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DNA decontamination of fingerprint brushes

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ABSTRACT

Genetic profiling of DNA collected from fingerprints that have been exposed to various enhancement techniques is routine in many forensic laboratories. As a result of direct contact with fingermark residues during treatment, there is concern around the DNA contamination risk of dusting fingermarks with fingerprint brushes. Previous studies have demonstrated the potential for cross-contamination between evidentiary items through various mechanisms, highlighting the risk of using the same fingerprint brush to powder multiple surfaces within and between crime-scenes.

Experiments were performed to assess the contamination risk of reused fingerprint brushes through the transfer of dried saliva and skin deposits from and to glass surfaces with new unused squirrel hair and fiberglass brushes. Additional new unused brushes and brushes previously used in casework were also tested for their ability to contaminate samples. In addition, the ability to eradicate DNA from used squirrel hair and fiberglass fingerprint brushes was assessed using a 1% sodium hypochlorite solution and a 5% solution of a commercially available alternative, Virkon. DNA profiling results from surfaces contacted by treated and untreated brushes were compared to determine the effectiveness of the devised cleaning protocol. Brush durability was also assessed over multiple wash/rinse/dry cycles with both agents.

Varying amounts of DNA-containing material were collected and transferred by squirrel hair and fiberglass brushes, with detectability on the secondary surface dependent on the biological nature of the material being transferred. The impact of DNA contamination from dirty fingerprint brushes was most apparent in simulations involving the transfer of dried saliva and brushes previously used in casework, while minimal transfer of touch DNA was observed. Alarmingly, large quantities of DNA were found to reside on new unused squirrel hair brushes, while no DNA was detected on new unused fiberglass brushes or brushes sold as DNA-free.

Squirrel hair brushes were easily and effectively cleaned with both hypochlorite and Virkon, with no evidence of DNA transfer between exhibits by treated brushes. Brushes were still deemed useable after multiple cleaning cycles with either agent. In contrast, fiberglass bristles became tangled and matted when wet and could not be cleaned effectively using either method. It is recommended they are disposed of following use. Each laboratory should consider their current circumstances before adapting a cleaning method. The implementation of a program to monitor the effectiveness of the cleaning regime is also advised.

1. Introduction

Fingerprints and DNA are both valuable sources of forensic evidence. Today, the ability to generate DNA profiles from touched objects [1–4], resulting from the increased sensitivity of DNA typing methodologies, has enabled the routine collection of DNA from fingerprints. In cases where conventional fingerprint analysis is uninformative due to smeared or partial prints, DNA analysis provides an alternative means for obtaining probative evidence about the offender. In some laboratories, the collection of DNA from evidentiary items occurs prior to latent print processing, while in others collection occurs following exposure to various optical, physical and chemical fingerprint enhancement methods [5–7]. Considering the latter, there is concern around the DNA contamination risk associated with various enhancement methods as a result of direct contact with fingermark residues during treatment [8–10].

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Previous studies have demonstrated how easily DNA-containing material can be inadvertently picked up from objects and surfaces at the crime-scene or in the laboratory through contact with gloves or tools used during the collection or examination of exhibits, and transferred to other exhibits, tools or surfaces [11–13].The possibility of these tools and surfaces becoming vectors for subsequent DNA transfer substantially increases if protocols regarding their replacement or cleaning are not sufficiently followed [13,14], or cleaning methods are inadequate [15,16]. On-going monitoring and managing of contamination risks thorough the implementation of a laboratory Environmental DNA Monitoring (EDM) program is vital in minimizing contamination [15,17,18].

While improvements in laboratory cleaning protocols have been effective in decontaminating surfaces and tools previously deemed to be high-risk, there has not yet been a method devised and empirically tested regarding the cleaning of fingerprint brushes. Commercially DNA-free or disposable brushes are an available alternative, however the increased cost of these brushes compared to the standard variety are a limitation for many laboratories. Thus, to reduce costs, it is common for forensic investigators to use the same fingerprint brush to powder multiple surfaces within and between crime-scenes, with little to no cleaning in between uses.

Considering current decontaminating agents, sodium hypochlorite is known to be effective in eradicating DNA and is a commonly used cleaning agent within forensic laboratories. However, it's highly corrosive and toxic nature has been known to irritate handlers and cause damage to equipment. In addition to hypochlorite, Ballantyne et al. [17] assessed various concentrations of a commercially available, non-corrosive, alternative, Virkon®, for its ability to eradicate DNA-containing body fluids from a range of surfaces. While it was concluded that a 1% solution of sodium hypochlorite is effective in eliminating DNA and is safe to use, Virkon reached its maximum decontaminating ability at 5%, proving to be effective in removing touch deposits, but less effective on saliva, blood and semen stains [17].

Given the negative impact DNA contamination can have on criminal investigations, the current study evaluates the risk of contamination through the transfer of dried saliva and skin deposits from and to glass surfaces with new unused squirrel hair and fiberglass brushes. In addition, the DNA decontamination methods identified by Ballantyne et al. [17], namely, a 1% hypochlorite solution and a 5% Virkon solution, were assessed for their ability to clean and eradicate DNA from squirrel hair and fiberglass fingerprint brushes. DNA profiling results from surfaces contacted by cleaned and uncleaned brushes were compared to determine the effectiveness of the devised cleaning protocol.

2. Materials and methods

2.1. DNA-free status of new unused brushes

Two commercially available DNA-free fiberglass brushes by Sirche® (DNA122L, USA) and BVDA (B-55500, supplied by Pathtech, Australia) were tested for their DNA-free status, along with two types of conventional brushes routinely used at the Victoria Police Forensic Services Department (VPFSD), including fiberglass filament latent fingerprint brushes (No. 122L, Sirchie®, USA) and squirrel hair latent fingerprint brushes (Optimum Technology, Australia). Bristles from new unused squirrel hair (n = 3) and fiberglass (n = 3) brushes in routine use, and from new unused DNA-free brushes (one of each make) were cut onto separate sheets of Whatman™ filter paper using scissors before being placed directly into a 2 mL tube using forceps. Scissors and forceps were cleaned prior to and in between uses with 1% hypochlorite, wiped dry, followed by a water rinse and wiped dry.

2.2. Contamination of brushes

Imitating casework conditions, simulations were performed where new unused squirrel hair and fiberglass brushes in routine use at the VPFSD were used to dust a primary surface containing biological material with Opti-black powder (prepared by Sirchie® for Optimum Technology, Australia), with the assumption that this will contaminate the brushes with DNA-containing material from the surface. To ensure a variety of casework situations were explored, multiple primary surfaces, biological materials and conditions were examined (Fig. 1). These conditions are described over the following sub-sections.

All glass surfaces were cleaned with 1% hypochlorite and water (as previously described for scissors and forceps) prior to and in between uses, and samples were taken (wet/dry swabbing) to assess their DNA-free status (n = 14). Deposits on glass primary surfaces (Fig. 1A,B,C,E) were passed over 20 times with the same brush on all occasions. During dusting, the brush was rotated at a 45° angle to the surface, and a light to moderate pressure was applied. Brushes were intermittently dipped (roughly every 4 passes) in aliquots of unused black powder throughout dusting. Separate aliquots of powder were used for each brush.

2.2.1. Dried saliva

Primary surfaces containing dried saliva were prepared by evenly spreading 1 mL of saliva from a single donor (donor 1) over the surface of DNA-free glass plates (140 × 220 mm). Plates containing saliva were air-dried at room temperature for ~8 h before being dusted with new unused squirrel hair and fiberglass brushes (Fig. 1A).

In a separate simulation to determine how much dried saliva is collected by squirrel hair brushes, an additional three DNA-free glass plates deposited with dried saliva (as previously described) were dusted, without powder, using three new unused squirrel hair brushes. Following dusting, bristles were cut onto separate sheets of Whatman™ filter paper using scissors before being placed directly into a 2 mL tube using forceps. Scissors and forceps were cleaned prior to and in between uses, as previously described.

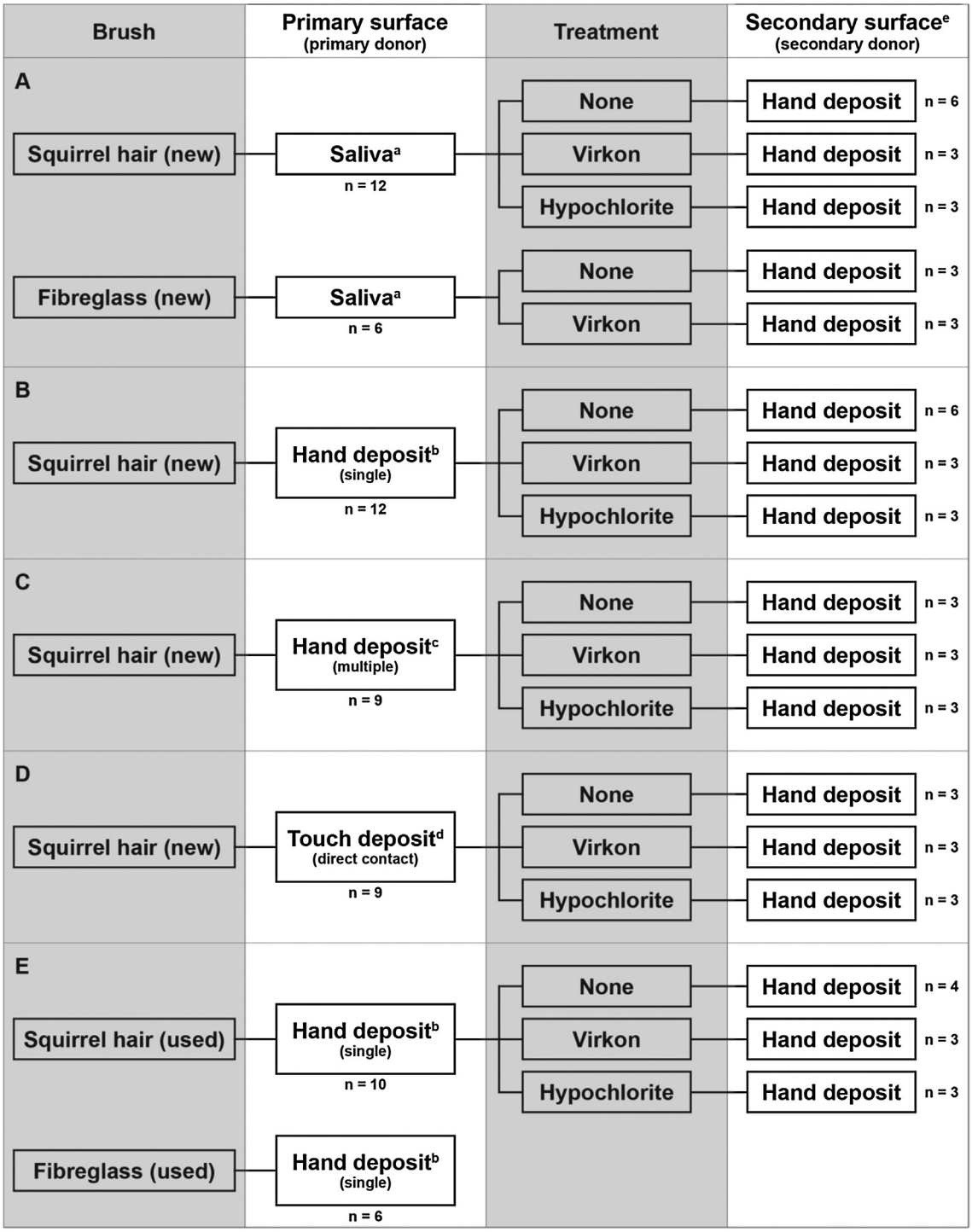
2.2.2. Touch DNA

Primary surfaces containing touch DNA from a single donor (donor 2) comprised of (a) single handprints on DNA-free glass plates (140 × 220 mm) deposited in pairs (left and right hands) over multiple days i.e. one pair of handprints per day, and (b) multiple handprints on DNA-free glass jars placed over a period of three days. All surfaces were dusted with new unused squirrel hair brushes (Fig. 1B,C). In addition, squirrel hair brushes were artificially contaminated by another donor (donor 3) through direct contact with brush bristles over a three day period (Fig. 1D). These brushes were reused from previous experiments involving hand deposits (Fig. 1B,C). Reused brushes were cleaned with hypochlorite and rinsed with water as per the cleaning protocol indicated herein, and samples were taken from bristles of three brushes (wet/dry swabbing) post-cleaning to determine if cleaning was effective.

Donors did not wash their hands prior to contact with the primary surface (glass plates and glass jars) or brush bristles. While donor 2 was primarily in contact with personal items immediately prior to depositing handprints, donor 3 was in contact with various personal and non-personal items.

2.2.3. Previously used brushes

Previously used squirrel hair and fiberglass brushes of various histories that were in current use by staff within the fingerprints group were brushed over primary surfaces comprised of a single handprint from a single donor (donor 1) on DNA-free glass plates



[a] dried saliva on a glass plate; no swabs taken.
[b] a single, 10 s hand deposit on a glass plate; glass plate swabbed following dusting.
[c] multiple hand deposits on a glass jar, placed over a period of three days; glass jar swabbed following dusting.
[d] multiple direct contacts with a squirrel hair brush over a period of three days; brush bristles swabbed following dusting of secondary surface.
[e] a single, 10 s hand deposit on a glass plate; glass plate swabbed following dusting.

Fig. 1. Schematic diagram of transfer experiments.

(140 × 220 mm) (Fig. 1E). Hands were washed 20 min prior to the first deposit, and handprints were placed in pairs (left and right) at 20 min intervals. Nitrile gloves (InControl, Australia) were worn by the handprint donor following hand-washing and in between deposits.

2.3. Treatment of contaminated brushes

Contaminated brushes were cleaned with a 5% solution of Virkon (DuPont, USA) or a 1% solution of hypochlorite, while other brushes remained "dirty" as controls for the transfer of DNA. As a

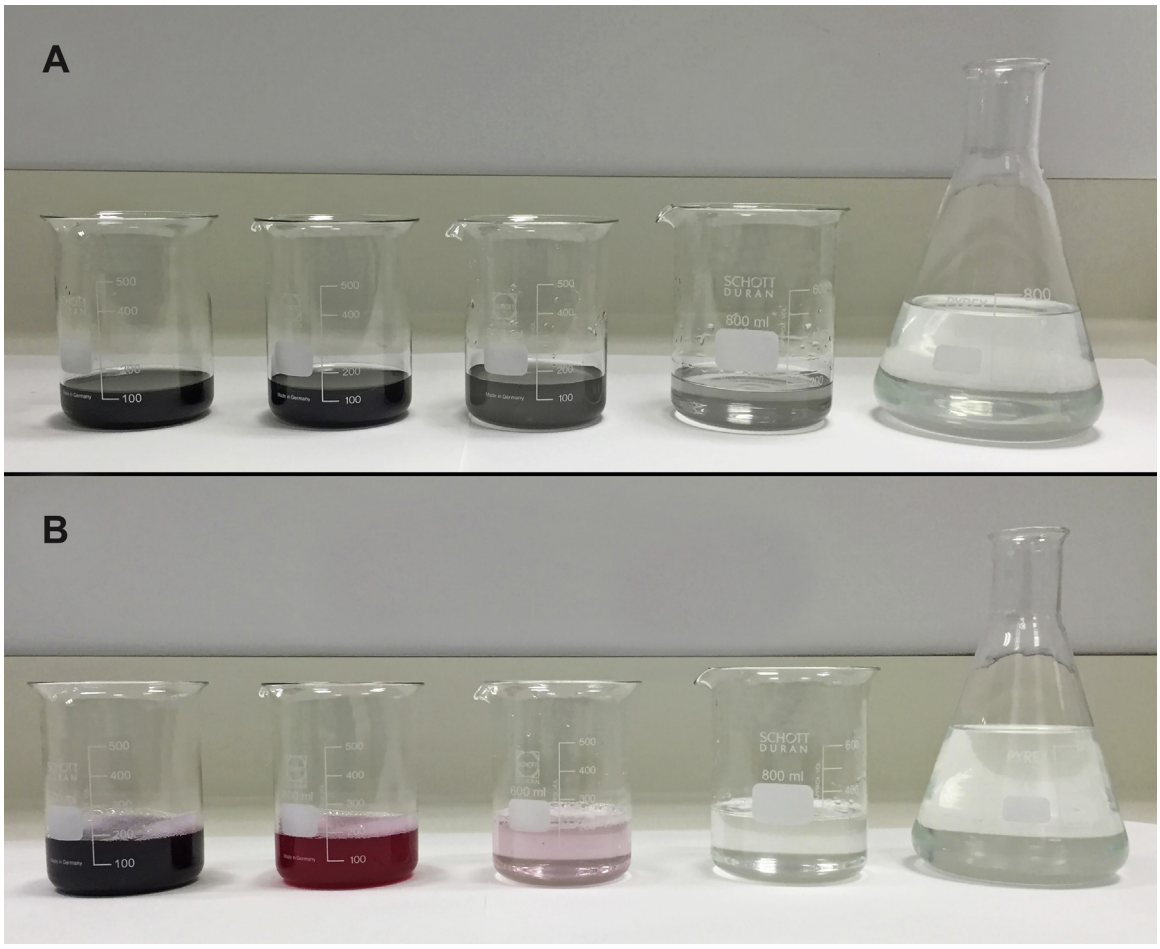


Fig. 2. Cleaning method devised for the decontamination of brushes with a 1% sodium hypochlorite solution (A) and a 5% Virkon solution (B). Each brush was submerged and agitated for ~1 min in beakers containing the decontaminating agent (beaker 1 then 2) and beakers of tap water (beakers 3 then 4). Note: the conical flask containing tap water demonstrates the increasing clarity of the liquid and did not form part of the cleaning method.

result of initial findings following the washing of fiberglass brushes with Virkon (Fig. 1A), previously used fiberglass brushes were not cleaned with either agent or dusted over a secondary surface (Fig. 1E).

Four 600 mL beakers were used to carry out cleaning of the brush bristles. The first two contained ~200 mL of the decontaminating agent (Virkon or hypochlorite) and the remaining two contained ~200 mL tap water (Fig. 2). Brushes were submerged in each of the four beakers for ~1 min while agitating and flexing the bristles during immersion. Before being transferred to the next beaker, the bristles of each brush were pressed and gently flicked against the side of the beaker to remove any excess fluid. In addition, brushes were shaken with force (into a sink) in between water rinses (third and fourth beakers), and following the final water rinse (fourth beaker) to aid the cleaning/drying process. All clean and dirty brushes were rested with bristles facing upwards for 24 h in separate racks.

2.4. Cleaning effectiveness

All brushes (dried and rested) were used to dust a secondary surface consisting of a single handprint (donor 4) on a DNA-free glass plate (140 × 220 mm). During dusting, separate aliquots of Opti-black powder were used for each brush. Handprints were deposited in pairs (left and right) on separate glass plates every 20 min, commencing 20 min post-hand washing, and nitrile gloves were worn following hand-washing and in between deposits. Secondary surfaces were dusted in the same manner as primary surfaces (brushed 20 times while each brush was intermittently dipped in a separate aliquot of unused black powder). Glass plates were cleaned with 1% hypochlorite and water (as previously described) prior to and in between uses, and samples were taken (wet/dry swabbing) to assess their DNA-free status (n = 16).

The entire dusted hand deposit on the secondary glass plates were collected using cotton swabs (150C, Copan) with a wet/moist swabbing protocol to ensure thorough collection. Where relevant (Fig. 1B,C,E), the entire dusted handprint on the primary surface was also collected to assess if sufficient DNA was present in the deposit to observe any transfer. The bristles of artificially contaminated brushes (Fig. 1D) were swabbed (wet/moist) following treatment and contact with the secondary surface. Swabs were stored in their tubes for up to 1 h following sample collection, before the tips were excised into 2 mL tubes and stored at −20 °C prior to processing.

2.5. Brush durability

Bristle degradation following cleaning with Virkon and hypochlorite was assessed. A new unused squirrel hair brush was submerged and vigorously agitated (~30 s) in either a beaker of hypochlorite or Virkon for a total of 20 wash/rinse/dry cycles over a period of two weeks.

During data collection, the Fingerprint Sciences Group at the VPFSD reported that new squirrel hair brushes with a higher

squirrel hair to synthetic fiber ratio were in use. A separate trial was performed to assess the durability of the new brushes, while also reassessing the previously tested brushes. As previously described, one brush of each type was immersed in hypochlorite or Virkon, rinsed with water and then dried over multiple cycles, however a more realistic submersion time of ~2 min was applied. In addition, one brush of each type was submerged in hypochlorite or Virkon (~2 min) and left to dry, with the rinsing step removed from each cycle.

2.6. Sample processing and analysis

DNA was extracted from swabs and cut bristles using DNA IQ™ (Promega, USA). Black powder has been shown to have no impact on the DNA extraction process using this system [7]. Sample processing methodology changed slightly during the study, so that sets A, B, C and E (Fig. 1) produced a final extraction volume of 50 μL and samples were quantified with Quantifiler® Trio (Life Technologies, USA), excluding set A which was quantified with Quantifiler® (Life Technologies, USA), while a final extraction volume of 60 μL was produced for set D (Fig. 1) and samples were quantified with Quantifiler® Trio. However, as results were analysed based on the detection of any transferred DNA or DNA from unknown persons, the alteration in methodology did not affect interpretation of the cleaning effectiveness. Samples were quantified using an ABIPRISM®7500 (Life Technologies, USA) with HID software. Amplification was carried out using PowerPlex® 21 (Promega, USA) as per manufacturer's instructions where the maximum number of alleles detectable for a heterozygous individual is 40 (excluding amelogenin). For amplification, 0.5 ng of template DNA was used or, in instances where the concentration of samples fell below 0.033 ng/μL, 15 μL of sample was used. Amplified product detection and sizing was performed on a 3500xL Genetic Analyser (Life Technologies USA). GeneMapper® ID-X software v1.4 (Life Technologies, USA) was used for genotyping with a baseline threshold of 175 RFU. The homozygous threshold was 2000 RFU, as per laboratory protocol.

2.7. DNA profile interpretation and data analysis

According to our validated DNA profile laboratory interpretation methods, peaks that were determined to be artifacts (e.g. stutter) were removed from the profiles. Reference profiles were obtained from the donors of biological material in this study, and each profile generated from the primary and secondary surface was compared to the relevant individual donor reference profiles. By omitting any shared alleles, unique allele and peak height contributions were determined for each of the donors to the profiles obtained. Amelogenin was not considered in the allele count. The average profile contribution (%) was determined by dividing the total unique peak height contribution for each donor by the total peak height contribution for all donors in the profile. In addition, the average peak height (RFU) of unique alleles was determined for the donors within each profile by dividing the total unique peak height contribution by the total number of unique alleles detected. Foreign DNA, in the form of alleles from unknown sources was also considered and the contribution assigned in the profiles obtained.

Due to limited sample sizes, the non-parametric, Kruskal–Wallis one-way analysis of variance test was used to determine whether the distribution of unique alleles corresponding to the transferred DNA observed in profiles generated from the secondary surface were the same across treatment groups. This was performed for simulations involving the transfer of dried saliva and for artificially contaminated brushes. A significance level of $p = 0.05$ was used. Statistical analyses were performed in IBM SPSS statistics v23 (IBM, USA).

3. Results

3.1. Quality control

Standard quality control procedures were followed during sample processing. Extraction positive (n = 11) and negative (n = 8) controls, and amplification positive (n = 8) and negative (n = 8) controls each performed as expected, with negative controls yielding no DNA and positive controls producing complete and correct profiles with no contaminating DNA detected. In addition, no DNA was detected in experimental negative controls taken from cleaned primary and secondary glass surfaces (n = 30) or from the bristles of cleaned brushes (n = 3).

3.2. DNA-free status of new unused brushes

No measurable quantities of DNA were detected in samples obtained from the two commercially available DNA-free brushes and from the conventional fiberglass brushes (n = 3); negative quantification (0.000 ng/μL) and profiling results were obtained from all samples (data not shown). In contrast, the conventional squirrel hair brushes (n = 3) produced DNA yields of ≤1 ng and resulted in profiles comprised of a minimum of three to four persons. The quantity of alleles detected within these profiles ranged from 17 to 94 with an average peak height of 461 RFU (data not shown).

3.3. Squirrel hair brush contamination and cleaning effectiveness

3.3.1. Dried saliva

On average, 95% of the DNA observed in profiles generated from cut bristles following the dusting of dried saliva deposits without powder, was derived from the saliva donor (Fig. 3). Full, 20-locus DNA profiles with alleles corresponding to the saliva donor were observed in all instances, equating to 22 unique alleles (Fig. 4). While the saliva donor was observed as the major contributor of DNA to all three profiles obtained from the bristles, additional alleles from unknown sources were also observed contributing 5% of the DNA profile (Fig. 3). The observation of extraneous alleles (avg. 8; Fig. 4) was somewhat unexpected in these simulations, although, as demonstrated, it is likely that DNA from various sources already present on the unused brushes resulted in the observed profiles.

Further experiments involving the dusting of secondary surfaces (comprising a single handprint) with untreated brushes resulted in the transfer of DNA from the saliva deposit on the primary surface to the hand deposit on the secondary surface via squirrel hair brushes on all occasions. On average, 46% of the DNA retrieved from these secondary glass plates was derived from the transferred DNA of the saliva donor, with further contributions from the handprint depositor (53%) and unknown sources (1%) (Fig. 3). Following treatment with Virkon and hypochlorite, the detection of contaminating DNA from the saliva donor decreased substantially in profiles obtained from the secondary surface. The saliva donor contributed to only 3% of the DNA detected when brushes were treated with Virkon, while for brushes treated with hypochlorite, the contribution was 1% (Fig. 3). The handprint depositor was the main contributor to these profiles (97%), while DNA from unknown sources contributed ≤2% (Fig. 3).

When considering the profiles obtained from secondary surfaces, the distribution of unique alleles corresponding to the transferred saliva donor was significantly ($p < 0.05$) different for the three treatment groups. Dusting with untreated brushes

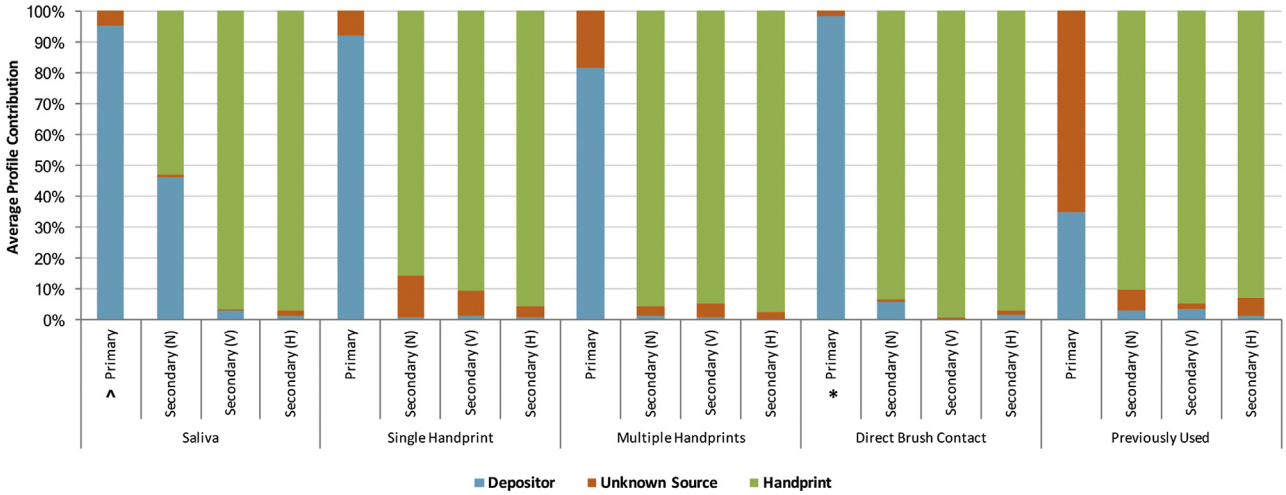


Fig. 3. Average DNA contributions (%) attributable to donors in the profiles generated from primary and secondary surfaces in simulations involving squirrel hair brushes. Unless indicated (*^), profiles were generated following the dusting of a primary surface deposited with biological material, followed by the subsequent dusting of a secondary surface containing a handprint with Virkon (V) or hypochlorite (H) treated brushes, or those that remained untreated (N).
^Profiles generated from cut bristles following dusting of the primary surface without powder.
*Profiles generated from swabs of untreated (none) bristles following dusting of the primary and secondary surfaces with powder.

resulted in the saliva donor contributing an average of 16 (sd. = 9) unique alleles, while the handprint depositor and unknown sources contributed 19 (sd. = 0) and 3 (sd. = 2) respectively (Fig. 4). Following treatment of brushes with Virkon and hypochlorite, an average of 1 (sd. = 1) unique allele corresponding to the saliva donor was detected in profiles generated from the dusted secondary surface (Fig. 4).

3.3.2. Touch DNA

Minimal transfer was observed in simulations involving the transfer of DNA from a primary surface comprising of singular or multiple handprints, to a secondary surface containing a hand deposit. While samples collected from the primary surface post-brushing indicate sufficient quantities of DNA for detection were deposited on singular and multiple occasions, brushing of the secondary surface with untreated and treated brushes similarly resulted in minimal transfer; on average $\leq$1% of the overall DNA retrieved could be attributed to the transferred handprint donor (Fig. 3). This contribution corresponds to an average of 1 unique allele on all occurrences (Fig. 4). As a result of the minimal transfer observed across treatment groups, it could not be inferred whether treatment of brushes with Virkon and/or hypochlorite had any impact in decreasing contaminating DNA.

Contrasting the limited transfer of DNA observed from handprints on primary glass plates (single and multiple), transfer was observed following direct contact with squirrel hair bristles. 6% of the DNA detected in profiles obtained from the secondary surface following dusting with untreated brushes that had been contacted directly corresponded to the transferred touch deposit, while 93% of the profile comprised of DNA from the handprint donor (secondary surface) and the remaining 1% from unknown sources (Fig. 3). The transferred contribution decreased to 1% following cleaning with Virkon, and to <1% following cleaning with hypochlorite.

Within the profiles obtained from the secondary surface, the distribution of unique alleles corresponding to the transferred touch donor was significantly ($p < 0.05$) different for the three treatment groups. Dusting with untreated brushes resulted in the touch donor contributing an average of 6 (sd. = 5) unique alleles, while the handprint depositor and unknown sources contributed 26 (sd. = 2) and 1 (sd. = 2) respectively (Fig. 4). One profile displayed a total of 12 unique alleles corresponding to the touch donor. Following treatment of brushes with Virkon and hypochlorite, an average of 1 (sd. = 1) unique allele corresponding to the touch donor was detected in profiles from the dusted secondary surface (Fig. 4).

In addition, the bristles of treated and untreated brushes that had been directly contacted were swabbed following dusting of the secondary surface. For untreated brushes, sufficient quantities of DNA from the touch donor were present for detection (26 unique alleles on average; Fig. 4), demonstrating that even after dusting, bristles retain DNA-containing material. For treated brushes, this confirmed that the cleaning method was successful, with no contaminating DNA detected from the donor directly contacting the brush bristles. DNA from the handprint donor was also collected from the secondary surface by the brushes, with an average of 2 unique alleles (sd. = 3) detected within the nine profiles obtained from the bristles. In one profile, 7 unique alleles corresponding to the handprint donor were detected, further demonstrating that DNA is collected through regular dusting, which can accumulate if brushes are not cleaned following use.

3.3.3. Previously used brushes

Dusting of primary surfaces with previously used brushes of unknown history resulted in the transfer of considerable volumes of DNA from unknown sources. 65% of the overall DNA contribution was unable to be attributed to the handprint donor, who contributed to the remaining 35% of the profile (Fig. 3). While majority of the unknown contribution is derived from the dirty brushes, it is also likely that DNA residing on the hand of the handprint donor has contributed to the profile.

As observed for singular and multiple hand deposits, the transfer of the handprint donor from the primary to the secondary surface via untreated brushes was minimal (3% of the profile contribution). In addition, decreased levels of DNA from unknown sources were also detected in these samples, contributing to 7% of the overall DNA detected (Fig. 3). This is surprising given the large contribution to the primary surface. As result of limited transfer from the primary surface and the decreased detection of unknown sources from untreated brushes, it cannot definitively be established whether the cleaning methods used were effective in the removal of DNA.

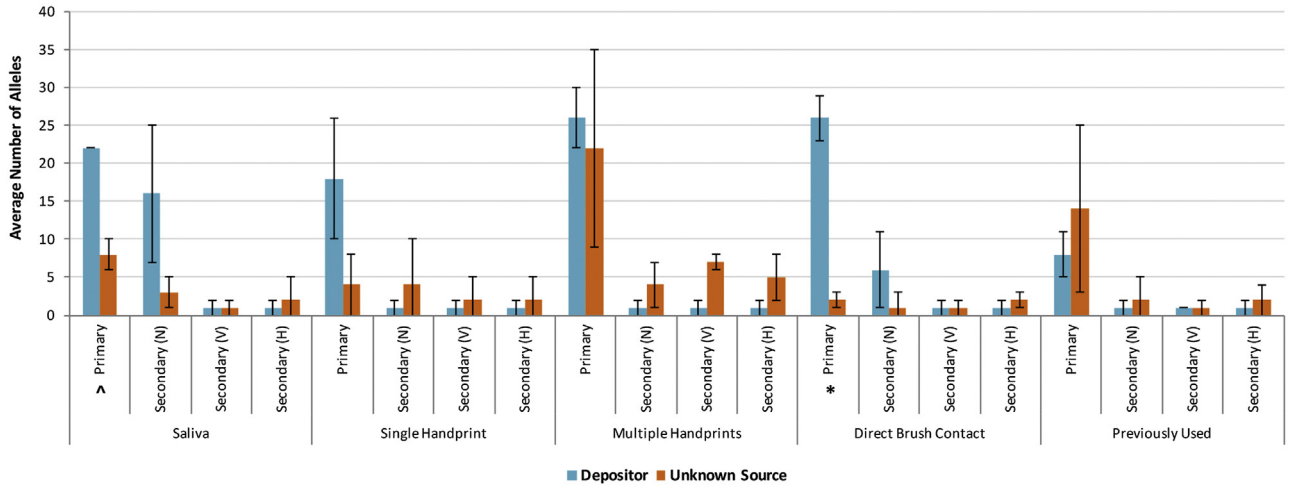


Fig. 4. Average number of alleles attributable to donors in the profiles generated from primary and secondary surfaces in simulations involving squirrel hair brushes. Unless indicated (*^), profiles were generated following the dusting of a primary surface deposited with biological material, followed by the subsequent dusting of a secondary surface containing a handprint with Virkon (V) or hypochlorite (H) treated brushes, or those that remained untreated (N).
^Profiles generated from cut bristles following dusting of the primary surface without powder.
*Profiles generated from swabs of untreated (none) bristles following dusting of the primary and secondary surfaces with powder.

3.4. Fiberglass brush contamination and cleaning effectiveness

3.4.1. Dried saliva

Preliminary simulations involving the transfer of dried saliva from a primary surface to a secondary surface containing a single hand deposit with new unused fiberglass brushes demonstrated that considerable quantities of DNA could be transferred through standard dusting of the surfaces. On average, the relative contribution of DNA from the saliva donor to the three profiles generated from secondary surface following dusting was 10% (Fig. 5), with further contributions by the handprint depositor (84%) and unknown sources (6%). However, one particular sample displayed substantially higher levels of transfer compared to the others, where the profile generated from the secondary surface displayed 17 unique alleles corresponding to the saliva donor, along with 17 corresponding to the handprint depositor and 13 from unknown sources.

Cleaning of contaminated fiberglass brushes was initially attempted with Virkon, however the brushes were not suitable for cleaning as the bristles become tangled and matted when wet.

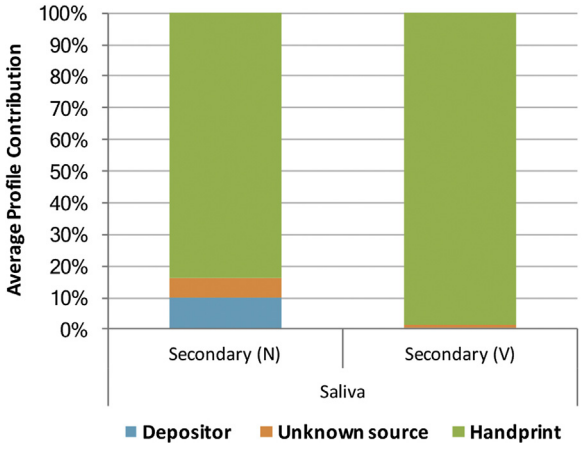


Fig. 5. Average DNA contributions (%) attributable to donors in the profiles generated from secondary surfaces in simulations involving fibreglass brushes. Profiles were generated following the dusting of a primary surface deposited with dried saliva, followed by the subsequent dusting of a secondary surface containing a handprint with Virkon (V) treated brushes, or those that remained untreated (N).

Although the transfer of DNA was decreased post-cleaning with Virkon, with less than 1% of the saliva donor's DNA detected in the three profiles generated (Fig. 5), the brushes became unusable.

3.4.2. Previously used brushes

Minimal transfer was observed with previously used fiberglass brushes of unknown history. On average, 2 (sd. = 1) unknown alleles were observed in the six profiles generated (data not shown). This suggests that either fiberglass brushes retain more DNA than is transferred, or there was not sufficient DNA present for transfer. The handprint depositor was detected in all profiles generated.

3.5. Brush durability

Though some periodic loss of bristles was observed, minimal degradation of bristle quality was observed after 20 wash/rinse/dry cycles of squirrel hair brushes with either hypochlorite or Virkon when submerged for ~30 s (Fig. 6B). When the submersion time increased to ~2 min, all treated brushes started to periodically loose bristles after 3 wash/rinse/dry cycles, and some loss of pigment was observed for brushes immersed in hypochlorite. Bristle and pigment loss noticeably increased after 7 cycles of 2 min treatment with hypochlorite (Fig. 6F), particularly for brushes with a greater proportion of synthetic fibers, however the overall structure remained intact. In contrast, little to no loss of bristles and pigment was observed for brushes immersed in Virkon (2 min) over the 12 cycles, and no substantial difference between the two brush varieties.

Only three wash/dry cycles were achieved for brushes submerged in hypochlorite for ~2 min and left to dry without rinsing, before the bristles became weak and wiry (Fig. 6E), while brushes submerged in Virkon were not affected (not shown). This indicates that brushes may remain submerged in Virkon for longer periods of time with less damage.

All treated brushes were presented for visual inspection to staff in the Fingerprint Sciences Group. While all brushes submerged in hypochlorite or Virkon for 12 or 20 cycles (Fig. 6B,C,D) were deemed 'useable', there was a preference for the brushes cleaned with Virkon as they remained softer and would be less abrasive when dusting the surface. Brushes submerged in hypochlorite without rinsing were deemed unusable (Fig. 6E).

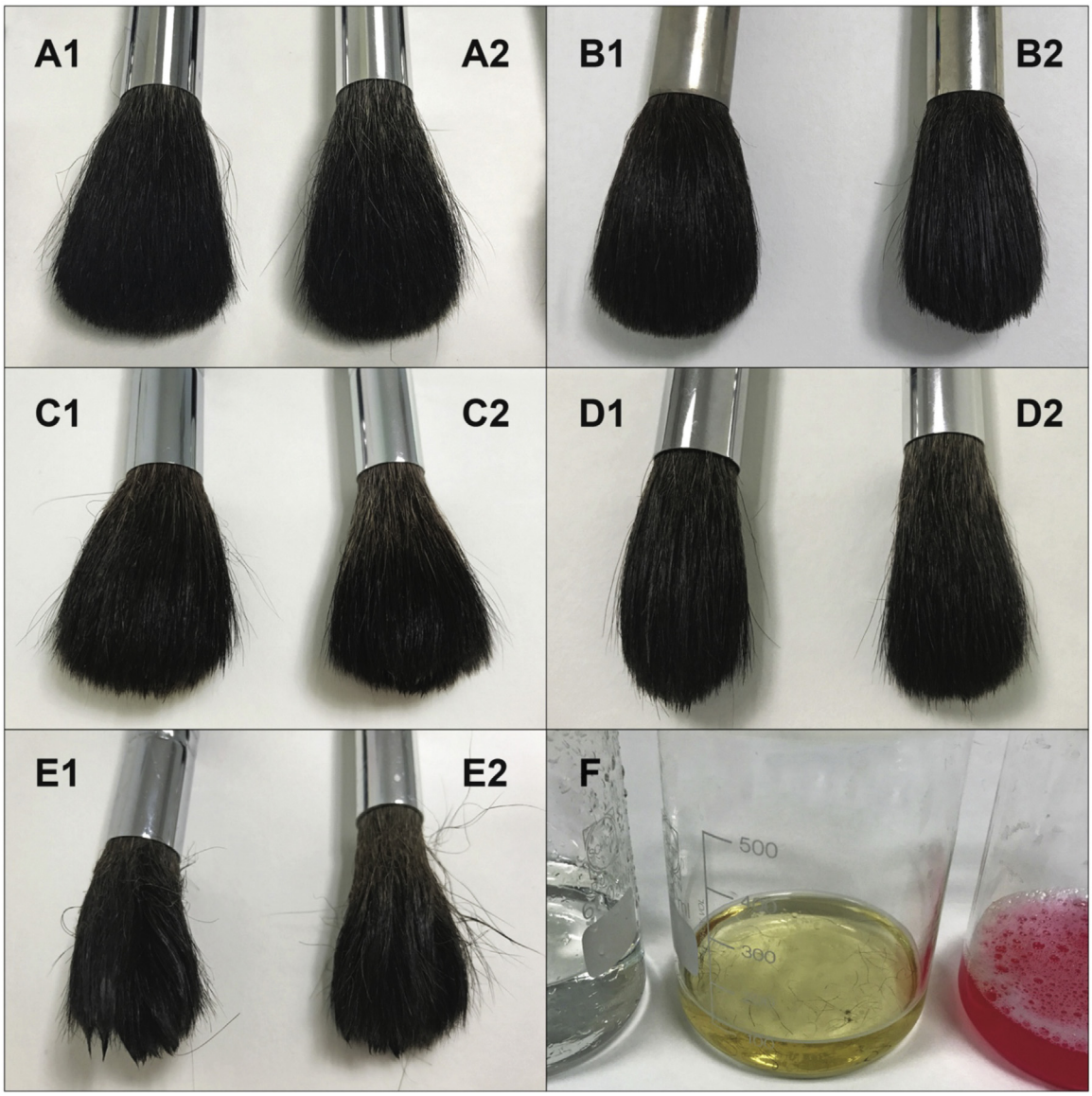


Fig. 6. Photos of squirrel hair brushes with increased synthetic (A1) or hair (A2) fibres, prior to any treatment; squirrel hair brushes with increased synthetic fibres after 20 wash/rinse/dry cycles with Virkon (B1) and hypochlorite (B2) using an immersion time of ~30 s; brushes with increased synthetic (C1) or hair (C2) fibres after 12 treatment cycles with Virkon using an immersion time of ~2 min; brushes with increased synthetic (D1) or hair (D2) fibres after 12 treatment cycles with hypochlorite using an immersion time of ~2 min; brushes with increased synthetic (E1) or hair (E2) fibres after 3 wash/dry cycles with hypochlorite and no rinsing; (F) beakers containing water, hypochlorite and Virkon (L-R) following immersion of a squirrel hair brush with increased synthetic fibres after the 7th wash cycle.

4. Discussion

Varying amounts of DNA-containing material were collected and transferred by squirrel hair and fiberglass brushes, with detectability on the secondary surface dependent on the biological nature of the material being transferred. The impact of DNA contamination from dirty fingerprint brushes was most apparent in simulations involving the transfer of dried saliva from a primary surface (glass plate) to a secondary surface containing a single hand deposit, with both squirrel hair and fiberglass brushes transferring DNA of the saliva donor. This is not surprising given the high quantities of DNA found in saliva. These findings are consistent with those of van Oorschot et al. [9], where significant quantities of DNA were collected from recently dried saliva and subsequently transferred to a clean surface. The detection of such a large portion of contaminating DNA from the saliva donor has a significant impact on the interpretation of the DNA profile, with additional contributors needing to be postulated and accounted for in the profile. While in casework it is uncommon to knowingly dust for fingerprints in saliva deposits, saliva traces are usually not visible to the human eye and may inadvertently become part of the print being dusted, whether already present on the surface or transferred by the hand during contact, or through general speaking.

In contrast to dried saliva, minimal to no transfer was observed via squirrel hair brushes following contact with hand deposits (single and multiple) on glass plates/jars. Previous studies investigating the DNA contamination risk via fingerprint brushes have also demonstrated minimal transfer of touch deposits [8,10], suggesting that there may be a limited risk of contamination via fingerprint brushes that have come into contact with touched surfaces. While this may be true for surfaces known to be deposited with biological material containing low quantities of DNA, under casework conditions large quantities of DNA may accumulate and be collected through the dusting process.

Transfer increased when brush bristles were artificially contaminated through direct contact, and while this situation is unlikely to occur in casework, it demonstrates the possibility of transfer if touch DNA is allowed to accumulate on used brushes. The large amount of foreign DNA present on, and subsequently transferred by, the casework brushes demonstrates the occurrence of this in operational practice. Within these DNA profiles the contribution of DNA from unknown sources far exceeded the contribution from the handprint donor, with 65% of the DNA detected unable to be attributed to any known individual/s (Fig. 3). In a practical context, if a handprint is found at a crime-scene and dusted with a used/dirty brush, the profile generated from downstream DNA analysis is likely to be contaminated with DNA from sources other than the targeted DNA. If the brush has been used across multiple items and/or crime scenes, there is the potential for DNA transfer to cause incorrect linking of cases or offenders due to contamination of the brushes. This risk rises with increasing use of the brush, where initial contamination is low when used on single or few handprints, and increasing as the amount of skin residue and cellular material contacted increases.

In addition to used squirrel hair brushes transferring DNA from one surface to another, this study has somewhat surprisingly shown that the bristles of new unused, conventional squirrel hair brushes contain large quantities of DNA. It is likely that DNA from various sources is collected by the brushes during the manufacturing, packaging and/or distribution process, whether transferred directly from the handler/s or indirectly though contact with surfaces on which DNA is already present [2,4]. This finding not only highlights the need to clean brush bristles prior to use, along with the brush handle and any packaging or tubing encasing the brush, especially if this is to be re-used in the storage of decontaminated brushes, but to perform cleaning within a DNA-free environment using appropriate personal-protective-equipment (PPE). The powder used during dusting may also be a potential source of contamination. In a study by van Oorschot et al. [9], aliquots of powder from regularly used containers were sampled, and indicated the presence of DNA. This reiterates the need to use separate aliquots of powder for different exhibits and scenes.

A decrease in the detectability of contaminating DNA was observed following cleaning of squirrel hair brushes with Virkon and hypochlorite. Since the transfer of DNA from the primary to the secondary surface was observed with untreated brushes, a decrease in the transfer of contaminating DNA was most evident in simulations involving dried saliva and brushes that were artificially contaminated through direct contact. On average, transfer of the saliva donor's unique alleles decreased from 16 to 1 following cleaning with both Virkon and hypochlorite, and from 6 to 1 for artificially contaminated brushes (Fig. 4). However, given the low weight of evidence obtained from the cleaned brushes (1 allele), there is also a possibility that the source of this allele was from an unknown donor, and represented an adventitious match with the primary deposit donor.

Contrasting the present study, which found no substantial difference between cleaning with sodium hypochlorite and Virkon®, Ballantyne et al. [17] established that a 1% solution of sodium hypochlorite was the most effective DNA decontamination method, while Virkon (5%) was successful in removing touch DNA, but less effective on both wet and dry saliva, blood and semen stains. It is possible that the increased effectiveness of Virkon observed in the present study is due to smaller quantities of dried saliva residing on the surface being decontaminated, the brush bristles, compared to the larger quantities (10 μL) of biological material deposited on surfaces by Ballantyne et al. [17]. This indicates that hypochlorite is more effective in eradicating DNA from surfaces comprising large quantities of DNA-containing material, such as blood or semen, as opposed to Virkon, which reaches its maximum decontaminating ability.

In further studies considering the effects of both agents on the durability of the squirrel hair bristles, it was demonstrated that neither Virkon or hypochlorite compromised the brushes beyond use after 20 wash/rinse/dry cycles when submersion in the agent was limited (~30 s), or 12 wash/rinse/dry cycles when submersion increased to ~2 min. Following removal of the water rinsing step when cleaning with hypochlorite, severe bristle degradation was observed. While in this instance, the impact of residual hypochlorite on the brush bristles would have minimal impact on downstream DNA analysis if direct contact is made with an exhibit containing DNA [19], this reinforces the importance of rinsing the brushes to prolong durability. Although no substantial difference was observed between the two brushes tested, those with more squirrel hairs and those with less, degradation of the bristles depends on the quality of the brush. In addition, the treatment cycles indicated do not take into account general wear-and-tear on the brushes through regular use. Users should confirm the impact of the cleaning regime on their own brushes when determining the most cost-effective option to adapt for cleaning fingerprint brushes.

While squirrel hair brushes could be easily and effectively cleaned with both hypochlorite and Virkon, fiberglass bristles became matted when wet and were unusable. A potential alternative for fiberglass brushes and other water-sensitive equipment, is ethylene oxide (EO) treatment. This gas-phase, DNA decontamination method is currently used to treat commercially available DNA-free brushes, and it has been shown to be an effective decontamination method for forensic consumables [20,21]. While EO treatment of used fingerprint brushes may be a substitute to the proposed cleaning methods with hypochlorite and Virkon, and is an area that deserves further research, due to the highly toxic nature of EO, commercial companies are required to carry out the procedure. As such, the practicalities of implementing such a method may be a limiting factor.

In general, DNA-containing material can be transferred via latent fingerprint brushes and regular cleaning or replacing of brushes is advocated to minimize the risk of contamination, especially in high risk situations such as (a) when it is unknown if downstream DNA analysis may be required, (b) in between items from different cases, and (c) if the brush has come into contact with biological material containing large quantities of DNA such as blood, saliva or semen. Similarly, using fresh aliquots of powder between scenes and exhibits should also be considered to minimise transfer during powdering. Nevertheless, using a clean brush or separate aliquots of powder on every item/exhibit may not be practical or cost-effective, especially if downstream DNA analysis is not required. Each laboratory should consider their own procedures in order to determine when cleaning or replacing is necessary.

5. Concluding remarks

This study has reiterated that DNA can be transferred via latent fingerprint brushes and the detection of transferred DNA can affect profile interpretation. The rate of transfer is dependent on the biological material being contacted by the brushes, with material containing higher quantities of DNA such as saliva likely to transfer DNA at a higher rate than those with less DNA such as touch deposits. Both Virkon (5%) and sodium hypochlorite (1%) are shown to remove fingerprint powder and contaminating DNA from squirrel hair brushes, while fiberglass brushes should be disposed of following use, or used only post-DNA collection. Each laboratory should consider their current circumstances including current

DNA decontamination methods, the sensitivity of PCR and amplification procedures, and their policy of when to replace or clean dirty brushes before adapting a cleaning method. The implementation of a program to monitor the effectiveness of the cleaning regime is advised.

5.1. Cleaning method

Recommended cleaning method for squirrel hair brushes:

1. Submersion and agitation of bristles in a 1% solution of sodium hypochlorite or a 5% solution of Virkon for 1–2 min. If the agent appears murky, dispense of liquid and rinse again with selected agent.
2. Shake/flick bristles against the side of the cleaning vessel (or into a washbasin) in between repetitions to remove the bulk of any retained liquid and to aid the cleaning process.
3. Submersion and agitation of bristles in water ~1–2 min. If the water appears murky, dispense of liquid and rinse again. Ensure the water is virtually clear in the final rinse.
4. Shake/flick bristles against the side of the cleaning vessel (or into a washbasin) in between repetitions and after the final rinse, to remove the bulk of any retained fluid. This will aid the cleaning and drying process, and increase the longevity of the brushes.
5. Using Kimwipes®, or similar, wipe the brush handle with hypochlorite or Virkon, followed by wiping with water.
6. Place brushes on a rack, or similar, in a protected environment to dry prior to use or storage. Brush bristles should not contact one another or another surface while drying.
7. Store each brush separately in a protective environment to prevent contamination during storage. If storing brushes in their original packaging (i.e. plastic tubes), ensure the packaging is cleaned/decontaminated with an appropriate agent prior to the storage of cleaned brushes.

Note: if the brushes are heavily soiled or multiple brushes are being cleaned at the same time, consider repeating steps 1 (cleaning with agent) and 3 (water rinse) multiple times, using fresh solutions each time.

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