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DNA transfer by examination tools – a risk for forensic casework?

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ABSTRACT

The introduction of profiling systems with increased sensitivity has led to a concurrent increase in the risk of detecting contaminating DNA in forensic casework. To evaluate the contamination risk of tools used during exhibit examination we have assessed the occurrence and level of DNA transferred between mock casework exhibits, comprised of cotton or glass substrates, and high-risk vectors (scissors, forceps, and gloves). The subsequent impact of such transfer in the profiling of a target sample was also investigated. Dried blood or touch DNA, deposited on the primary substrate, was transferred via the vector to the secondary substrate, which was either DNA-free or contained a target sample (dried blood or touch DNA). Pairwise combinations of both heavy and light contact were applied by each vector in order to simulate various levels of contamination.

The transfer of dried blood to DNA-free cotton was observed for all vectors and transfer scenarios, with transfer substantially lower when glass was the substrate. Overall touch DNA transferred less efficiently, with significantly lower transfer rates than blood when transferred to DNA-free cotton; the greatest transfer of touch DNA occurred between cotton and glass substrates. In the presence of a target sample, the detectability of transferred DNA decreased due to the presence of background DNA. Transfer had no impact on the detectability of the target profile, however, in casework scenarios where the suspect profiles are not known, profile interpretation becomes complicated by the addition of contaminating alleles and the probative value of the evidence may be affected. The results of this study reiterate the need for examiners to adhere to stringent laboratory cleaning protocols, particularly in the interest of contamination minimisation, and to reduce the handling of items to prevent intra-item transfer.

1. Introduction

The presence, accumulation and transfer of extraneous DNA within a forensic laboratory is a fundamental issue as contaminating DNA can be detrimental to profile analysis and subsequent investigative outcomes. Although the existence of background DNA in a laboratory setting is deemed to be low [1,2], its mere presence provides the opportunity for DNA transfer and the potential to contaminate evidentiary samples. Furthermore, the detection of previously undetected trace elements of DNA has increased due to the recent introduction of more sensitive DNA typing systems [3]. In such instances, the level of DNA in the targeted sample influences the detectability of any contaminating DNA, with contamination more likely to be detected in cases where the target sample is present in only trace quantities.

Whilst some understanding of key variables affecting transfer has been acquired, especially in relation to biological sample type, sample moisture, substrate type and manner of contact [4–7], it is clear that a greater understanding is required to assist evaluations of potential scenarios where transfer plays a role [8]. Laboratory-based simulations reconstructing crime-scene scenarios are useful in the assessment of DNA transfer and the probability of attaining positive DNA profiles in such instances [9], however very little has been done to investigate transfer within the laboratory and its potential to contaminate evidentiary samples.

Contamination within the laboratory may take place as a result of unprotected speaking [10], through the presence of DNA on unused laboratory gloves [11], and ineffective cleaning procedures [3,12,13]. A recent preliminary study observed the transfer of dried blood from and to cotton via tools used during examination

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(scissors, forceps and gloves), and indicated that if these instruments are not adequately cleaned, they pose a high contamination risk [14]. Here we extend this study by repeating preliminary tests (transfer of dried blood to DNA-free secondary substrates) to improve the assessment of the level of risk, and by performing additional tests that explore the transfer of touch DNA, a greater variety of heavy and light transfer scenarios, an additional substrate type (hard/non-porous glass), and transfer to non-DNA free secondary substrates, deposited with blood or touch DNA, and the effect this has on the profiling of a target sample.

2. Materials and methods

2.1. Deposition of blood

Venous blood (25 μL) from Person A (male) or Person B (female) was deposited on cotton drill (Lincraft, Australia), as per Szkuta et al. [14]. An additional 25 μL of blood from Person A was deposited onto 1.5 × 1.5 cm areas of glass slides (7.6 × 2.6 cm, MENZEL, Germany). Blood deposits were air-dried at room temperature (18–24 h). The same donors were used throughout the course of the experiments. Donors were not analysts within the laboratory where the experiments were carried out.

2.2. Deposition of touch DNA

2–12 h prior to transfer experiments, male (Person A) or female (Person B) subjects were asked to (a) vigorously rub unwashed hands over the surface of an A4 piece of cotton drill, adhered to a clean plastic transparency and further adhered to a bench, or (b) press with medium pressure the surface of glass slides, adhered side-by-side to a plastic transparency, multiple times in different directions. Contacts were for 30 s. Following initial contact with the cotton drill, the process was repeated on the alternate side before 1.5 × 1.5 cm cotton swatches were excised with a scalpel and placed in plastic zip-lock bags. For consistency, only designated 1.5 × 1.5 cm areas on the exposed surface of glass slides were used in transfer experiments. Donors varied across experiments, however, those selected were not analysts within the laboratory where the experiments were carried out.

2.3. High-risk vectors

Twenty uniform pairs of metal scissors were sourced for use in experimentation, along with disposable forceps (Multigate, Australia) and nitrile, powder-free gloves (NI-TEK, Australia). Contact with the cotton swatches occurred as previously described [14]. As gloved hands tend to come into contact with both hard and soft surfaces, whilst scissors and forceps are mainly used on light-weight, fabric-based exhibits, experiments concerning transfer from and to glass slides were investigated via gloves only. Only the exposed surface of glass slides were sampled in the present study as DNA sampling generally occurs on one side of exhibits composed of hard surfaces; slides were contacted with medium pressure by a gloved thumb (distal segment), with a single contact lasting 2–3 s. This is in contrast to cotton, where the entire swatch was included for analysis; any DNA deposited by contact with both sides of the swatch was extracted and formed part of the generated profile.

2.4. Experimental design

Within each set of transfer experiments (Fig. 1), the primary substrate (PS) contained a biological sample (primary deposit) and transfer occurred to the secondary substrate (SS). As previously described in Szkuta et al. [14], a single cut through, or touch of, the PS represented light contamination of the vector, and multiple (8) cuts or touches, represented heavy contamination of the vector. Immediately following interaction with the PS, the secondary substrate (SS) was similarly cut (or touched) once or multiple (8) times by the vector, corresponding to light or heavy contact respectively. Pairwise combinations of heavy and light transfer were explored including heavy/heavy, heavy/light, light/heavy and light/light. Four replicates were performed for each individual transfer scenario.

In experiment 1, to assess the levels of DNA potentially transferred, the SS was DNA-free in initial transfer events (Fig. 1, Set 1a–d). Further testing exploring the effect on the profiling of a target sample was performed in experiment 2, by depositing blood or touch DNA (regarded as the target sample) on the SS which was similarly subjected to the transfer scenarios previously outlined (Fig. 1, Set 2a–d). In all instances, blood or touch DNA from Person A (male) were deposited on the PS and from Person B (female) on the SS. Transfers involving blood deposited on cotton (PS) to blood on cotton (SS) were only performed via scissors under heavy/heavy conditions ($n = 3$) as it was predicted that the quantity of DNA within the target sample would overwhelm the detection of any transferred DNA (Fig. 1, Set 2a). Each set of tests in Fig. 1 were performed on separate occasions in order to keep high-level and low-level DNA samples separate.

2.5. Quality control

Scissors and disposable forceps were cleaned prior to use as per laboratory protocols; immersion in 1% hypochlorite followed by cleaning with 70% ethanol. Gloves were assumed to be DNA-free. While scissors were cleaned (1% hypochlorite and 70% ethanol) and reused between each set of tests, forceps and gloves were single use. Additional layers of gloves were worn to avoid skin exposure and potential contamination during changing of gloves. Following cleaning and prior to use in each set of transfer tests, negative control swabs were taken from the vector applied; scissors ($n = 17$), forceps ($n = 8$) and gloves ($n = 44$).

Cotton substrate (1.5 × 1.5 cm swatches or A4 pieces) and glass slides were exposed to UV-light (>1 h, both sides) to degrade any DNA present prior to use in experimentation. Cotton swatches and swabs of glass slides were further processed as negative controls in each set of tests; 4 replicates per vector were performed in each set of transfer tests (Fig. 1, Set 1a–d and 2a–d).

Cotton swatches and swabs of glass slides deposited with dried blood (4 replicates) or touch DNA (6 replicates) from Person A or B were processed as positive controls for each set of tests to determine the presence and amount of DNA.

2.6. Sample processing

Cotton SS was placed directly into extraction tubes for processing. The wet–dry swabbing technique (150C swabs, Copan, USA) was used to collect samples from glass slides. Individual swabs were packaged within envelopes and dried at room temperature for up to 12 h prior to extraction. DNA was extracted using an automated DNA IQ™ (Promega, USA) extraction system as per manufacturer's instructions, to a final volume of 50 μL. Samples were quantified with Quantifiler® (Life Technologies, USA) on an ABIPRISM®7500 (Life Technologies, USA) using Sequence Detection System (SDS) software (Life Technologies, USA). Amplification and profiling was carried out using the PowerPlex® 21 System (Promega, USA) as per manufacturer's instructions, where a full profile is identified by the presence of 42 alleles. Amplified product detection and sizing was performed with an ABIPRISM® 3500xL Genetic Analyser (Life Technologies USA). For the purpose of amplification, 0.5 ng of template DNA was used or, in instances where the concentration of samples fell below

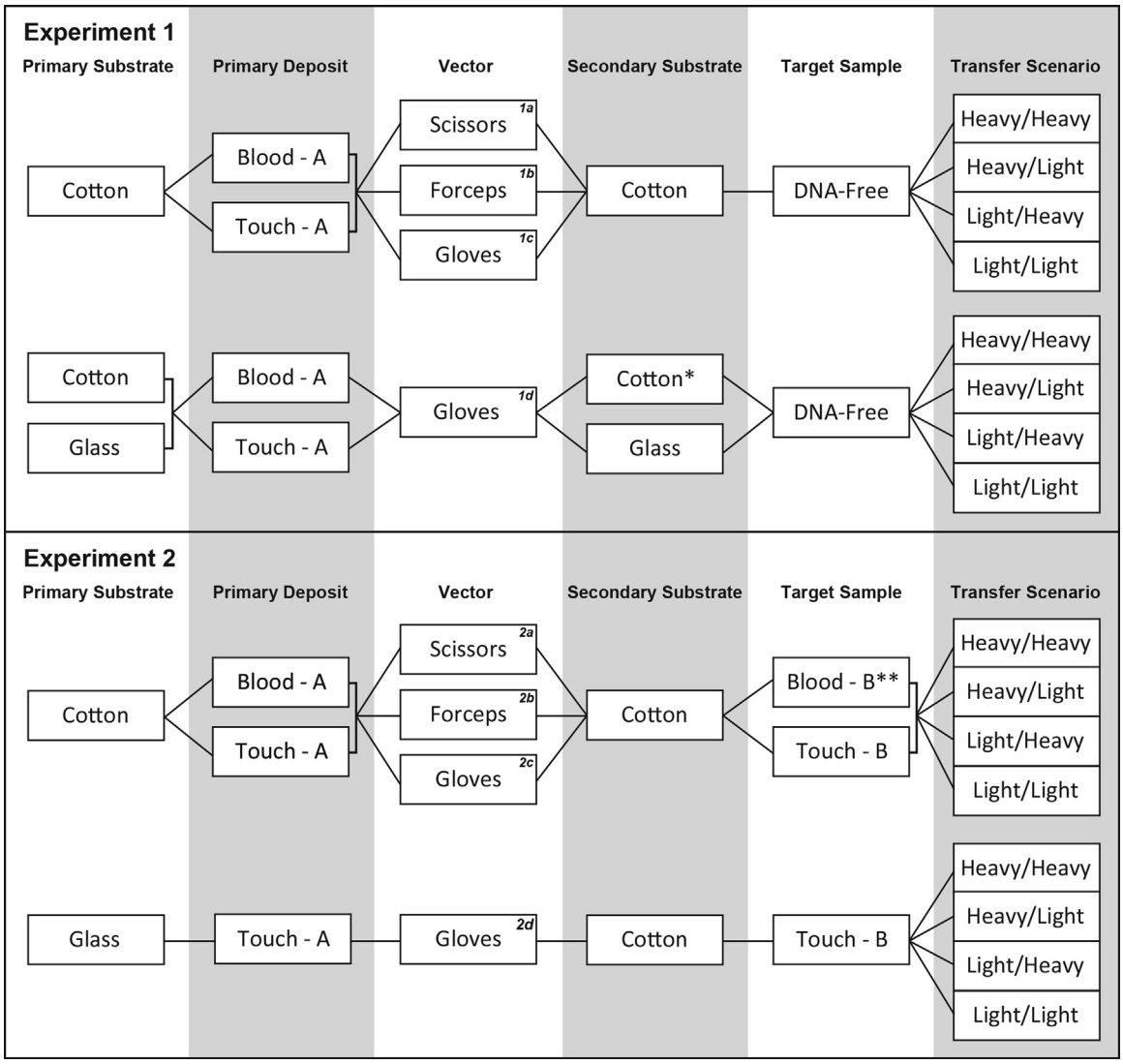

Fig. 1. Schematic diagram of transfer experiments. As indicated in the vector column, transfer experiments were performed in sets of tests (1a–d and 2a–d). A = Person A (male); B = Person B (female); *Results from cotton used as both primary and secondary substrates were taken from Set 1c. **Transfer of blood (primary deposit) to blood (target sample) performed via scissors under heavy/heavy conditions only.

0.033 ng/μL, 15 μL of sample was used. Automated processing was performed robotically on Biomek® NX^P automated liquid handling platforms (Beckman Coulter, USA).

2.7. Data analysis

Due to the PowerPlex® 21 amplification kit having a greater sensitivity than Quantifier®, a number of samples within this study produced low (≤0.010 ng/μL) quantification results and provided a full profile. Supplementary Table 1 provides a comparison of Quantifier® and PowerPlex® 21 results. Due to unknown DNA quantities being used during amplification, a definite amount of DNA could not accurately be calculated for samples. As such, the percentage of alleles transferred (%) was used as a comparison of experimental variables affecting transfer.

GeneMapper ID-X software (Life Technologies, USA) was used for genotyping with a baseline threshold of 175 RFU. Single source profiles (experiment 1) were compared to individual reference profiles from donors and the percentage of observed alleles transferred, from a total of 42 transferable alleles (Person A), was determined along with a total of their corresponding peak heights (RFU).

In the analysis of multiple source profiles (experiment 2), alleles shared by the two donors were omitted, as the donors' relative contribution could not be accurately determined. By omitting shared alleles from contributor profiles, the potential allele contribution for each donor was reduced from 42 (Supplementary Table 2). The remaining alleles provided a unique profile for each contributor. The total number of transferable alleles (Person A) is indicated in the figure descriptions for each set of tests. Using these unique profiles, the percentage of observed unique alleles within sample profiles was determined for both Person A and B, along with an average of their corresponding peak heights (RFU), enabling the major and minor contributors to be observed. The total peak heights obtained in single source profiles (DNA-free SS) were also converted to average peak height for comparative purposes.

An average of the four replicates performed for each transfer scenario was used in statistical analysis. Replicate profiles displaying zero alleles and indicating no detectable transfer, or profiles affected by methodological malfunctions, were omitted from analyses.

Statistical analyses were performed in SPSS v17.0 (SPSS Inc., Chicago). To examine the relationship between the various

experimental factors, allele transfer (%) and corresponding peak heights (RFU), linear mixed model analysis was performed, as substantial correlations between the variables (body fluid, substrates, vectors and levels of contact) were found, and missing data prevented the use of a standard repeated measures approach. Model suitability was evaluated with Akaike's Information Criterion. Fixed effects were evaluated with *F*-statistics, and covariance parameters with the Wald statistic. Pairwise comparisons of factor levels were performed on estimated marginal means to account for covariance with related factors, with Bonferroni adjustment performed. Results from the transfer of dried blood on cotton (PS) to blood on cotton (SS) via scissors (Fig. 1, Set 2a) were omitted from statistical comparisons as not all transfer scenarios were explored.

3. Results

3.1. Transfer of primary deposit from cotton to DNA-free cotton (experiment 1)

In general, transfer was significantly higher for dried blood compared to touch DNA ($P < 0.001$), with transfer observed for all vectors in all heavy and light transfer scenarios explored. Gloves facilitated the greatest amount of transfer (87–99%), with little impact of contact level on transfer amounts. In contrast, transfer was low for forceps in all scenarios, and particularly when contact was light (Fig. 2).

On average the transfer of touch DNA was minimal with 53% fewer alleles transferred than dried blood. The greatest transfer of touch DNA was observed via forceps, with 76% of alleles transferred under heavy conditions (Fig. 2). However, this is considered an outlier as it is the result of a single replicate, with the other replicates and transfer scenarios displaying no detectable transfer. The quantity of touch DNA in the positive control samples associated with forceps tests were lower than expected (Supplementary Table 3) and may have resulted in the low levels of transfer observed. Similarly to dried blood, the transfer of touch DNA was consistently higher via gloves with little variation between transfer scenarios (10–18%, Fig. 2).

Overall the various heavy and light transfer scenarios applied were found to have a significant effect on the transfer of alleles ($P = 0.006$) and the total peak heights of alleles transferred ($P = 0.044$, Supplementary Table 4). Pairwise comparisons of allele transfer revealed that transfer was significantly higher for heavy/heavy combinations compared to light/heavy ($P = 0.026$) and light/light ($P = 0.007$). This was not unexpected given the variation in contact between heavy and light contamination, i.e., eight touches of the PS compared to one touch. These trends were not observed in the corresponding analysis of total peak heights, but this may be skewed by the transfer of touch DNA between glass substrates; the total peak heights were greater when light/heavy and light/light combinations were applied, compared to heavy/heavy (Supplementary Table 4).

3.2. Transfer of primary deposit from cotton to target sample on cotton (experiment 2)

As indicated, transfers involving blood deposited on primary cotton to blood on secondary cotton were only performed via scissors under heavy/heavy conditions ($n = 3$). The results were as expected, with the target sample (Person B) overwhelming any DNA of Person A, resulting in single source profiles from Person B (data not shown).

The transfer of dried blood deposited on cotton (Person A) to cotton containing touch DNA (Person B) was observed for all vectors and transfer scenarios (Fig. 3), with the greatest and most consistent transfer occurring via gloves (78–94%, Fig. 3E). In contrast, the transfer of touch DNA was considerably less (Fig. 4). While the transfer of touch DNA via forceps and gloves was observed in all scenarios, only a small number of unique alleles were detected on the SS following transfer via scissors; 7% in heavy/light and light/light scenarios and none in the other scenarios (Fig. 4A). Gloves consistently transferred a greater amount (13–26%) (Fig. 4E).

There were significantly fewer unique alleles detected (−6%, $P = 0.008$) and the corresponding average observed peak heights were lower (−112 RFU, $P = 0.046$) when the target sample (Person B) was present on the SS compared to DNA-free, regardless of the biological material (blood or touch) being transferred. Person B was the major component in the all of the profiles obtained and Person A the minor component, or absent. Statistical analysis revealed the various transfer scenarios had no significant effect on the percentage of alleles detected for Person B, or the corresponding peak heights (all $P > 0.05$).

3.3. Influence of substrate on the transfer of biological material via gloves (experiments 1 and 2)

Decreased transfer of dried blood occurred via gloves when glass was used as either the primary or secondary substrate (0–19%, Table 1), compared to cotton (87–99%, Table 1 and Fig. 2). In contrast, the greatest transfer of touch DNA occurred between

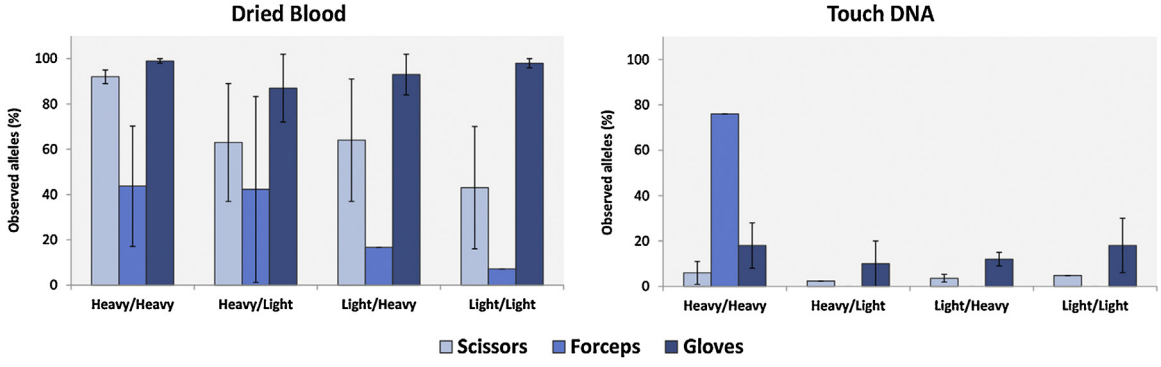

Fig. 2. Observed allele transfer (%), from a total of 42 transferable alleles (Person A), via scissors, forceps and gloves in experiment 1 (Fig. 1, Set 1a–c); transfer of dried blood and touch DNA deposited on cotton, to DNA-free cotton, through the application of various heavy and light transfer scenarios. Error bars represent the 95% standard deviation between replicate analyses. Dried blood $n = 42$ (of 48); touch DNA $n = 23$ (of 48); remaining samples did not display detectable transfer. Additional data for other substrate combinations is presented in Table 1.

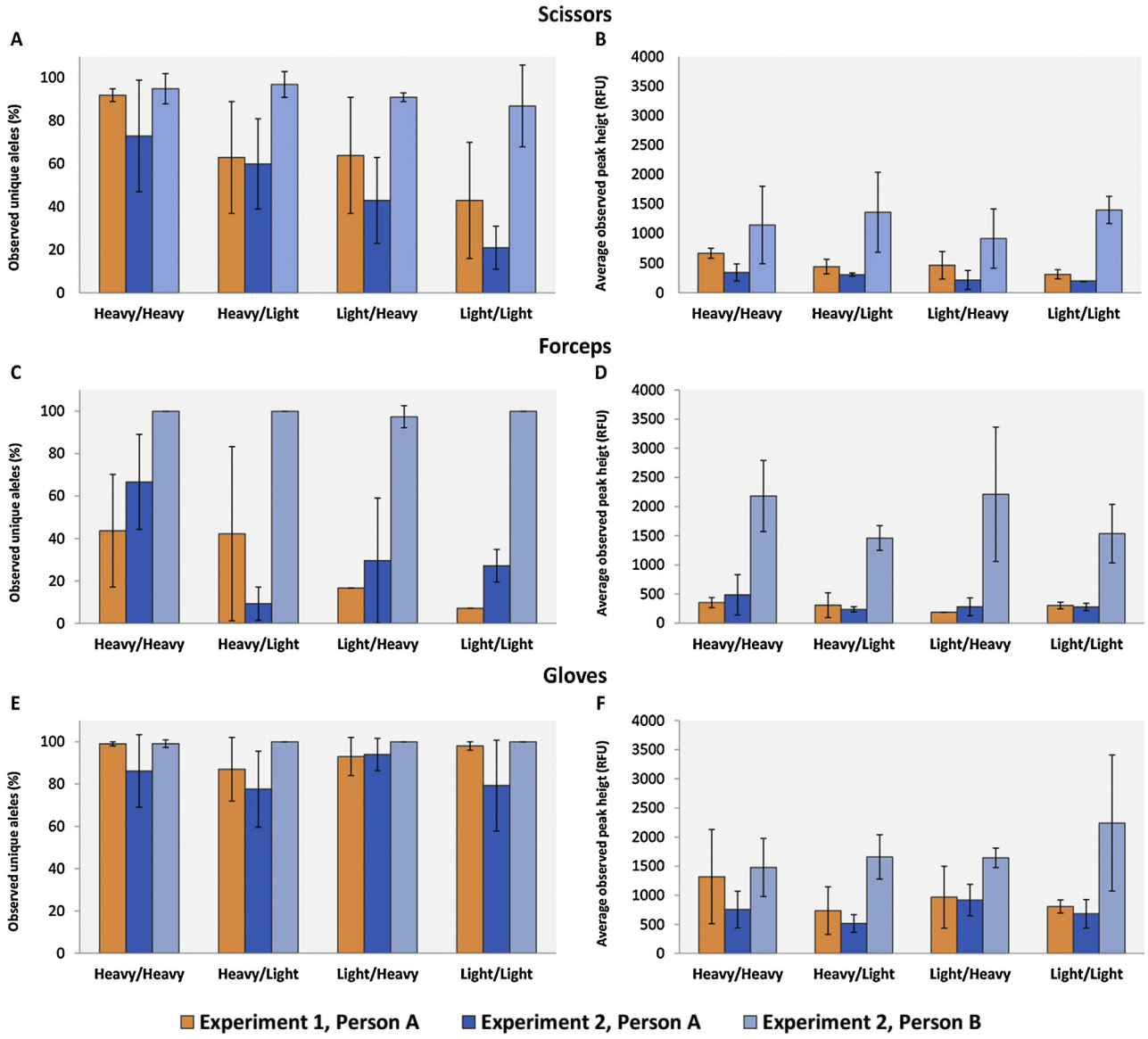

Fig. 3. Observed unique alleles (%) and an average of their corresponding peak heights (RFU), in DNA profiles generated from the transfer of dried blood (Person A) deposited on primary cotton substrate, to a secondary cotton substrate deposited with touch DNA (Person B), through the application of various heavy and light transfer scenarios via scissors, forceps and gloves in experiment 2 (Fig. 1, Set 2a–c respectively); total number of unique transferable alleles (Person A) scissors = 15, forceps = 27 and gloves = 29. Results from the transfer of dried blood (cotton) to DNA-free cotton in experiment 1 are included for comparative purposes. Error bars represent the 95% standard deviation between replicate analyses. Scissors $n = 11$ (of 16); forceps $n = 12$ (of 16); gloves $n = 16$ (of 16); remaining samples did not display detectable transfer.

primary glass and secondary cotton substrates (82–99%, Table 1). Other combinations of cotton and glass substrates revealed considerably lower transfer rates (0–25%, Table 1 and Fig. 2).

Overall, transfer rates were considerably higher for touch DNA than dried blood ($P = 0.031$), but this was highly skewed by the high transfer of touch DNA between glass and cotton substrates ($P < 0.001$). Touch DNA resulted in a 1% increase in allele transfer compared to blood in cotton to glass transfers; a 2% increase in glass to cotton transfers; and an 82% increase in glass to glass transfers. In contrast, blood showed 75% higher allele transfer compared to touch DNA in cotton to cotton situations. These patterns are consistent with the corresponding allele peak heights ($P < 0.001$, Supplementary Table 4). This indicates that the transfer of dried blood occurs more readily from porous cotton, in comparison to touch DNA where transfer rates are greater from non-porous glass. In addition, cotton acquires more DNA from both dried blood and touch DNA compared to glass.

With the greatest transfer of touch DNA observed between glass and cotton substrates, the equivalent substrate combination was explored in experiment 2 with the added presence of a target sample (touch DNA, Person B) on the SS (Fig. 1, Set 2d). This resulted in the reduced detectability of Person A when the target sample was present compared to DNA-free (Fig. 5). The detectability of the target sample (Person B) was not affected (99–100%, Fig. 5).

3.4. Controls

No DNA was detected in the profiles obtained from scissor negative control samples ($n = 17$), a single pair of control forceps ($n = 8$) displayed 1 allele (187 RFU) and 1–4 alleles (178–559 RFU) were detected in seven profiles obtained from unused gloves ($n = 44$). Within the negative substrate controls, a single cotton control profile ($n = 20$) displayed 1 allele (175 RFU), whilst three

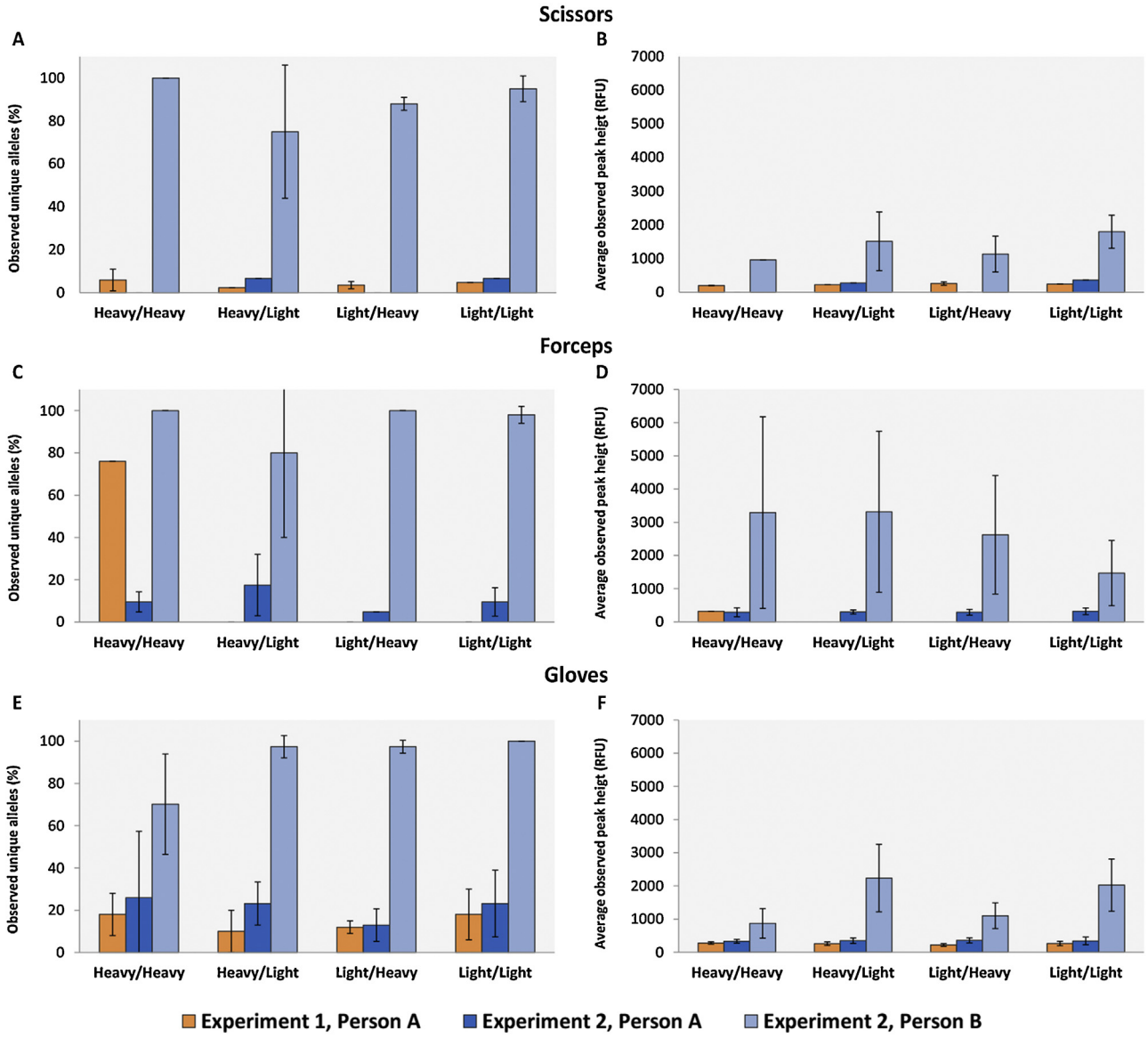

Fig. 4. Observed unique alleles (%) and an average of their corresponding peak heights (RFU), in DNA profiles generated from the transfer of touch DNA (Person A) deposited on primary cotton substrate, to a secondary cotton substrate deposited with touch DNA (Person B), through the application of various heavy and light transfer scenarios via scissors, forceps and gloves in experiment 2 (Fig. 1, Set 2a–c respectively); total number of unique transferable alleles (Person A) for scissors = 15, forceps = 21 and gloves = 27. Results from the transfer of touch DNA (cotton) to DNA-free cotton in experiment 1 are included for comparative purposes. Error bars represent the 95% standard deviation between replicate analyses. Scissors $n = 2$ (of 16); forceps $n = 10$ (of 16); gloves $n = 14$ (of 16); remaining samples did not display detectable transfer.

profiles obtained from glass slides ($n = 12$) displayed one allele each (209–333 RFU). Due to robotic error, no results were obtained from four cotton swatches ($n = 20$) processed in the forceps experiments (Fig. 1, Set 1b and 2b). None of the observed alleles in control profiles matched the contributors in transfer experiments.

Positive control samples obtained from dried blood deposited on cotton provided full profiles in all instances (Supplementary Table 3). Slightly fewer alleles (93%, Supplementary Table 3) were observed in samples obtained from blood deposited on glass slides. As expected, substantial variation was observed between the various replicates of touch deposits, on both cotton and glass (35–100%, Supplementary Table 3).

Non-donor alleles from unknown sources were observed in some of the positive control profiles (Supplementary Table 3). The non-donor alleles deposited via touch were expected as donors did not wash their hands. However, the additional alleles of low RFU present in blood deposits were unexpected. Their presence suggest either non DNA-free substrates, although substrate negative controls indicate these are DNA-free, or post-deposit contamination, although DNA contamination minimization procedures were followed. The investigator was excluded based on profile comparison. In some instances unknown alleles detected in positive control profiles obtained from Person B matched alleles from Person A. These alleles were omitted from the analysis of sample profiles in experiment 2 (Supplementary Table 2). The relevant number of unique alleles is indicated within figure descriptions.

4. Discussion

4.1. Transfer of primary deposit from cotton to DNA-free cotton (experiment 1)

Experimental findings from the transfer of dried blood via scissors, forceps and gloves are concordant with the results of

Table 1
Observed allele transfer (%), from a total of 42 transferable alleles (Person A), via gloves in experiment 1 (Fig. 1, Set 1a–d); transfer of dried blood and touch DNA deposited on cotton or glass, to DNA-free cotton or glass, through the application of various heavy and light transfer scenarios. Dried blood $n = 51$ (of 95); Touch DNA $n = 54$ (of 96); remaining samples did not display detectable transfer.

Substrate combination		Contact type (transfer scenarios)		Observed alleles % (s.d.)	
PS	SS	PS	SS	Dried Blood	Touch DNA
Cotton	Cotton*	Heavy	Heavy	99 (1)	18 (10)
		Heavy	Light	87 (15)	10 (10)
		Light	Heavy	93 (9)	12 (3)
		Light	Light	98 (2)	18 (12)
Cotton	Glass	Heavy	Heavy	0 (0)	11 (0)
		Heavy	Light	7 (0)	0 (0)
		Light	Heavy	0 (0)	6 (0)
		Light	Light	10 (0)	0 (0)
Glass	Cotton	Heavy	Heavy	5 (0)	89 (9)
		Heavy	Light	0 (0)	82 (19)
		Light	Heavy	7 (0)	93 (9)
		Light	Light	8 (2)	99 (1)
Glass	Glass	Heavy	Heavy	0 (0)	15 (12)
		Heavy	Light	19 (0)	10 (5)
		Light	Heavy	0 (0)	25 (18)
		Light	Light	2 (0)	13 (3)

PS, primary substrate; SS, secondary substrate; s.d., standard deviation.
* Data for this substrate combination is also presented in Fig. 2.

Szkuta et al. [14], although direct comparisons can only be made regarding heavy/heavy and light/light transfer scenarios. Whilst the previous study demonstrated the greatest transfer of dried blood via scissors, in the current study, gloves facilitated the greatest transfer with an average of 21% more alleles compared to scissors and 42% more than forceps. Variation in the characteristics of the dried blood deposit and the substrate (hardening of the cotton or the production of lint) may be a potential cause for the differing results.

When cotton was applied as the primary and secondary substrates, the transfer of dried blood was substantially higher than touch DNA, as predicted from the higher DNA content of blood. Furthermore, experiments conducted with blood used fixed amounts (25 μL) of deposits whilst the quantity of touch DNA varied due to factors including the amount of time since subjects last washed their hands and their individual shedder status [15]. The variation observed between replicates can be attributed to (i) contact with the substrate occurring with different parts of the hand, (ii) the length of contact time, and (iii) the manner of contact (passive, pressure or friction) [5].

Gloves were a highly efficient transfer vector, with higher rates from both blood and touch DNA across all transfer scenarios. The flexible nature of the glove surface may be part of the reason for the greater transfer via gloves, with the contours of the fingertip enabling the substrate to slightly wrap around the fibers, making it capable of contacting a larger surface area. A non-flexible surface is not able to do this, contacting only the tips of the fibers. Furthermore, the dimensions of the gloved thumb and index finger are similar to the cotton swatches (1.5 × 1.5 cm), facilitating greater transfer. In contrast, the comparative size of the scissor blades and forceps tips meant that only a fraction of the vector made contact with the cotton substrate (on both sides).

4.2. Transfer of primary deposit from cotton to target sample on cotton (experiment 2)

Similar transfer patterns were exhibited when transfer occurred to DNA-free cotton (experiment 1) and to cotton containing a target sample (experiment 2), with dried blood transferring a higher percentage of alleles compared to touch DNA,

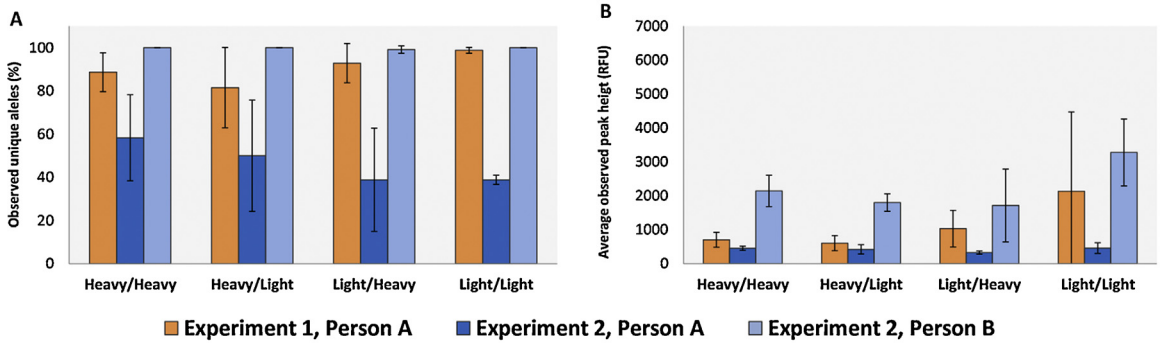

Fig. 5. Observed unique alleles (%) and an average of their corresponding peak heights (RFU), detected in the DNA profiles generated from the transfer of touch DNA (Person A) deposited on primary glass substrate, to a secondary cotton substrate deposited with touch DNA (Person B), through the application of various heavy and light transfer scenarios via gloves in experiment 2 (Fig. 1, Set 2d); total number of unique transferable alleles (Person A) = 27. Results from the transfer of touch DNA (glass) to DNA-free cotton in experiment 1 are included for comparative purposes. Error bars representing the 95% standard deviation between replicate analyses. 4 replicates of each transfer scenario were included in the analyses of experiment 2 data ($n = 16$).

increased transfer from heavy contact compared to light for scissors and forceps, and little variation in percentage transfer between heavy and light contacts with gloves.

In considering the detectability of Person A representing the contaminant sample, allele transfer and the corresponding average peak heights were marginally lower in the presence of a target sample compared to DNA-free (−6% and −112 RFU, respectively). Whilst the detectability of transferred DNA decreased slightly, the presence of any contaminating alleles within these profiles is still of concern. The target sample (Person B) was consistently the major component within the profiles obtained. As such, it can be inferred that the level of contamination and subsequent contact by the vectors had no impact on the target sample (Person B). However, in casework scenarios where the suspect profiles are not known, profile interpretation becomes complicated by the addition of contaminating alleles and the probative value of the evidence may be affected.

Results from this study indicate that inter-exhibit transfer, i.e., the transfer of DNA between exhibits via intermediary vectors, is a possibility. However, if laboratory cleaning protocols are followed the potential for inter-exhibit transfer/contamination is minimised. Despite this, there is considerable potential for intra-exhibit DNA transfer – the movement of DNA from one part of an exhibit to another. Such transfer can be detrimental to the quality of profiles, especially when transfer occurs to an area further sampled for trace DNA, as it can lead to the creation of misleading DNA mixtures and potentially alter the interpretation of the DNA profile/evidence. Furthermore, the possible occurrence of intra-exhibit transfer is greater with touch DNA and trace samples as they are less visible, thus increasing the probability of accidental contact during examination.

4.3. Influence of substrate on the transfer of biological material via gloves (experiments 1 and 2)

Unlike previous studies on the impact of substrates and DNA transfer [4,5,7] which analysed quantities of DNA to determine transfer rates, it was hypothesised and later confirmed that the majority of samples in the current study would fall below the amount accurately detectable using Quantifiler®. As yield could not be determined, this study used percentage allele transfer determined from profiles generated using the PowerPlex® 21 system. In addition, the use of examination tools as transfer vectors, rather than direct substrate-to-substrate contact [4,5] means that past and present studies are not absolutely comparable, although some comparisons can be made on the influence of substrate in the transfer of biological material via gloves due to subtle similarities; the relative contact area on the substrate by the vector and the surface composition of the glove (rubber).

A substantially greater transfer of alleles (%) was observed with porous cotton as the primary and secondary substrate in the transfer of dried blood, contrasting the findings of Goray et al. [4] who found non-porous plastic as the primary substrate facilitating greater transfer. It is possible that the flexible plastic resulted in increased flaking compared to the rigid glass substrate [7], although flaking was not reported by Goray et al. [4]. Within this study, flaking of blood was not observed on glass with the sample remaining adhered to the surface, which may explain the reduction in transfer when comparing the two studies.

The composition of the vector (gloves) is another factor to consider when assessing transfer. In this case, the greatest transfer of dried blood was observed by applying pressure contact between porous cotton and non-porous rubber (glove), followed by transfer between non-porous rubber (glove) and porous cotton.

The transfer of touch DNA was highest with non-porous glass as the primary substrate followed by porous cotton as the secondary substrate. These results are concordant with the findings of Goray et al. [5] where the combination of non-absorbent primary and absorbent secondary gave the greatest transfer of touch DNA. However, when taking the vector into consideration, the greatest transfer of touch DNA was observed between non-porous glass and non-porous rubber (glove), followed by transfer between non-porous rubber (glove) and porous cotton.

Examination of the positive control samples obtained from the glass substrate (Supplementary Table 3) revealed a substantial decrease in the percentage of alleles retrieved compared to cotton for both dried blood (7% loss) and touch DNA (28–33% loss). Whilst it has previously been shown that the amount of DNA retrieved from touch deposits on cotton is higher than that from glass [16], the decrease in alleles retrieved from the large volume of blood deposited on glass in this experiment (25 μL) is surprising. This indicates that DNA loss is possibly occurring during sample collection [17] and/or the DNA extraction process [7]. DNA-containing material could also be transferred, and therefore lost, during excision of the swab tip via scalpel (onto the sterile surface of the scalpel packaging). Future investigations exploring transfer between exhibits and surfaces during casework examinations would be beneficial in assessing how much DNA is lost from an exhibit during examinations.

A concerning finding of this study was the detection of quantifiable DNA, and the ability to recover profiles, from the swabbing of unused laboratory gloves. This is not an uncommon occurrence as gloves have previously been shown to contain trace levels of human DNA [11]. This not only highlights the risk of manufacturer-based contamination but reiterates the need for DNA free gloves and swabs. To help remove any DNA that is potentially on the gloves one could consider cleaning the outer surface after putting them on just prior to use. Such a cleaning agent may be an appropriate concentration of hypochlorite [18]. However, one would need to perform tests to be assured that there are no negative effects due to potential transfer of residual hypochlorite to the exhibit being examined.

5. Conclusion

Results from this study demonstrate that DNA-containing material can be transferred from exhibit to exhibit by scissors, forceps and gloves. It is clear they pose a significant contamination risk if not DNA-free before contact is made with the targeted sample during exhibit examination, although the contamination risk of this source is reduced/eliminated if the deposit area is not touched. The reuse of instruments and further contact with other areas of an exhibit, could potentially relocate DNA, which could negatively affect the interpretation of relevant activities in some instances.

With the introduction of more sensitive DNA typing systems such as PowerPlex 21® the detection of contaminating DNA has become more noticeable. The following recommendations should be considered by laboratories to limit the risk of contamination by DNA transfer during examination via scissors, forceps and gloves.

- Encourage awareness amongst staff of the potential sources of contamination within the laboratory and during examination.
- Use of DNA-free scissors, forceps and gloves.
- Preferably use disposable forceps and scissors. When this is impractical, ensure that they have been cleaned prior to reuse using a validated method.
- Clean gloves with an appropriate cleaning agent prior to use.
- Change gloves every time after touching items or surfaces, prior to touching the exhibit.
- Wear multiple layers of gloves to avoid skin exposure during the changing of gloves.

- When touching the exhibit with gloves or forceps, avoid touching areas that are to be targeted for sampling.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2015.02.004.

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