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Criminalistics

DNA transfer in packaging: Investigation of mitigation strategies

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Abstract

Crime scene exhibits are often packaged at a crime scene and transported to a laboratory for DNA analysis. DNA-containing material may be lost from the sampling site of the exhibit to the inside of the packaging, preventing identification of a suspect, or may transfer to other parts of the exhibit complicating the interpretation of results. We sought to mitigate this DNA transfer by testing packaging that reduced direct contact with the exhibit, limited the exhibit's movement, or contained physical barriers to separate areas of the exhibit. Blood, saliva, or touch DNA were deposited onto mock exhibits that were packaged by one of four methods: unsecured, secured to bottom, secured suspended, or secured suspended with barrier separating areas. Packaged exhibits were then transported in a manner resembling casework, after which the location and amount of DNA on the exhibit and packaging were assessed. Control samples, which were not transported, were also tested. Touch and saliva deposits appeared to transfer by direct contact with the packaging and this transfer could be mitigated by suspending and/or securing the exhibits within packaging to minimize contact. Blood flaking from the exhibits meant the transfer of blood was inevitable under the conditions tested. While limiting direct contact between the exhibit and packaging minimized relocation of blood on the exhibit, the use of physical barriers prevented its transfer to other parts of the packaging. We show that while DNA transfer in packaging is not uncommon, there are strategies to mitigate this.

KEYWORDS

contamination mitigation, DNA loss, DNA transfer, exhibit handling, packaging, trace DNA

Highlights

- The manner and type of packaging influences the degree of DNA transfer/loss.
- Methods of handling and transporting packaged exhibits may influence how DNA transfers.
- Different biological materials transferred differently under a specific set of conditions.
- Minimizing direct contact between packaging and biological materials reduced DNA transfer.
- The use of physical barriers within packaging limited the transfer of blood flakes.

1 | INTRODUCTION

Highly discriminating DNA profiles are a key element in many forensic investigations. Current routine methodologies allow DNA profiles to be generated from minute traces of biological material, though less informative profiles may be generated when insufficient DNA is available or collected [1]. Forensic DNA analyses of crime scene exhibits may identify individuals associated with a crime scene [1, 2], while the relative locations of individuals' biological material on an exhibit may assist in the reconstruction of a criminal event [2–11] and guide the formation/evaluation of activity level propositions [12–18]. Preserving the integrity of DNA on an exhibit at a crime scene is therefore of critical importance to downstream forensic analyses and interpretations.

Exhibits are often packaged at a crime scene to maintain the integrity of the exhibit before transport to a laboratory for DNA analysis. It is demonstrated that DNA may frequently transfer within exhibit packaging [19–23]. Factors including the substrate of the exhibit or packaging [19, 20], as well as the type of pressure or handling applied to the packaging [19, 22], appear to influence the extent of DNA transfer. In many cases, DNA appears to transfer from an exhibit to the inside of its packaging through direct contact, representing a loss of DNA from the exhibit [19–22]. Commonly, only limited DNA quantities are available for recovery from an exhibit [24–29], so that any loss of DNA from an exhibit may prevent a usable DNA profile from being generated and subsequently preclude the identification of a person of interest.

Further, DNA transfer from one site of an exhibit to another, or between exhibits within the same packaging, may complicate activity level evaluations. Probabilistic methods are often utilized to evaluate evidence given competing hypotheses in criminal investigations [13, 17]. The location of DNA on an exhibit can be an important consideration when constructing such models [11, 14, 15], with the location of DNA potentially indicative of the activity that led to its deposition. Movement of DNA within packaging may present a further consideration for competent probabilistic modeling [30], as the movement of biological material within packaging would occur prior to the analysis of an exhibit in a laboratory, and may be difficult to distinguish from DNA transfer that occurred prior to the exhibit being packaged. As variation may exist in how DNA transfers in different packages with seemingly similar histories of handling [19, 20, 22], further understanding of DNA transfer within packaging and potential mitigation strategies would be beneficial.

While there are industry standards and guidelines within forensic science that make reference to packaging, these focus primarily on maintaining safety, quality, and the consistency of practices across jurisdictions; none provide specific guidance on how DNA should be preserved within packaging while these other aims are met [20]. As such, none seek to address DNA transfer within packaging. Stella et al. [20] suggested the following strategies for mitigating DNA transfer in packaging:

(i) Avoiding direct contact between an exhibit and its packaging, as demonstrated by various devices developed for the collection and packaging of fired cartridge cases [31, 32], the use of swab containers for packaging fired cartridge cases [33], particular weapon tubes for collecting knives [34, 35] or specifically designed packaging for bottles [35–37].
(ii) Reducing the movement of an item within its packaging, as demonstrated by Meakin et al. [35] when exhibits were secured within boxes using wire or cable ties to preserve fingermarks on exhibits.
(iii) Using barriers to prevent transfer of biological substances between parts of an item.

Here, we assessed the transfer of blood, saliva, and touch DNA from a nonporous exhibit within different types of packaging. In this conceptual study, the packaging types were designed to utilize features that test each of the above mitigation strategies, and the transfer of DNA within each package was assessed.

2 | MATERIALS AND METHODS

2.1 | Exhibits

A standardized mock exhibit was employed for this study, manufactured using sections of hollow PVC tubes (33 mm diameter). PVC was selected as representing a common nonporous substrate, with the long, rounded shape of the tube selected as representing a relatively common general object. Previous studies have demonstrated that biological materials typically transfer more readily from nonporous (including plastics) than porous substrates [19, 38–40]; therefore, use of a nonporous substrate for the mock exhibit within this study was predicted to result in the greatest losses of DNA-containing material to facilitate the investigation of DNA transfer in packaging. Tubes of length 16.8 and 19.5 cm were sealed at each end by affixing electrical outlet covers (Perma, Australia) using general purpose glue (Tarzan's grip, Selleys, Australia). Longer tubes of length 20.8 cm were not sealed at the ends, as the increased length meant the ends were in contact with the edges of the packaging when placed inside the containers, and were therefore considered much less likely to have DNA loss to the inside of the tube.

Permanent marker was used to mark areas of the tubes where biological material was to be deposited, and where DNA collection was to occur (Figure 1). Tubes were then decontaminated by washing in 1% sodium hypochlorite and rinsing with deionized water.

2.2 | Packages

A standardized packaging container was also utilized for this study (Figures 2 and S1). Packages were developed using commercially available plastic 2.3 L containers (Anko, Australia), ca. 23 cm long,

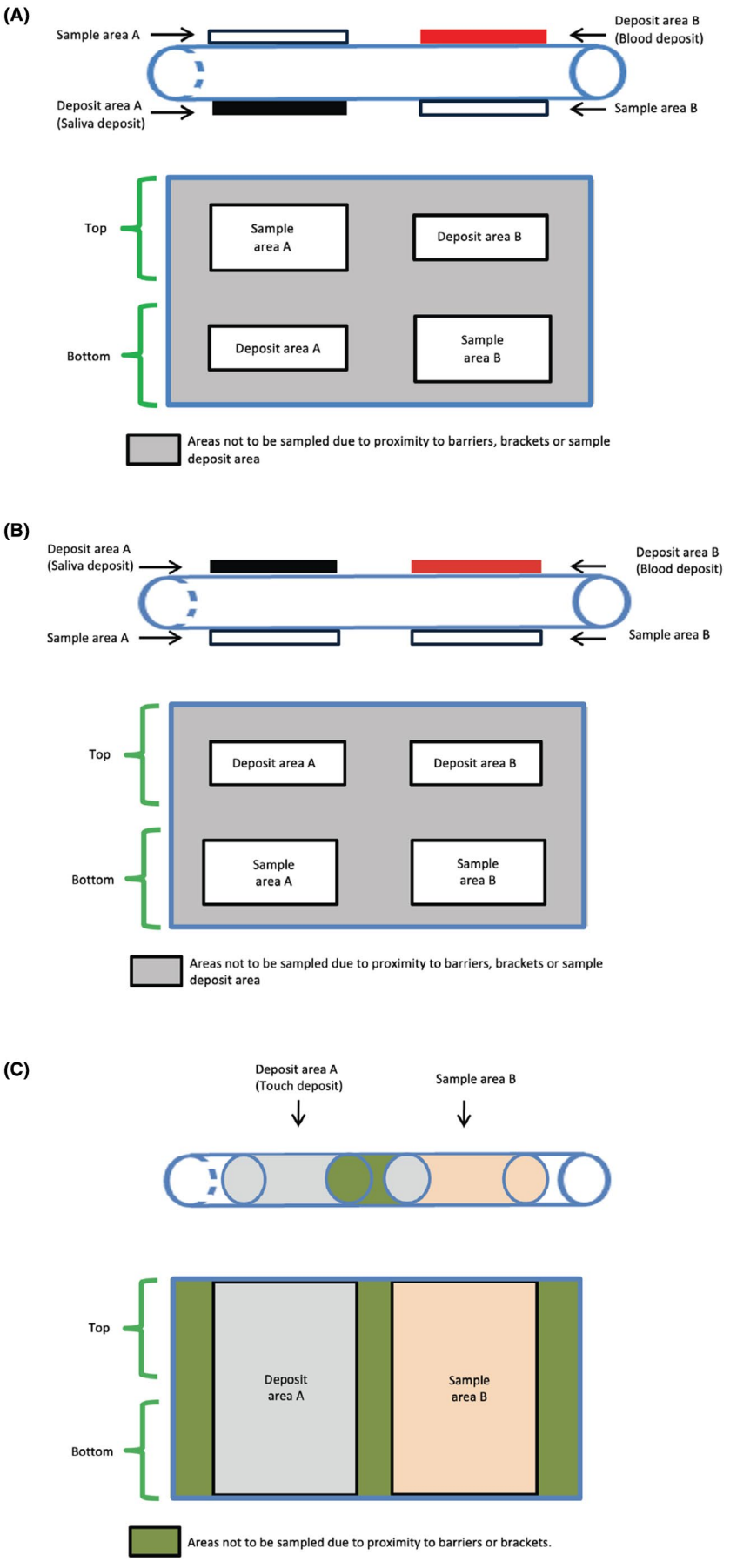

FIGURE 1 (A) Schematic diagram of tubes used for blood and saliva in methods 1, 3, 4, and 5, (B) tubes used in method 2, and (C) tubes used for deposits of touch DNA.

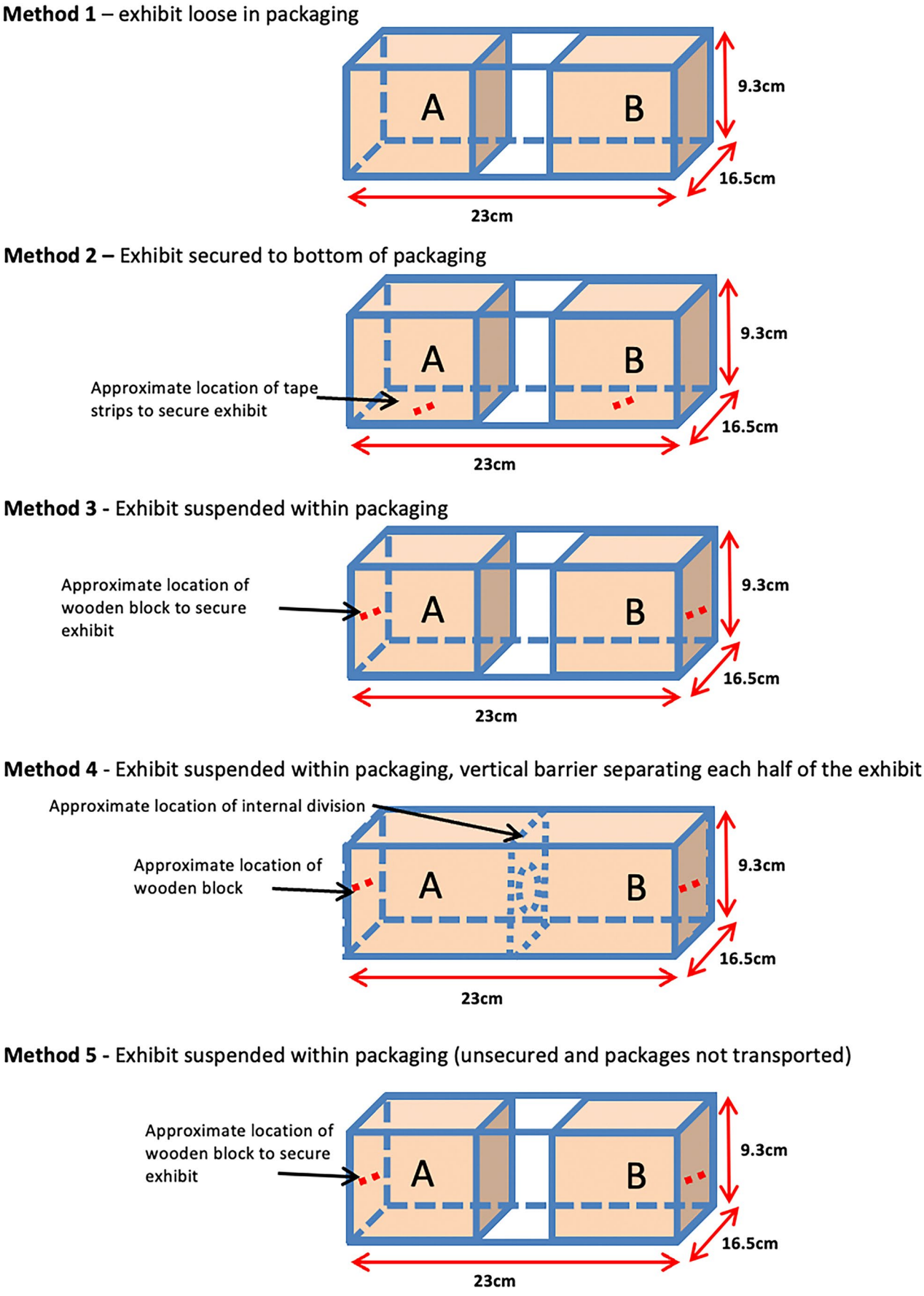

FIGURE 2 Schematic diagrams of packages used for each of the five methods. Sampling area A is the left shaded area of each container and sampling area B is the right shaded area of each container, each denoted by A and B in the figure.

16.5 cm wide and 9.3 cm high, that contained clip-sealed lids. All internal surfaces were flat (no contours or rough areas) with rounded edges. Plastic and paper bags and containers of different shapes are often used as packaging; however, for the purposes of this study, we used this form of container to enable configuration of alternative internal structures within the same packaging to test the impact of the different packaging features. Using nonporous, plastic packaging allowed efficient recovery of any biological material transferred to it [21, 41, 42], and thus the ability to assess the impacts of the different packaging types to be tested.

For methods 1, 2, 3, and 5, a section of 0.5 cm was marked on either side of the center of each container and its lid as not to be sampled, so that each half of the package was clearly defined and movement of DNA to the other side of the package could be avoided as packages were sampled during DNA collection. Marking was done to the outside of the container and lid using a permanent marker, and markings were visible through the transparent plastic. The insides of the plastic containers and their lids were cleaned with 1% sodium hypochlorite and deionized water.

A different type of packaging was used in each of five packaging methods (Figures 2 and S1). These are as follows:

For method 1, a plain plastic container was used that was not modified. An exhibit (i.e., tube) of length 16.8 cm was placed into the container without being secured.

For method 2, a plain container was again used, but exhibits (i.e., tubes) of length 19.5 cm were secured to the base using strips of PVC electrical insulating tape (Deta, Australia) of 0.18 mm thickness. Before using the tape, the edge of the tape roll was wiped with 1% hypochlorite followed by water and the top layers of tape were removed. Sections of the tube containing biological deposits touched the base of the packaging.

For methods 3, 4, and 5, exhibits (tubes of length 20.8 cm) were suspended approximately within the middle of the packaging by resting them on small blocks secured to two shorter sides of the containers. These wooden blocks were ca. 4.2 cm high and 4.4 cm wide, with a semicircular section removed of an approximate 3.85 cm diameter, to allow PVC tubes to be placed. The blocks were shaped by sawing and sanding to fit tightly to the sides of the containers. These blocks were secured to the packaging using double-sided tape (3 M, USA), after they were wiped with 1% hypochlorite and water prior to securing within the packaging. In methods 3 and 4, the tubes were placed onto the blocks, before a length of PVC electrical insulating tape was placed over the tube, with each end of the tape secured to either side of the block. For method 5, the tubes were not secured to the blocks, but were left resting on the blocks.

For method 4, exhibits were suspended and secured on wooden blocks as per method 3, and a barrier was constructed in the middle of the container to divide it into two equally sized separate areas of the packaging. The barriers were sections of a corflute sheet (Corex, Australia) cut using a new, prepackaged scalpel (Swann-Morton, England). The corflute sheet was first cleaned using 1% hypochlorite and water, and sections of the decontaminated sheet were retained for use as a negative control. Cleaned corflute inserts were secured within the packaging using PVC electrical insulating tape. Holes were cut in the center of the corflute barrier to provide space for the exhibits to fit, and a section of corflute was removed above the hole so that the exhibit could be inserted into the package. This piece of corflute was then reattached to the barrier using PVC electrical tape once the exhibit was in place.

Packages for method 5 were constructed in the same manner as those in method 3; however, tubes were not secured to the wooden inserts using tape as in method 3, and these packages were not exposed to handling and transportation. Method 5 was assessed as a control.

Individual tubes containing both blood and saliva deposits were used for each method, while tubes containing only touch deposits were utilized in methods 1, 3, and 5 only. This was due to differences in the expected transfer of touch deposits as opposed to blood or saliva. It was anticipated that blood and saliva would dislodge from the exhibit without contact more readily than touch deposits [40, 43–45]; hence, it was anticipated that minimizing direct contact with packaging, rather than seeking to minimize indirect transfer, would be most productive for exhibits containing touch DNA. For this reason, touch DNA was not tested in method 2 where exhibits were secured to the base of the packaging, or method 4 where the use of a barrier was assessed to restrict movement of biological materials in packaging.

2.3 | Biological material deposition

All volunteers participated in this study under organizational ethics approval and with informed consent.

Blood was collected from donor A by a qualified phlebotomist and stored in EDTA at 4°C until use. Blood was collected the day before experiments were conducted. A reference sample from donor A was obtained using blood taken directly from this sample.

Saliva from donors B or C was collected when donors secreted their saliva directly into a falcon tube. Donors did not consume anything by mouth for at least 45 min prior to saliva collection. Saliva was either used immediately, or after being stored overnight at 4°C. Reference samples from donors B and C were available from previous related studies, also performed under the same ethics approval and with informed consent [20, 22], which utilized blood of donor B and saliva of donor C.

Where blood and saliva were deposited onto a single exhibit tube, four specific areas were marked on each tube (Figure 1). These were deposit area A (1.5 cm × 4 cm) to which 100 µL of saliva from either donor B or C was deposited; and deposit area B (1.5 cm × 4 cm) to which 100 µL of blood from donor A was deposited. Sample area A (2.5 cm × 5 cm) was adjacent to deposit area A and no sample was deposited; and sample area B (2.5 cm × 5 cm) was adjacent to deposit area B and no sample was deposited. The deposit and sampling areas for blood and saliva were in the

same relative positions for tubes used in methods 1, 3, 4, and 5 (Figure 1A). For method 2, the relative locations of each area differed (Figure 1B) to allow the deposit sites of both blood and saliva to directly contact the packaging, while the sample areas were on the opposite sides of the tube and not in direct contact with the packaging.

A bespoke trestle was utilized to suspend tubes in the air for depositing blood and saliva. Saliva was deposited first using a pipette while deposit area A was facing directly upwards. Saliva was spread evenly within the deposit area using a pipette tip and left for ca. 1.25 h to air dry. For tubes used in methods 1, 3, 4, and 5, the tube was then turned 180 degrees so that sample area B was now facing directly upwards. Blood was pipetted on the deposit area and spread evenly within the deposit area using a pipette tip before being left for ca. 5.5 h to air dry. Tubes used in method 2 were not rotated between the deposition of blood and saliva, as the deposit sites were on the same side of the tube. The deposition and drying of biological materials were performed in a laboratory precleaned with 1% hypochlorite and water, and only accessed by the researcher during the testing period.

Where only touch DNA was deposited, two specific areas were marked on each tube (Figure 1C). These were deposit area A (6 cm length, entire circumference of tube) on which touch DNA was deposited by donor B, and sample area B (6 cm length, entire circumference of tube) to which no biological material was deposited. There was an area of 3 cm between areas A and B to avoid accidental collection of directly deposited DNA from area A when sampling from area B.

For touch DNA deposits, PVC tubes were held upright by a researcher wearing a hairnet, face mask, gown, and new gloves. The researcher held sample area B of the tube, as past studies demonstrated new gloves to be DNA-free [22]. The tube was held so that sample area B was above sample area A. The lower half of the PVC tube was then handled by the donor who wore a hairnet, facemask, and gown. The donor handled the lower half of the tube to limit DNA transfer to the upper half of the tube, which could potentially occur without contact [40, 46], and twisted their hand on the tube for 30 s in a manner derived from Reither et al. [47]. Donors handled two tubes at a time, one in each hand. After handling a total of four tubes, two with each hand, the donor waited a minimum of 2 h prior to handling the next four tubes, and deposited DNA onto a total of 12 tubes on the same day. The donor did not wash their hands for a minimum of 45 min prior to handling each set of PVC tubes.

Following DNA deposition, tubes were placed into their packages by lifting the tubes over a side edge of the container, so that each biological deposit on the tube was only held over one side of the packaging and not the other. Tubes were secured as described in Section 2.2. Tubes containing both blood and saliva were placed in the packaging, so that either deposit area faced a side of the packaging to ensure each biological material experienced the same forces. Containers were then sealed using the clip-seal lids.

2.4 | Transport of packages

Once exhibits were secured in the packaging, packages for all methods, other than method 5, were transported as follows to recreate the movement and handling that would occur during routine casework.

Packages were picked up, carried to the other side of the laboratory and placed down. Packages were then placed together into a plastic bag of size 68 cm × 55 cm (Wayne Richardson Sales, Australia) for ease of carrying. One container from each method was placed into each bag to ensure consistency of handling across the different packaging methods. Containers were then carried ca. 80 m and placed into the trunk of a car and driven for ca. 30 min at speeds of up to 60 kph on asphalt roads. Bags were carried ca. 30 m and stored at an indoor location before being driven by the same route to the laboratory (i.e., an additional ca. 30 min). Containers were then carried ca. 80 m back to the laboratory. Packages were carried into an adjacent laboratory, where they were picked up and placed down 10 times, before being transported by trolley over asphalt, linoleum flooring, carpeted flooring, and a concrete path. Packages were then placed in one laboratory before being carried to an adjoining laboratory for analysis.

Packages in method 5 were not removed from the laboratory following DNA deposition, but remained in the laboratory overnight and were sampled the following day at the same time as other packages were sampled and in the same manner.

2.5 | DNA collection and extraction

For all packages that were transported, upon their return to the laboratory and following the handling steps, exhibits were removed from their packaging and placed on the trestle for sampling. New gloves were used to handle each exhibit, and care was taken to ensure each half of the exhibit remained on the half of the packaging in which it was originally placed. Exhibits were removed by picking them up and lifting them over the side edge of the container, so that biological material would not fall to the other side of the packaging.

Where tape was removed in methods 2, 3, and 4, a clean, prepackaged, disposable scalpel was used for each side of each package to cut the tape. In method 4, the scalpel was also used to cut tape on the corflute barrier on that side of the packaging. Each scalpel was discarded after use on one half of the package.

Relevant areas of exhibits and packages were sampled for DNA using a wet/dry swabbing technique using viscose swabs (Sarstedt, Australia). Wet swabs were moistened using approximately four to five drops of water (water for injections BP, Fresenius Kabi, Australia) and the swab tip traversed the sample area multiple times, while rotating the swab and applying mild pressure. The same area was then sampled using a dry swab in the same manner. The wet and dry swab tips from each sample were stored together in AutoLys tubes

(Hamilton®, Bonaduz, Switzerland) at −20°C prior to processing, and were not dried prior to freezing.

For packages, the bottom and vertical walls of the container, as well as the lid, were sampled for each side of the package (packaging areas A and B). Where a barrier was inserted into the package (method 4), the corflute was also sampled as part of each half of the packaging. For all packages that were transported, sample areas A and B were sampled for exhibits with both blood and saliva deposits. For exhibits with touch deposits, both the deposit area and the sampling area of the exhibit were sampled separately. This was to ensure sufficient amounts of touch DNA were available to transfer from each exhibit, as touch DNA might be expected to be present at lower levels than blood or saliva [2], and failure to detect the transfer of touch DNA on sample areas of the exhibit or packaging could possibly be because so little was present and available for transfer from the exhibit. For method 5, where packages were not transported, deposit sites of blood and saliva were also sampled along with each half of the packaging and the two sampling sites. Deposit sites of blood and saliva were sampled in method 5 to ensure the biological materials had been successfully deposited and to facilitate any inferences of DNA amounts that may have been made throughout the study.

DNA was extracted from frozen swab tips using the DNA IQ™ System (Promega, USA) according to the manufacturer's instructions and eluted into 60 µL volumes. DNA was quantified by real-time PCR using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher USA) on an ABIPRISM® 7500 real-time PCR instrument (Thermo Fisher, USA) according to the manufacturer's instructions. Extracted DNA was then amplified for 30 cycles using the PowerPlex® 21 System (Promega, USA), according to the manufacturer's instructions. An amount of 0.5 ng of extracted DNA was used for amplification, except where insufficient DNA was obtained, in which case 15 µL of extracted DNA was used for amplification. All samples were committed to the amplification step, even where no DNA was detected during the quantitation step. DNA was typed using a 3500xL Genetic Analyzer (Thermo Fisher, USA) (1.2 kV/24 s) and GeneMapper ID-X v1.6 (ThermoFisher Scientific, USA) with a threshold of 175 RFU. DNA profiles were assessed visually and where it was clear that only a single donor contributed to a profile, the presence of donor alleles within test samples was determined by comparing the profile to the known reference profile.

2.6 | Data analysis

Total DNA amounts in each sample were obtained by multiplying the DNA concentration obtained in ng/µL by real-time PCR by the elution volume of 60 µL. DNA was considered to be detected if more than one allele was detected by PCR profiling, or if any quantity was detected by real-time PCR. The requirement for more than one allele to be detected by PCR profiling assisted in matching the detected profiles to those of the expected contributors with greater confidence and distinguishing results from any potential minor contaminant or nonspecific amplification. In most cases, where DNA was detected, it was detected by both methods.

The number of contributors to DNA profiles was determined based on the maximum allele count and peak height information, once artifacts were identified and removed from the process. Mixture deconvolution, mixture proportion assignment, and likelihood ratio calculations were performed using STRmix™ (v.2.9) (New Zealand Institute for Public Health and Forensic Science and Forensic Science South Australia).

2.7 | Quality control

Negative controls were collected by wet/dry double swab using viscose swabs with water as the wetting agent as described in Section 2.5. Control samples were taken from clean PVC tubes and caps, the insides of cleaned containers and their lids, cleaned wooden inserts, cleaned PVC tape, and cleaned corflute pieces. All controls were negative for the presence of DNA.

3 | RESULTS

3.1 | General results and controls

A summary of the frequency with which DNA transferred to each area of the exhibit or packaging is described in Tables 1–3. DNA amounts recovered from each area and the success of DNA profiling are summarized in Tables S1 and S2, while Table S3 shows the number of contributors, mixture proportions, likelihood ratios to each person of interest and average relative fluorescence unit contributions per contributor as determined through STRmix™ for each sample. A total of three samples returned only one allele where no DNA was detected by real-time PCR (Table S3) and these observations were not recorded as transfer events.

Blood was observed to flake from the exhibit in each of the controls (method 5) and fall to the base of the packaging as the exhibit was placed into the packaging. In three out of four replicates, blood was observed to transfer to both sides of the packaging, and in one

TABLE 1 Number out of total samples where blood was detected on part of the exhibit or packaging.

	Deposit area		Sample areas			
	Exhibit		Exhibit		Packaging	
Method	A	B	A	B	A	B
1			6/6	6/6	6/6	6/6
2			0/4	0/4	4/4	4/4
3			0/4	0/4	2/4	4/4
4			0/4	0/4	0/4	4/4
5	0/5[a]	5/5	0/4	0/4	3/4	4/4

[a]Saliva was deposited in deposit area A while blood was not.

TABLE 2 Number out of total samples where saliva was detected on part of the exhibit or packaging.

	Deposit area		Sample areas			
	Exhibit		Exhibit		Packaging	
Method	A	B	A	B	A	B
1			2/6	0/6	3/6	0/6
2			0/4	0/4	1/4	0/4
3			0/4	0/4	0/4	0/4
4			1/4	0/4	0/4	0/4
5	5/5	0/5[a]	0/4	0/4	0/4	0/4

[a]Blood was deposited in deposit area B while saliva was not.

TABLE 3 Number out of total samples where touch DNA was detected on part of the exhibit or packaging.

	Exhibit		Packaging	
Method	Deposit area	Sample area	A	B
1	4/4	0/4	4/4	0/4
3	4/4	0/4	0/4	0/4
5	4/4	0/4	0/4	0/4

case was observed to flake to only the side of the packaging directly below the deposit site on the exhibit. Blood-derived DNA was detected in the areas where the flakes were visible and was not detected on one half of the packaging in the one replicate where blood flaking was not observed (Table 1). Flaking was not visible for the touch DNA or saliva deposits, and DNA analysis did not detect the presence of either on the packaging (Tables 2 and 3). No DNA of any source was detected on the sample areas of the exhibits. DNA was detected in each of the deposit areas of the exhibit for touch (0.18–0.66 ng), blood (2.58–231.90 ng) and saliva (69.60–157.98 ng). For one replicate of blood in the control samples (replicate 3, Table S1), a greater amount of DNA appeared to adhere to the exhibit than in other replicates; however, a lower proportion of the total DNA in that replicate was located on the packaging suggesting that less DNA transferred from the deposit site to other sampling sites in this instance.

3.2 | DNA transfer from unsecured exhibits

When exhibits containing both blood and saliva were placed into packaging in method 1, flaking of blood was again observed in each replicate. In five out of six cases, blood flakes were visible on both sides of the packaging following transport, while in one of these cases the flakes pooled on one side. In all cases, DNA from blood deposits was detected on both sides of the packaging by PCR profiling (Table 1). DNA from saliva deposits was detected in area A of the packaging in three out of six replicates, but was not detected in area B of the packaging on any occasion (Table 2). No flaking of saliva was observed. DNA from blood was detected in both sampling areas of all exhibits in all cases (0.48–65.28 ng). DNA from saliva was found in sample area A of the exhibit in two out of six cases, but was not detected in sample area B of any exhibit. Touch DNA transferred from the exhibit to side A of the packaging in each replicate (Table 3) and was detected by PCR profiling, but not real-time PCR. No DNA transfer from the donor was detected on side B of the packaging, or sampling area B of the exhibit. Touch DNA was detected in deposit areas in each case. In one case, DNA attributed to the researcher was located on side B of the packaging (method 1, touch DNA, replicate 2) and this was excluded from the results.

3.3 | DNA transfer from secured exhibits

In a similar manner to the controls, blood flaked to the base of packaging as exhibits were placed into packaging on each occasion (methods 2 and 3). Following transport, blood flakes were observed on both sides of the packaging in each case in method 2. Transfer of saliva or touch deposits was not visible.

DNA profiling demonstrated that where the exhibits containing both blood and saliva were secured to the base of the packaging (method 2), saliva transferred to area A of the packaging in one out of four cases, but did not transfer to any other part of the packaging or exhibit (Table 2). Blood transferred to both sides of the packaging in each replicate, but did not transfer to sample sites of the exhibit on any occasion (Table 1).

When the exhibits were secured and suspended in packaging (method 3), blood was again transferred to at least one side of the packaging on each occasion, but not to other areas of the exhibit. Blood flakes pooled on side A of the packaging in replicate 2, resulting in an increased DNA recovery from that side over side B. Neither saliva nor touch DNA was detected on the packaging, or on the sample areas of the exhibit. Touch DNA was detected on the deposit area of each exhibit (1.20–3.72 ng). The amounts of touch DNA detected in the deposit areas of exhibits in method 3 were greater than those detected in the deposit areas of exhibits in methods 1 and 5 (Table S2).

3.4 | Use of a barrier within packaging

Where the corflute barrier was utilized, DNA did not transfer from one side of the packaging to the other. Blood again flaked when placing the exhibit into the packaging, as per previous replicates, and was detected by PCR profiling on side B of each package (32.04–62.04 ng), but not on side A which contained the saliva deposits. DNA from blood did not transfer to sampling areas of the exhibit (Table 1). Saliva did not transfer to the packaging in any replicate, and on one occasion saliva-derived DNA was detected in sampling area A (replicate 4, 0 ng and 6 alleles detected). No DNA from saliva was detected on side B of the packaging (Table 2).

4 | DISCUSSION

4.1 | Summary

Touch DNA and saliva were observed to transfer to areas of the packaging with which the biological deposit on the exhibits appeared to have direct contact, and also to DNA-free parts of the exhibit that directly contacted areas of the packaging to which saliva had transferred from the deposit area of the exhibit. This DNA transfer could be mitigated by eliminating direct contact between areas of exhibits containing biological material and packaging. Blood also transferred to areas of the packaging which directly contacted the deposit area of the exhibit, or to areas of the exhibit which were expected to have had direct contact with those areas of the packaging to which blood transferred from the deposit site of the exhibit. In contrast to touch DNA and saliva, blood was observed to flake frequently and also transferred to areas of the packaging with which the deposit area of the exhibit did not have direct contact. Eliminating direct contact between the exhibit and packaging mitigated the relocation of blood-derived DNA on the exhibit. In cases where flaking occurred and the movement of blood could not be prevented, physical barriers within the packaging contained the transfer of blood to specific areas of the packaging and appeared to reduce the total amount of DNA transferred to packaging.

4.2 | Control samples

The results of method 5, the control samples, suggest that in some cases the transfer of blood within packaging is inevitable. Blood flaked readily from PVC tubes in all controls upon placing them into packaging, and in three out of four cases, more blood was recovered from the packaging than from the deposit site of the exhibit. These observations are perhaps due to the nature of the biological deposit and/or the physicochemical properties of the exhibit [43–45], and indicate that consideration should be given to ensuring blood is adequately preserved on exhibits of specific materials. Blood flaking from nonporous materials has been described previously and is not uncommon [40, 48–51]. In this study, we used EDTA to preserve blood rather than using whole blood, as with the blood volumes required for this study, whole blood would have clotted prior to depositing sufficient amounts on each exhibit. While its use was unavoidable, EDTA may have further exacerbated the observed blood flaking, through altering the nature of the biological deposit [51]. Less flaking and transfer of blood may have been observed had an exhibit of a porous material or a different nonporous material been assessed [19, 20, 40, 45], while a differently shaped exhibit may have better retained flaked blood and limited its transfer to packaging. The results demonstrate that there are occasions where DNA loss from an exhibit may be unavoidable and this should be considered when developing packaging, sampling for evidence, or interpreting the results of DNA analyses.

Flaking was not observed for saliva or touch deposits, and DNA from these saliva or touch deposits was not detected on the packaging when exhibits were suspended within the packaging (methods 3 to 5). This indicates that touch or saliva deposits will transfer differently from blood under this specific set of conditions, where there was no direct contact between the biological deposit and other surfaces, which is similar to the observations of others [40]. The data suggest that to preserve the integrity of an exhibit and the evidence derived from it, different means of packaging may be suitable for exhibits containing different biological materials.

No DNA transfer was observed for any biological material to sample areas of the tubes in the controls (method 5) and neither touch DNA nor saliva transferred to the packaging. These results demonstrate the overall success of DNA decontamination measures employed in this study and suggest that any DNA transfer to these areas in other methods resulted from either an aspect of the different packaging types and/or the movement that occurred during the handling steps.

On one occasion, DNA of an unknown single source was detected on packaging area A of a replicate containing touch DNA (method 5, packaging area A, replicate 4), but not in any other part of the tube or packaging. DNA from this source was not derived from the donor of the DNA deposited on the tube, or any other known donor or researcher in the study and was not observed in any other sample. As a full single source profile of the known depositor was detected in the sample collected from the sample deposit area of the tube in this replicate, this does not appear to be the source of the DNA detected on the packaging and hence appeared to result from a contamination event or from insufficient decontamination of this individual package. This result was excluded from the study.

4.3 | Unsecured exhibits

Where tubes were unsecured within the packaging, DNA transferred to the packaging in experiments with every biological material. Saliva and touch DNA transferred only to the half of the packaging with which the deposit area of the exhibit made direct contact, while blood flaked on each occasion and transferred to both sides of the packaging. This further highlighted the differences in how different biological materials transferred, with blood flaking appearing to be an important mode of transfer in addition to transfer by direct contact, while the saliva and touch deposits appeared to transfer only by direct contact as described earlier. As transfer was detected across a wider area of the exhibit and packaging than that detected in the stationary packages of method 5, it suggested that direct contact between exhibit and packaging, and possibly also movement of the exhibit within packaging during transport, increased DNA transfer and indicated the need for mitigation strategies.

While it might be expected that saliva would transfer from the deposit site in each replicate of methods 1 and 2, as in each case there was direct contact between the saliva deposit and packaging, there were examples of blood being detected during

these methods when saliva was not. While the reasons for this remain unclear, it has previously been suggested that DNA from a more prolific contributor can swamp that deposited from a poorer source, precluding detection of DNA from the lesser contributor [52, 53]. It may be that a further potential implication of the relocation of DNA within packaging from one part of an exhibit to another was observed, which is that extensive transfer from one source (blood) may have swamped the DNA of another source of lower quantity (saliva) in that area of the packaging, meaning the second source of DNA was not detected. This is apparent in samples including method 2, replicate 2, package area A and method 1, replicates 5 and 6, packaging area A, where saliva-derived DNA was detected in a mixture containing more blood than saliva. Swamping is a possible consequence of DNA transfer in packaging and could have implications including inhibiting the identification of a suspect whose DNA constitutes the less prevalent profile, or complicating interpretations of data that are based on the relative locations of the DNA of each contributor. While software including STRmix™ can deconvolute complex mixed profiles, it cannot aid in analysis where a contributor is not detected during DNA typing, presenting difficulty in assessing whether saliva transferred from the deposit site in these cases. Further studies involving source identification methods [54] or cell separation techniques [55], and deeper analysis of the biological materials [56], may be beneficial in identifying the composition of biological materials when both blood and saliva deposits are present within the same packaging.

As expected, lower DNA amounts were recovered from both the exhibit and packaging from touch deposits than from other biological materials. Touch DNA transfer was observed to the packaging in each replicate, such that sufficient touch DNA was deposited to enable detection of DNA transfer. Increased DNA amounts may have been deposited and recovered from the exhibit had each tube been handled by a donor on multiple occasions [22, 47, 57], handled by a donor of greater shedder status [58–61], or had greater pressure applied [62, 63]. With respect to the amounts transferred to the packaging, these may have increased with greater pressure between the package and exhibit [38, 39], which may have been achieved through the use of a heavier exhibit. Previous attempts to increase pressure within packaging by increasing the mass of the exhibit by 68 g were insufficient to alter the proportion of DNA transfer [19], however, further investigation of an exhibit's mass may demonstrate the amount of pressure that would increase DNA transfer in this situation. Further, the precise nonporous material or the shape of the exhibit may also have altered the interaction between the exhibit and packaging and therefore influenced DNA transfer.

In contrast to saliva and blood, the relocation of touch DNA on the exhibit was not detected. This may have been in part due to the design of the exhibit, with the sampling site of the exhibit not coming into direct contact with parts of the packaging that had contacted the deposit site of the exhibit. The method of depositing touch DNA used in this study, including only a single handling step and a deposit site at the opposite end of the exhibit from the sampling site, sought to ensure the donor's touch DNA did not contaminate the sample site prior to the experiment being conducted. The absence of the donor's touch DNA from the sample site on each tube demonstrated the success of this method of avoiding donor contamination. However, this meant that the relocation of touch DNA on the other half of the exhibit may not have occurred under the conditions tested, as touch DNA appeared to transfer at least primarily by direct contact rather than transferring indirectly without contact. Nominating a deposit site in a central area of the tube, so that sampling sites were on either side of this and at each end of the tube, may have allowed the deposit site to contact each side of the packaging, facilitating DNA transfer to each side of the packaging and subsequently secondary transfer to the sampling sites of the exhibit. However, this would have complicated DNA deposition by the donor, exposing each sampling site to the donor and increasing the risk of contaminating the sampling areas. The same could be said for moving the deposit site along the length of one side of the tube, as although this may have facilitated detection of DNA relocation on the tube, the deposition of DNA on the tube would have been complicated and the potential for contamination events increased. Although tubes of slightly shorter length were used in this method to allow increased movement of the exhibit within packaging, this alone was insufficient to observe the relocation of touch DNA on the exhibit and the use of a longer container with these shorter tubes may have further increased the potential for movement within packaging and enabled testing of the relocation of touch DNA on the exhibit. While the results demonstrated transfer of touch DNA to packaging, the relocation of touch DNA on the exhibit was not detected.

4.4 | Secured exhibits

Securing the exhibit to the base of the packaging (method 2) resulted in DNA transfer to the packaging, but not to sampling areas of the tubes. This method secured the sampling sites away from the packaging and deposit sites, which ensured no contact between the sampling sites and packaging, and reduced movement of the exhibit within the packaging. This was sufficient to prevent transfer/relocation to sampling sites of the exhibit on these occasions; however, it did not entirely mitigate the loss of DNA to packaging when compared with the loose tubes in method 1.

Where exhibits were secured and suspended within packaging (method 3), blood flaked from the exhibit and transferred to the packaging in each replicate. No other biological material was detected on the packaging, and no biological material transferred to the sample site of an exhibit. In contrast to other packaging methods (methods 1 and 2), eliminating direct contact between the saliva or touch deposits and packaging resulted in no detectable transfer of either saliva or touch DNA to the packaging. Under the conditions tested, this was sufficient to prevent transfer of these DNA sources. Although blood was not detected on the sampling sites of the exhibits, the presence of numerous blood flakes on packaging created the potential for transfer to the exhibit. This may not have occurred with an exhibit constructed of a different material, if packages were

handled differently, or if environmental factors such as heat or humidity altered the nature of the blood flakes in packaging so that their potential for transfer changed. Under the conditions tested, however, securing and suspending the exhibit prevented relocation of blood on the exhibit.

In one replicate involving touch DNA (method 3, sample area, replicate 4), though 0 ng of DNA was detected during DNA quantitation, six alleles of the researcher's DNA were detected by PCR profiling. Its deposition may have occurred when that part of the tube was handled by the researcher during touch DNA deposition by the donor to the other side of the tube. This may have been avoided had touch DNA deposition occurred differently, such as by securing tubes on a trestle in a similar manner to those on which blood and saliva were deposited, rather than being held by the gloved researcher. Although gloves were assumed to be DNA-free and were frequently changed to minimize contamination risk, the risk could have been further reduced by wiping gloved hands with hypochlorite and deionized water prior to handling the tubes.

4.5 | Use of a physical barrier within packaging

The use of a barrier within the packaging successfully prevented blood transfer to the other half of the packaging. Blood did flake and fall to the base of the packaging in each replicate, but interestingly, the amounts of blood that transferred to the packaging were generally lower than in other methods. This may be because the barrier, used in conjunction with the wooden blocks, limited the movement of the exhibit and provided greater stability, decreasing blood flaking to some extent.

On one occasion (method 4, replicate 4), saliva transferred to a sample area of that side of the exhibit, but did not cross the corflute barrier. Three alleles were detected by PCR profiling, but no DNA was detected by real-time PCR. The reasons for saliva transfer to the sample site are unclear and this was not observed in any other replicate that was suspended on the wooden inserts.

While the nature of the barrier could be improved for ease of manufacture and use, we have provided proof of principle that barriers within packaging can restrict DNA to specific areas of the package when the exhibits are solid, inflexible items such as the tubes used in this study. A similar principle has been used by others for the packaging of flexible clothing items [64], where paper sheets are placed around clothing before placing the clothing into packaging. In doing so, specific areas of flexible clothing do not come into direct contact with one another and DNA transfer between these parts of the clothing item is mitigated. However, some areas of the same clothing item may still contact one another, such as the inner areas of a clothing item which are laid flat on the paper sheet before being wrapped, so that the areas of clothing to be preserved must be considered prior to packaging in this manner. The use of physical barriers to prevent DNA transfer between areas of an exhibit is worthy of further investigation, but features of these barriers may differ between flexible and inflexible items. The shape of the item and areas to be prioritized for sampling may also influence the design of the barriers.

4.6 | Further considerations

DNA may transfer differently within packaging, if the packaged exhibit or the package itself were of different materials [43–45]. This study used nonporous exhibits and packages to investigate DNA transfer and recovery when most likely to occur [38, 39]. Results may have differed had exhibits of different substrates, shapes, or sizes been assessed, or packaging of different shape, rigidity, or material been utilized. The mitigation strategies required for other exhibit or packaging types may vary, given that biological materials are likely to adhere to or transfer from each differently [40, 43–45]. Further analysis of the effects of specific handling and transportation methods would also be beneficial. The orientation of packages varied during transportation and handling in this study, and we observed, in some cases, that blood flakes pooled in specific parts of the packaging, increasing DNA amounts recovered from those areas (e.g., method 3, replicate 2, packaging side B). The handling events therefore appear to have influenced how DNA transferred within the packaging, so that greater focus on controlling the packages during transport may further aid management of DNA transfer. Further testing would benefit from maintaining the same orientation of packaging during handling and transport.

Greater consideration of DNA transfer in packaging during probabilistic modeling may also assist in mitigating its effects [30]. While the extent to which this transfer influences such modeling depends on the specific scenario under consideration, there may be cases where its effects are significant. The results presented here, and the results of previous studies [19, 20, 22], demonstrate DNA can transfer differently in packages with seemingly similar handling histories, and the extent of transfer in individual cases can be difficult to evaluate when the precise handling history of an exhibit is unknown. While this study utilized current commonly used methodologies to collect and process samples for DNA quantitation and profiling, that are considered reasonably efficient and sensitive, further transfer detectability studies will be required as more sensitive methodologies are developed and implemented in casework. Greater standardization of packaging and consideration of DNA transfer mitigation strategies will facilitate the incorporation of DNA transfer within packages into probabilistic models and strengthen the confidence of such models.

Further studies are required to consider the aspects associated with: (a) optimizing design features to further mitigate the loss and transfer of biological material from exhibits, that do not introduce risks to the preservation of the integrity of the packaged exhibit, and maintain other necessary/desired features of packaging within forensic casework contexts (e.g., features associated with the visibility of the packaged exhibit, security and continuity recording); (b) manufacturing and creating packaging that includes the features relevant to the packaging of a wide array of exhibit shapes, sizes,

and materials routinely packaged for examination during casework investigations; (c) the practicalities and costs of implementing optimal or improved packaging principles.

5 | CONCLUSION

DNA transfer may occur frequently in forensic exhibit packaging, either through direct contact between the exhibit and packaging, or through flaking of biological materials, such as blood. DNA from a prominent biological source may swamp a lesser source and prevent the generation of a DNA profile from that contributor. Eliminating direct contact between areas of the exhibit containing biological material and its packaging can prevent DNA loss from the exhibit in many cases. Where this fails to assist, such as when blood flaking occurs, physical barriers within the packaging can limit DNA transfer within the packaging.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Tables S1–S3 and Figure S1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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