Effect of swabbing technique and duration on forensic DNA recovery

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Conflict of Interest statement

None.

Highlights

- Substrate type, recovery technique and swabbing duration all impact DNA recovery
- Significantly more DNA was recovered from matte tiles than cotton fabric
- Maximum DNA recovered from cotton with a wet-moist double swabbing technique
- Maximum DNA recovered from matte tiles with a single wet or moist-dry swab
- More DNA recovered from cotton when swabbing for 30 sec than 15 sec per swab

Abstract

Various factors have been shown to affect performance of the conventional wet-dry double and single wet swabbing techniques to recover DNA, such as pressure and angle of application, volume and type of wetting agent, and swab type. However, casework laboratories in some jurisdictions have recently adopted different swabbing techniques that include wet-moist double swabbing and moist-dry single swabbing. Factors affecting the effectiveness of these recent techniques in maximising DNA recovery therefore need to be investigated. Here, the performance of traditional and recent swabbing techniques was compared and the impact of swabbing duration on DNA recovery was investigated. Ten µl aliquots of a known concentration of DNA extracted from human blood were deposited on pre-cleaned DNA-free cotton swatches (porous) and porcelain tiles (non-porous). Five swabbing techniques were used, of which three were double swabbing techniques: wet-moist, wet-wet and wet-dry, and two were single swabbing techniques: wet and moist-dry. For a 'wet' or 'moist' swab, 100 or 50 µl water was added, respectively. For a moist-dry swab, water was applied to one side of the swab, leaving the other side drier. Each swabbing technique was applied for two durations, 15 and 30 sec per swab, with 5 reps of each combination (n=100 plus controls). All samples were extracted and quantified, and a sub-set was profiled. The results showed that the wet-moist double swabbing technique with a swabbing duration of 30 sec maximised DNA recovery from cotton. From tile, a single wet or moist-dry swab maximised DNA recovery, but increasing swabbing duration from 15 to 30 sec had no impact. These data can be used to inform standardisation of DNA collection protocols across casework laboratories.

Key words: DNA recovery, DNA collection, Swabbing

1. Introduction

In the last two decades, forensic genetics has focused on improving DNA extraction and the robustness of profiling techniques, with minimal focus on recovery methods of biological material [1]. A successful DNA profile does not only rely on a laboratory's analytical process, but also on the sampling procedure used to collect biological material [2]. Recovering the maximal amount of available DNA through appropriate collection methods is critical in predicting whether a usable profile can be derived from an exhibit [1, 3].

A range of general collection methods has been described in the literature, including: excision [4], taping [5], swabbing [6], soaking [7] and scraping [4]. While excision, scraping and soaking are destructive processes that directly impact the state of an exhibit, taping and swabbing are generally classified as non-destructive methods [5, 8], as they have less of an impact on the state of an exhibit. Tapes and swabs are commonly used to collect biological material, as they are efficient, relatively inexpensive, and simple to use and transport [5, 9].

Several DNA transfer studies have used a single wet swab technique to collect biological material from touched items [10-12]. In 1997, Sweet *et al.* compared the performance of single wet swabbing to the wet-dry double swabbing technique [6]. In their study, a theory

was presented explaining that epithelial cells and leukocytes are dehydrated when exposed to the air and are, therefore, in need of a wetting agent to rehydrate the cells. The double swab technique, they suggested, allows for moisture to be left behind that rehydrates the dried epithelial cells, causing them to adhere to the swab head and the second dry swab collects the moisture, therefore, additional cells [6]. Since then, the wet-dry double swabbing technique has commonly been considered an optimal method for collection of biological material for DNA analysis, as it has demonstrable improvements in DNA recovery [13, 14] and is employed by casework laboratories and police forces in many jurisdictions.

In the last decade, the use of the double swabbing technique for DNA recovery in casework has evolved in some jurisdictions to include alterations, such as wet-moist double swabbing, wet-wet double swabbing, and moist-dry single swabbing. The wet-moist double swabbing technique, in which a wet swab is applied to the substrate first, followed by a moist swab to which a smaller volume of wetting agent is added, has been used for DNA recovery from clothing and fabric [15, 16]. The wet-wet swab combination, where a wet swab is applied to the substrate followed by a second wet swab, has been examined for a range of porous and non-porous substrates [17]. Finally, the moist-dry single swabbing technique involves use of a single swab, with one side moistened with water and applied to the substrate first and then the other side, which is drier, is applied to the same area on the substrate. This technique has been used to recover DNA from firearms [18] and a range of different substrates [19]. However, the effectiveness of these swabbing techniques in maximising DNA recovery has not previously been compared in published research. These three techniques are therefore compared within this study, along with the more conventional wet-dry double swabbing and single wet swabbing techniques, for their effectiveness of recovering DNA from porous cotton fabric and non-porous matte tiles.

Although various factors impacting DNA recovery, such as substrate type, swab material, selected wetting agents and volume of wetting agent, have been explored [9, 17, 20-25], there are many unknown factors that may impact recovery, such as the duration of swabbing. Whilst 15 seconds per swab has been identified as an appropriate duration when using the wet-dry double swabbing technique [26], this study will explore the impact of swabbing duration on DNA recovery, specifically comparing 15 seconds per swab to 30 seconds, for all aforementioned techniques. Investigation of swabbing duration in this study, along with different swabbing techniques, may lead to the development of a more standardised swabbing protocol to enhance DNA collection, making available a greater percentage of starting DNA, thereby increasing the likelihood of obtaining successful quantification and profiling data.

2. Materials and methods

2.1 Materials and their preparation

One metre of 100% cotton fabric (Spotlight, Australia) was selected as the porous substrate and 36 mosaic porcelain matte tiles, 48 mm x 48mm in size (Bunnings, Australia), were used as the non-porous substrate. To remove any extraneous DNA, all utensils (including scissors, forceps, pipettes etc), tiles and cotton (cut to 12cm x 12cm swatches) were placed under

ultraviolet light (UV) for 20 minutes on both sides. The tiles were further cleaned with 1% sodium hypochlorite and 70% ethanol, and dried with a clean paper towel prior to DNA deposition.

The swabs used within this study were cotton-tipped swabs (150C, Copan) and, where required, autoclaved milli-Q water was used as the wetting agent (referred to simply as water throughout). To initially examine the performance of different swabbing techniques, fingerprints were deposited on to the tiles by a consenting donor and visualised using Sirchie Opti Black latent fingerprint powder (Optimum Technology) with Squirrel Hair Brush No. 4 (Optimum Technology), prior to the tiles being swabbed. Swabs and tiles were photographed using a handheld camera with general room lighting. For the DNA recovery experiment, DNA for deposition on to the substrates was extracted from a screened blood sample. This had been taken from a single consenting donor and stored in at 4°C until processed. The use of human participants in this project was approved by the [***redacted for peer review***] Human Research Ethics Committee (***redacted for peer review***).

2.2 Visualisation of swabbing performance using black fingerprint powder

To visually observe any differences among swabbing techniques, black fingerprinting powder was used. Using the index finger, three fingerprints were deposited on each tile. Between each fingerprint deposition, the index finger was dabbed on multiple areas of the face to generate oils. Using Squirrel Hair Brush No. 4, black powder was carefully dusted on the tiles until the fingerprints were fully saturated.

Initial tests were performed to visualise the impact of swabbing duration on powder recovery. A single wet swab, wetted with 100µL of water, was used to collect the black powder from each fingerprint to determine the average number of swabbing strokes made in 15 seconds. This was determined to be 25 strokes in 15 seconds, and the number of strokes was doubled for 30 seconds. A single swabbing stroke consisted of a back-and-forth motion in a diagonal direction.

Further tests were performed to visualise the differences between five different swabbing techniques on powder recovery including: wet-moist double swabbing (WM), wet-wet double swabbing (WW), wet-dry double swabbing (WD), single wet swabbing (W), and moist and dry single swabbing (MD); these five swabbing techniques were also applied in DNA deposition experiments. Fingerprints were deposited on tiles and dusted using the method above. Three replicates were performed for each of the five swabbing techniques at two durations, 15 and 30 seconds per swab, through use of the above determined number of strokes. One hundred µL of DNA-free water was deposited onto the swab head for a ‘wet swab’ and 50µL for a ‘moist swab’.

2.3 Impact of swabbing technique and duration on DNA recovery

To create stock solutions of known concentrations to deposit onto the surfaces, DNA was extracted from 40µL aliquots of the blood sample (as per Section 2.4) and pooled into one tube to maximise homogeneity. To minimise the number of freeze-thaw cycles, this pooled

stock solution was then separated into five tubes and stored at -20°C until required, with each tube used per day to generate all the samples required. Each of these five stock solutions, labelled A-E, were quantified (as per Section 2.4) in triplicate to give average concentrations of 2.63, 3.07, 2.35, 2.44, and 2.22 ng/µL for A to E, respectively.

Ten µL of an aliquot of DNA stock solution was deposited within a $2cm^2$ area on each prepared cotton swatch and tile and left to dry for 40 minutes. Each cotton swatch and tile were then swabbed with one of the aforementioned swabbing techniques. Each of the five swabbing techniques had five replicates each at 15 and 30 seconds duration per swab giving a total of 100 samples (Table 1).

Table 1. Breakdown of samples (n=100) across substrate type, swabbing technique, swabbing duration, and number of replicates per stock solution aliquot used (A-E).

Substrate	Swabbing technique	Duration per swab	No. of replicates (stock solution aliquot used)
Cotton	Wet-moist double	15 sec	4 (A); 1 (B)
	Wet-wet double		4 (A); 1 (B)
	Wet-dry double		4 (A); 1 (B)
	Wet single		4 (A); 1 (B)
	Moist-dry single		4 (A); 1 (B)
Cotton	Wet-moist double	30 sec	3 (B); 2 (E)
	Wet-wet double		3 (B); 2 (E)
	Wet-dry double		3 (B); 2 (E)
	Wet single		3 (B); 2 (E)
	Moist-dry single		3 (B); 2 (E)
Tile	Wet-moist double	15 sec	5 (C)
	Wet-wet double		4 (C); 1 (D)
	Wet-dry double		5 (C)
	Wet single		5 (C)
	Moist-dry single		5 (C)
Tile	Wet-moist double	30 sec	4 (D); 1 (E)
	Wet-wet double		4 (D); 1 (E)
	Wet-dry double		5 (D)
	Wet single		5 (D)
	Moist-dry single		5 (D)

2.4 Processing of DNA samples

The PrepFiler *Express*™ Forensic DNA Extraction Kit was used for DNA extraction from blood (to create the stock solution) and swab samples using the 'Body Fluids' protocol on the AutoMate Express Nucleic Acid Extraction System, as per manufacturer's instructions (ThermoFisher Scientific). For the blood, 40µL aliquots were extracted with an elution volume of 100 µL. For the swabs, depending on the swabbing technique employed, either one swab or two swabs were added to each column/tube assembly, to which 500µL lysis buffer was added. A final elution volume of 50µL was used for all swab techniques. An extraction blank

was run with each batch of extractions which included a single unused swab placed into a column-tube assembly and processed for extraction. A negative control was created for each substrate by swabbing the presumed clean substrate with a wet swab which was then processed. No DNA was detected in all extraction blanks and negative controls.

All samples were quantified using the Quantifiler™ Trio DNA Quantification Kit (ThermoFisher Scientific) with the QuantStudio™ 6 instrument, according to the manufacturer's instructions. The amount of DNA in each sample was calculated by multiplying its concentration by the relevant elution volume. The amount of DNA deposited on to each substrate was calculated by multiplying the concentration of the relevant stock solution by the volume deposited on the substrate (10 µL). For each substrate, the amount of DNA recovered was then compared with the amount of DNA initially deposited to calculate the percentage of DNA recovered. A sub-set of samples were selected to be profiled. These comprised samples that had the median total DNA across each set of replicates, the stock solution of DNA extract (for the reference profile), and two negative substrate controls, one for each substrate. DNA profiling was conducted by the NSW Forensic and Analytical Science Service using the PowerPlex® 21 Amplification System (Promega Corporation). Amplification was performed using an Applied Biosystems GeneAmp® PCR System 9700 thermal cycler instrument (ThermoFisher Scientific) for 29 cycles and an input of 0.7 ng of DNA in a 15µL volume per reaction, as per internal validation. Amplified product detection and sizing were performed on an Applied Biosystems 3500xL Genetic Analyser (ThermoFisher Scientific) with an injection voltage of 1.2kV and an injection time of 24 seconds, as per internal validation and manufacturer's instructions. Profiles were analysed using GeneMapper™ ID-X Version 1.6 Software (ThermoFisher Scientific), with an analytical threshold of 175 RFU and a homozygous threshold of 700 RFU, as per internal validation.

2.5 Data analysis

Statistical tests were performed using R Studio version 1.3.1093 or IBM SPSS Statistics version 28, with all data points included in the calculations. The Shapiro-Wilk Normality Test (fBasics package) was used to determine the distribution of recovery data which was confirmed by data transformation. As all datasets were non-parametric, the Kruskal-Wallis rank sum test was used to compare percentages of DNA recovery between different swabbing techniques within each substrate type (porous or non-porous). Significant differences, if any, were then confirmed using Dunn's test (dunn.test package) using the Bonferroni correction method. DNA recovery percentages between the two surfaces, irrespective of swabbing technique, were compared using the Mann-Whitney U test (N = 50 for each substrate). Percentages of DNA recovery between swabbing durations (15 and 30 sec) were compared for each of the five swabbing techniques, and for each substrate, also using the Mann-Whitney U test (N = 10 for each comparison). Effect size of significant differences was interpreted from r^2, determined by squaring the value of r, calculated from $r=Z/\sqrt{N}$.

3. Results

3.1 Visualisation of swabbing performance using black fingerprint powder on tile

A preliminary study was initially conducted to visualise performance of the different swabbing techniques through the swabbing of powdered fingerprints on tile. This revealed no noticeable visual differences in the residual powder left on the tiles between a swabbing duration of 15 seconds per swab and 30 seconds per swab, irrespective of swabbing technique, illustrating that the duration of swabbing did not visually impact black powder recovery (Supplementary Table 1). However, differences were seen in the saturation of the swabs with the black powder, illustrating potential differences in the recovery of black powder by different swabs within a technique. It was observed that all first swabs of double swabbing techniques, as well as the single wet swab and the moist side of the moist-dry swab, were heavily saturated with black powder (Supplementary Table 1). In contrast, the second moist and second wet swab of the double swabbing techniques showed moderate levels of saturation, whereas the second dry swab had a low saturation of black powder and the dry side of the moist-dry swab showed high saturation (Supplementary Table 1).

3.2 Impact of swabbing technique and duration on DNA recovery from porcelain matte tiles

DNA recovery from the matte tiles across the swabbing techniques and durations ranged from 30 to 80% (Figure 1). The percentage of DNA recovery from tile was not significantly impacted by an increase in swabbing duration from 15 to 30 seconds for any of the techniques tested ($Z = -1.057$, $p = 0.29$). In addition, there were no significant differences in DNA recovery among the five techniques when swabbing for 15 seconds ($\chi^2 = 4.56$, $p = 0.34$) or 30 seconds ($\chi^2 = 2.20$, $p = 0.70$). The samples from the tiles that were profiled all returned a complete DNA profile that matched that of the donor.

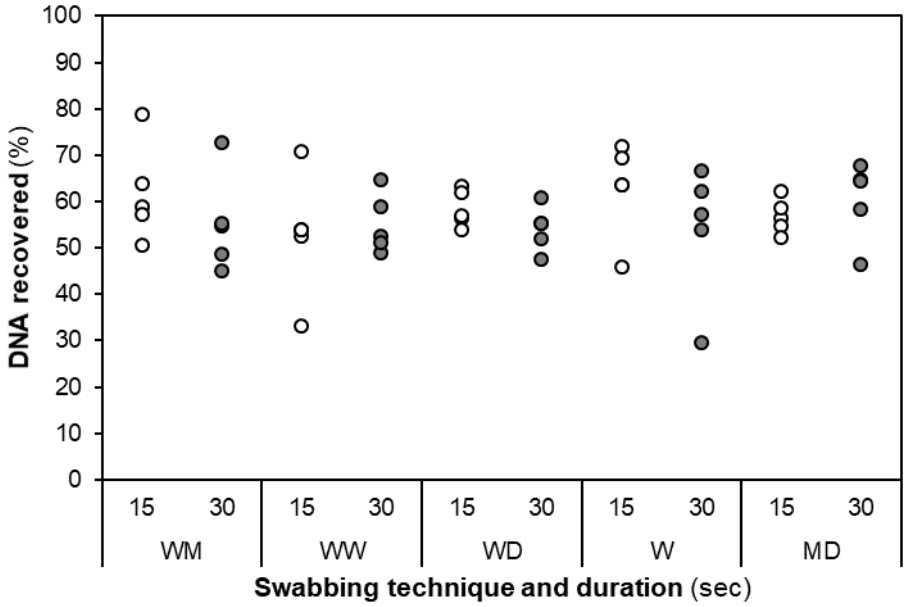

Figure 1. *Percentage of DNA recovery for each swabbing technique and duration (15 (white) and 30 (grey) seconds per swab) used on the non-porous substrate (tiles). Data points for all five replicates per swabbing technique and duration are shown.*

3.3 Impact of swabbing technique and duration on DNA recovery from cotton swatches

The percentage of DNA recovered from cotton swatches using the five swabbing techniques ranged from 0 to 10% across the techniques (Figure 2), which was significantly lower than the DNA recovery from the tiles ($Z = -8.617$, $p < 0.001$, $r^2 = 0.74$). There were no significant differences in percentage of DNA recovery across the five swabbing techniques when swabbing for 15 seconds ($\chi^2 = 3.11$, $p = 0.54$), whereas swabbing for 30 seconds introduced more variation among the techniques (Figure 2). For example, at a swabbing duration of 30 seconds, wet-moist double swabbing provided significantly greater DNA yields than moist-dry single swabbing ($Z = -3.14$, $p < 0.01$, $r^2 = 0.99$). In addition, swabbing for a duration of 30 seconds overall allowed for significantly more DNA recovery than when swabbing for 15 seconds (Figure 2; $Z = -3.463$, $p < 0.001$, $r^2 = 0.24$). This was particularly observed for the wet-moist double swabbing technique (Figure 2; $Z = -2.611$, $p < 0.01$, $r^2 = 0.68$). Overall, the highest DNA recovery was obtained when using the wet-moist double swabbing technique for a

duration of 30 seconds (median = 7.0%; IQR = 6.3 - 7.4%), whereas the lowest recovery was achieved with wet-dry double swabbing at 15 seconds swabbing duration (median = 1.8%; IQR = 1.8 - 2.1%). The samples from the cotton swatches that were profiled all returned a complete DNA profile that matched that of the donor.

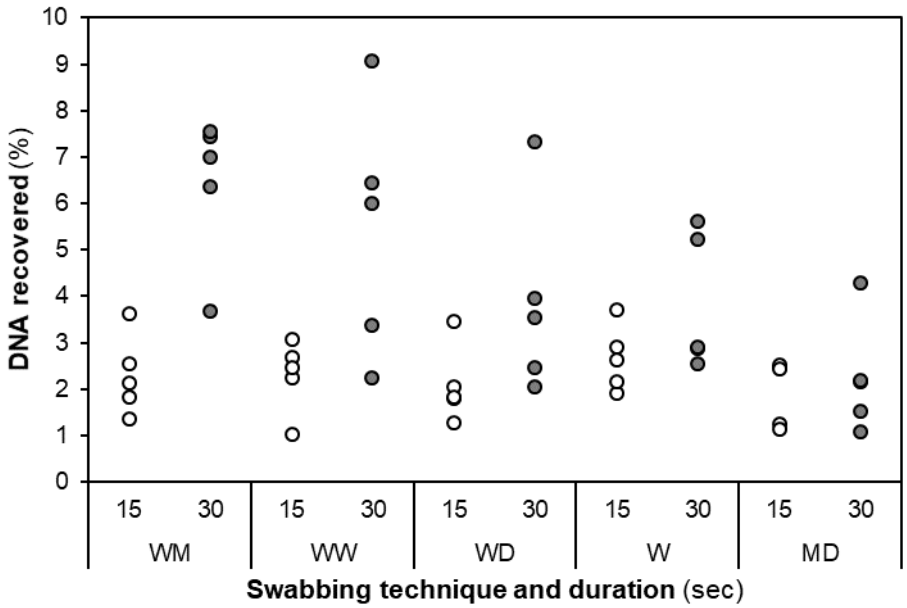

Figure 2. *Percentage of DNA recovery for each swabbing technique and duration (15 (white) and 30 (grey) seconds per swab) used on the cotton swatches (porous substrate). Data points for all five replicates per swabbing technique and duration are shown.*

4. Discussion

When using swabs to recover DNA from both porous and non-porous substrates, this study is the first to demonstrate that a combination of different techniques and durations should be used to maximise the efficiency of DNA recovery, depending on the substrate type. Previous studies have also shown the influence of other factors, such as pressure and angle of application [21], volume and type of wetting agent [21, 23, 24], and swab type [9, 23, 27]. The data herein therefore builds on previously published studies to contribute to informing a standardised forensic DNA collection procedure across global casework laboratories to achieve reliability of forensic operating procedures while maximising DNA recovery.

This preliminary study illustrated that whilst a single swab collected a large amount of black powder visually from powdered fingermarks on tile, there was potential for more to be collected with the application of a second swab. Since this echoed the recommendations by Sweet *et al.* [6] and Pang & Cheung [13] for using the double swabbing technique to recover DNA, it was hypothesised that swabbing fingerprint powder could be a simple visual way to compare swab performance and technique, especially as non-magnetic black fingerprint powder does not interfere with the downstream DNA processes used in this study [28]. However, when recovering DNA from dried DNA aliquots on tiles, there was no statistically

significant difference in the percentage of DNA recovered between the single and double swabbing techniques, disputing using fingerprint powder for this purpose. Although in contrast to Sweet *et al.* [6] and Pang & Cheung [13], these DNA findings support those of Hedman *et al.* [17] who observed that far greater amounts of DNA were recovered by the first swab than the second from similar non-porous substrates. This was due to their observation that for non-absorbing surfaces, such as the tile used in our study, the first wet swab recovered almost all of the cells from dried stains, leading to their suggestion that a second swab is not always required, depending on the substrate and efficiency of sampling technique [17].

Also for the tiles (Figure 1), our data showed that swabbing for 30 seconds over 15 seconds did not significantly increase DNA yield, indicating that investigators could spend less time with each swab, which may allow multiple samples to be taken from a surface or item in a shorter timeframe, especially when only one swab is used instead of two. Although using a single swabbing technique does not differ in DNA yield from a double swabbing technique, it would be beneficial to use a single swab per sample, since using an extra swab increases cost and time, increases the risk of introducing contamination and may not be suitable for certain automated extraction protocols. This is supported by Hedman *et al.* [17], who state that using only one swab per sample would lower workload and streamline workflow from crime scene to laboratory. Since not significantly different in DNA yield, our data also suggest that the single swab could be a wet swab or a moist-dry swab, depending on the current protocols already employed within a casework laboratory. Further research is required to verify this recommendation for a range of non-porous substrates.

From cotton swatches, a representative porous substrate, significantly more DNA was recovered overall when swabbing for 30 seconds than 15 seconds. This increase in duration had a medium effect on DNA yield, with 24% of the variability being accounted for by the increase in swabbing duration. Increase in duration had a larger effect on DNA yield for the wet-moist swabbing technique, with 68% of the variability being accounted for by the duration increase, and maximal DNA recovery from cotton was achieved with this technique and duration. In particular, at this swabbing duration, changing the swabbing technique from moist-dry single swabbing to wet-moist double swabbing had a large effect on the increase in DNA yield, with the majority of the variability being accounted for by the change in swabbing technique. Whilst wet-moist double swabbing technique is employed by some laboratories, such as in an Australian jurisdiction [15, 16], this study provides the first empirical data to underpin the large effect its use over other swabbing techniques can have on DNA yield. The use of a second dry swab in DNA recovery has been routinely used by many casework laboratories for a variety of surfaces since first recommended by Sweet *et al.* [6] and Pang & Cheung [13]. However, the results of more recent research by Hedman *et al.* [17] revealed that the application of a second wet swab allowed for higher DNA yields than a second dry swab when double swabbing DNA from a hard absorbing substrate (wood) and other non-porous surfaces. Whilst they did not examine this for a soft absorbing substrate (fabric), our results indicate that using a second moist swab recovers more DNA than a second dry swab when double swabbing a porous substrate, such as cotton. Nevertheless, it is common for casework laboratories in other jurisdictions, such as in the UK and other Australian jurisdictions, to employ taping to recover DNA from fabrics [15, 19]. Whilst it has been demonstrated that taping can recover significantly more DNA from cotton than moist-

dry single swabbing [19], it has been previously suggested that wet-moist double swabbing may have a better DNA recovery efficiency from cotton than taping [15]. Further research is therefore required to verify this, along with comparing these recovery methods on a range of porous and semi-porous substrates.

Our data also support previous research demonstrating that substrate type impacts DNA recovery [9, 17, 21, 29, 30], as a significantly greater DNA yield was recovered from tiles than cotton substrates. Calculation of effect size showed that substrate type had a large effect on DNA yield, with 74% of the variability being accounted for by substrate type. As discussed by de Oliveira Francisco *et al.* [29], this is likely due to differences in porosity and other features of the substrates, particularly as it has been shown that DNA will transfer more readily from a non-porous to porous substrate (e.g. from tile to swab) than from porous to porous substrate (e.g. from cotton swatch to swab) [31]. Substrate type is a therefore a substantial impacting factor on DNA recovery, further emphasising the need to build on this study by comparing the swabbing techniques on a range of porous, semi-porous and non-porous substrates. Since prior research has shown that swab type is also a key factor in DNA recovery [9, 23, 27], the potential impact of swabbing technique and duration should also be explored with different swab types. Furthermore, whilst pre-extracted DNA was used in this study to allow accurate calculations of percentages of DNA recovery and because it may behave similarly to cellular material [32], this study should also be expanded to test the swabbing techniques on recovery of different biological fluids and other sources of cellular DNA.

5. Conclusion

A successful DNA profile does not only rely on a laboratory's analytical process, but also on the sampling procedure used to recover the biological material. Maximising the recovery of available DNA from items via appropriate collection methods is therefore critical in determining whether a usable profile can be derived from an exhibit. Herein, we have built on previously published research to demonstrate that the swabbing technique employed and duration of swabbing can impact DNA recovery, depending on the type of substrate from which the DNA is being recovered. Our data indicate that, of the swabbing techniques and durations tested, using a single wet or moist-dry swab for 15 seconds is optimal for DNA recovery from tile and the double wet-moist swabbing technique with 30 seconds per swab maximises DNA recovery from cotton. Whilst these findings need to be explored across a wider range of non-porous and porous substrates and different swab types, these data can be used with previously published data to enable the standardisation of forensic DNA collection protocols across laboratories to achieve reliability of forensic procedures while maximising DNA recovery.

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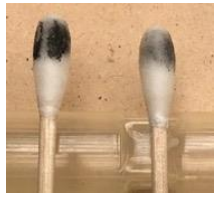
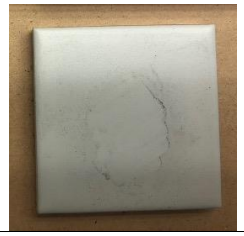
490

Supplementary Table 1. *For each combination of swabbing technique and duration, photographs of the swabs and tiles taken after swabbing the powdered fingerprints from the tiles.*

Swabbing duration/technique	Wet-moist double	Wet-wet double	Wet-dry double	Wet single	Moist-dry single
15 sec					
					
30 sec