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ORIGINAL PAPER

Criminalistics

A study of DNA transfers onto plastic packets placed in personal bags

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Abstract

The ability to detect low level DNA brings with it the uncertainty of whether the detected DNA is a result of transfer. To address this uncertainty, a simulation study was conducted in which a mock illicit drug packet was placed into the personal bags of individuals. When the average transit time of the packets was increased from around 2 h to more than 14 h, the percentage of the DNA profiles recovered from the packets which could be attributed to the individuals increased greatly from 5.3% to 48.6%. We found that drug packers who were poor shedders could not be included as contributors to the DNA profiles from the drug packets at all and there was a higher chance that individuals other than themselves could be included as contributors to the DNA profile recovered from drug packets. We also found that it was equally likely that the drug packers who had direct contact with the drug packets and bag owners who did not, could be included as contributors to the DNA profiles recovered from the packets. The results in this study highlight the importance of taking into consideration the transit time of drug packet, the shedder status of the alleged packer and the history of an item, when evaluating DNA evidence in the context of illicit drug activities.

KEYWORDS
DNA deposition, DNA persistence, DNA transfer, drug baggy, personal items, shedder status, trace DNA

Highlights

- DNA transfer onto drug packet more likely over a longer transit time with drug packet in bag.
- Equally likely to find DNA profiles of packers and bag owners depending on their shedder statuses.
- Need to consider exhibit history and suspect's shedder status when evaluating DNA evidence.

1 | INTRODUCTION

The ability to generate DNA profiles from smaller amounts of DNA has enhanced the detection and identification of the contributors to DNA evidence recovered from a crime scene. However, this increase in the sensitivity of DNA profiling techniques has its downsides with mixed DNA profiles being recovered and the detection of more low-level contributors in the mixed DNA profiles [1]. Taking

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into consideration the persistence and indirect transfer of DNA, this also means that some of the observed contributors may have deposited their DNA in circumstances unrelated to the crime.

The DNA recovered on the surface of an item may have been directly deposited or indirectly transferred. Indirect transfer was first demonstrated by van Oorschot and Jones [2], when they found DNA on the inside of gloves that did not belong to the wearer. Since then, extensive studies have been conducted regarding DNA transfers and the associated fields of DNA persistence, prevalence, and recovery (DNA-TPPR), in particular for touch DNA, which we define as DNA deposited via physical contact. DNA transfer is dependent on numerous factors including the type of substrates involved, duration and nature of contact, activity prior to deposition, shedder statuses of the persons involved, biological source of DNA, and the initial amount of DNA. The numerous studies in the field of DNA-TPPR have been summarized in several review papers [3–6].

The DNA profile recovered on the exterior surface of a packet of illicit drugs may be used by the Court to draw inferences on the guilt of the donor. However, the existence of indirect transfer makes it possible that the DNA of a person may be recovered even if the person had not come into direct contact with the item. Furthermore, studies have demonstrated that the dominant DNA profile recovered from an item handled by numerous persons may not necessarily correspond with the last person who touched it [7, 8]. In drug trafficking offenses where the drug packets are often recovered from personal bags (e.g., haversacks, sling bags, handbags) belonging to the suspect, it would also be necessary to take into consideration the possibility of DNA transfer between the bag and the drug packet. Several studies on personal items have been conducted with a similar finding that the dominant DNA profile obtained could either belong to the owner of the personal item or the last handler, depending on factors such as duration of usage [7–11].

Due to the complexity of DNA-TPPR issues and its impact on activity-based touch DNA evidence evaluation, some members of the forensic community hold the opinion that trained DNA scientists should provide guidance to the legal system, when the point of contention is not who the DNA belongs to but how it got there [12–17]. To date, there have been few studies on DNA transfers with specific regard to illicit drug activities. One study in 2008 looked at the probability of obtaining identifiable DNA results from resealable plastic packets in drug-related cases in Norway and the success rate was found to be 14% [18]. Another study looked at the use of Bayesian analysis to evaluate DNA transfer and persistence on resealable plastic packets and rolls of tapes commonly encountered in drug activities [19], while two other studies looked at DNA deposition, transfer, and persistence on the packaging of illicit drug capsules and found the DNA of the person who prepared the capsule [20, 21]. In a more recent study that evaluated DNA transfer within a residential setting where the resealable plastic packets (mimicking illicit drug packets) were placed on the surface of the kitchen bench or bedroom drawer, it was found that direct or longer duration of contact led to greater DNA transfer than passive and short duration contact [22].

Building on the study by Reither et al. [22] where the resealable plastic packets were generally static (i.e., not moving) on the surfaces in a home setting, this present study examines (a) DNA transfers to resealable plastic packets (commonly used to contain illicit drugs) from personal bags that the packets are placed in; and (b) the impact of this transferred DNA from the bag on the DNA that had been previously deposited on the packets (by the drug packer) through direct contact. This study design is premised on the "packer" (i.e., person who fills the resealable plastic packet with illicit drugs for sale) making use of a "courier" (i.e., uninvolved person) to transport the drug packet in his bag. We first investigated DNA transfer from personal bags to "clean" resealable plastic packets to determine transfer probabilities and then looked at the effect of possible DNA depositions by packers of different shedder statuses on the transferred DNA from personal bags and vice-versa, to more closely mimic actual events. The data presented contributes to the dataset on DNA-TPPR in the context of illicit drug activities.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Informed consents were obtained from all participants who were at least 18 years old before commencing the study. Blood samples were collected, dried, and stored on FTA™ cards (Qiagen) for subsequent DNA profiling analyses. The Domain Specific Review Board of the National Healthcare Group, Ministry of Health, Singapore, has determined that this study does not require Institutional Review Board review and approval. The types of personal bags in this study included backpack, sling bag, handbag, and tote bag with volumes ranging from 0.7 to 32 L. Before using the commercially purchased resealable plastic packets (5 cm by 7.6 cm) to construct mock illicit drug packets, three packets were randomly selected and assessed to be DNA-free. The remaining resealable plastic packets were then filled with approximately 50 g of fine salt each. The salt-filled packets were placed into the personal bags of the participants either by the participants (phase 1) or by staff members (phase 2) or by individuals with known shedder statuses (phase 3). When participants arrived at work, the packets were collected by staff members wearing new disposable gloves. The packets were placed on new sheets of A3 size printing paper before DNA deposited on the surface of the packets was collected through double swabbing of the entire surface with a single pair of wet and dry sterile cotton swabs (all-purpose cotton tips purchased from pharmaceutical drugstore). For all the samples collected, the entire swab heads were cut into an Eppendorf tube and stored in −20°C freezer prior to extraction. Each of the participants then completed a questionnaire providing details regarding their bag and commute (Table S1).

2.1.1 | Phase 1

Salt-filled packets were prepared and placed into another larger, new resealable plastic packet by staff members who wore new disposable gloves and masks to ensure that they did not deposit their DNA onto the packets. Before commuting to work, participants ($n = 38$) were asked to leave their usual items in their personal bags and to empty the salt-filled packet out from the larger resealable plastic packet

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into the main compartment of their personal bags without contacting the salt-filled packets. The average transit time of the packet, that is, duration of the packet was in the personal bag, was approximately 2 h in this phase and ranged from 20 to 160 min (Table 1).

2.1.2 | Phase 2

Salt-filled packets were prepared and placed in the main compartment of the personal bags of the participants ($n = 37$) by staff members wearing new disposable gloves and masks before participants commuted home from work. The transit time for the packets was between 14 and 20 h (Table 1).

2.1.3 | Phase 3

Four individuals (two good shedders and two poor shedders) prepared salt packets with their bare hands. Their shedder statuses were determined from our earlier study [23] where the participants' DNA deposition (number of alleles) was analyzed. Each of them was asked to wash hands and minimize contact with shared items at the workplace 1 h before holding packets, adding salt into them, and sealing them. For each round of washing hands and minimizing contact for 1 h, each individual was able to prepare three to four salt packets ($n = 33$). Each salt packet was placed in the main compartment of the personal bag of each of the participants ($n = 33$) by the four individuals. For the four individuals who prepared the salt packets, measures were taken to ensure that the salt packets placed in their bags were not prepared by the bag owners. The transit time for the packets was between 14.5 and 17 h (Table 1).

2.2 | DNA processing

DNA extraction was performed on the cotton swabs using the DNA IQ™ Casework Extraction Kit for pre-processing and the DNA IQ™ Casework Pro Kit for extraction on a Maxwell® FSC instrument (Promega) as per manufacturer's protocol to obtain an elution volume of 56 μL. DNA yield was estimated using the Quantifiler® Duo DNA Quantification kit (Applied Biosystems) on the QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems) as per manufacturer's protocol. DNA extract was amplified (29 cycles) with GlobalFiler™ kit (Applied Biosystems) with a maximum of 15 μL or 1 ng input DNA. Capillary electrophoresis was performed on the ABI PRISM® 3500xL Genetic Analyzer (Applied Biosystems) with 3 μL of amplified product injected at 1.2 kV 24 s. Results were analyzed using GeneMapper® ID-X v1.2 software. DNA profiles were interpreted according to laboratory guidelines (analytical threshold 110 relative fluorescence unit [RFU]; stochastic threshold 535 RFU) and considered as "reportable" if they had a minimum of 16 alleles above analytical threshold. Interpretability of a profile was determined according to a set of laboratory guidelines that assesses several factors, including peak heights, number of contributors, peak height proportions of major to minor contributors, etc. In general, the proportion of major to minor contributors should be at least 3 to and any profile with three or more contributors at an indistinct level would be deemed not interpretable. Interpreted profiles were compared with the reference profiles of the participants. Participants will be included if their profiles match the profiles obtained from the salt packets. As such, no likelihood ratios were calculated. For further analysis of results in phases 1 and 2, any allele present was first assigned to the bag owner before assigning it as a foreign allele if it did not match to that of the bag owner. For the results in phase 3, any allele present was first assigned to the individual who prepared the salt packet followed by the bag owner.

3 | RESULTS

3.1 | Profiles and DNA yield

Results from phase 1 showed that of 38 samples, only two samples had interpretable single source DNA profiles with DNA yield of 0.11 and 2.32 ng. These two samples made up 5.3% of the total samples in phase 1 (Figure 1). Results from phase 2 showed that of 37 samples, 14 samples had interpretable single source DNA profiles with DNA yield from 0 to 7.51 ng and four samples had mixed DNA profiles with interpretable major contributors and DNA yield from 0.44 to 1.74 ng. These 18 samples made up 48.6% of the samples in phase 2 (Figure 1). Results from phase 3 showed that of 33 samples, seven samples had interpretable single source profiles (DNA yield from 0 to 8.64 ng), two samples had interpretable mixed DNA profiles with two co-contributors (DNA yield from 3.93 to 6.49 ng) and 11 samples had mixed DNA profiles with interpretable major contributors (DNA yield from 0.2 to 6.34 ng). These interpretable profiles made up 60.6% of the samples in phase 3 (Figure 1).

TABLE 1 Experimental design.

	Phase		
	1	2	3
Participant/sample number	38	37	33
DNA-free packet?	Yes	Yes	No
Packet packed by	Staff members with gloves	Staff members with gloves	High and low shedders, no gloves
Transit time	20–160 min	14–20 h	14.5–17 h

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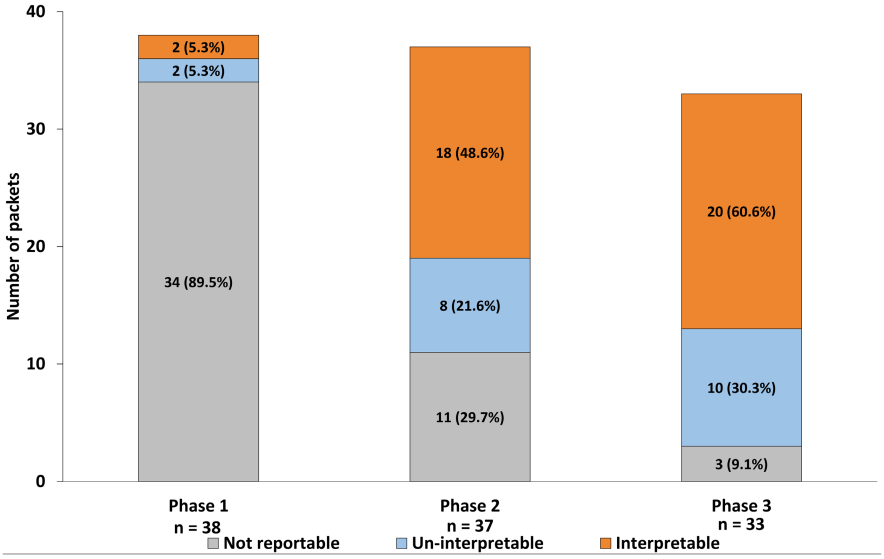

FIGURE 1 Types of DNA profiles obtained from resealable plastic packets. Numbers within brackets are percentages of the total number of packets in each phase. ■ Not reportable ■ Uninterpretable ■ Interpretable.

3.2 | Increase in transit time in bag is related to increase in DNA transfer

Comparing the results of phase 1 and phase 2, an increase in transit time of the packets in personal bags from around 2 h on average to at least 14 h was found to increase the number of interpretable DNA profiles obtained from 5.3% to 48.6% (Figure 1). The number of DNA profiles which were not reportable decreased from 89.5% to 29.7%. In terms of DNA yield, the maximum DNA quantity recovered from a packet increased from 2.32 to 7.51 ng (Table 2), along with the average and median DNA quantities. The average and median number of DNA alleles detected also increased with transit time. Such increases were in agreement with the significant difference in bag owners' allele count between phase 1 and phase 2 (Mann–Whitney $U = 271.5$; $p < 0.01$).

3.3 | Increase in direct contact is related to increase in DNA transfer

In phase 3, when packers did not wear gloves, there was a decrease in the number of non-reportable DNA profiles compared to phase 2, from 29.7% to 9.1% (Figure 1). The number of un-interpretable profiles (indistinguishable mixtures) and interpretable profiles both increased from phases 2 to 3. The number of un-interpretable profiles increased from 21.6% to 30.3% while the number of interpretable profiles increased from 48.6% to 60.6%. The increase was also reflected in the amount of DNA and the number of alleles detected (Table 2). Maximum DNA yield increased from 7.51 ng in phase 2 to 8.64 ng in phase 3, and the median number of alleles detected rose from 28 to 40. These changes were expected since more DNA would have been deposited by packers without the use of gloves in phase 3 compared to phase 2 where gloves were worn.

In phase 3, where both the packer's hands and bag interior were in contact with the packet, as compared to only the bag interior in phase 2, an increase in DNA contribution was observed with a reduction in the number of non-reportable profiles since the laboratory's minimum reporting threshold of 16 alleles was more easily exceeded. There was a corresponding increase in the number of indistinguishable mixtures, due to the mixing of DNA transferred from the packer's hands and from the interior of the bag. The sources of DNA were increased by the increase in the number of possible indirect transfer events through the presence of non-self-DNA on the hands of the packers and pre-existing DNA from the interior of the personal bags. This is reflected both in the increase in the proportion of packets with alleles from unknown contributors from 14 in 37 (37.8%) to 15 in 33 (45.5%) (Table 2) and the increase in DNA attribution to unknown persons from 1 in 37 (2.7%) to 3 in 33 (9.1%) (Figure 2). The increase in the proportion of packets with alleles from unknown persons is likely underestimated as alleles were only counted if they were different from both the packer and bag owner's profile though there could be unknown contributor(s) who shared the same allele(s).

The attribution rate of the DNA profiles to the bag owners versus that of the packers was approximately equal in phase 3 of our study. Profiles in which bag owners alone could be included as contributors constituted 24.2% (8 of 33) of the samples, packers as contributors alone constituted 21.2% (7 of 33), and profiles in which both the bag owner and packer could be included as contributors constituted 6.0% (2 of 33) (Figure 2). The low attribution rate of a mixture profile to both the bag owner and packer is likely due to the approach adopted in this study of not interpreting mixture profiles with three or more contributors.

3.4 | Shedder statuses of packers were associated with the DNA profiles recovered

When results were categorized by packers' shedder statuses, it was observed that the shedder status of a packer corroborated with his attribution to the DNA profile recovered from a packet. Packers who were classified as good shedders according to a previous laboratory study [23] were included as contributors to the DNA profiles obtained from the surface of packets 9 of 17 times (52.9%) (Table 3).

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	Phase		
	1 ($n=38$)	2 ($n=37$)	3 ($n=33$)
DNA yield range (ng)	0–2.32	0–7.51	0–8.64
Average DNA yield (ng)	0.13	0.77	1.34
Median DNA yield (ng)	0.00	0.11	0.26
Average no. of alleles	6.79	25.26	34.58
Median no. of alleles	1	28	40
No. of packets with alleles from unknown	9	14	15

TABLE 2 DNA yield and alleles obtained from resealable plastic packets.

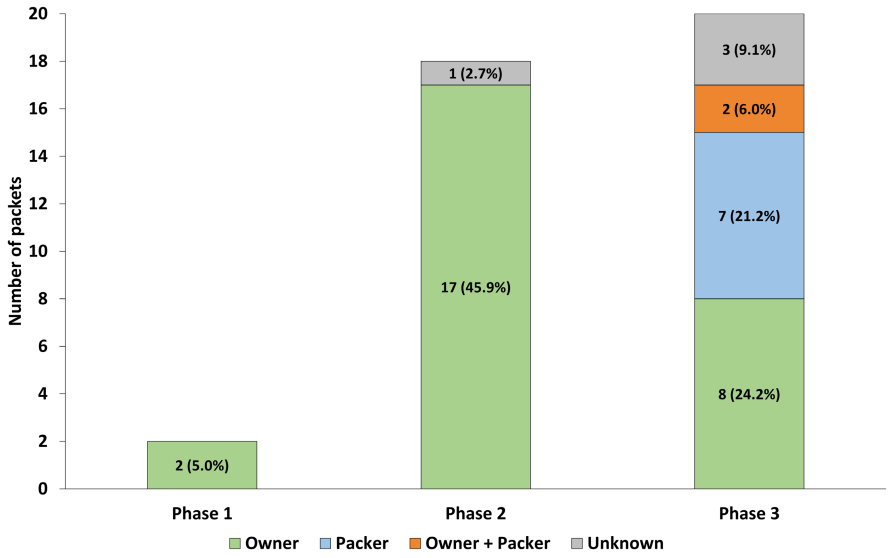

FIGURE 2 Contributors to interpretable DNA profiles from resealable plastic packets. Numbers within brackets are percentages of the total number of packets in each phase. Owner Packer Owner + Packer Unknown.

Packer	Not reportable	Not interpretable	Packer included	Bag owner included	Unknown included
Good shedder 1 ($n=9$)	1	5	2	1	0
Good shedder 2[a] ($n=8$)	0	0	7	3	0
Poor shedder 1 ($n=9$)	1	4	0	3	1
Poor shedder 2 ($n=7$)	1	1	0	3	2

[a]Total exceeded 8 due to 2 profiles being a mixture of packer and bag owner.

TABLE 3 Detailed breakdown of the DNA profiles from resealable plastic packets in phase 3.

The percentage was higher for good shedder 2 alone, at 7 of 8 times (87.5%). In contrast, packers who were classified as poor shedders were not included as contributors to the DNA profiles obtained from the surface of any of the packets (0 of 16 times). When poor shedders were packers, there is also a higher chance that an individual other than themselves could be included as contributor of the DNA on the packet (9 of 16 times, 56.3%) compared to if the packer was a good shedder (4 of 17 times, 23.5%).

4 | DISCUSSION

The DNA yield in phases 2 and 3 in this study was similar to the DNA yield obtained for the active contact scenario in the earlier study by Reither et al. [22] This is expected as the duration of the contact between the exterior of the salt-filled packets and other surface was both 1–2 days. However, the transfer rates of about 50% in phase 2 and 24%–50% in phase 3 of our study were much lower, even though our study involved much greater physical movement. A possible reason could be the difference in the way the two studies defined DNA transfer—the current study excluded samples that yielded uninterpretable profiles in the count of DNA transfer whereas the earlier study had included such samples.

Similar to the study by Reither et al. [22], some of the DNA on the packets in the current study were found to originate from participants' spouses or children. This observation could be explained by the next-of-kin having access to the participants' bags, which further demonstrated the persistence of this 'foreign' DNA in the participants' bags and the ease with which it is transferred onto the resealable bags.

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The different probabilities of DNA transfer obtained when the packers of different shedders statuses directly deposited their DNA on the packets can be used in scenario when the defense proposition claimed that the accused is only the courier and not involved in the packing of the drug. It is also important to consider the shedder status of the possible packer, which if not available, we could use the probabilities as a guide when trying to draw inference from the DNA evidence.

There was no obvious trend when results were classified by the shedder statuses of the bag owners. Besides shedder status, several factors such as bag owner's walking duration, number of times bag was accessed, volume of the bag, and its level of fullness were investigated. However, no strong correlation or trend was observed with respect to the number of bag owner's alleles detected on the packet. The study by Fonneløp et al. [19] found higher DNA quantity on the resealable plastic packets in their study when the personal bags used were purses compared to other bag types, which the authors reasoned as likely due to the greater accumulation of user DNA on the interior. We were unable to verify their hypothesis as no purses were used in our study. The study by Fonneløp et al. [19] also showed more DNA deposition on packets that were handled directly compared to indirect transfer from personal bags. Our study has, however, shown that packers who were poor shedders directly deposit so little of their DNA, that their contribution could be masked by indirectly transferred DNA from the interior of personal bags.

5 | CONCLUSIONS

This simulation study demonstrated that the probabilities of DNA transfer to the drug packet from a regularly used personal bag were related to (a) the duration the packet was left in the bag, (b) shedder status of both packer and bag owner, as well as (c) the persistence of DNA in the bag. Therefore, when presented with DNA evidence in similar scenarios involving personal items and resealable plastic packets, it is pertinent to understand the history of the item, to establish the possible activities and chain of events that may account for the evidence. Only in doing so can the investigator distinguish false DNA associations (e.g., bag owner) from actual criminal activities (e.g., drug packer) that led to the deposition of the observed DNA profile on the drug packet.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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