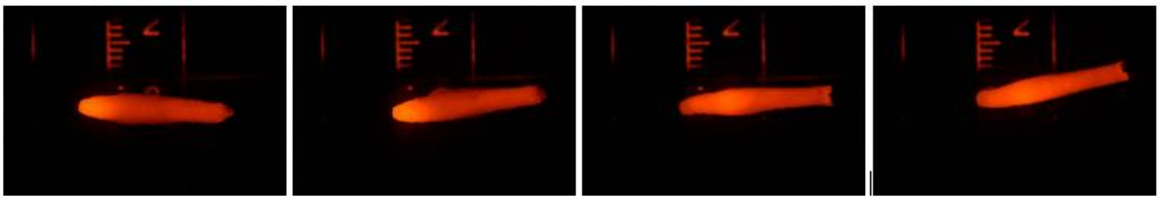

Figure 37. Remaining DNA from Can 2 on Cotton Swabs Post-Extraction

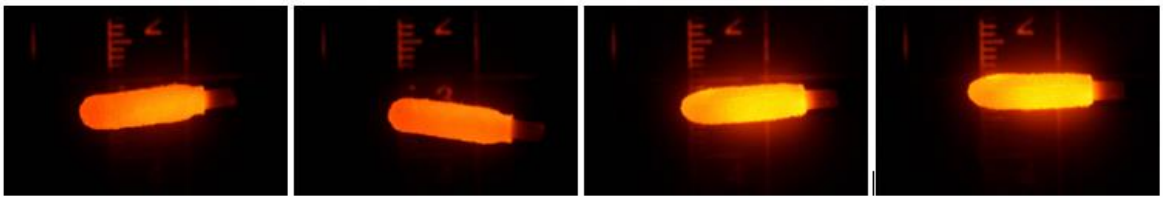

Figure 38. Remaining DNA from Can 3 on Foam Swabs Post-Extraction

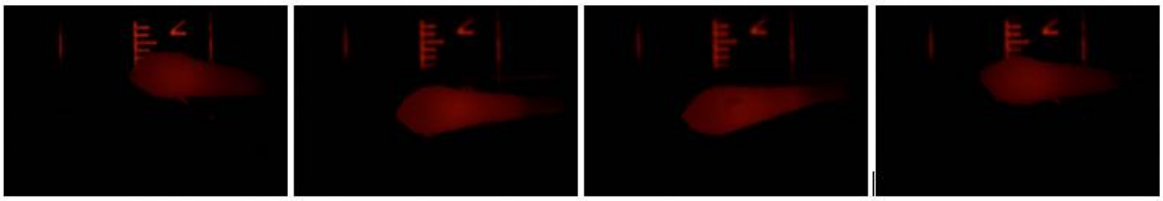

Figure 39. Remaining DNA from Can 4 on Rayon Swabs Post-Extraction

Appendix 5. Images of Touch DNA Deposits Staining upon Drinking Glasses with Diamond™ Nucleic Dye.

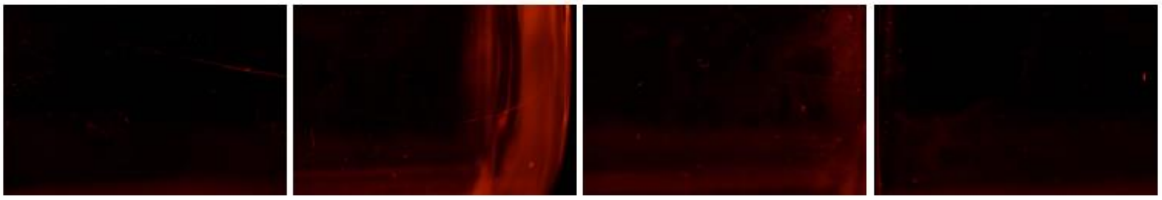

Figure 40. Touch DNA Deposits on Glass 1

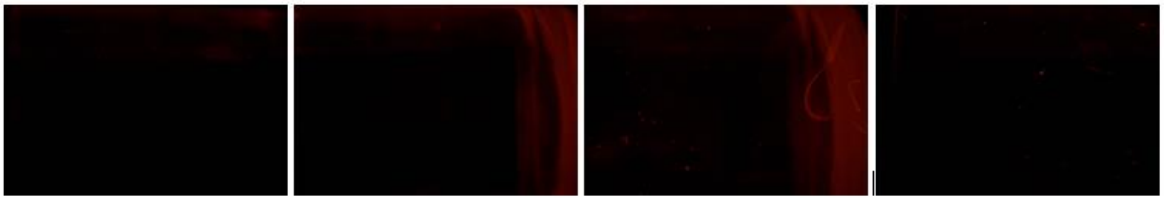

Figure 41. Touch DNA Deposits on Glass 2

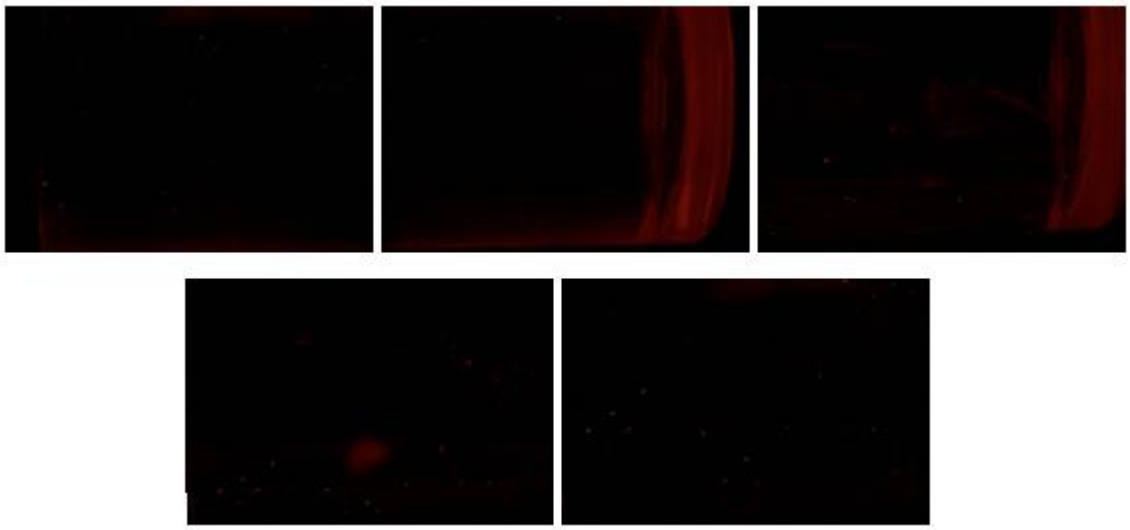

Figure 42. Touch DNA Deposits on Glass 3

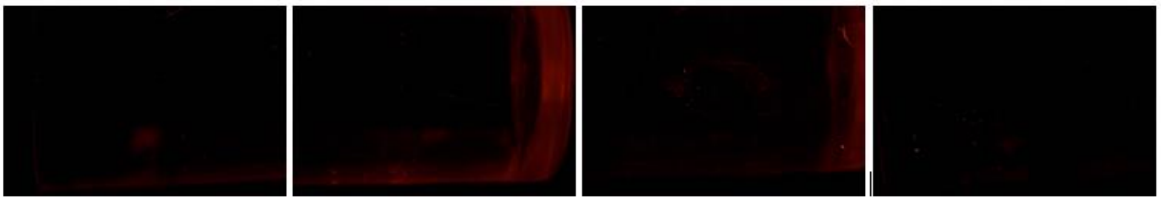

Figure 43. Touch DNA Deposits on Glass 4

Appendix 6. Images of Remaining Stained Touch DNA on Drinking Glasses Post-Recovery.

Figure 44. Touch DNA on Glass 1 After Swabbing

Figure 45. Touch DNA on Glass 2 After Swabbing

Figure 46. Touch DNA on Glass 3 After Swabbing

Figure 47. Touch DNA on Glass 4 After Swabbing

Appendix 7. Images of Recovered Touch DNA on Swabs from Drinking Glasses Stained with Diamond™ Nucleic Dye

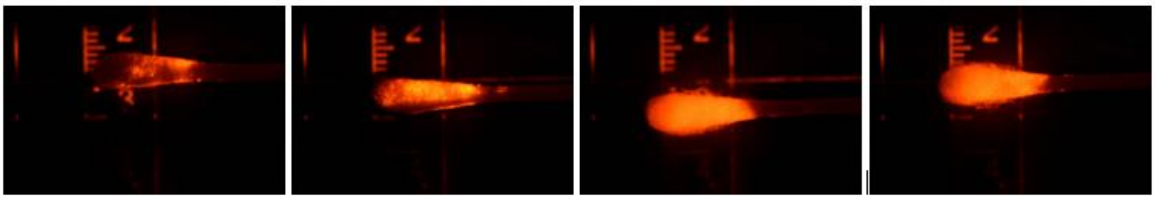

Figure 48. Recovered Touch DNA from Glass 1 on Polyester Swabs

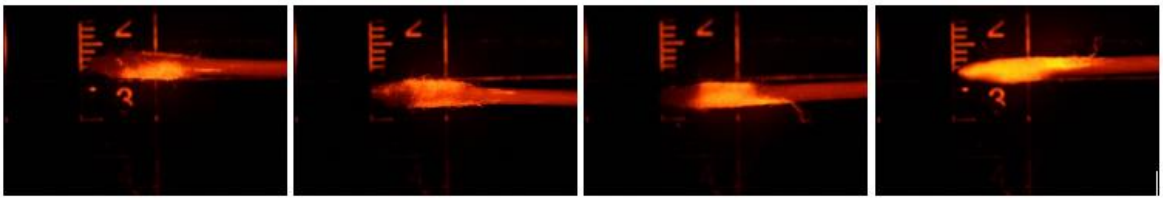

Figure 49. Recovered Touch DNA from Glass 2 on Cotton Swabs

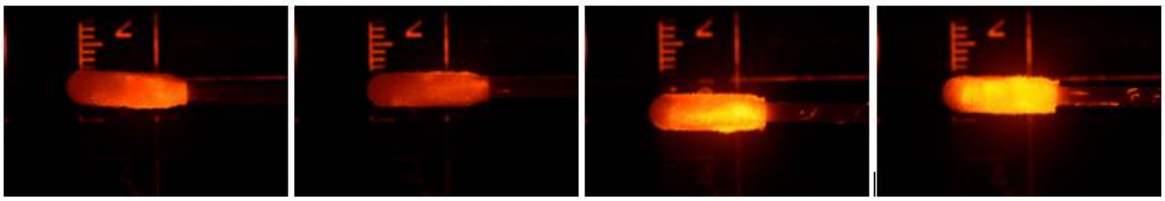

Figure 50. Recovered Touch DNA from Glass 3 on Foam Swabs

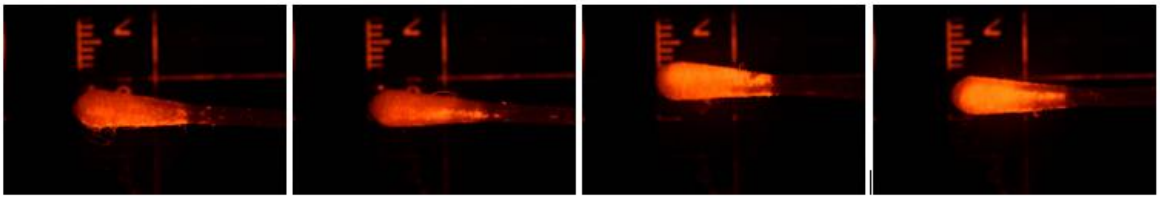

Figure 51. Recovered Touch DNA from Glass 4 on Rayon Swabs

Appendix 8. Images of Remaining DNA on Swab Materials Used on Drinking Glasses Post-Extraction

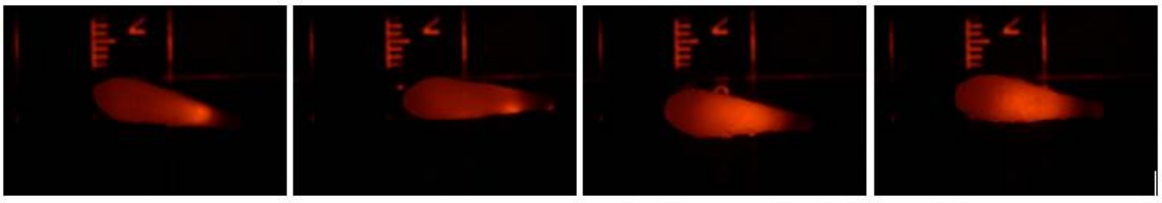

Figure 52. Remaining DNA from Glass 1 on Polyester Swabs Post-Extraction

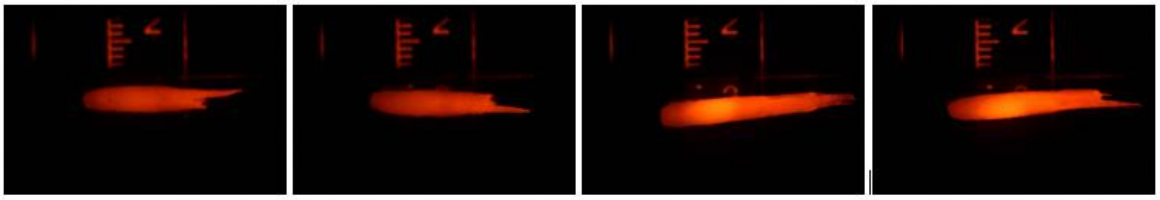

Figure 53. Remaining DNA from Glass 2 on Cotton Swabs Post-Extraction

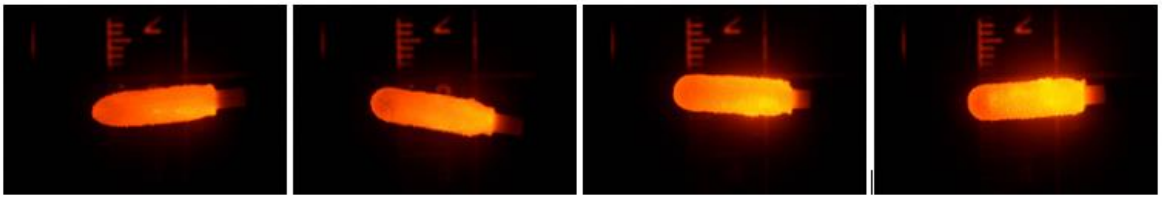

Figure 54. Remaining DNA from Glass 3 on Foam Swabs Post-Extraction

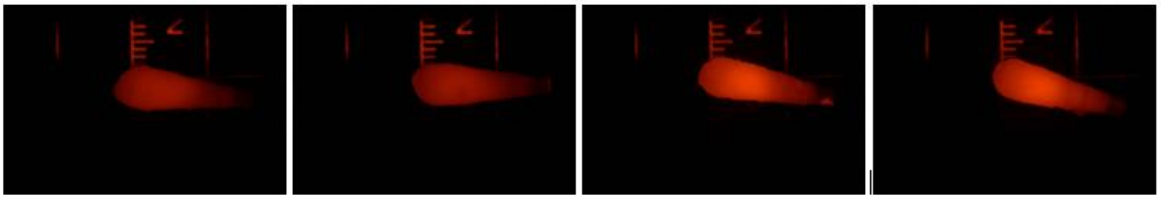

Figure 55. Remaining DNA from Glass 4 on Rayon Swabs Post-Extraction

Appendix 9. Images of Touch DNA Deposits Staining upon Plastic Handled Screwdrivers with Diamond™ Nucleic Dye.

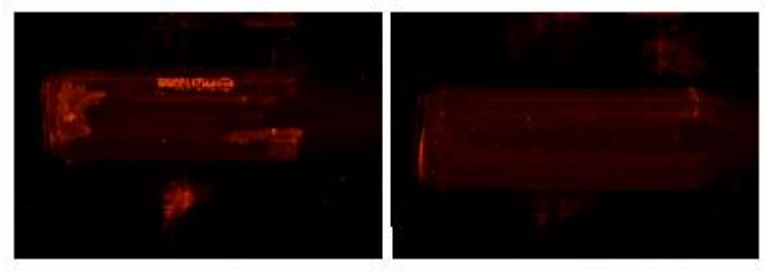

Figure 56. Touch DNA Deposits on Screwdriver 1

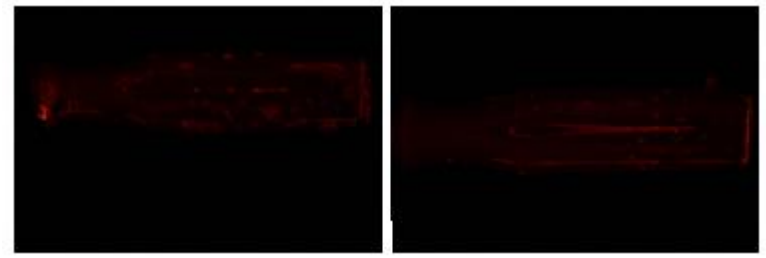

Figure 57. Touch DNA Deposits on Screwdriver 2

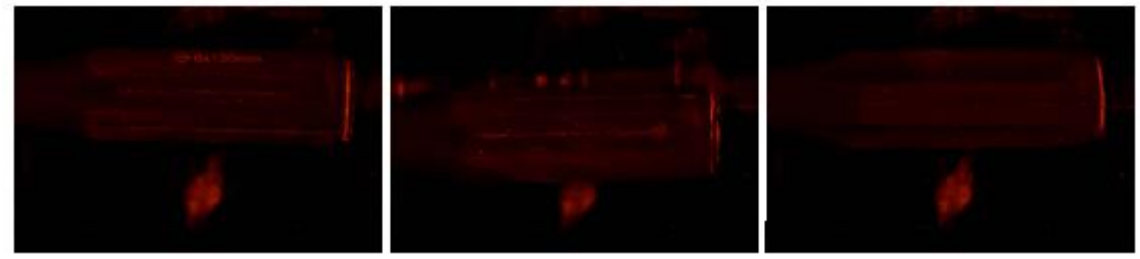

Figure 58. Touch DNA Deposits on Screwdriver 3

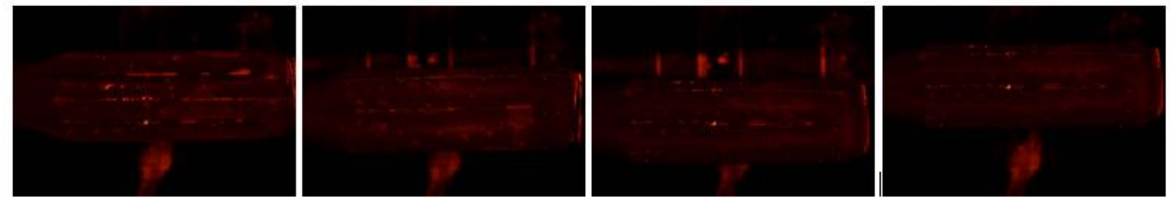

Figure 59. Touch DNA Deposits on Screwdriver 4

Appendix 10. Images of Remaining Stained Touch DNA on Plastic Handled Screwdrivers Post-Recovery.

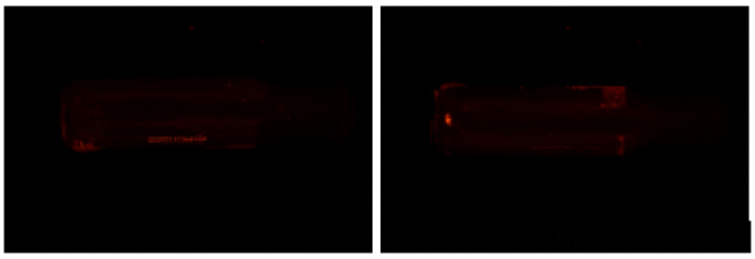

Figure 60. Touch DNA on Screwdriver 1 After Swabbing

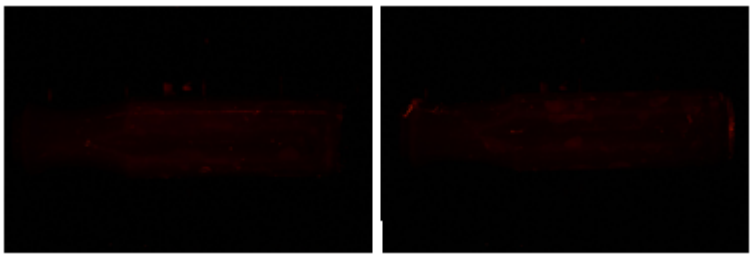

Figure 61. Touch DNA on Screwdriver 2 After Swabbing

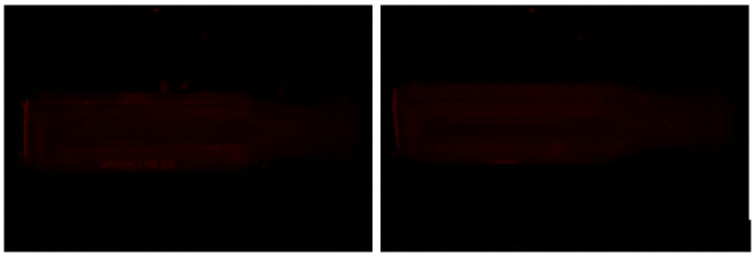

Figure 62. Touch DNA on Screwdriver 3 After Swabbing

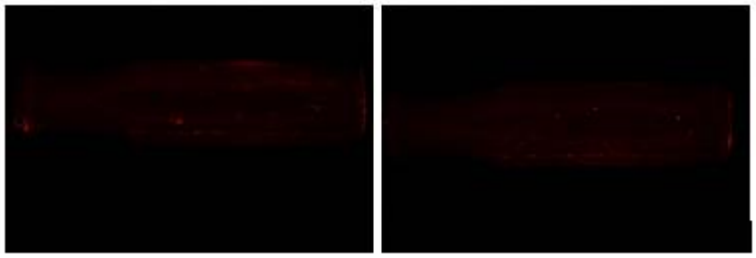

Figure 63. Touch DNA on Screwdriver 4 After Swabbing

Appendix 11. Images of Recovered Touch DNA on Swabs from Plastic Handled Screwdrivers Stained with Diamond™ Nucleic Dye

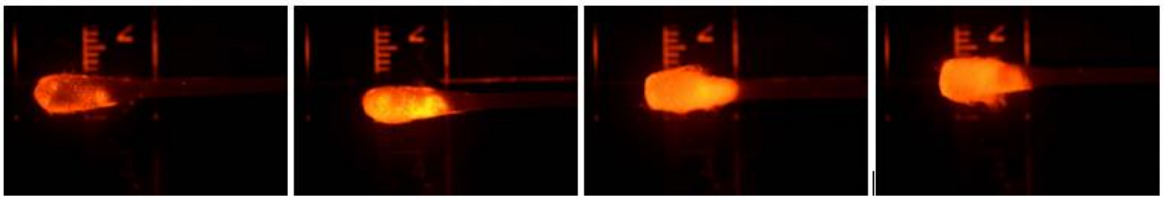

Figure 64. Recovered Touch DNA from Screwdriver 1 on Polyester Swabs

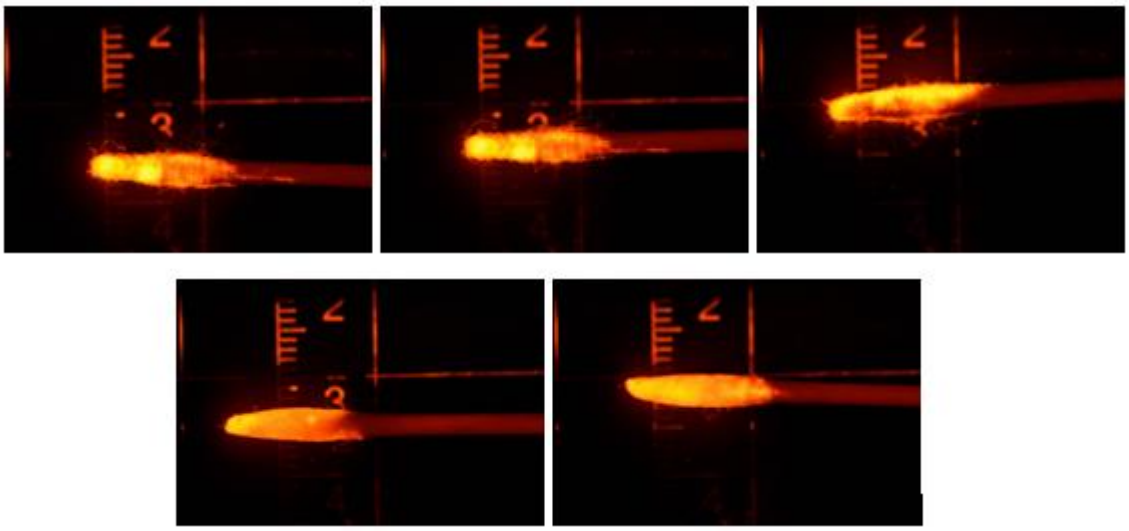

Figure 65. Recovered Touch DNA from Screwdriver 2 on Cotton Swabs

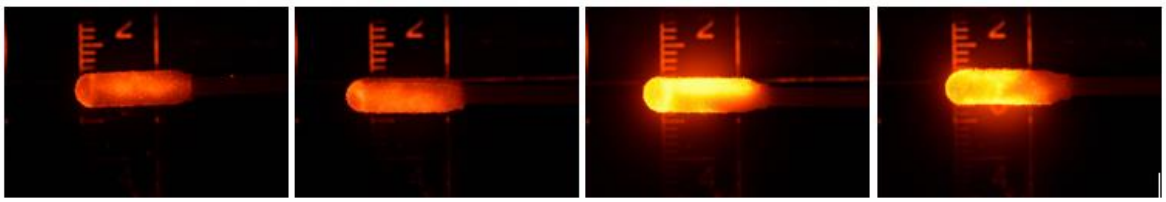

Figure 66. Recovered Touch DNA from Screwdriver 3 on Foam Swabs

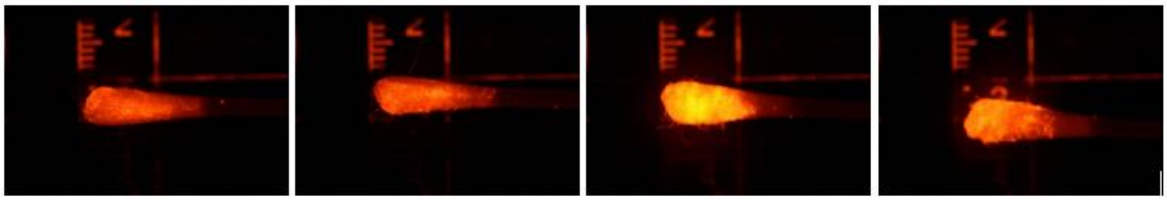

Figure 67. Recovered Touch DNA from Screwdriver 4 on Rayon Swabs

Appendix 12. Images of Remaining DNA on Swab Materials Used on Plastic Handled Screwdrivers Post-Extraction

Figure 68. Remaining DNA from Screwdriver 1 on Polyester Swabs Post-Extraction

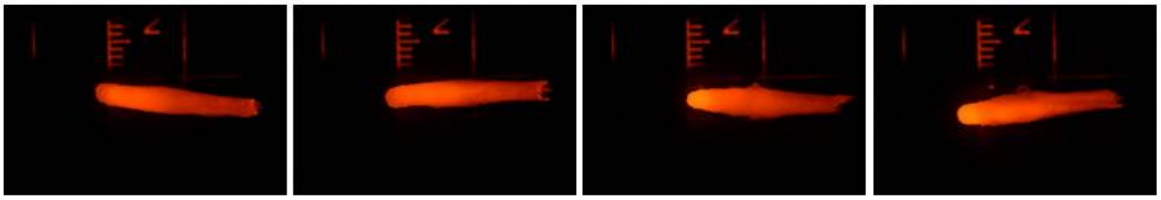

Figure 69. Remaining DNA from Screwdriver 2 on Cotton Swabs Post-Extraction

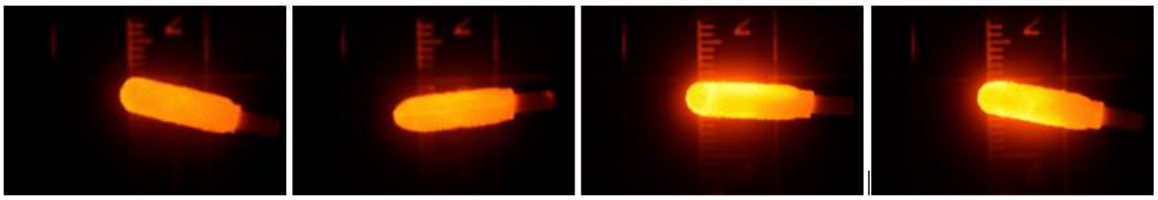

Figure 70. Remaining DNA from Screwdriver 3 on Foam Swabs Post-Extraction

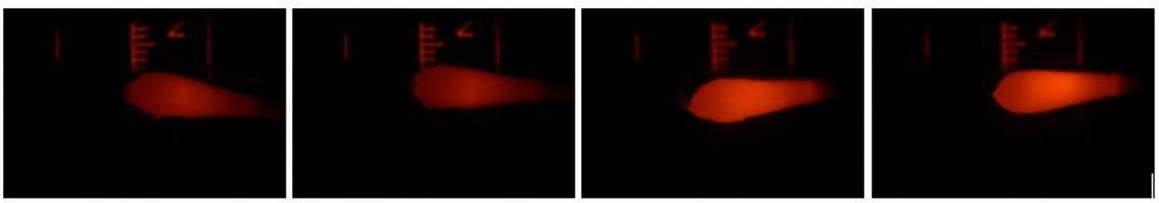

Figure 71. Remaining DNA from Screwdriver 4 on Rayon Swabs Post-Extraction

Appendix 13. Images of Touch DNA Deposits Staining upon Wooden Handles with Diamond™ Nucleic Dye.

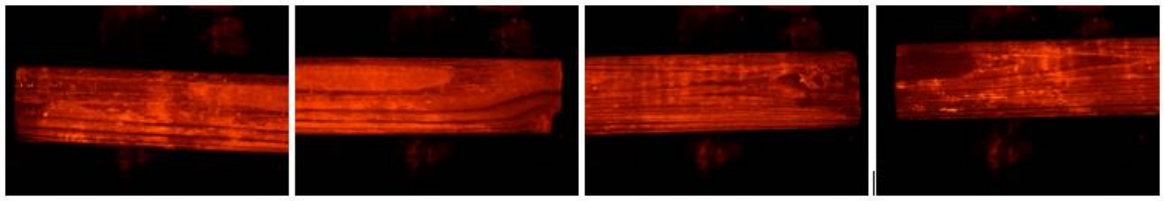

Figure 72. Touch DNA Deposits on Wooden Handle 1

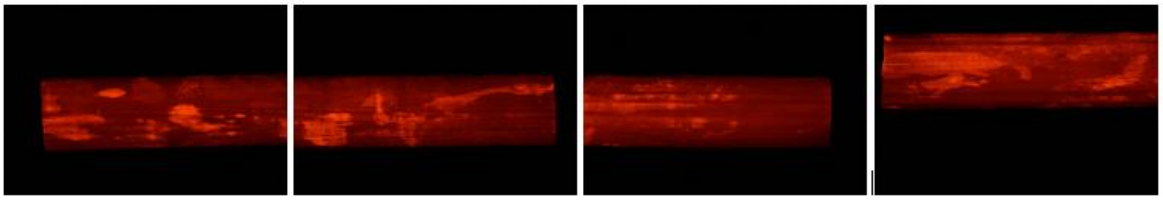

Figure 73. Touch DNA Deposits on Wooden Handle 2

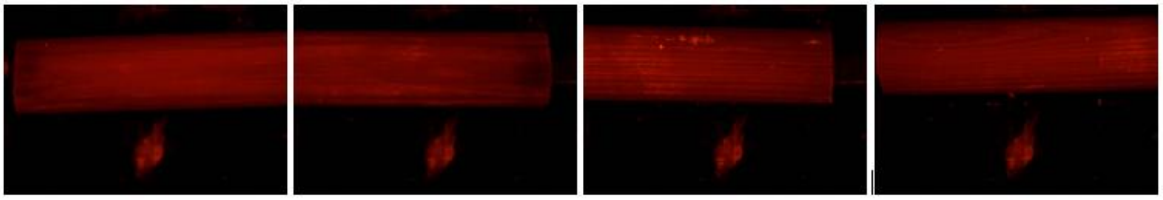

Figure 74. Touch DNA Deposits on Wooden Handle 3

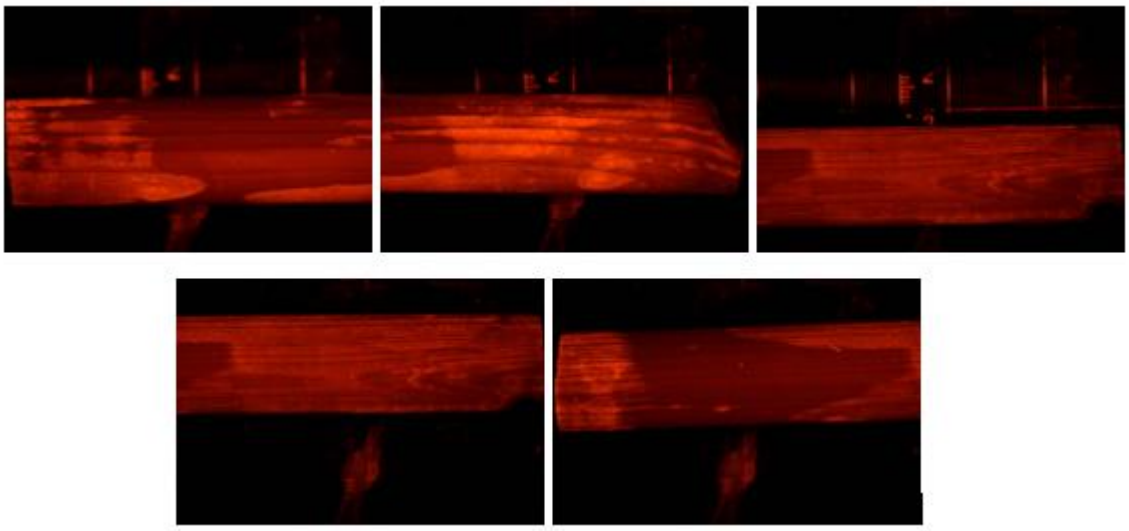

Figure 75. Touch DNA Deposits on Wooden Handle 4

Appendix 14. Images of Remaining Stained Touch DNA on Wooden Handles Post-Recovery.

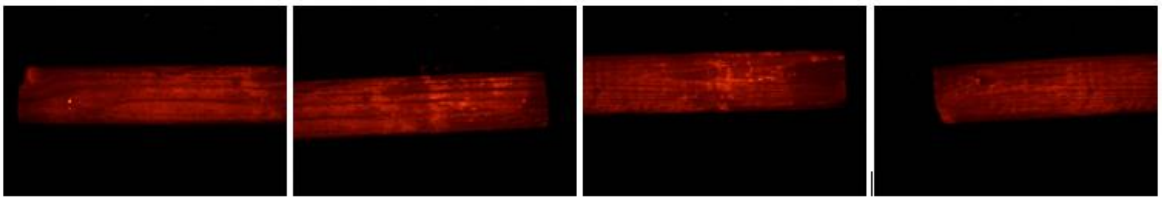

Figure 76. Touch DNA on Wooden Handle 1 After Swabbing

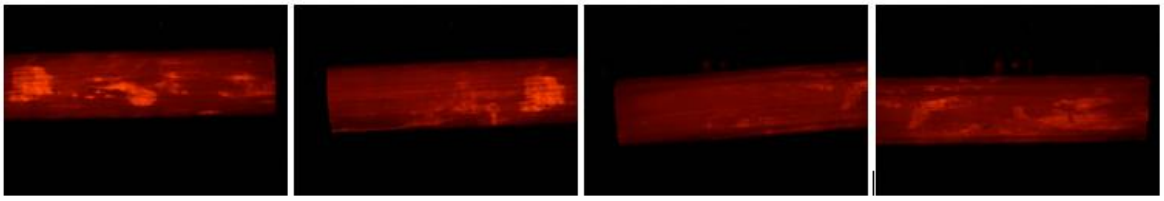

Figure 77. Touch DNA on Wooden Handle 2 After Swabbing

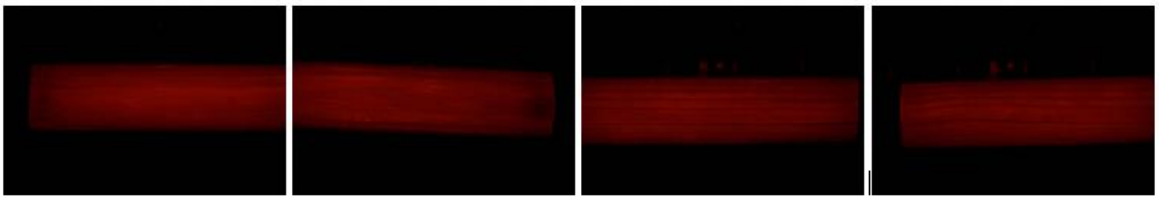

Figure 78. Touch DNA on Wooden Handle 3 After Swabbing

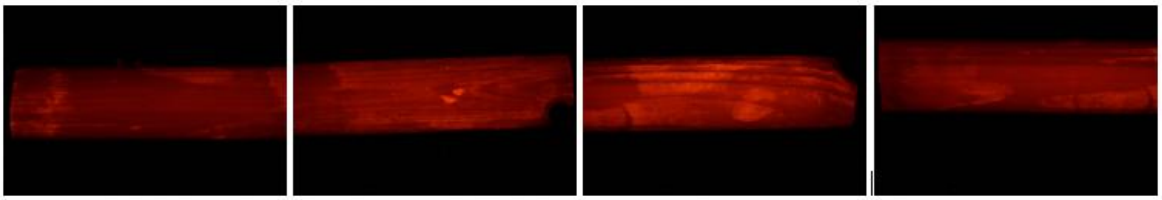

Figure 79. Touch DNA on Wooden Handle 4 After Swabbing

Appendix 15. Images of Recovered Touch DNA on Swabs from Wooden Handles Stained with Diamond™ Nucleic Dye

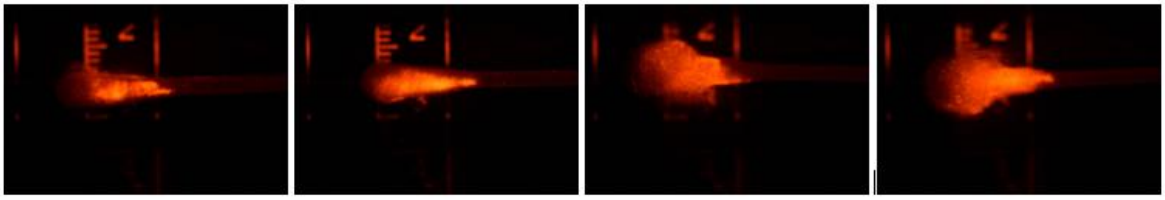

Figure 80. Recovered Touch DNA from Wooden Handle 1 on Polyester Swabs

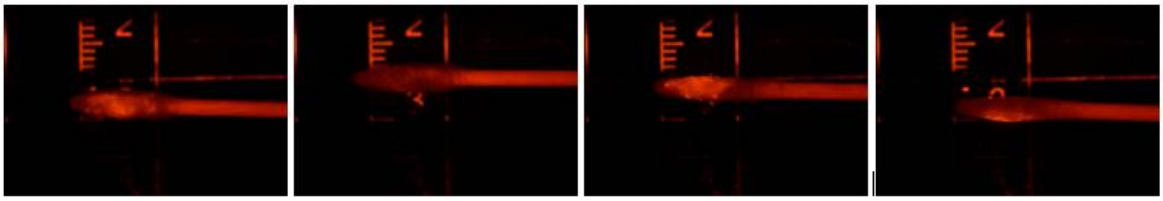

Figure 81. Recovered Touch DNA from Wooden Handle 2 on Cotton Swabs

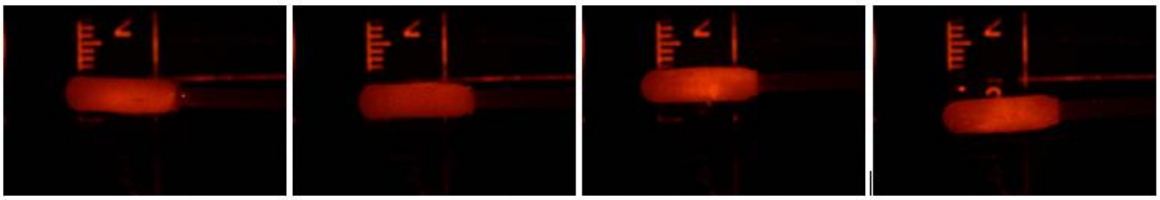

Figure 82. Recovered Touch DNA from Wooden Handle 3 on Foam Swabs

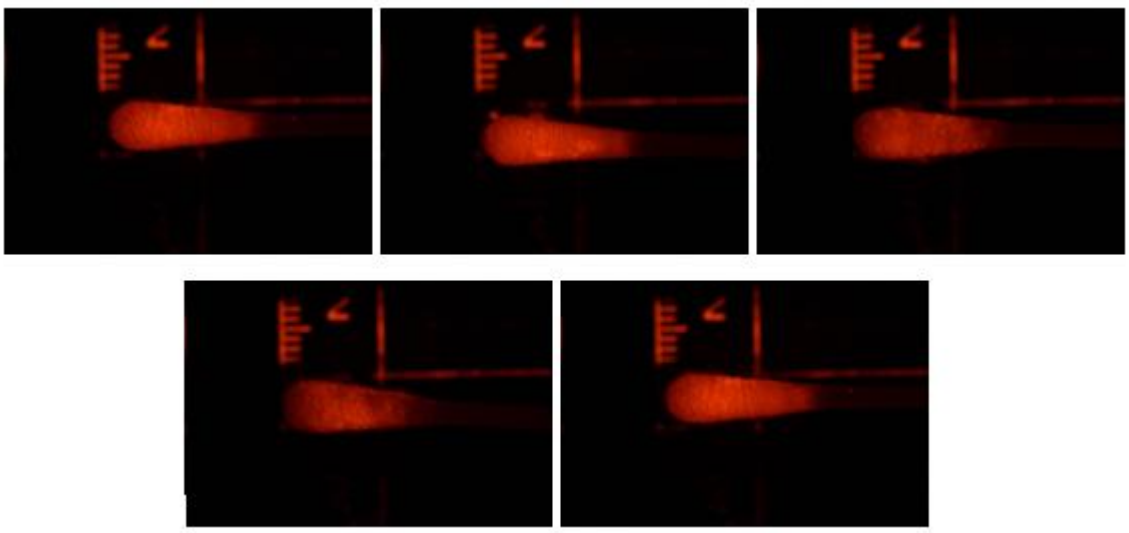

Figure 83. Recovered Touch DNA from Wooden Handle 4 on Rayon Swabs

Appendix 16. Images of Remaining DNA on Swab Materials Used on Wooden Handles Post-Extraction

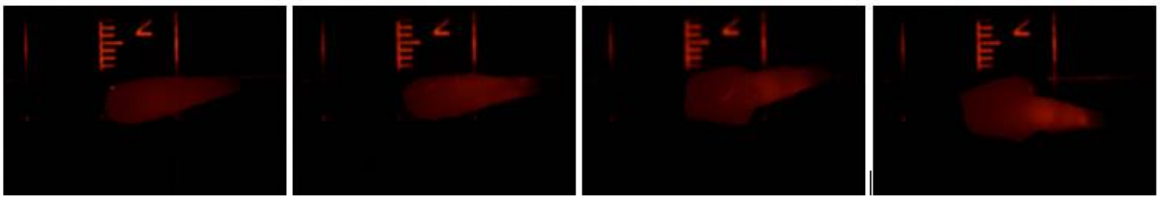

Figure 84. Remaining DNA from Wooden Handle 1 on Polyester Swabs Post-Extraction

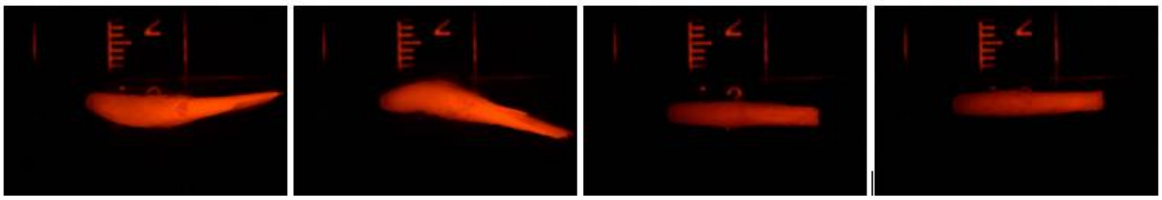

Figure 85. Remaining DNA from Wooden Handle 2 on Cotton Swabs Post-Extraction

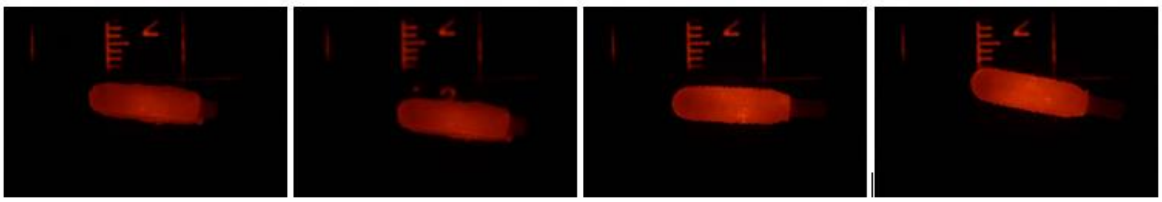

Figure 86. Remaining DNA from Wooden Handle 3 on Foam Swabs Post-Extraction

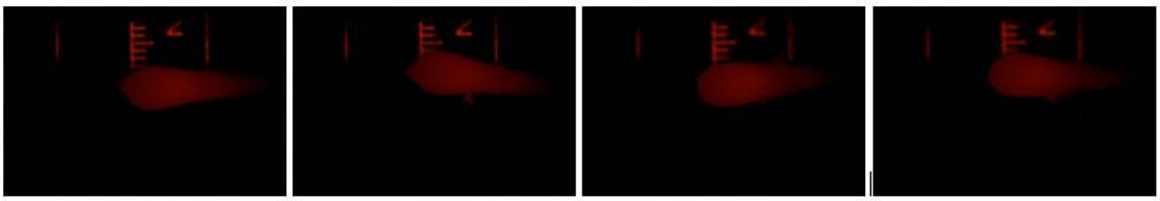

Figure 87. Remaining DNA from Wooden Handle 4 on Rayon Swabs Post-Extraction