

1 **The deposition and persistence of indirectly-transferred DNA on**
2 **regularly-used knives**

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11 **Abstract**

12 Considerations concerning how DNA recovered from a crime scene was deposited
13 are of increasing significance to forensic casework. While the possibility of indirect
14 DNA transfer is well established, research into such transfer is limited and focused
15 mainly on the handling of DNA-free items. This study investigated whether
16 secondarily-transferred DNA can be detected on regularly-used items, and if so, for
17 how long might it persist. Volunteers each used a set of knives regularly over a period
18 of two days, after which, each of these ‘regular users’ shook hands with another person
19 (‘handshaker’) and then immediately, without touching anything else, repeatedly
20 stabbed one of their own regularly-used knives into foam for 60 seconds. DNA was
21 recovered from the knife handles using mini-tapes approximately one hour, one day,
22 and one week after the stabbings. In three of the four pairings of volunteers, complete
23 and partial DNA profiles matching those of the regular user and handshaker
24 respectively, at ratios of ~10:1, were recovered from the knives within one hour.
25 Alleles attributed to the handshaker were still detected after one week, but were
26 significantly reduced in number and peak height for two of the three pairings. Unknown
27 alleles were also recovered from the knives, suggesting other indirect DNA transfer
28 events. These included repeated detection of alleles attributed to the DNA profile of
29 a volunteer’s partner. For the fourth pairing, only complete single-source DNA profiles
30 matching the regular user’s profile were recovered. This study demonstrates that, on
31 regularly-used items, secondarily-transferred DNA can be detected and can persist for
32 at least a week; this has implications for forensic reconstructions.

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Keywords

Trace DNA, DNA transfer, DNA persistence.

1. Introduction

Considerations concerning how DNA recovered from a crime scene was deposited are of increasing significance to forensic casework [1]. Of critical importance is the possibility of indirect DNA transfer, a phenomenon which has been demonstrated by empirical research. For example, it has been shown that handling a plastic tube or glass beaker after shaking hands with another person can leave DNA from the handler and/or the handshaker, and the proportions of these DNA contributions can vary from major handler to major handshaker [2, 3].

However, such experiments use items that have been cleaned of DNA, but in casework, items are unlikely to have been free of DNA before coming into contact with DNA from the offender. Items may already have a background layer of DNA present; this is particularly true of regularly-used items, such as, items of clothing and some types of weapons. This study therefore investigated whether DNA transferred indirectly via a handshake, could be detected on knife handles that had been artificially set up as ‘regularly-used’, and if so, for how long it might persist if the knife is not used subsequently. Such studies are important to assist the interpretation of trace DNA evidence in cases, such as R v Reed and Reed (2009) [4].

2. Materials and Methods

2.1 Experimental design

Four volunteers each prepared a set of ‘regularly-used’ knives by handling the knife handles in a prescribed manner for 1 minute twice a day for two consecutive days. Then once a day, over the next three days, each person shook hands with another volunteer for 10 seconds and then immediately, without touching anything else, repeatedly stabbed one of their regularly-used knives into a foam apparatus for 1

1 minute. A different knife was used each day for the stabbing to give triplicate results. This 5-day schedule was repeated across three consecutive weeks to allow DNA to be recovered from the knife handles using mini-tapes at approximately one hour, one day, and one week after the stabbings.

Prior to starting the experiments, the knives and stabbing apparatus were cleaned of DNA using MicroSol3+ or 10 % bleach, followed by 70 % ethanol and UV-irradiation for 20 minutes. The foam and foil covering of the apparatus were replaced and cleaned after each stabbing session. Cleaned knives and a sample of the regularly-used knives for each volunteer were mini-taped to provide negative and positive controls, respectively. Volunteers were denoted W, X, Y and Z and buccal swabs were taken from each to provide reference DNA samples. A buccal swab was also taken from the partner of Volunteer X to provide an additional reference DNA sample.

2.2 Processing of DNA samples

DNA was extracted from the mini-tapes using the swab protocol of the QIAamp® DNA Investigator Kit and eluted into 35 µl. Extracts were quantified and then profiled using AmpF/STR® NGM SElect™ (10 µl template in 25 µl reactions, 30 cycles) and interpreted using GeneMapper® 4.0 software (peak height threshold of 100 RFU). Profile percentages were determined on the basis of unique alleles that, for each pairing of volunteers, could be attributed to the reference profile of the handshaker and not to that of the regular user. SPSS® Version 22 software was used to identify significant trends in or differences between the data sets.

3. Results

3.1 Controls

No DNA was detected on the knife handles prior to use in these experiments. The average total quantities of DNA recovered from the regularly-used knives, prior to handshaking and stabbing, were 3.4 ± 0.5 , 0.9 ± 0.8 , 1.2 ± 0.5 and 10.4 ± 3.7 ng for Volunteers W, X, Y and Z, respectively. The amounts of DNA deposited by Volunteers

1 W and Z were significantly different from each other and significantly greater than
2 those deposited by Volunteers X and Y (ANOVA, $F = 30.79$, $p < 0.01$).

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4 *3.2 Indirect transfer of DNA to knife handles*

5 For three of the four sets of regularly-used knives, profiles of DNA from at least three
6 people were recovered from the knife handles an hour after the handshaking and
7 stabbing sessions. These mixtures could be attributed to DNA from the specific
8 regular user and handshaker for each set of knives, and to DNA from unknown
9 sources. DNA contributions from the handshakers were detected as minor profiles, at
10 ratios to regular user DNA of approximately 1:10, 1:7 and 1:11 for the pairings of W:Y,
11 X:Z and Y:W, respectively. Notably, the same unknown alleles were repeatedly seen
12 in the profiles from knives handled by Volunteer X; these were found to match those
13 in the DNA profile from the volunteer's partner. For the fourth pairing of Z:X, only
14 complete single-source DNA profiles matching the regular user's DNA profile were
15 recovered.

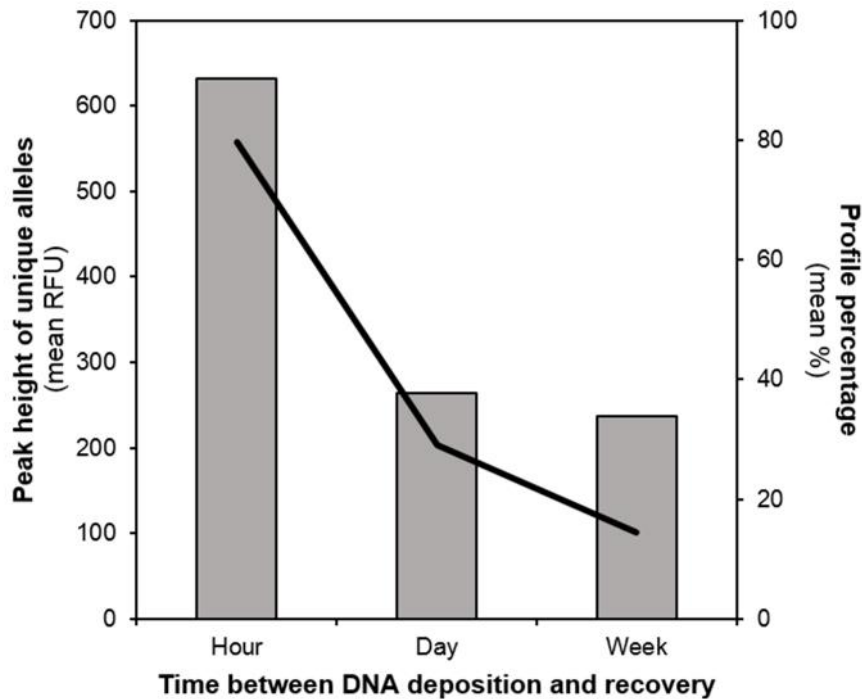
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17 *3.3 Persistence of indirectly-transferred 'handshaker' DNA on knife handles*

18 For the knife handles on which DNA had been indirectly transferred, the DNA
19 attributed to the handshaker was observed as partial profiles. These were still
20 detected after one week, but the numbers of alleles and the heights of those allele
21 peaks were significantly reduced for the pairings of W:Y and Y:W, with only the peak
22 heights being significantly reduced after one week for X:Z. To illustrate, reductions in
23 unique allele peak height and profile percentage of the handshaker's DNA in the
24 pairing of W:Y were significantly correlated with increasing time between DNA
25 deposition and recovery (Pearson's $r = -0.55$ and -0.87 respectively, $p < 0.01$; Fig. 1).

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 2 **Fig. 1.** Average allele peak heights (bar) and profile percentages (line) for DNA attributed to the
 3 handshaker, Volunteer Y, recovered from knife handles at various time points after use by Volunteer W.
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 6 **4. Discussion**

7 The results of this study demonstrate that, on regularly-used items, indirectly-
 8 transferred DNA can be detected and can persist for at least a week. Although the
 9 regularly-used knives were artificially set up, the amounts of DNA recovered (~1-
 10 10 ng) prior to handshaking and stabbing were comparable to those previously
 11 reported for regularly-used items [5]. However, these amounts of DNA significantly
 12 varied among the individual volunteers, supporting the concept that different people
 13 deposit different amounts of DNA [3].
 14

15 When DNA from the handshaker was recovered from the knife handles, it was
 16 consistently seen as a partial minor profile, contributing approximately 10 % to the total
 17 profiles recovered. Unknown DNA was also recovered from these knives, suggesting
 18 the occurrence of other indirect DNA transfer events, such as the finding of DNA from

1 a volunteer's partner who had never been in the laboratory. This supports the previous
2 observation of indirect DNA transfer from a spouse to a worn item of clothing [6].

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4 In summary, these findings suggest that, when items already have a background level
5 of DNA present from a regular user, indirectly-transferred DNA may only be detected
6 as a partial minor profile, if at all, when transferred via the regular user immediately
7 after contact with another person. This is in contrast to previous studies using DNA-
8 free items and a longer handshake, in which indirectly-transferred handshaker DNA
9 could occasionally be detected as the major or only profile [2, 3]. This study also
10 showed that the indirectly-transferred handshaker DNA could persist for at least a
11 week, but with significantly smaller allele peak heights corresponding to the detection
12 of fewer alleles than samples taken one hour after indirect transfer. This implies that
13 there is a greater chance of recovering secondarily-transferred DNA the sooner an
14 item is sampled after an offence. These results contribute to the body of empirical
15 data, which can provide an evidence base that can assist casework scientists in their
16 interpretation of the finding of trace DNA at crime scenes.

17 18 **Acknowledgements**

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21 22 **Conflict of interest statement**

23 None.

24 25 **References**

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