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Research paper

What's on the bag? The DNA composition of evidence bags pre- and post-exhibit examination

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ABSTRACT

Current forensic DNA profiling kits and techniques enable the detection of trace amounts of DNA. With advancements in kit sensitivity, there is an increased probability of detecting DNA from contamination. Research into DNA transfer within operational forensic laboratories provides insight into the possible mechanisms that may lead to exhibit contamination. To gain a greater understanding of the potential for evidence bags to act as DNA transfer vectors, the level of DNA accumulating on the exterior of evidence bags during the exhibit examination process was investigated. The exterior of 60 evidence bags were tapelifted before and after the examination of the exhibit inside of the bag resulting in 120 DNA profiles. These DNA profiles were compared to DNA profiles of staff working within the building and samples taken from the exhibit inside the bag. Common DNA profile contributors from each sample were also identified through STRmix™ mixture to mixture analysis. The average DNA quantity and number of profile contributors was higher in samples taken from the bag before exhibit examination than after examination. Fifty six percent of all samples taken identified a match between DNA recovered from the evidence bag and at least one staff member. On 11 bags, a common contributor was identified between the exhibit in the bag and the exhibit package post-examination. In one instance a DNA profile, matching that of a donor, on the exhibit bag before examination was also detected on a sample taken from the exhibit, raising the possibility of outer bag-to-exhibit DNA contamination. This study demonstrates that operational forensic laboratories must consider exhibit packages as a potential source of DNA contamination and evaluate their exhibit handling and storage procedures accordingly.

1. Introduction

Transfer of DNA between two substrates through direct contact is a well-established concept [1–5]. Further transfer of this DNA through subsequent contacts with other substrates, referred to as indirect transfer, has also been demonstrated [3–6]. With DNA technologies becoming increasingly sensitive, the probability of detecting DNA amounts originating from higher order transfers increases. Hence, the risk of detecting DNA that has been transferred to exhibits during the forensic process, and is not related to an alleged offence, has also subsequently increased. Such contamination events can have severe consequences for the outcome of criminal investigations. If a contamination goes undetected, it can mislead investigations, which may result in wasted resources and miscarriages of justice [7]. Additionally, contaminating DNA can create a mixed DNA profile or mask the offender's profile from a sample, which can decrease the evidential value of a match with a person of interest or result in the loss of information that could have been used to identify an individual [7,8]. Accredited forensic laboratories have procedures in place to prevent person to sample contamination, which include the use of personal protective equipment (PPE) in areas where DNA exhibits and samples are processed and frequent cleaning of equipment and workspaces. Some laboratories also use environmental monitoring programmes to assess the levels of background DNA in the laboratory environment to ensure that cleaning regimes are effective [9,10]. It is also standard practice for laboratories to establish a register of staff DNA profiles to identify contamination by an operator during examination or sampling of an exhibit [11,12]. While procedures exist to minimise and identify contamination events in forensic DNA laboratories, it is important to understand how or when DNA is transferred so that high-risk practices can be identified and modified. Many studies have investigated the potential for exhibit contamination through inadvertent transfer of DNA

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from laboratory equipment and workspaces [3,13–17]. Gloves and examination tools, such as forceps and scissors, have been identified to be efficient DNA transfer vectors, and therefore pose a high contamination risk [3,13–16,18–20]. In multiple studies, DNA from mock exhibits was observed on secondary substrates after contact with previously used scissors, forceps and gloves [14,15]. Residual DNA could still be detected on these items after subsequent contacts with secondary surfaces [13]. DNA from examination staff and case-associated persons of interest has also previously been detected in samples taken from the gloves used during the examination of casework exhibits [19]. Mercer et al. [21] observed that significant levels of DNA could accumulate on the exterior surface of an exhibit bag through routine handling, movement or storage of an exhibit. This is unsurprising as packages are typically handled without PPE and come into contact with numerous surfaces which are not free from DNA. More concerning is the demonstration that DNA present on the external bag surface can be subsequently transferred to the exhibit inside the bag [17] and from the exhibit inside the bag to the external bag surface [21]. Fonnelop et al. [17] observed one instance where a full profile from an individual who handled the outside of an evidence bag was generated from a swab inside, even though it was handled far from the bag. Additional contamination events were observed, where full or partial profiles from the bag handler were detected on items, when the bag was handled above the exhibit [17]. Demonstration of indirect DNA transfer, with the exhibit bag as the intermediary vector, is of concern as it creates the potential for cross-contamination between exhibits leading to false inclusions. However, data regarding the risk that DNA transfer from exhibit bags pose to the integrity of DNA evidence are limited and further research is required. Research which identifies the composition and origin of DNA on exhibit bags is also required to understand how it comes to be there and develop work practices which minimise it. This study explored the potential for evidence bags to act as DNA transfer vectors by examining DNA quantity and composition on the exterior of evidence bags before and after the exhibit examination process. Additionally, DNA transfer between an exhibit and the exterior of its package, were identified by determining the source of accumulating DNA. Information about the origin of DNA accumulating on evidence bags, and the transfer mechanisms involved can be used to assess and improve current exhibit handling and storage procedures to minimise the potential for exhibit contamination.

Table 1
The type of exhibits inside each of the evidence bags sampled. The biological material sampled on each exhibit is indicated. Numbers that are underlined indicate exhibits which were sampled for trace DNA in addition to another biological material.

Exhibit type	Number of bags containing each exhibit type	Number of exhibits sampled for each biological material				
		Trace only	**Blood**	**Saliva**	**Semen**	**Tissue**
Jacket / Jumper	6	4	2			
Glove	10	10				
Headwear (beanie, cap, hat, bicycle helmet)	13	13				
Face mask	3	1	1	1		
Underwear	2	1			1	
Bag (plastic, cloth)	4	4				
Pillowcase	1		1			
Plastic bottle	1			1		
Sock	1	1				
Shoe (sand shoe, thong, sandal)	3	3				
Paper towel/ Tissue	1	1				1
Wooden frame	1	1				
Handkerchief	1	1				
Tools (Hammer, Crowbar)	2	1	1			
Heat sealing machine	1	1				
T-shirt	3	2		1		
Pants (trousers, jeans, leggings)	3	3				
Nightie	1	1				
Clear tape	1	1				
Mattress Cover	1	1				
TOTAL	60	50	5	3	1	1

2. Materials and methods

2.1. Exhibit bags

Sixty casework evidence bags from routine case examinations at Forensic Science SA (FSSA) were sampled, before and after the examination of the exhibit inside of the bag. Samples were taken before the exhibit was removed from the bag and directly after the exhibit was repackaged, and the bag was resealed. Evidence bags were received from different police local service areas (LSA) and contained different types of exhibits (Table 1). All bags were composed of brown paper, but their size and brand varied depending on the police LSA and size of the exhibit. The approximate size of brown paper bags sampled in this study was 30 $\times$ 40 cm. An example of one of the exhibit bags sampled in this study is shown in Supplementary Fig. S1. Samples were collected in designated evidence recovery laboratories, by examiners wearing a face mask, laboratory gown, hair net and gloves.

2.2. Sampling of exhibit bags

2.2.1. Method 1 (primary sampling method)

As indicated in Fig. S1, the top half (including front, back and sides) of the bag exterior, which included the evidence tape seal, was sampled on 50 bags, using DNA Tapelift Kits (Lovell Surgical Solutions, Melbourne, Australia) which were repeatedly pressed onto the bag's surface until the designated area was sampled, or adhesiveness of the tapelift was lost. The same area of the bag was tapelifted before and after exhibit examination.

2.2.2. Method 2 (alternative sampling method)

When sampling using the primary sampling method there was a risk that the sampling that occurred prior to exhibit examination was removing DNA, and this could lead to apparently lower amounts of DNA in the samples taken post-examination. To determine if the amount of DNA recovered from the bag post-examination was being reduced by the pre-examination sampling, a second approach was employed where the top half of the bag was tapelifted pre-examination and the bottom half post-examination. With the variation in exhibit packaging received there is not always a clearly defined 'top' and 'bottom' of the bag which is predominantly gripped during handling. The packaging of exhibits is not consistent and bags can be labelled and stored in any orientation. Bags are extensively handled and may be contacted in differing regions, depending on the orientation of the bag and the shape of exhibit inside. For this reason, it was assumed that the manner of contact between the designated top and bottom half of the bag did not differ. An additional 10 bags were sampled using the alternative sampling approach.

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2.3. DNA analysis

DNA analyses were performed using in-house validated protocols at FSSA. DNA was extracted using the DNA IQ system (Promega, Madison, WI, USA) on a Microlab® AutoLys STAR Liquid Handling Platform (LHP) (Hamilton Company, Reno, NV, USA), with an elution volume of 60 μL. Samples were quantified using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA, USA), on an ABI PRISM® 7500 Sequence Detection System (Thermo Fisher Scientific). DNA was amplified using the GlobalFiler™ PCR amplification kit (Thermo Fisher Scientific) on a ProFlex™ Dual 96-Well PCR System (Thermo Fisher Scientific). Cycling conditions were as per manufacturer's recommendations for 29 cycles with either 400 pg of DNA or 15 μL DNA extract (where 400 pg DNA was not available). Amplified DNA fragments were electrophoresed on a 3500*xl* Genetic Analyser (Thermo Fisher Scientific).

2.4. DNA profile analysis and comparison

All 120 DNA profiles taken from the bags were analysed using GeneMapper® ID-X Software v1.6 (Thermo Fisher Scientific) with an allele analytical threshold (AT) of 50 relative fluorescence units (RFU). The minimum number of contributors required to reasonably explain each profile was determined, using a minimum allele count and taking into consideration peak heights. STRmix™ V2.7 was used to deconvolute DNA profiles into individual contributor profiles using in-house derived settings. Due to limitations in computing power, profiles containing more than four contributors (N = 82) could not be analysed in STRmix™ and were re-analysed in GeneMapper® ID-X using an AT of 250 RFU, to capture the major profile contributors. This is not a practise carried out at FSSA in casework but was applied here to obtain the maximum amount of useful information. This is the same methodology employed in another study [9] and in practise is similar to the process of carrying out a top-down analysis [22,23]. When the elevated AT was used, the number of contributors were assessed in the re-analysed profiles, and those which still contained more than four contributors were not analysed further (N = 21). Profiles generated from the evidence bags were compared to de-identified DNA profiles on the FSSA staff elimination database (N = 1801). The staff elimination database contains DNA profiles of all individuals who work at FSSA (including contractors, students and laboratory visitors), crime scene examiners and other individuals who may attend crime scenes or be involved in the handling of exhibits (police officers and doctors). Of the 1801 individuals on the database, 81 provided informed consent to be identified from their DNA profile within this study. For each comparison, a likelihood ratio (LR) was calculated using the opposing H1 and H2 propositions:

H1) the staff member and (N-1) unrelated individuals are the sources of DNA.

H2) N unknown individuals, unrelated to the staff member are the sources of DNA.

(where N is the number of profile contributors). An account for co-ancestry was not applied to the LR calculations and the point estimate value was used. The Australian Caucasian allele frequency data was used for LR calculations [24]. A minimum LR cut-off of 10,000 was used to minimise adventitious matches, as per standard FSSA protocol. To better resolve the genotypes of unknown individuals, profiles with staff matches were re-analysed in STRmix™ with the matched individual assumed as a contributor [25]. The mixture to mixture comparison function of STRmix™ was used to compare DNA profiles from bag samples taken before and after exhibit examination. For each comparison, a LR was produced using the propositions:

H1) the two mixtures share a common donor or.

H2) there are no common contributors within the two mixtures.

Again, a minimum LR threshold of 10,000 was also used in mixture-to-mixture comparisons. There were 18 exhibit bags where a mixture comparison could not be performed, due to the complexity of profiles generated from the bag samples. Mixture-to-mixture analysis was also used to compare the DNA profiles in both samples taken from the bag and the 67 profiles from samples taken from the exhibit inside of the bag. Exhibit profiles that were previously determined to contain more than four contributors were re-analysed in GeneMapper® ID-X using an allele detection limit of 250 RFU (N = 17). Sixteen of these re-analysed exhibit profiles were able to be further used, one could not be due to its complexity.

2.5. Statistical analysis

Mann-Whitney *U* Tests were used to compare the DNA quantities and minimum number of profile contributors in samples taken before and after exhibit examination. The quantities of DNA and number of profile contributors recovered from the bags using the 'primary' and 'alternative' sampling approach were also compared using a Mann-Whitney *U* Test. There were not enough bags which contained exhibits with different biological materials to determine whether there was any correlation between the quantity of DNA recovered from the exterior of the bag and each type of biological material. Instead, the DNA quantity and number of contributors for bags containing exhibits with trace DNA (typically low amounts of DNA) were compared to those containing exhibits with non-trace biological material (typically high amounts of DNA) using a Mann-Whitney *U* Test. Regression analysis was also performed, to determine whether there was a correlation between exhibit storage time and the DNA quantity or number of profile contributors in samples taken from the bag before exhibit examination. In all statistical tests, a significance level of $p < 0.05$ was used.

3. Results

3.1. DNA yield and profile contributors

Casework exhibit bags were sampled to investigate the composition of DNA on the exterior of evidence bags and explore how it may be altered during the exhibit examination process. DNA profiles were produced from all 120 tapelifts taken within this study. Minimum numbers of contributors to all profiles were assessed and these values are displayed in Fig. 1, along with the DNA quantities.

Highly variable quantities of DNA were recovered from evidence bags. DNA quantities between 0.4 and 36 ng were observed pre- examination, while values between 0.07 and 37 ng were detected post-examination. The sampling method did not have a significant effect on the magnitude of differences in DNA amount seen before and after examination ($p = 0.71$). For each sampling method, the difference in numbers of profile contributors observed pre- and post- exhibit examination were also compared, and no statistically significant difference was identified ($p = 0.26$). As there was no difference in the DNA quantities or number of profile contributors between sampling methods, results from all tapelifts have been combined in Figs. 1, 2, 3, 5 and 6, Table 2 and further analysis. On 44 of the 50 bags tapelifted using the primary sampling method, there was a higher DNA quantity before exhibit examination, compared to after examination. For this sampling approach, the average quantity of DNA recovered from the bag before exhibit examination was significantly higher than samples taken post-examination ($p = 0.00016$). On 34 of the 50 exhibit bags tapelifted using the primary sampling method, there was a higher number of contributors observed before exhibit examination compared to after examination. On eight bags, the same number of contributors were observed both before and after exhibit examination. On the remaining eight bags, a higher number of contributors were observed after exhibit examination, compared to before examination. For samples taken using the primary sampling method, the average number of contributors observed on bags before exhibit examination was significantly higher than after examination ($p = 0.0022$). On eight of the ten bags sampled using the alternative sampling method, a higher DNA quantity was

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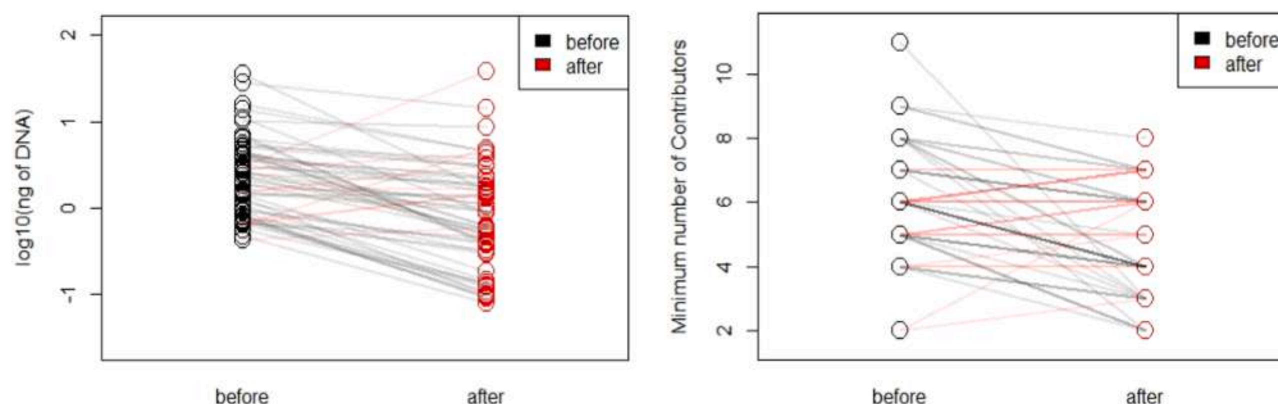

Fig. 1. The log10 DNA quantities (ng) recovered from evidence bags before and after exhibit examination are displayed on the left side of the figure. The number of contributors to profiles generated from bag samples taken before and after exhibit examination are displayed on the right side of the figure. Numbers of profile contributors were assessed using a peak detection threshold of 50 RFU. Red lines represent instances where the DNA quantity or number of profile contributors in the sample taken after exhibit examination was higher than the sample taken before examination. Grey lines represent a decrease or no change in the DNA quantity or number of contributors between the samples taken from the bag before and after examination. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

observed before exhibit examination, compared to after examination. This decrease in DNA was also observed in the primary sampling approach but was expected to be a result of the initial tapelift removing DNA from the sampled area. As this same trend was observed within the alternative sampling approach, it is apparent that other factors may also have influenced the result. The average quantity of DNA recovered from the bag before exhibit examination was significantly higher than samples taken post-examination ($p = 0.038$) within the alternative sampling approach. Due to the smaller sample size within the alternative method, it was expected that p values may be less significant, compared to the primary method. Within this method there was no significant difference in the number of contributors on bags before and after examination ($p = 0.26$). On four bags, a higher number of contributors were observed pre- examination, compared to post-examination. The opposite trend was observed on three exhibit bags, and on the remaining three bags, the same minimum number of contributors were observed both before and after examination. Bags containing exhibits with non-trace materials (blood, semen, saliva, tissue) did not have significantly higher amounts of DNA recovered from the outer bag compared to exhibits with trace DNA. This was observed both before ($p = 0.61$) and after ($p = 0.46$) exhibit examination. There was no correlation between pre-examination exhibit storage time (displayed in Fig. 4 and Supplementary material Fig. S4) and the DNA quantity ($p = 0.72$) or number of contributors ($p = 0.48$) on the bag before examination.

3.2. Staff database and exhibit chain of custody comparisons

To determine the source of DNA accumulating on the exterior of evidence bags, profiles which were deemed to be analysable with STRmix™ were compared to DNA profiles on the FSSA elimination database. This allowed us to further investigate exhibit bags as a potential vector for DNA transfer and explore the general concepts of DNA transfer and persistence. The results from staff database comparisons are displayed in Fig. 2.

Of the 120 DNA profiles produced, 41% generated an inclusionary LR to at least one individual on the elimination database, 41% did not generate any matches and 18% were too complex for analysis. At least one staff match was generated to 43% of samples taken before exhibit examination and 40% of the samples taken after exhibit examination. The breakdown of matches to individuals in specific workgroups is shown in Fig. 3.

By comparing the FSSA chain of custody records to staff matches, mechanisms whereby an individual's DNA came to be on the exhibit bag were investigated. The chain of custody for all exhibit bags is depicted in Fig. S4. For reasons of brevity, the chain of custody for four of the 60 exhibit bags sampled within this study has been depicted in Fig. 4.

Twenty-one individuals generated an LR which favoured their inclusion to at least one bag sample. Six of these individuals provided informed consent to have their DNA profile identified. For individuals who were present on the exhibit chain of custody and provided consent to have their DNA identified, a summary of the bags handled by the individual and the bags on which their DNA was detected, are shown in Table 2.

Most commonly, individuals who yielded the largest LRs had not contacted the bag most recently. Some individuals who were known to handle exhibit bags did not deposit their DNA at all or in high enough quantities to produce inclusionary LRs. Some individuals were detected on exhibit bags more frequently than others. For example, an inclusionary LR was generated between 12 samples and individual A3, which was the highest number of matches observed to a single individual. Of the 17 samples that were matched to an individual who provided consent to be identified, 16 were taken from a bag that the individual directly handled. There was one instance where an individual was not listed on the chain of custody for an exhibit, but their DNA was detected on the bag. This instance was a match between the sample taken from bag 11 before exhibit examination and individual A2. With the chain of custody records, some inferences about possible DNA donors could also be made for unknown staff members who yielded an inclusionary LR. There was one instance where DNA from an individual who did not consent to identified, but is on the laboratory elimination database, was matched to a bag that they did not handle according to the chain of custody. This individual was a laboratory visitor, who was matched to the sample taken from bag 50 after exhibit examination. Instances where DNA from an individual was detected on a bag which they had not handled suggests that in-direct DNA transfer mechanisms are also responsible for some of the DNA accumulating on exhibit bags. Common DNA profiles of unknown individuals (i.e., individuals who were on the elimination database but had not given consent for their identity to be known) were also detected on multiple bags. While the chain of custody provides some insight into possible sources of unknown staff DNA, it could not be determined with certainty whether the other 15 individuals who generated an LR directly contacted the bags, as their DNA could not be identified. This made it difficult to make inferences about the most likely explanation for the detection of these individual's DNA within a sample.

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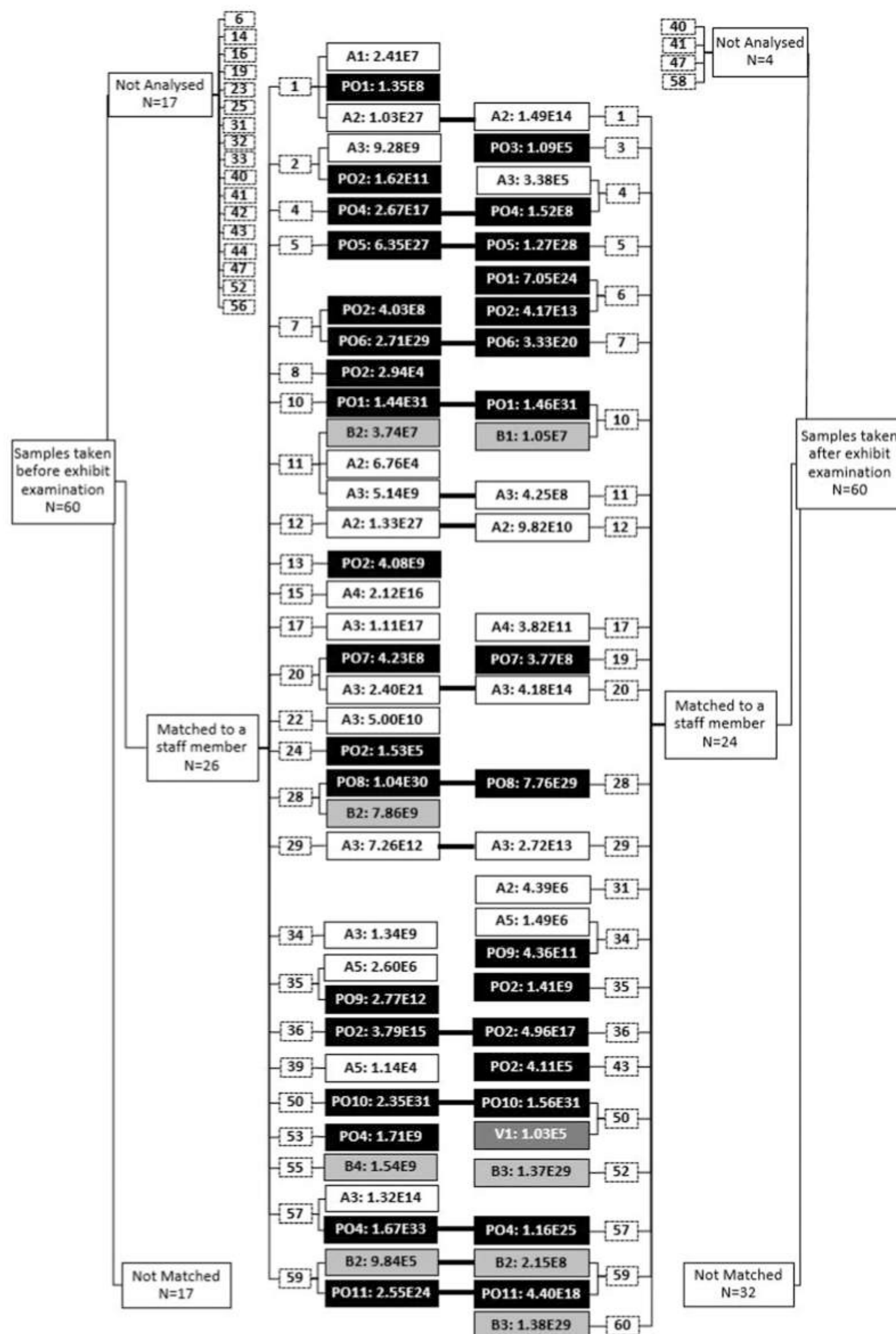

Fig. 2. Samples taken from bags before exhibit examination are displayed on the left side of the figure, while samples taken after exhibit examination are displayed on the right side. On both sides, samples have been divided in three categories to indicate the samples which were not analysed due to profile complexity, those which did not generate any matches to the staff database, and those that yielded a staff match with an LR > 10,000. The values in boxes with a dashed border represent the exhibit bag number. Individuals matched to a profile have been indicated using the following letters and shades for each workgroup: A = Admin (white box with black text), B = Biology (light grey box with black text), PO = Police (black box with white text) and V = Visitor (dark grey box with white text). Each individual has been assigned a number to differentiate multiple individuals within the same workgroup. The corresponding LR for each inclusion is also shown after the individual. Connecting lines have been used to indicate staff DNA that was on the bag before exhibit examination and persisted on the bag after examination. Bags 1–50 were sampled using the primary sampling method and bags 51–60 were sampled using the alternative sampling method.

3.3Mixture to mixture comparisons between samples taken from the same bag

To further investigate the possible origin of DNA, which was detected on bags, but could not be attributed to staff members whose DNA is on the laboratory elimination database, a comparison was performed between the contributors to profiles generated from samples taken pre- and post- exhibit examination, and to the DNA profiles generated from the exhibit inside the bag. The bags where a common non-staff contributor was identified between samples taken before and after exhibit examination are displayed in Fig. 5. No comparison between samples taken before and after examination was performed for 18 bags, due to the complexity of the resultant profiles. Of the remaining bags, 18 contained at least one common non-staff contributor between the samples taken before and after exhibit examination. Pre- and post-examination samples taken from seven of these bags did not yield any LRs to staff members or have a common contributor identified within the profile generated from the exhibit.

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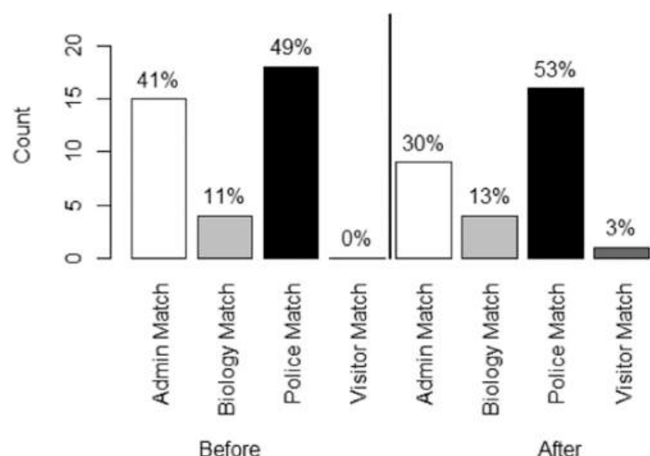

Fig. 3. Breakdown of the 41% of comparisons of the 120 profiles generated from bag samples, showing proportions of individuals from each FSSA department that were matched to the samples taken from bags before and after exhibit examination.

3.4. Mixture to mixture comparisons between bag samples and exhibit profiles

Diagrams which map each distinguishable DNA transfer or persistence event for bags where a common contributor was identified between a sample taken from the bag and the exhibit inside of the bag are displayed in Figs. S5 and S16. For reasons of brevity, a summary diagram that contains the total number of staff matches observed, common contributors between samples, and common profile contributors identified between bag and exhibit samples are displayed in Fig. 6.

Due to a low DNA quantity being detected within samples, a profile was not generated from nine of the exhibits inside of the bags sampled. With the absence of these exhibit profiles and the portion of profiles generated from the bags that were too complex for analysis, 84 of the 120 bag samples were compared to the exhibit inside of the bag (36/60 samples before and 48/60 samples after). There was at least one common contributor observed between the profile generated from the exhibit and a sample taken from the outside of its packaging for 12 bags. In eight of the 15 instances where a common donor was observed between the exhibit and the bag sample, the donor was the major contributor to both profiles. Only one of the 36 samples taken from an exhibit bag before examination was identified to have a common donor with the sample taken from the exhibit inside of the bag. As the bag was sampled before the exhibit was removed from its package, this DNA could not have been transferred to the bag because of the examination process. This result raises the question of whether the DNA was transferred from the exterior of the evidence bag to the exhibit inside of the bag. The same donor was also identified in the sample taken from the exhibit bag after examination. Through comparison to the staff database, the common donor was determined to be a police employee. In this case, it cannot be determined whether DNA transfer occurred between the exhibit and the bag, or whether the DNA from this donor was transferred to the exhibit and the bag separately through unrelated transfer events. At least one common donor was only observed between the exhibit and the sample taken from the bag after examination on the remaining 11 bags. For two of these bags, it could not be determined whether the DNA was also on the bag before examination, as the samples yielded a profile that was too complex for analysis. Since no common donors were identified between the exhibit and pre-examination samples from the remainder of bags, these results indicate that DNA from the exhibit was transferred to the exterior of these bags as a result of the examination process. The alternative is that the DNA was present on the outer surface of the bag initially, not detected during the initial sampling, then transferred to the exhibit during examination. Detection of exhibit DNA in post- examination bag samples raises the question of what mechanisms could be responsible for the transfer of DNA from an exhibit to its exterior package and whether this DNA is being further transferred to workspaces and other items within the laboratory.

4. Discussion

Experiments that investigate the accumulation of DNA on evidence bags, and the potential for exhibit packaging to act as DNA transfer vectors, provide valuable insight into the possible risk of exhibit contamination. The consideration of this information is important to assess and improve current exhibit handling and storage procedures. There are many variations in the size and composition of exhibit packaging used, and while the movement and handling histories of all exhibits differs, this study provides insight into the levels of DNA

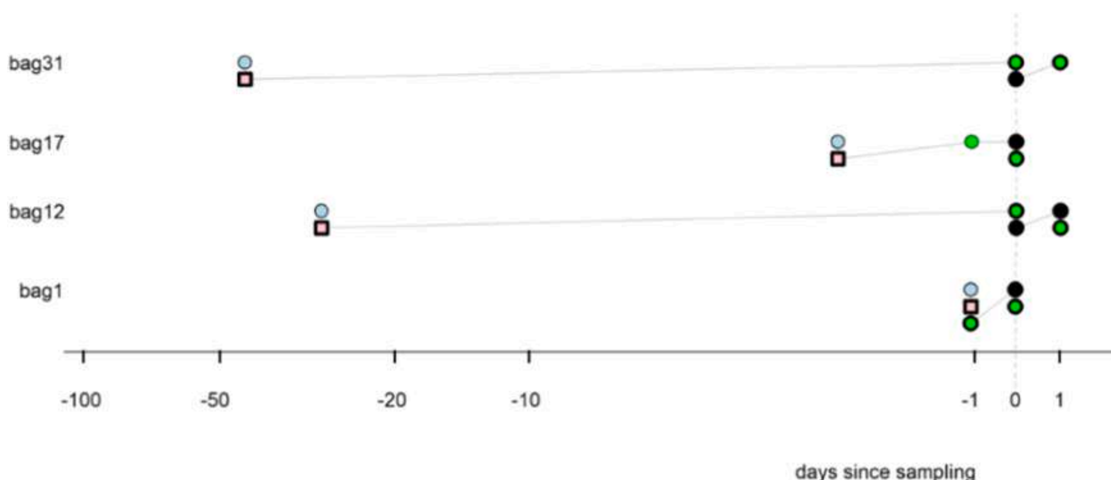

Fig. 4. Chain of custody for four exhibit bags sampled within this study. The bag number is shown on the vertical axis and the horizontal axis represents the number of days relative to the first sample being taken from the bag. Individuals who handled the bags have been represented with a symbol. Circles indicate individuals who were identified as contacting the bag in the chain of custody but were not detected on the bag. Squares indicate individuals who were identified as contacting the bag in the chain of custody and detected on the bag either before or after. Symbols with bold borders represent individuals who provided consent to have their DNA identified. The colour of the symbols represents individuals from different departments (blue = police, red = admin, green = biology). Black circles indicate the bag sampling points. For bags where multiple movements occurred on the same day, symbols are vertically stacked with the first movement at the top of the stack. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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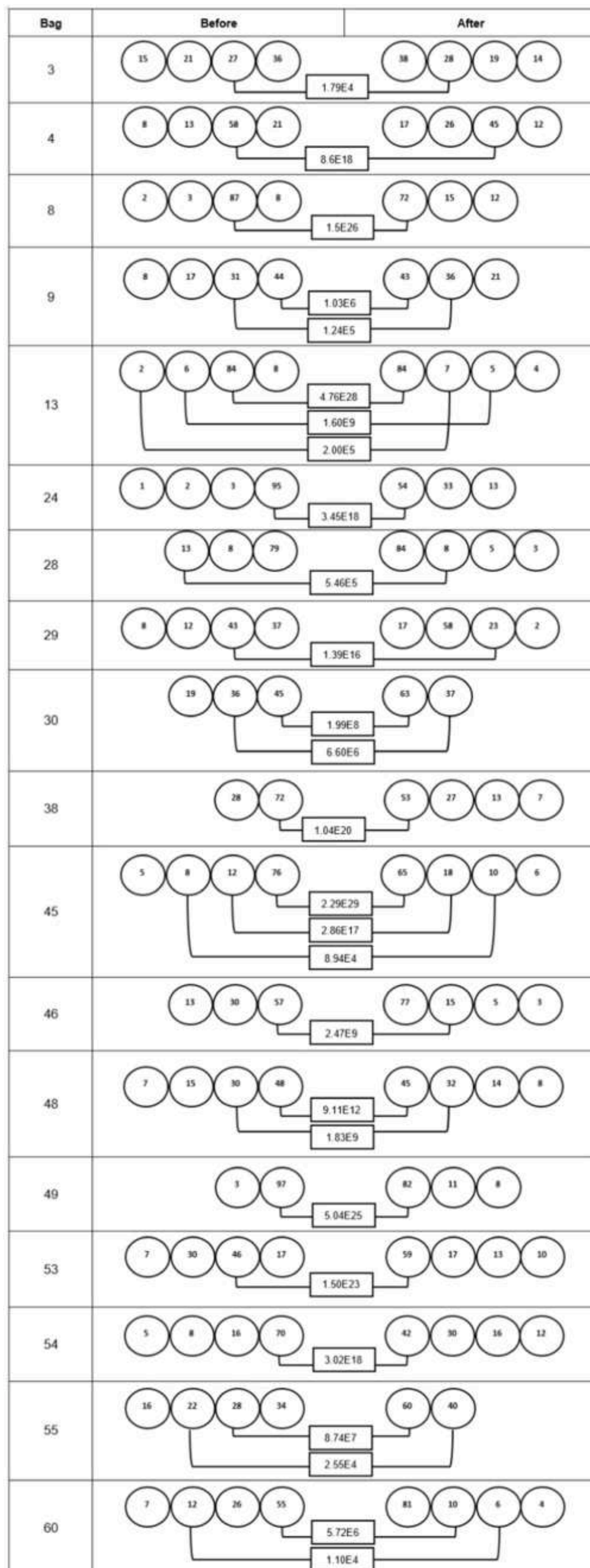

(caption on next column)

Fig. 5. Mixture to mixture analysis results, where the number of contributors to profiles generated from samples taken from the bag pre-examination are displayed on the left and post- examination are displayed on the right. Circles have been used to indicate profile contributors and the contributor mixture proportion (rounded to the nearest whole number) has been written inside of each circle. Common contributors between each of the profiles are indicated using a connecting line with the corresponding LR for the inclusion in the attached box. Common contributors who could be accounted for through a staff member being matched to both samples from pre- and post- exhibit examination have not been displayed.

accumulating on evidence bags. This study demonstrates that DNA can be transferred to evidence bags, via direct and in-direct mechanisms, throughout the various stages of exhibit handling, which occurs during criminal investigations. As contact between substrates provides the opportunity for bidirectional DNA transfer to occur [26,27], it was expected that each exhibit bag would have a different DNA composition because of its unique movement history. This explains the variation in DNA quantities and the number of profile contributors observed between bag samples and the results are consistent with findings from other studies [17,21]. Most frequently, there was a decrease in DNA quantity and the number of contributors between the samples taken from the bag before and after examination. Between sampling points, bags were handled in designated 'DNA-free' laboratories by examiners wearing PPE. Gloves are known to be efficient DNA transfer vectors [15,16,18–20], therefore it is possible that DNA was removed from the bags through contact with the examiner's gloves while unpacking the exhibit. It is also possible that DNA was transferred from the bag to the surface or workspace where it was stored during exhibit examination, which reinforces the importance of cleaning laboratory surfaces. These concepts could be tested by sampling the gloves worn by examiners after handling evidence bags and the surfaces where exhibit packaging is stored during the examination of the exhibit. The ability for DNA to accumulate on the exterior of gloves reinforces the importance of frequently changing gloves throughout the examination process and particularly after contact with items which are not 'DNA-free'. Changing gloves immediately before and after removing exhibits from their package may reduce the risk of DNA being transferred between the exterior of the bag and the exhibit. For the remaining few samples, there was an increase in DNA quantity and numbers of profile contributors between the samples taken before and after examination. For one of these instances, DNA transferred from the exhibit to the bag could explain this trend. In the other instances, DNA that was transferred into the sampling area via the examiner's gloves, from an area of the bag that was not sampled, may explain this result. It is also possible that DNA was added to the bag, from a surface where it was stored during exhibit examination. As PPE is only required for handling exhibit packaging during the laboratory examination stage in our laboratory, it was expected that police and certain forensic employees may be a source of accumulating DNA. Previous studies show that DNA from individuals who have directly handled an exhibit bag without PPE can be detected on the bag's exterior [17]. Comparison of the exhibit chain of custody with staff inclusions provided some indication of the mechanisms, which may have resulted in DNA from a donor being detected on the bags. However, the exhibit chain of custody is not always a representation of all individuals who have contacted an exhibit bag, as entries are not present for instances where an individual has handled the exterior of an exhibit bag but not taken custody of it. One example of a situation where this may occur is when an individual contacts an exhibit bag while searching for another exhibit which is stored on the same shelf. The unknown occurrence of contact between exhibit bags and other substrates, such as shelves and workspaces, also makes it difficult to assess the exact origin or transfer mechanism of accumulating DNA. LRs that favoured the inclusion of a staff member generally corresponded with the individual's regular work duties. All individuals from Biology who were matched to a sample, collected the exhibit for examination and therefore handled the

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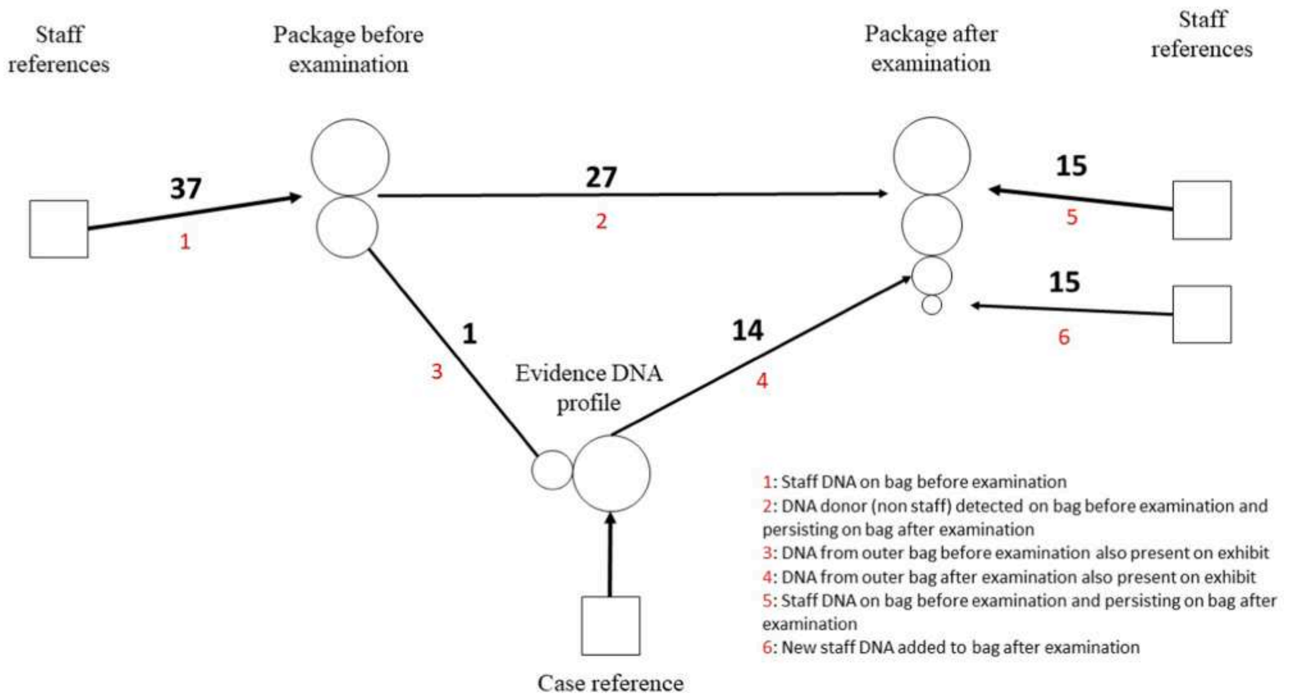

Fig. 6. Summary DNA transfer diagram that indicates the DNA transfers observed within this study. Circles represent a mixture where the number of circles represent the number of contributors to DNA profiles generated from the bag before and after exhibit examination and from the exhibit inside of the bag. The square boxes represent staff references (as per Fig. 2 and Table 2) or case reference profiles. Arrows are used to indicate common contributors between profiles with the direction of the DNA transfer indicated (if known). There is no arrowhead for arrow 3, as the direction of transfer is unknown. Numbers displayed in red indicate the type of each observed event, as described by the text in the bottom right corner of the figure and bold black numbers indicate the number of instances of each event observed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Shaded bag numbers represent samples which were matched to the individual. Black shading represents a match between the individual and both samples taken from the bag. Light grey shading indicates a match between the sample taken from the bag before examination. Dark grey shading represents a match between the individual and the sample taken after examination.

STAFF REFERENCE	BAGS DIRECTLY HANDLED BY THE INDIVIDUAL
B1	4 **10** 20 22 49
B2	1 2 3 4 5 6 7 8 9 10 **11** 12 13 14 15 16 17 18 19 21 22 23 24 26 27 **28** 29 30 33 38 48 51 52 53 54 55 56 57 58 **59** 60
B3	25 30 40 50 51 **52** **60**
B4	6 8 14 23 25 31 32 33 38 41 45 47 48 49 50 54 **55** 57 58
B5	35 36 37 39 42 43 44 46
A2	**1** **12** **31**
A4	**15** 16 **17** 19 30 33 33 55 58 59
A6	50 60

bag without PPE prior to sampling. Although a Biology employee handled all bags prior to examination, there were fewer inclusions to members of this workgroup, compared to Administration and Police. This could be due to a shorter contact time with the bags, as a basket is commonly used to move items from the storeroom to the laboratory and then PPE is used from that point onwards. The frequent handwashing by

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individuals from the Biology workgroup, as part of their normal work practices, may mean that they have less 'self' DNA on their hands compared to other individuals. It is unknown whether frequent glove use may also influence the amount of DNA deposited by individuals from Biology. In comparison, Police and FSSA Administration employees extensively handle exhibit bags during bag labelling, recording of item details and throughout the process of transporting items between storage areas. As the departments of all individuals who matched to a sample were known, inclusions to unknown individuals could be accounted for with the assumption that an individual will deposit DNA within areas they frequent [9,28], which can be transferred to other substrates through vectors [3,5,6,29–32]. All unknown individuals, except for the laboratory visitor, are either police employees or work within the FSSA Administration department. These matches therefore correspond with frequent evidence bag handling, which is involved in the regular work duties of individuals from these workgroups. As it is not routine for a laboratory visitor to handle an evidence bag, it is expected that DNA from the individual was most likely detected on the bag as the result of an in-direct transfer mechanism. One explanation for this inclusion could be that DNA was deposited onto a laboratory surface, and then transferred to the bag through contact with that surface. Instances where individuals who yielded the largest LRs but had not contacted the bag most recently indicate that there may be variation in the amounts of DNA being deposited by different individuals. Some individuals were known to handle a bag but either did not deposit any DNA or enough DNA to generate an inclusion to those samples. This result suggests that some individuals may be more prone to depositing larger amounts of DNA than others and supports the concept of 'shedder status' [2,33–37]. This concept was further supported by the result that some individuals generated inclusions to samples more frequently than others. However, it is possible that these individuals were inadvertently involved in the handling of more of the bags sampled, and therefore had more of an opportunity to directly deposit their DNA onto the bag than others. Much of the DNA detected did not generate an inclusion to any individuals on the staff database or contain a common donor with the exhibit. Possible sources of the unknown DNA are 'non-self' DNA [36] that was transferred from individuals who have handled the exhibit bags or police employees who are not on the laboratory elimination database. As exhibit bags are not 'DNA free' before use, it is possible that DNA from individuals who are involved in the packaging or production of the bags is also present. These data demonstrate the importance of all individuals who are involved in the handling of exhibit bags, or work within workspaces where exhibits are handled, being included in a laboratory elimination database. This would allow the detection of any staff contaminations, which may occur because of DNA transferred from the bag to the exhibit. There were instances where DNA from the exhibit inside of the bag was detected on the exterior of the bag after exhibit examination. The mechanism that facilitated this transfer is unknown, but one possible explanation is that the exhibit very briefly contacted the exterior of the bag during the re-packaging process. Another possible explanation is that DNA from the exhibit is transferred onto the examiner's gloves, as in [19], and then onto the bag exterior, during repackaging. It is also possible that DNA containing material is dislodged from the exhibit and falls onto the exterior of the bag while repackaging or removing the item. This result emphasises the opportunity for evidence bags to act as transfer vectors that facilitate the transfer of DNA from an exhibit to other exhibit bags and workspaces. Additionally, this result raises the question of whether exhibit DNA can accumulate within forensic workspaces, if transferred to the bag exterior during examination. Precautions should be taken to prevent DNA from the exhibit being transferred to the exterior of its packaging, as this creates the potential for DNA from case related persons of interest to be transferred to other non-related exhibits via exhibit packaging. To prevent the accumulation of exhibit DNA on the exterior of evidence bags, examiners should change gloves before repacking an exhibit and minimise contact with the outside of the bag. As there is limited research which investigates the transfer of DNA between exhibit bags and other substrates, further research is required to better understand this risk. A previous study demonstrated the ability for DNA to be transferred from the exterior of the exhibit bag to the exhibit itself [17]. In this study, DNA transfer from the bag to the exhibit was observed in instances where the exhibit contained high quantities of DNA, which implies that the higher the amount of DNA on the bag exterior, the higher the risk there is of exhibit contamination [17]. While the risk of transfer from a bag to other surfaces is unknown, these findings reinforce the risk of bringing evidence bags, which are observed to accumulate DNA, into 'DNA-free' examination laboratories. The extensive movement and handling of exhibits, both external and internal to an operational forensic laboratory, makes it difficult and likely unrealistic to implement procedures which result in exhibit bags being free of contaminating DNA before they enter an examination laboratory. Whilst introducing a requirement for exhibit bags to be handled by a person wearing PPE (or at least gloves) may reduce DNA transfer, this would be easier to implement in an operational environment where these practices are commonplace, compared to a crime scene or police station, where compliance would be often impractical. Frequent cleaning of areas where exhibits are handled and stored may also reduce the amount of DNA accumulating. While preventing contact between the bags of different exhibits during storage and transport may reduce the risk of contamination from direct contact between evidence bags, the required space to transport and store exhibits in this manner may also be unrealistic and impractical. 'Double bagging' exhibits and removing the outermost layer once the item reaches the laboratory may reduce the amount of contaminating DNA brought into examination laboratories, however regardless of the exhibit packaging method used, at some point there must be an outermost surface that is considered 'unclean' as it is free to accumulate DNA from its surroundings in an uncontrolled and unmonitored way. It is unclear whether 'double bagging' would improve the situation, as the exhibit is still required to be taken out of the packaging and then placed back in after examination and this seems likely to be the event of greatest contamination risk.

5. Conclusion

Classically in forensic science we assume that a single brown paper bag is a sufficient barrier to contamination, that we can consider the inside being free of extraneous DNA and the outside being contaminated with multiple sources of DNA from its environment. To make this assumption it is important to test various aspects of potential transfer, such as whether it is possible for DNA to be transferred from the outer surface of the bag to the inner surface (and on to the exhibit within), or from the exhibit (inner surface of the bag) to the outer surface of the bag. If the latter has occurred due to primary contact between the exhibit and the outer surface of the bag, the risk of bi-directional DNA transfer may mean that the exhibit has now been contaminated, and further examinations could result in compromised DNA profiles. The levels of DNA detected on the exterior of evidence bags sampled within this study, reinforce the risk of exhibit contamination that is posed by contact with exhibit packaging. Much of the accumulating DNA was not able to be attributed to individuals on the laboratory elimination database or the exhibit inside of the bag. In all samples taken there was only one potential case of DNA transferring from the outer exhibit bag to the exhibit, however this may be due to the contaminating DNA being deposited in two separate primary transfer events rather than a primary transfer to the bag and then secondary to the exhibit. This indicates that (at least in the exhibits and packaging examined in this study) the risk of contaminating exhibits via the outer surface of the exhibit packing is minimal when appropriate forensic procedures are in place. If we considered this single observation out of 60 as a sample-to-sample contamination event, then this would equate to a rate of approximately 1.7%. In 13 instances, it was shown that DNA from an exhibit can be transferred to the outside of its exhibit bag, during the examination process. Again, we cannot

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determine whether this has occurred due to direct contact with the exhibit and the outer packaging (which would have then potentially contaminated the exhibit) or due to a secondary transfer through the examiner's gloves (which would avoid exhibit contamination). The study highlights the importance of assessing exhibit handling procedures, to ensure that current practices are suitable with the introduction of highly sensitive DNA profiling systems.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigen.2021.102652.

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