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TECHNICAL NOTE

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Beware of the Possibility of Fingerprinting Techniques Transferring DNA*

ABSTRACT: Fingerprinting brushes have the potential to collect and transfer DNA during powdering. Squirrel-hair fingerprint brushes exposed to specific sets of saliva stains and brushes used in routine casework were tested for their ability to collect and transfer DNA containing material using standard DNA extraction procedures and AmpF*l*STR® Profiler Plus™ amplification and typing procedures. The tests found that the risk of transferring DNA during powdering and having a detrimental impact on the analysis increases if the examiner powders over either biological stains (such as blood or saliva) or very fresh prints and uses more sensitive PCR amplification and typing procedures. We advocate caution when powdering prints from which DNA may also be collected and provide options for consideration to limit the risk of transferred DNA contamination while fingerprinting.

KEYWORDS: forensic science, DNA, trace, fingerprint brushes, transfer

Increasingly, DNA is being collected for genetic profiling from touched objects that have been exposed to fingerprinting techniques. While it is clear that the fingerprint itself can't be transferred from one object to another, it may be possible to transfer DNA-containing material (1). This is especially a possibility when using the same brush to powder different objects within and between crime scenes since this is common practice within many forensic jurisdictions.

Sutherland et al. (2) examined the fingerprint brushes and powder from kits used by Scene of Crime Officers at their laboratory (number unknown) and reported obtaining a full multiplex profile from one of the brushes. Furthermore, the potential for detection of any transferred material may increase as some forensic laboratories increase the number of amplification cycles beyond standard protocols to acquire probative genetic profiles (3–6).

Here, we report the outcome of some preliminary tests performed, using squirrel-hair fingerprint brushes, to ascertain to what extent DNA transfer may actually occur.

Materials and Methods

General

The following general methods were used in the experiments presented below: the DNA was extracted using Chelex® 100 (Bio-Rad) 5% DNA extraction (7); concentrated or purified using Centricon® devices (Amicon), Microcon® devices (Amicon), and/or QIAquick® (Qiagen); quantitated using Quantiblot® (Applied Biosystems) methodologies; amplified for genetic profiling using AmpF*l*STR® Profiler Plus™ Amplification kit (Applied Biosystems) and a GeneAmp® PCR System 9600 thermal cycler (Applied Biosystems) as recommended by the manufacturer (50 μL reactions, 28 cycles) unless specified otherwise; and typed using an ABI PRISM® 310 Genetic Analyser (Applied Biosystems) (5 sec injection of typing mix: 12 μL Hi-Di formamide, 0.2 μL Genescan Rox 400-HD size standard, and 2 μL amplified trace DNA) unless specified otherwise and GeneScan® Analysis and Genotyper (Applied Biosystems) software (100 RFU cut-off) unless specified otherwise. The plastic sheets used were taken from packages of single-use sterile sheets from Defries Industries (Australia). Plastic sheets were cut into thin strips prior to DNA extraction. The bristles of a brush were recovered for DNA extraction by cutting them all from the base of the brush. Samples of powder taken for the purposes of DNA extraction were of approximately 0.03 g taken from the upper central portion of the jar of powder to be tested.

Many sheets from the same batch were used for these and other experiments not shown. Sheets within these experiments that were not exposed to human biological substances (as well as some that were) and subsequently committed to DNA extraction and amplification did not provide Profiler Plus™ profiles. The collective data suggested that the sheets of plastic were unlikely to have been contaminated prior to use in the experiments reported here. It was assumed that new brushes and reagents used within all these experiments were free of human DNA-containing material.

Experiment 1

Thirteen squirrel-hair fingerprint brushes, of various history (see footnotes to Table 1), were each used over two clean sheets of

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TABLE 1—*AmpF*l*STR® Profiler Plus™ typings from clean sheets of plastic brushed with used squirrel-hair brushes, the brush itself, and powder that was used.*

Brush No.	History	% Ext Amp'd‖	1st Sheet	2nd Sheet	Brush	Powder	Comment
1	Casework^{B*}	50	N	N	P (6)	P (1-3)	Some shared alleles
2	Casework^{W*}	50	N	N	P (3)	N	
3	Casework^{B*}	50	N	P (2)	P (7)	P (1-3)	Some shared alleles
4	Casework^{W*}	50	N	N	N	N	
5	Casework^{W*}	50	N	P (4)	N	N	
6	100 handprints^{B†}	100	N	N	N	N	
7	100 handprints^{W†}	100	N	N	N	N	
8	100 saliva stain^{B‡}	100	N	N	N	N	
9	100 saliva stain^{W‡}	100	N	N	N	N	
10	108 partial handprints^{B§}	100	N	N	N	N	
11	153 partial handprints^{W§}	100	N	N	N	N	
12	112 partial handprints, 2 saliva stain + 1 blood stain^{1B§}	100	N	N	N	N	
13	86 partial handprints, 1 saliva stain + 1 blood stain^{2W§}	100	N	N	N	N	

N = no profile, P = partial profile with number of observed alleles in parentheses.
W = white powder, B = black powder.
[1] = The saliva stains were the 11th and 105th areas and the blood stain was the 72nd area within the series of 115 areas that were brushed.
[2] = The saliva stain was the 22nd area and the blood stain was the 81st area within the series of 88 areas that were brushed.
* = These brushes had been used frequently over a long period by forensic caseworkers. Details on the history of the brushes were not available.
† = Twenty full hand prints from each of five individuals collected over a 24 h period.
‡ = Twenty saliva stains (40 μL spread over 1 to 1.5 cm^2 and dried at room temp for a minimum 24 h) from each of five individuals.
§ = All prints and stains of various size and origin.
‖ = The percentage of the total volume of DNA extracted that was used as template DNA in the amplification mix.

plastic using a separate aliquot of powder per brush. All samples were extracted using Chelex-Centricon®, and the DNA was not quantified prior to amplification. For samples associated with brushes numbered 6 to 13, only 1 μL of amplified product was added to the typing mix and a RFU cut-off of 150 applied. A small number of fibreglass brushes (filament brush No 122L, Sirchie®) were similarly examined.

Experiment 2

Saliva stain samples were prepared by spreading 40 μL saliva over an area of 2 × 2 cm on 19 sheets of plastic and allowed to dry overnight at room temperature. All saliva stains within a series were from the one saliva sample from one individual. Samples from different individuals were used for different series. Brushes were dipped in an aliquot of corresponding unused white powder at the start and after every 4th stain. This was done to insure there was sufficient powder present on the brush and for consistency. A different container was used per series. The brush was then brushed over the remaining 20 clean sheets without being dipped in powder again. The amounts of DNA placed on each of the initial 19 sheets of plastic were determined by adding an aliquot of 40 μL of saliva used in each series into separate Eppendorf tubes for DNA extraction. The DNA concentration and amounts within these were determined using Quantiblot.

The samples were extracted using Chelex-Centricon®-QIAquick®-Microcon®, and all available DNA was used as template during amplification. (Some sheets were not examined due to resource constraints and thus were not reported.)

Experiment 3

Test A—Different amounts of powder (0.005, 0.01, 0.015, 0.02, 0.025 and 0.03 g), were added to separate amplification mixes of AmpF*l*STR® Profiler Plus™ (50 μL) and 1 ng of DNA (AmpF*l*STR® control DNA). This was repeated for both types of powder routinely used to dust prints, i.e., white and black powder (Optimum Technology). Preliminary investigations had found that the amount of powder left on a surface area of approximately 10 × 10 cm after powdering with white or black powder fluctuated between 0.003 and 0.027 g. The amounts tested thus represent potential amounts present in casework.

Test B—Six sheets of plastic were powdered (white powder), in numerical order, with a squirrel-hair brush known to be heavily contaminated with DNA from a single source (the brush had been dipped in liquid saliva four months previously). Two methods of DNA extraction were employed. Two samples were extracted using Chelex-Centricon®-Microcon® and four extracted using Chelex-Centricon®-QIAquick®-Microcon®. All available extract was used as template in an AmpF*l*STR® Profiler Plus™ amplification together with 1 ng positive control DNA. (see Table 3).

Experiment 4

Seven-three squirrel-hair brushes that were in current use by staff within our Department were tested for their potential to transfer DNA to a surface in the process of collecting a fingerprint. They were brushed (using white powder) over a series of five sheets of plastic of which the 1st, 2nd, 4th and 5th were clean, and the 3rd contained a fresh handprint (deposited <5 min prior to powdering). Each brush was dipped once in a separate, fresh, unused aliquot of powder and brushed over the five sheets without repowdering the brush.

Sheets 1 and 2 were tested to see if any DNA that may have been on the brush had been transferred to a subsequently powdered clean surface. The 3rd sheet was used to check if the presence of a handprint attracted DNA from the brush. This 3rd sheet also functioned as a control for the sensitivity of the detection. Analysis of the 4th and 5th sheets checked if the brush had collected DNA from a fresh print (i.e., sheet 3) and transferred it to clean sheets brushed immediately afterwards.

Details on the history of each of the 73 brushes (when last used, how long used for, whether used over biological stains or skin,

TABLE 2—*AmpFlSTR® Profiler Plus™ profiles (from saliva stain) transferred to 20 sheets of plastic by a brush immediately after brushing over 19 saliva stains.*

Series	Clean Sheets Brushed After Brushing Over 19 Saliva Stains																Peak Area (RFU)			Brush	Saliva ng DNA/40 μL
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	14th	16th	18th	20th	1st Sheet	20th Sheet	General		
1	F	F	F	F	F	F	F	F	F	F	F	P19	P11	F	F	F	61,615	5,120	Grad decline	F[a]	82
2	F	F	F	F	F	F	F	F	F	F	F	P18	P16	P14	N	P18	17,596	1,114	Grad decline	P18[a]	30
3	F	F	F	F	F	F	F	F	F	F	F	F	P19	F	F	P18	1,822	1,344	Even	F[a]	240
4	F	F	F	F	F	F	F	F	F	F	F	P5	P3	P1	P1	P6	8,242	186	Grad decline	P19*	26

F = full profile of originating saliva sample, P = partial profile of originating saliva sample (with the number representing the number of alleles observed of the possible 20 in the target DNA), N = no profile.

* = peak heights and area were very high with peak areas in excess of 60,000 RFU (i.e., over-amplification due to too much template DNA).

and whether periodically cleaned) were also recorded to determine possible reasons for the results obtained and to explain any variation among them. Mouth swabs from most users/owners of the brushes were collected to check if any of the profiles observed on the sheets or the brush may have been derived from the user. All samples were extracted using the Chelex-Centricon®-QIAquick®-Microcon® process shown to be effective in the inhibition Experiment 3-Test B. All extracted DNA from the sheets of plastic was utilised as template in the PCR amplification. One set of 37 series of samples (Set 1) were tested using standard amplification and typing techniques (28 amplification cycles, 5 sec injection on ABI 310, only peaks above 100 RFU called) while a second set of 36 series (Set 2) was "pushed" beyond what is commonly performed in routine casework (32 amplification cycles and 10 seconds injection on the ABI 310, only peaks above 100 RFU called). (Some sheets were not examined due to resource constraints and thus were not reported.)

Results and Discussion

Experiment 1: Initial Observations

An initial investigation of brushes used in casework or mock casework situations indicated that DNA is present in some used fingerprinting brushes (those used in casework) and in the powder containers that are regularly used. On some occasions DNA could be transferred to brushed surfaces (see Table 1).

Fibreglass brushes treated similarly to squirrel-hair brushes 6, 7, 10 and 11 in Table 1 did not provide any profiles from the two sheets of plastic, brushes or powders. Fibreglass brushes treated similar to brushes 8, 9, 12 and 13, however, provided full profiles from the two sheets of plastic and the brushes but not from the powders. This finding suggests the possibility of differences in transfer of DNA-containing material dependent on the type of brush used. Given the overwhelming use of squirrel-hair brushes in our jurisdiction, further studies concentrated on these. Further research on other types of brushes would be desirable.

It should be noted that the level of deposit has been reported to not only be dependent on the material and area touched but also on the amount of material deposited by the donor of the fingerprint and his/her immediate history (6,8–10).

Experiment 2: Accumulation and Transfer

Further investigation showed that brushes can pickup DNA from recently dried saliva stains and subsequently transfer it to several brushed clean sheets of plastic (see Table 2). Testing of six additional series resulted inthe detection of full, overamplified, AmpF*l*STR® Profiler Plus™ profiles from the brushes (extracts from touched clean sheets of plastic were not recorded for these repeats).

TABLE 3—*Effectiveness of QIAquick® to improve ability to generate AmpFlSTR® Profiler Plus™ profiles from powdered samples (Test B).*

Plastic Sheet	QIAquick® Used	Profile Powdered Saliva	Profile Pos Control	Average Peak Area* (RFU) Pos Control
1	No	P10	P12	10,000
2	No	P10	P12	10,000
3	Yes	F	F	18,100
4	Yes	N	F	19,000
5	Yes	F	F	17,000
6	Yes	F	F	17,900

F = full profile, P = partial profile (with the number representing the number of alleles observed of the possible 20 in the target DNA), N = no profile.

* Note: the standard AmpF*l*STR® positive control of 1 ng for this series of amplifications (i.e., that was not part of any mixtures) gave a full profile with an average peak area of 17,900.

Given the results of this experiment, one may have expected to obtain profiles from samples generated from brushes 8 and 9 used with saliva stains identified in Table 1. The reason for this difference is unclear but may be due to the different DNA extraction processes, the different amount of amplified DNA used in the typing mix, and/or the higher RFU cut-off applied.

Experiment 3: Inhibition

It has previously been reported that fingerprinting techniques (including those using powder) do not hinder the obtaining of DNA profiles from treated samples (6, 11–15). If, however, fingerprinting powder were to remain in the DNA sample after extraction (as has on occasion been noted when performing Chelex extractions followed by using Centricons® and/or Microcons® to concentrate the sample) and inadvertently added to the PCR mix, would it inhibit the amplification?

No profiles could be generated from any of the Test A samples tested. The positive control (the same components but without the addition of any powder) gave the expected full profiles. The results of Test A and Test B (see Table 3) illustrated that the presence of even a small amount of fingerprinting powder in the PCR amplification mix can inhibit amplification. The reason for the inability to generate a genetic profile from one of the six plastic sheets brushed with the contaminated brush is unclear but may be due to lack of transfer of a detectable amount of DNA containing material to this sheet. Test B also illustrated that the use of QIAquick, to remove the powder, can increase the number of alleles observed and their peak areas. To ensure proper PCR amplification, care should be taken

TABLE 4—*Pickup and transfer of alleles by brushes that are detected using standard methods (Set 1).*

Sheet/Brush	No. Tested	No. Pos Result*	Series id† Providing Pos Result* (Number of Alleles > 100 RFU)	Comments
1st	37	1	Z(P3)	The observed alleles did not match the user or examiner
2nd	37	1	M(P3)	The observed alleles did not match the user or examiner
3rd	14‡	5	E(P4), G(P17), I(P8), M(F), U(P9)	The profiles detected match that of the depositer of print, several other alleles from the depositer were present below 100 RFU. No additional alleles were observed. (The 14 sheets included those of the series for which sheets 1 or 2 contained a profile.)
4th	14‡	1	G(P1)	The observed allele matched that of the depositer of print on the 3rd sheet
5th	14‡	0		
Brush	8	0		Quantiblot revealed that sufficient quantities of DNA were retrieved from each of the brushes for typing (16, 3.2, 16, 10, 2.4, 4.8, 3.6, 3.6 ng) yet amplifications of 25% and 2.5% of total extract did not provide a Profiler Plus profile

* Full and partial profiles (even when only one allele is observed) are considered as positive results.
† Each series of five sheets and corresponding brush were given an unique alphabetical identification code (A, B, C, etc.) (different sheets/brushes with the same letter ID are from the same series).
‡ Sheets from the same 14 series were examined and included sheets from series M and Z that provided a profile from sheets 1 or 2.
F = full profile, P = partial profile (with the number representing the number of alleles observed of the possible 20 in the target DNA), N = noprofile.

to remove the powder during the DNA extraction process and/or avoid any powder being added to the amplification mix.

Experiment 4: Operational Brushes

Table 4 shows that there is a very limited pickup and transfer of alleles by brushes when using standard amplification and typing techniques (Set 1). Interestingly, DNA was retrieved directly from the brushes but was unable to be amplified (Table 4).

Significant pickup and transfer of alleles by brushes was, however, detected when "pushing" the system (Set 2). This was illustrated by the following observations (specific data not shown):

- All but one of the first sheets and all of the second sheets contained alleles of unknown origin transferred by the 36 brushes tested.
- When the number of alleles of unknown origin on these sheets was considered in the context of the last time the brushes were used, it is clear that the more recently used brushes transferred more alleles. For example, brushes used less than 24 h prior to the experiment versus those last used >7 days before; $p = 0.0016$ (1st sheets), $p = 0.0011$ (2nd sheets) and brushes last used 1 to 7 days prior to the experiment versus those last used >7 days before; $p = 0.0202$ (1st sheets), $p = 0.0690$ (2nd sheets).
- While there appeared to be fewer alleles transferred from brushes that had at some stage been washed versus those that had not, and between those that had at some stage been brushed over biological samples such as blood, saliva, or fingers versus those that had not, the differences were not statistically significant. It should be noted that most of the brushes that had been washed had not been washed recently. Similarly, many of the brushes that at some stage had been brushed over biological samples had not done so recently. Also, the vast majority of brushes used in these experiments had been in use for over 1 year, with only 6 (16.7%) having been in use for less than 6 months.
- The 2nd sheets tended to provide more alleles than the 1st sheets (the reasons for this are unclear, but are possibly associated with one-off powdering procedure adopted)
- Whereas alleles shared by the user of the brushes were found on the 1st and 2nd sheets to varying degrees (up to a maximum of 79% of a user's profile), no full DNA profiles of the user were found on any of the sheets. Presence of shared alleles may, in some cases, be adventitious (due to their frequency within general population).
- The DNA profile of the handprint depositor on the 3rd sheet was observed in full 20 times, partially 14 times, and not at all twice. A proportion of these alleles was also observed on the 4th and 5th sheets, in descending number. Thus, material from fresh handprints is picked-up by a brush and transferred to other surfaces brushed immediately after.
- A few 3rd, 4th, and 5th sheets had some alleles additional to those of the handprint, that were observed on the associated 1st and 2nd sheets (less on the 5th than the 4th than the 3rd).
- Four of the brushes associated with these series were tested for the presence of DNA (using standard amplification and typing procedures). Like those for Set 1, Quantiblot results for each brush (using 25% of the DNA extract) indicated that there were amplifiable amounts of DNA retrieved. We were however unable to generate DNA profiles, which may be due to either the inability of the extraction process to remove excess inhibitors or degradation of the DNA still present.

Limiting Risk

Consideration should be given to limiting the risk of contamination of a target sample by transfer of foreign DNA, via the use of fingerprint brushes. Some aspects to consider include:

- Use of alternative fingerprinting techniques in which the equipment does not come into contact with the print.
- DNA bearing material deposited onto a surface by touch may be lying loose on that surface (especially on flat, hard surfaces) that could potentially be easily dislodged by a brushing action. Such dislodged cells may be brushed away from the target area

or adhere to the brush, not only leaving less DNA to collect post fingerprinting but also providing a vehicle to transport this material from object to object/scene to scene.

- Other fingerprinting processes exist (including light sources, cyanoacrylate fuming and metal deposition techniques) that may be less destructive of the deposited material. Wickenheiser (8) indicates that the application of a very thin layer of acrylic through the fingerprint fuming process may help seal DNA-containing material, to be removed later through swabbing. Such a process would, however, only be practical in specific situations.
- If available, use of a separate cheap, disposable brush for the powdering of each object to avoid the possibility of transfer.
- Where practical, provide each fingerprinting staff member with a number of sets of good brushes. Each brush is to be used only on one object then placed in a separate container for cleaning (and sterilisation). Alternatively, establish a clear procedure that dictates when and how brushes are to be cleaned and how this is to be monitored.
- Rather than repeatedly dipping a brush into a single container for an extended period, consider preparing and using separate small aliquots of powder for each object.
- Avoid contact of the brushes with biological samples such as blood, saliva, semen, and skin where possible. Alternative printing methods are likely to be in routine use for prints in blood but possibly not for prints in or near saliva. Use of alternative printing methods should also be considered in such situations. If alternative printing methods can not be used, do not use the brush again, clean it in a manner that rids it of any human DNA (and test that this has occurred) prior to reuse, or consider using cheaper disposable brushes for these situations. Also, refrain from powdering hands to obtain their prints or use a dedicated brush to do this.
- Where possible, avoid areas distinct from the print area that may be swabbed for the collection of DNA (e.g., the rim of a cup is more likely to contain saliva/contact DNA than the rest of the cup that may contain fingerprints).
- Apply an alternative sequence of collection in specific situations (e.g., swab the rim of the cup prior to powdering the remainder of the cup).
- Assess the likely ability to collect fingerprints versus DNA from a specific object and their relative evidentiary worth. There may be situations where it would be more appropriate to attempt to collect a sample for DNA extraction rather than attempt to acquire a fingerprint.
- The potential transfer of prints may be related to the freshness of the print and the time between brushing a fresh print and a subsequent second surface. In casework, prints are unlikely to be as fresh as those used in the above experiments with the operational brushes.
- Moist areas are likely to facilitate transfer (10) so they should be avoided. Use of alternative printing methods should be considered in such situations if not already in use.
- Sticky and rough surfaces may more readily attract material from the brush than non-sticky hard flat surfaces. Thus subsequent swabbing of such areas for DNA may be more prone to being contaminated by DNA from the brush (7,10). Use of alternative printing methods should be considered in such situations if not already in use.
- If a brush were to add DNA-containing material to a surface containing a handprint, the proportion of the added DNA is likely to be less than that retrieved from the depositor of the print. If the target print contains a reasonable amount of DNA, the minor component of the mixture derived from the brush may not be detectable.
- Consider the possibility of contamination by brushes when analysing DNA profile results obtained from brushed samples.

Conclusion

The results of the tests described above demonstrate that fingerprint brushes can accumulate DNA from surfaces they come into contact with and that they can also redeposit DNA-containing material to a number of subsequently brushed objects. The chance of this occurring is increased after powdering biological samples, such as blood, saliva, skin, or fresh prints. Use of alternative printing techniques and/or procedures should be considered in such situations. While there is little risk of transferring detectable quantities of DNA from and to most surfaces under examination via fingerprinting brushes, the possibility of detecting transferred DNA will increase as more sensitive DNA typing methods are utilised. It should be noted that the detection of the transferred material would also be influenced by the presence of the target DNA in the amplification. Any transferred material in many cases will only form a small proportion of the total DNA and may remain undetected.

Further assessment of the risk of transfer is required, as is consideration of alternative methods and procedures associated with exposing and collecting fingerprints from surfaces that may also be subjected to sampling for DNA.

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