DNA transfer in packaging: attention required

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Abstract

Items from crime scenes are frequently packaged and transported to laboratories for forensic examination. The packaging primarily maintains the integrity of forensic evidence associated with the item by protecting it from contamination and environmental impacts, plus limiting and recording access. There is diversity in the types of packaging used. We are aware that DNA-containing material may transfer from an item to the internal side of packaging and/or from one area of an item to another area, potentially limiting generation of good quality DNA profiles and/or affecting their interpretation. However, the level of transfer risk is unclear, as are the main impacting factors. It is thus highly relevant to improve our understanding of these. Here we explore what commonly applied standards prescribe regarding packaging requirements to maintain the integrity of DNA on a packaged item, conduct further studies confirming the risk of transfer, and suggest packaging features requiring research considerations that may limit the risk of DNA transfer within packaging.

Keywords

DNA transfer, Packaging, Contamination, DNA loss, Exhibit

1. Introduction

Exhibits to be sampled for DNA are often packaged at crime scenes for transfer to/from storage at a laboratory. Each exhibit is typically packaged separately in its own package. A range of types of packaging are available to collect each item. Many Australian laboratories use bags of various sizes to collect the bulk of exhibits and may use cardboard tubes to collect sharp items such as knives. The same type/size of packaging is often used for a wide variety of exhibit types.

Primary requirements of packaging for forensic exhibits include ensuring the integrity of traces/marks, including DNA, protection of exhibits/traces from external contaminants, sealable to allow continuity and breathable to prevent mould growth. Key standards within forensic science [1-6], developed to ensure the safety, quality and consistency of practices across laboratories and jurisdictions, that relate to packaging tell us:

- Items should be made safe, secure and in a state they can be preserved before packaging [1].
- Loss, degradation, contamination, or alteration of an item should be minimised [1,4,5].
- Items should be packaged separately [1,2,4].
- Packaging should be sealed to prevent contamination/loss [2,4,6].
- Package seal should be tamper-evident [2].
- Appropriate packaging should be used [1,2,4].
- Examples of appropriate packaging for particular items [2].
- Packaging and/or item should be labelled [1,2,4].
- Chain of custody should be maintained and recorded [1,2,5,6].
- Security and integrity of the item must be maintained [1,5,6].
- Items should be repackaged in original packaging once examined [3].

However, none specifically indicate how DNA should be preserved while achieving these aims. Instructions are not explicit on the type of packaging required to ensure the integrity of DNA on an item.

Goray et al. [8] showed that DNA derived from dried saliva and blood can readily transfer from an item contained in packaging to the internal side of the packaging, from one item to another contained in the same packaging, and from one area of the item to other areas of the same item. To ensure the integrity of the quantity, quality and location of DNA associated with an exhibit, the risk of DNA loss and transfer within packaging needs to be kept low. Implications of DNA loss/transfer include reduced opportunity to generate a full DNA profile from targeted sampling areas when dealing with small quantities of DNA, generation of more complex DNA results from collected samples thus limiting the identification of a POI, and/or altered interpretations of generated data when conducting activity level assessments. However, the degree to which DNA transfer within packaging occurs in routine casework has yet to be addressed. Causal factors and what can be done to mitigate DNA transfer in packaging remain unclear. Here we conduct two small experiments to further assess the risk of DNA loss and transfer within packaging, specifically commonly-used paper bags. Transportation and handling that packaged items commonly encounter were also simulated.

2. Materials and methods

This study was conducted under institutional ethics approval with informed consent of all volunteers.

2.1. Experiment 1

Personal items belonging to, and recently used by, person A were carefully placed in brown paper bags (43x30.5 cm) commonly used to package items of this type (Table 1). Small quantities of saliva from person A and/or blood from person B had been added to some items, and allowed to dry, prior to packaging (Table 1). Appropriate PPE was worn, and fresh gloves were used for each item. The packages were handled and transported to mimic handling in real casework (including securing the packaging with tape, adding labels, carrying to and from car and different rooms, and two short trips in a car). Six days after being packed the items were removed from the packaging and bottom third of the bag (sides plus base) double swabbed with wet viscose swabs (Forensic Swab L, Sarstedt).

2.2. Experiment 2

Polyvinyl chloride (PVC) capped tubes (length: 20 cm, diameter: 33 mm) were used as mock exhibits, either as is or covered with cotton tape. All tubes were pre-cleaned using 1% hypochlorite followed by water. Clean, new unexposed cotton tape (Premium elastic adhesive bandage, BDF Leukoband) was used to tape up one set of tubes. Each tube was placed on a bespoke trestle platform to elevate and suspend it in the air, ensuring no contact with relevant areas of the tube while adding biological materials to allocated areas and prior to placement within the packaging.

Each tube was marked to indicate four specific areas: Bottom left – sample deposit area A to which saliva (100 µl) of person A was added to 1.5x4 cm area, or touch (fingertip rubbed) of person C was added to 2x5 cm area; Bottom right – non-deposit area 1 (3x5 cm); Top right – sample deposit area B to which blood (100 µl) of person B was added to 1.5x4 cm area, or touch (fingertip rubbed) of person D was added to 2x5 cm area; Top left – non-deposit area 2 (3x5 cm). Zones of ~2.5 cm were avoided between the four areas to prevent contamination during sample deposition and/or collection. Biological samples were first deposited on to area A while it was facing up. After ~1.25 h, the tubes were gently rotated and the samples added to area B. After a further ~4 h, the tubes were carefully placed in their separate paper bags. Three repeats of each tube type and combination of saliva (person A) and blood (person B) or touch (person C) and touch (person D) were packaged. Representative negative control samples of tubes prior to use gave results as expected. Samples collected from the touch deposit sites (areas A and B) showed that 0.06 to 0.18 ng DNA had been deposited.

Appropriate PPE was worn, and fresh gloves were used for each item. The packages were handled and transported as indicated for experiment 1. Five days after being packaged, the items were removed from the packaging and placed on a cleaned trestle platform. Separate samples were collected from the non-deposit areas 1 and 2 of each tube using double swabbing technique (wet and dry viscose swabs), as well as a sample from the bottom third of their corresponding bags samples as per experiment 1.

2.3. Sample processing

DNA was extracted (DNA IQ, Promega, USA), quantified (Quantifiler Trio, Life Technologies, USA), amplified (PowerPlex 21, Promega, USA), run on a 3500xL Genetic Analyzer (Life Technologies, USA) and genotyped using GeneMapper ID-X software (Life Technologies, USA).

3. Results

In experiment 1, DNA quantities from the internal sides of packaging bags were 0.06-0.48 ng and profiles showed all expected donors (Table 1). In experiment 2, several of the tubes exhibited DNA transfer to the internal sides of the packaging bags with varying degrees of transfer to other non-deposit areas; the extent of which appears to depend on the biological material and substrate type (Table 2). Together, the results demonstrate that DNA transfers readily from an item to the internal side of its packaging (Tables 1 and 2) and can transfer from one area of an item to another whilst packaged (Table 2).

Table 1

DNA quantities and profiles generated from samples taken from internal sides of bags after storing various items used by person A. The item user, source of biological material and profile contributor are in brackets.

Item	DNA (ng)	Profile
Pillowcase (A)	0.18	Single (A)
Cap (A)	0.36	Single (A)
Glasses case (A)	0.24	Single (A)
Book (A)	0.12	Single (A)
Spoon (A)	0.06	Single (A)
Water bottle (A)	0.06	Single (A)
Paper cup (A) with blood smear (B)	0.12	Mixed (A & B)
Sock (A) + 100 µL saliva (A) + 100 µL blood (B)	0.48	Mixed (A & B)
Sock (A) + 20 µL saliva (A) + 20 µL blood (B)	0.24	Mixed (A & B)
Cup lid (A) with blood smear (B)	0.24	Mixed (A & B)
Nitrile glove (A) with external blood smear (B)	0.06	Single (B)

Table 2

Frequency of DNA transfer detected to non-deposit areas of tubes and internal side of bags (% of three replicates per combination of area, tube type and biological material). The source of biological material and profile contributor are in brackets.

Biological deposit		Non-deposit PVC tube			Non-deposit cotton-covered tube		
Area A	Area B	Area 1	Area 2	Bag	Area 1	Area 2	Bag
Saliva (A)	Blood (B)	100 (B)	67 (A,B)	100 (B)	0	0	100 (B)

Touch (C)	Touch (D)	0	0	0	0	0	100 (C)

4. Discussion

The results of these casework realistic experiments confirm the earlier results of transfer within packaging [7]. The apparent differences in transfer due to the type of biological material and type of substrate has been observed previously [8] and may be explained by their physiochemical properties [9]. More research is required towards improving fit-for-purpose types of packaging to mitigate loss and transfer of DNA within packaging. Mitigation may possibly be achieved by:

a) Reducing contact between the item and its packaging. For example, Bille et al. [10] designed a device for collection of fired cartridge cases with such a feature.
b) Reducing movement of the item within the packaging. See [11] for illustration of packaging with such a feature.
c) Using barriers to prevent movement of biological material between parts of the item.
d) As transportation may also be contributing to the risk of loss and transfer [12], this aspect should also be considered further.

Greater awareness of the extent of DNA loss and transfer within the various types of packaging, and further consideration of the potentially impacting factors, are required.

5. Conclusion

More research is needed to identify, measure, and mitigate risk factors of DNA transfer within packaging.

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Conflict of interest

None

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