Forensic Science International: Genetics 17 (2015) 155–162

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Secondary and subsequent DNA transfer during criminal investigation

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ARTICLE INFO

Article history:
Received 24 October 2014
Received in revised form 4 May 2015
Accepted 12 May 2015

Keywords:
DNA
Forensic
Transfer
Touch DNA

ABSTRACT

With the introduction of new multiplex PCR kits and instrumentation such as the Applied Biosystems 3500xl, there has recently been a rapid change in technology that has greatly increased sensitivity of detection so that a DNA profile can routinely be obtained from only a few cells. Research to evaluate the risks of passive transfer has not kept pace with this development; hence the risk of innocent DNA transfer at the crime-scene is currently not properly understood. The purpose of this study was to investigate the possibility of investigator-mediated transfer of DNA traces with disposable nitrile-gloves used during crime-scene examinations. We investigated the primary transfer of freshly deposited DNA from touched plastic, wood or metal substrates and secondary and tertiary transfer by a person wearing disposable nitrile-gloves and onto a third object. We show that with use of the new highly sensitive technologies available in forensic DNA analysis there is an enhanced probability to obtain a DNA-profile which has not been directly deposited on the object but is an outcome of one or more transfer events. The nitrile-gloves used by investigators during exhibit examination can act as a vector for DNA transfer from one item to another. We have shown that the amount of DNA deposited on an object affects the probability of transfer. Secondly, the type of substrate material that DNA is deposited onto has an impact on transfer rates.

1. Introduction

The transfer of DNA to a crime scene or items related to the crime event can happen in several ways. Transfer may be described as “active” or “passive” [1]. Active transfer of DNA traces originating from the perpetrator occurs during the crime event itself; DNA is transferred via direct contact or aerosol e.g. from saliva spray to the surroundings. Passive transfer can be completely unrelated to the crime-event. Via this route, DNA can be transferred to crime related objects by a vector (secondary transfer) or by aerosol transfer of cells already present in the surroundings (e.g. in house-dust). Because there is an unfortunate tendency, to associate a crime-stain profile with direct evidence of the crime-activity, there are considerable dangers associated with lack of understanding of the various risks of alternative (innocent) means of transfer. This concern is gaining increased attention. Several studies have been conducted to investigate secondary transfer [2–5]. Goray et al. [6] found that the types of primary and secondary substrates, the level of moistness of the sample and the manner of contact, all played important roles in transfer of DNA. The initial deposit of DNA must be of sufficient quantity and quality to be detected and a good shedder is more likely to deposit significant amounts. Both Lowe et al. [7] and Farmen et al. [8] observed the event of secondary transfer of “touch” DNA via an individual to a second object when the first individual involved was classified as a good shedder. The surface of the substrate is a factor that was observed to have an effect on DNA depositions during contact, comparing items held for 60 s, Daly et al. [9] found that more DNA was recovered from wood than from fabric, and the least was recovered from glass. It is expected that the way the object is handled (light, force and friction) can affect the transfer rate [3]. During a study on transfer during social interactions, Goray and van Oorschot [5] observed that a jug passed between the participants acted as an efficient vector for secondary transfer. In addition they found that the individuals acted as vectors for multiple transfer events of foreign DNA. Lehman et al. [10] performed another study on multiple transfer events. With glass or cotton as a substrate they attempted to transfer DNA six times. They found that “touch” DNA produced a full profile only on the first substrate, and partial profiles from the second to the fifth substrate when the substrate was glass. When the substrate was cotton only a partial profile on the first substrate was achieved.

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http://dx.doi.org/10.1016/j.fsigen.2015.05.009
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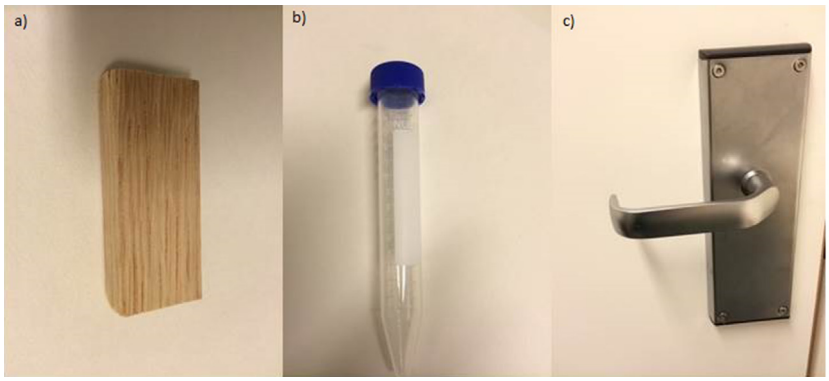

Fig. 1. The material used for initial deposition of DNA, (a) piece of wood, (b) plastic tube and (c) metal door handle.

1.1. Secondary transfer at the crime-scene

With the introduction of new multiplexes and instrumentation such as the Applied Biosystems 3500xl, there has recently been a rapid change in technology that has greatly increased sensitivity of detection so that a DNA profile can routinely be obtained from only a few cells [11]. Research to assess the risks of passive transfer has not kept pace with this development; hence the "hidden" risk of innocent DNA transfer at the crime-scene is currently not properly understood.

Crime scene investigators always use extensive protective clothing to secure the integrity of the crime scene from inadvertent contamination of their own DNA. Although disposable clothing and gloves prevent the investigator from inadvertent contamination of the crime-scene, there is potential for the investigator to act as an unwitting vector of DNA transfer between items within the crime-scene itself. This is termed as "investigator-mediated" transferred by Gill [1]. Szkuta et al. [12] studied the possibility of investigator-mediated DNA transfer via high risk vectors – latex gloves, scissors and forceps commonly used during exhibit examination. They tested the transfer of a dry blood spot under light and heavy transfer conditions and successfully demonstrated the transfer of DNA adequate for identification purposes in both heavy and light scenarios for scissors and gloves. Gloves as a medium for secondary transfer was also observed by Poy and van Oorschot [13] where they observed DNA transfer from an examined exhibit and onto a magnifying lamp with no direct contact between the two.

The purpose of this study was to investigate the possibility of investigator-mediated transfer of DNA traces with disposable nitrile-gloves used during crime-scene examinations. We investigated the primary transfer of freshly deposited DNA from touched plastic, wood or metal substrates and secondary and tertiary transfer by a person wearing disposable nitrile-gloves and onto a third object. The procedure was designed to illustrate the situation where a person handles an object and deposits DNA. During examination of the crime scene or evidence an investigator wearing nitrile-gloves first handles an object and DNA is transferred to the investigator by secondary transfer. Next, still wearing the same gloves, the investigator handles a different object or surface and DNA is transferred from the gloves to this surface. If this occurs, tertiary transfer of DNA could lead to connection of an innocent person to the crime.

The background idea for the experimental design was based on the case of the murder of Meredith Kercher [1] where the defense proposed that an investigator in the case may have inadvertently transferred DNA of a suspect from a door handle[1] to another piece of evidence in the case (a bra-clasp recovered from the floor of the crime-scene). To our knowledge no experimental evidence has been presented to illustrate whether passive transfer in this particular example is possible.

2. Materials and methods

2.1. Material

The material used for the transfer events was pieces of wood (5 cm × 2 cm × 0.5 cm) cut from untreated oak lists, plastic disposable conical tubes with screw caps, a metal door handle, nitrile disposable gloves, pieces of fabric (cotton sheets) and Labsorb cellulose coted bench papers (Ahlstrom Munktell) with a surface similar to fabric. The fabric and paper had a drawn up placement for hand prints. The materials used for the initial (primary) deposition of DNA are shown in Fig. 1.

Treatment of material before experiments:

1. The pieces of wood were polished with sand paper and cleaned with a 70% ethanol wipe. The pieces of wood and the cotton sheets were autoclaved for 20 min at 121 °C and 2 atm.
2. The door handle was cleaned with RNase AWAY™ Surface Decontaminant (thermo scientific) and 70% ethanol wipes prior to all transfer chains.
3. The wood, plastic, fabric and paper items were UV irradiated for approximately 30 min on each side to degrade any extraneous DNA present on them.
4. The objects were stored in sealed bags until they were used in the experiments.
5. Control samples were taken from a specimen of all items before the transfer experiments in order to preclude any contaminant DNA.

2.2. Transfer procedure

Three donors, previously assigned to be good shedders (known to frequently provide full profiles from touched items) participated in the experiments. The donors were asked to wash their hands and the transfer procedure was then performed after 1–2 h. The donors picked up the first substrate (pieces of wood or plastic tube) and handled it with medium pressure and friction in their hands for 30 s. The wood/plastic piece was then placed onto a clean bench paper. A second person, the "investigator", wearing nitrile-gloves and full laboratory cover (laboratory coats, hair nets and face masks) picked up the same substrate and handled it in the right hand glove (moved around in the hand) for 30 s. The substrate was

[1] The proposition is that the suspect had unsuccessfully attempted to open a locked door; the investigator forced to open the door and forcibly touched the door handle wearing gloves and then touched the evidence without changing the gloves.

placed back onto the bench paper and the right hand glove was held with medium pressure and friction against a new pre-cleaned piece of bench paper or fabric. Only one substrate was handled by the donor at each sampling and the samplings took place over several weeks. In the case of the metal door handle the donors simulated the "suspect" trying to open a locked door by pulling and forcing a metal doorknob up and down for about 30 s. A second person impersonating the "investigator", dressed in full protective gear as previously explained, then attempted to open the door in the same manner. Directly after opening the door, the "investigator" picked up the piece of fabric with the right hand and examined it for 30 s, the fabric was then put back into place. The transfer chain is described in Fig. 2. Samples were then collected from all substrates in the transfer chain after the transfers were completed. Each single transfer chain was completed within at least 10 min. The procedure was repeated 11 times for wood, 9 for plastic and 10 for metal, giving a total of 30 transfer chains and 90 samples. Donor 1 participated in the procedure 8 times, donor 2 participated 15 times and donor 3 participated 7 times. All donors handled each initial substrate type (plastic, wood or metal) at least once.

2.2.1. Transfer procedure – reduced investigator handling time

Since the substrate handling time by the investigator (30 s) in each transfer might be longer than that expected in a real case scenario, the experiment was repeated 6 times with a shorter investigator handling time. All transfers were conducted as previously explained except that the time of handling by the investigator was reduced to 10 s (glove in contact with substrate 1 and glove in contact with substrate 3). The substrate handled by the donor was a plastic tube in four cases and a metal door handle in two cases.

2.3. Sample processing

DNA was recovered from all items by the use of DNA-free minilifting tapes (Scenesafe FAST™). The minitape was repeatedly pressed against the surface of the objects. One minitape was used per object. All samplings were performed in the same day that the transfer events took place. The minitape was cut into smaller pieces and placed into an extraction tube directly after the sampling. The samples were stored at room temperature until extraction.

DNA was extracted by the 5% Chelex® procedure where 250 μL Chelex® was added to the samples. All samples were quantified with Quantifiler® Duo Kit (Applied Biosystems) on the 7500 real-time PCR system (Applied Biosystems) with the standard in-house protocol which has a standard curve spanning from 0.0115 ng/μL to 16.7 ng/μL and a total volume of 27 μL for each reaction (4 μL sample). All samples were amplified using the PowerPlex® ESX 17 System kit (Promega) as recommended by the manufacturer (0.5 ng template, 25 μL reaction volume and 30 amplification cycles). Samples that had lower concentrations than the recommended template amount were amplified with the maximum template volume of 17.5 μL. The samples were amplified on a GeneAmp® PCR System 9700 (Applied Biosystems). Samples were injected on the Applied Biosystems 3500xl Genetic Analyzer at 1.2 kV for 10 s. The results were analyzed in the GeneMapper® ID-X Software (Applied Biosystems) and the limit of detection (LOD) for alleles was set to 200 RFU. Profiles were compared to reference samples of the donors.

2.3.1. Sample processing – reduced investigator handling time

Sample processing took place as previously explained except from the following changes: the samples were amplified using the PowerPlex® ESX 17 Fast System kit (Promega). Samples were injected on the Applied Biosystems 3500xl Genetic Analyzer at 1.2 kV for 24 s.

2.4. Data analysis

ANOVA was used to test if expected values were the same in different groups. In cases not fulfilling the assumption of normality, the non-parametric alternative Kruskal–Wallis was used.

2.4.1. Logistic regression model

A logistic regression was performed to model the probability that a high quality profile could be transferred during secondary or tertiary transfer based on the amount of DNA found on the first substrate (quant). The profile quality classes (Q, response variable in logistic regression) were defined by the number from 0 to 16 (Amelogenin is excluded) of observed full donor loci (l) in each sample as follows:

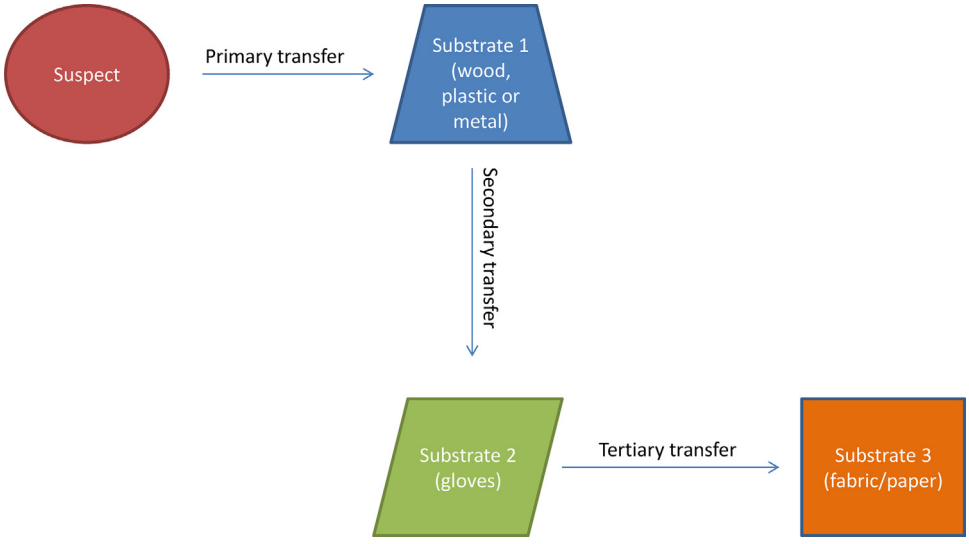

Fig. 2. The donor touches substrate 1 and transfers DNA to the substrate (primary transfer). An investigator wearing gloves touches substrate 1 and DNA is transferred to the gloves (secondary transfer). The investigator touches substrate 3 and DNA is transferred from the gloves to substrate 3 (tertiary transfer). Samples were collected from all substrates after the transfer chain was completed.

$Q = \text{High if } l \geq 10(10-16)$

$Q = \text{Low if } l < 10(0-9)$

The high profile class is considered usable for reporting in casework, whereas the low profile class may be borderline or too low-level to report.

The purpose is to model the probability of each of the groups given the "quantitation result" (quant, ng) as the predictor, to prepare an exploratory analysis to show how much the quant influences the probabilities.

The logistic model is thus defined as:

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 * \text{quant}$$

where $p = P(Q = \text{High/quant})$

3. Results

3.1. Controls

All quantitation results were negative and no profiles were retrieved from the control samples taken from the pre-cleaned objects used in the study.

3.2. Effect of substrates

The total amounts of DNA (ng) in each sample were calculated by multiplying the concentrations from the quantitation (per μL) with the total sample volume. The total amount of DNA deposited during primary transfer to substrate 1 was calculated by adding together the DNA concentrations found on substrate 1 (s1), substrate 2 (s2) and substrate 3 (s3) (see Fig. 2). This was repeated for all transfer chains. The percentage transfer of DNA during secondary transfer to s2 is the amount of DNA found on s2 and s3 divided by the total amount of DNA deposited, i.e., (s2 + s3/s1 + s2 + s3). The percentage transfer of DNA to the tertiary substrate is the amount of DNA found on the tertiary substrate divided by the amount found on the secondary and tertiary substrate, (s3/s2 + s3). The average total DNA quantity deposited during primary transfer to s1 and the average percentage transfer to s2 and s3 are presented in Table 1.

Less DNA was deposited on the metal than on the wood and plastic objects. A significant difference was observed between the objects from Kruskal–Wallis test ($p = 0.008$). Proportionally more DNA was transferred from the metal doorknobs to the gloves (substrate 1 → substrate2) than from plastic and wood. ANOVA showed a significant difference between groups ($p < 0.001$). A Tukey post hoc test showed that there was a significant difference in transfer proportions between metal and wood ($p < 0.001$) and between metal and plastic ($p < 0.001$). The difference between plastic and wood was not significant. For the tertiary transfer event (substrate 2 → substrate3), as expected (the surfaces of the fabric and bench paper are very similar), no difference was detected between the transfer from gloves to paper and fabric ($p > 0.05$). It was therefore valid to combine the results for paper and fabric.

ANOVA showed no significant differences between the initial DNA-depositions made by the three DNA-donors used in the study ($p = 0.552$).

The results from the initial deposits of DNA to each substrate and the transfer rates from each transfer are shown in Fig. 3.

3.3. Transfer of DNA

As explained in Section 2.4.1 the profiles were divided into high and low quality profiles (excluding Amelogenin).

For the samples collected from the first substrates (metal, plastic or wood, s1) 83% was of high quality and 17% was low quality profile. For the second substrates (gloves, s2) 53% was high quality and 47% was low quality. For the third substrates (fabric or paper, s3) 17% was high quality and 83% was low quality. This means that in 5 out of 30 transfer chains the result was tertiary transfer of a profile of good enough quality for case reporting and database searches given the previously mentioned quality standards. The quantitation results and proportion of donor allele's from the samples after all transfer events are provided in Fig. 4. The number of donor alleles observed in the samples collected from each substrate in each transfer chain is given in Table 2 (data includes Amelogenin).

3.4. Transfer in experiments with reduced investigator handling time

Regarding the four cases when substrate 1 was a plastic tube, all four experiments performed with reduced investigator handling time resulted in successful transfer of DNA and high quality profiles were found from samples collected in each step of the transfer chain. When substrate 1 was a metal door handle only a few alleles, or no alleles, could be observed on substrate 3. The results from the experiment are given in Table 3.

3.5. Logistic regression model

The probability of a high or low quality profile being transferred to the second or third substrate was estimated as explained in Section 2.4.1 and the results are given in Fig. 5. Performing the Hosmer–Lemeshow goodness of fit test indicated no reason to reject the models.

From the 95% confidence interval curves it was demonstrated that there is a high degree of uncertainty in the prediction curves and that for parts of the prediction area the curves are overlapping and therefore one should be cautious to conclude regarding the profile quality.

For some parts of the models the curves are not overlapping: from model a, if the DNA concentration on substrate 1 is more than 0.07 ng/μL it is more likely that a high profile will be recovered from substrate 2. If the concentration on substrate 1 is below 0.1 ng/μL it is more likely that a low profile will be recovered from substrate 3, based on model b.

Table 1
The average DNA quantity (standard deviation) from the first deposit on each substrate type and the average proportion of DNA transferred (standard deviation).

	Metal	Plastic	Wood	Paper/fabric
Average DNA quantity in ng transferred donor → substrate 1	5.55 (7.68)	17.3 (11.56)	18.42 (16.00)	–
Average DNA proportion transferred substrate 1 → substrate 2	64.23 (24.97)	29.13 (16.11)	15.95 (9.31)	–
Average DNA proportion transferred substrate 2 → substrate 3	–	–	–	32.04 (26.5)

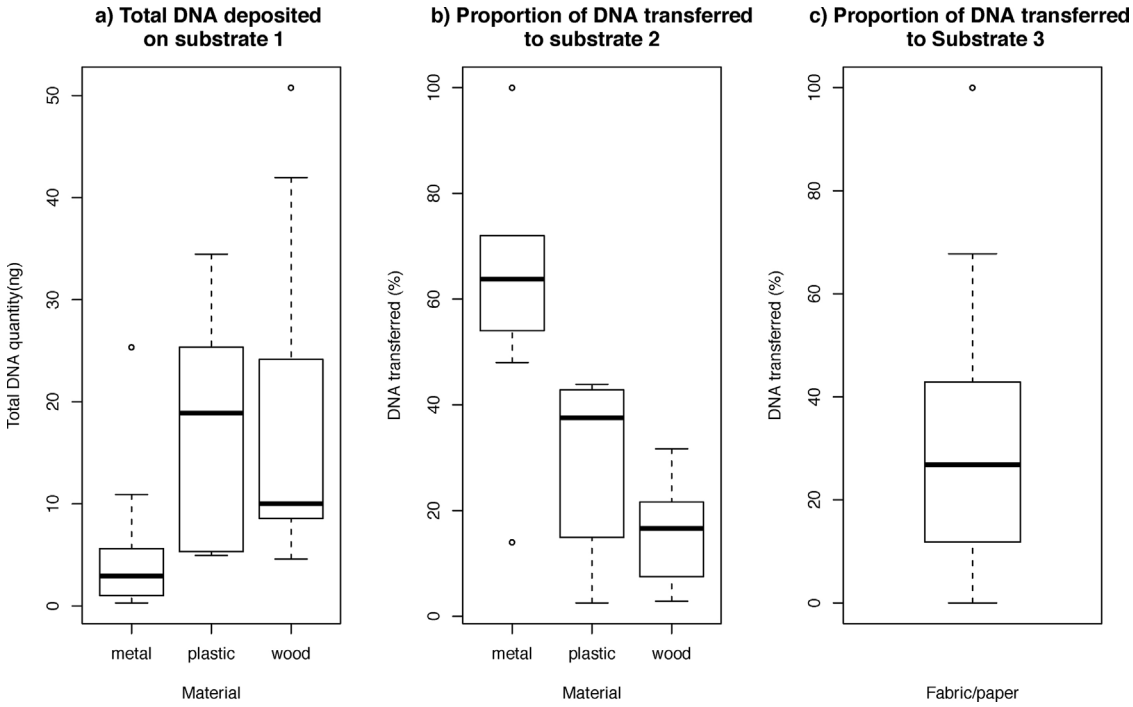

Fig. 3. (a) The total amount of DNA deposited on the three types of substrate 1 materials (metal, plastic or wood) after primary transfer from the donor. (b) The proportion of DNA transferred during secondary transfer to substrate 2 (gloves) after contact with metal, plastic or wood. (c) The proportion of DNA transferred during tertiary transfer to substrate 3 (fabric/paper) after contact with glove.

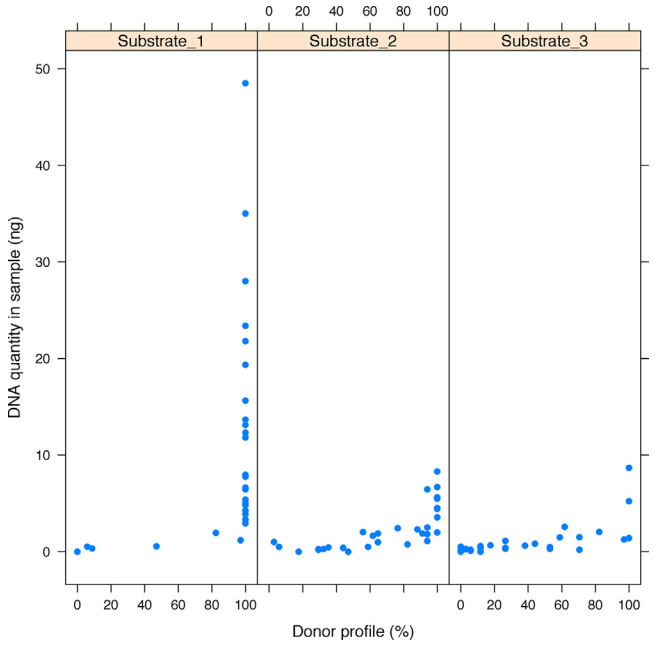

Fig. 4. Scatterplots showing the DNA quantity in the samples and the proportion of the donor profile recovered from (a) substrate 1 (wood, plastic or metal) primary transfer, (b) substrate 2 (gloves) secondary transfer and (c) substrate 3 (paper or fabric) tertiary transfer.

3.6. Evidence of quaternary transfer events

Unknown alleles were observed in 17 of the 108 analyzed samples. In 13 of the cases the alleles occurred only on the first substrate (s1), but unknown alleles were also observed on the second substrate (s2) in two cases and on the third substrate (s3) in two cases. The samples that contained most unknown alleles were all in the same transfer chain. From this transfer chain the unknown alleles were compared to a reference sample of the girlfriend of the donor, and a match was found in all substrates of the transfer chain. The girlfriend of the donor had never previously visited the office where the samplings were performed and they had not been in contact for 10 h prior to sampling. The donor had also washed his hands after their last contact.

The proposed chain of transfer is complex, comprising four events in this example, and can be explained as follows: donor1 to donor 2, to substrate 1, to substrate 2, to substrate 3.

4. Discussion

Our findings show that under the conditions used in this study "touch-DNA" can be transferred between multiple objects, and that disposable gloves can act as an efficient transfer vector.

In the first series of experiments an investigator handling time of 30 s was used. This might be longer than expected in a realistic transfer scenario. But we have seen that even when the investigator handling time was reduced to 10 s, full donor profiles could be obtained from samples in all steps of the transfer chains. This proves that this type of transfer is also possible with a much shorter handling time. The results may indicate that transfer is not crucially dependent on the handling-time as most of the transfer can happen with a few seconds of contact. This supports the findings by Van Oorschot and Jones [14] that a large proportion of DNA will be transferred during the initial contact. The transfer success in the reduced handling time experiment was actually higher than with the initial experiments when substrate 1 was plastic. This difference might be attributed to variation in hand-washing intervals which could vary between one and two hours. It is known that increasing the time since hand wash increases the amount of DNA that will be transferred when touching an object [7,15]. It is certainly not unrealistic that a person on a daily basis would have intervals of two hours or more between washing their hands. On the other hand, the experiments carried out when substrate 1 was metal demonstrated that the results were in the

Table 2
For each of the 30 transfer chains, the table records the donor (1, 2 or 3) that delivered the initial deposit, the material (substrate 1) the deposit was made on and the number of donor alleles (full loci) including Amelogenin observed in the samples collected from each step of the transfer chain (substrate 1, substrate 2, substrate 3).

Transfer chain	Donor	Material substrate 1	Donor alleles substrate 1	Donor alleles substrate 2	Donor alleles substrate 3
1	1	Metal	34 (17)	30 (14)	4 (0)
2	2	Metal	0 (0)	10 (4)	2 (1)
3	2	Metal	2 (1)	21 (9)	6 (2)
4	2	Metal	33 (16)	22 (10)	28 (11)
5	2	Metal	29 (13)	22 (9)	15 (6)
6	2	Metal	34 (17)	34 (17)	20 (8)
7	2	Metal	34 (17)	26 (11)	9 (4)
8	3	Metal	3 (1)	15 (5)	1 (0)
9	3	Metal	15 (4)	11 (3)	0 (0)
10	3	Metal	0 (0)	10 (3)	2 (1)
11	1	Plastic	34 (17)	34 (17)	33 (16)
12	2	Plastic	34 (17)	32 (15)	0 (0)
13	2	Plastic	34 (17)	7 (3)	0 (0)
14	2	Plastic	34 (17)	34 (17)	34 (17)
15	2	Plastic	34 (17)	32 (15)	3 (1)
16	2	Plastic	34 (17)	30 (13)	4 (1)
17	2	Plastic	34 (17)	34 (17)	18 (7)
18	3	Plastic	34 (17)	32 (15)	34 (17)
19	3	Plastic	34 (17)	34 (17)	21(8)
20	1	Wood	34 (17)	34 (17)	34 (17)
21	1	Wood	34 (17)	21 (9)	4 (1)
22	1	Wood	34 (17)	31 (15)	24 (10)
23	1	Wood	34 (17)	34 (17)	24 (10)
24	1	Wood	34 (17)	16 (7)	9 (2)
25	1	Wood	34 (17)	24 (10)	1 (0)
26	2	Wood	34 (17)	12 (5)	9 (4)
27	2	Wood	34 (17)	32 (15)	13 (5)
28	2	Wood	34 (17)	34 (17)	18 (6)
29	3	Wood	34 (17)	2 (1)	0 (0)
30	3	Wood	34 (17)	1 (0)	0 (0)

Table 3
For each of the 6 transfer chains in the experiments with reduced investigator handling time, the table records the donor (2 or 3) that delivered the initial deposit, the material (substrate 1) the deposit was made on and the number of donor alleles (DNA-quantity (ng)) observed in the samples collected from each step of the transfer chain (substrate 1, substrate 2, substrate 3).

Transfer chain	Donor	Material substrate 1	Donor alleles substrate 1	Donor alleles substrate 2	Donor alleles substrate 3
31	2	Plastic	34 (20.0)	34 (16.7)	34 (3.4)
32	2	Plastic	34 (26.3)	34 (13.2)	34 (2.4)
33	2	Plastic	34 (5.8)	34 (6.8)	32 (2.3)
34	2	Plastic	34 (9.3)	34 (25.8)	34 (7.9)
35	3	Metal	34 (1.7)	33 (3.8)	8 (0.2)
36	3	Metal	32 (1.8)	33 (1.0)	0 (0.5)

same range as previously shown. At the time of the experiment with reduced handling time there had also been made some minor changes in the routine analysis at the institute. The routine amplification method had been changed from PowerPlex® ESX 17 System kit (Promega) to PowerPlex® ESX 17 Fast System kit (Promega) and the injection time used for the new kit on the Applied Biosystems 3500xl Genetic Analyzer at 1.2 kV was 24 s while it had been 10 s for the previous used kit. This could also be the reason for some of the increase in success. But the quantitation results indicated that the amount of DNA present in the samples was adequate to achieve a full profile by the old standards in at least three of the tertiary transfer events. In addition these settings are the in-house validated standard routine analysis method found to be best suited for the kit and currently used on all case samples analyzed. Therefore this proves that this transfer scenario could be possible in current routine case analysis.

In this study we observed that DNA was readily transferred to wood and plastic, while less was transferred to a metal door handle. Although this is a relatively small study, the results confirm the observations of Daly et al. [9] that more DNA was transferred to wood and fabric than to glass. The hard and smooth surface of the metal could be compared to the surface of a glass vial, while the rough surfaces on the wood and screw top on the plastic tube will facilitate cell transfer by abrasive action. Goray et al. [3] observed that the initial deposit of DNA was 20 times greater when deposited to a porous cotton surface than when deposited onto a smooth and hard plastic surface. Although DNA was transferred more readily to porous rough surfaces in the initial primary transfer, less DNA was secondarily transferred from these surfaces to the gloves. On the other hand DNA transfer was much higher from the smooth metal surface to the gloves. These findings are consistent with observations of Goray et al. [3] who found that more DNA was transferred from plastic to cotton than in the reversed case example.

The transfer rates from gloves to fabric/paper are more variable. This might be due to lower concentration of DNA on the gloves and larger impact on stochastic effects from sampling and extraction process. The samplings were performed over several months and different “investigator” assistants were used to transfer DNA via gloves, although the assistants were given the same instructions there may have been some difference in the performance. In some cases more DNA was found on the paper/fabric than on the gloves, this was probably because the samplings were made after the transfer took place and most of DNA had transferred to the fabric.

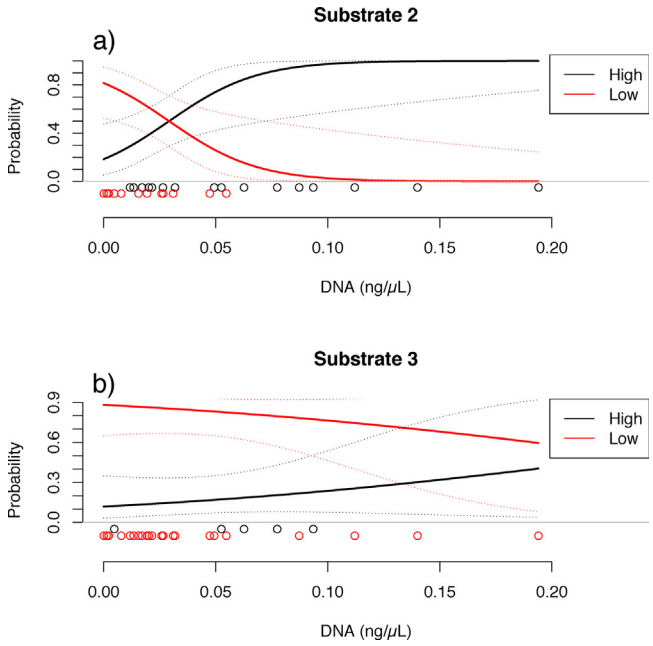

Fig. 5. The probability of a high or low quality profile being transferred given the DNA concentration found on substrate 1 (a) during secondary transfer from substrate 1 to substrate 2 or (b) during tertiary transfer from substrate 2 to substrate 3. The model is based on logistic regression. The 95% confidence intervals for the curves are given as the dotted lines in the plots.

Also in two transfer chains with the metal door handle as substrate 1, no DNA was found on the door handle itself, while DNA was recovered from the gloves and to some degree from the fabric in the same transfer chain. In this case it is possible that most of the DNA present could have been wiped of the door handle by the glove. There were in total 8 transfer chains where more DNA was measured on substrate 2 than on substrate 1. In some cases up to 70% of the sample had been transferred.

We observed a higher success in obtaining DNA profiles from low DNA quantitates than Daly et al. [9]. Some of the observed variation may be due to different quantitation methods used in the two studies (Quantifiler® and Quantifiler® Duo), and it is also expected that different labs will have different equipment and protocols that lead to different results. But the majority of this variation is most likely to be associated with the more sensitive methods used in the present study (PowerPlex® ESX 17 and 3500xl Genetic Analyzer) compared to the older less sensitive, AmpFLSTR® SGM Plus® (Applied Biosystems) and 3130 Genetic Analyzer (Applied Biosystems) used by Daly et al. [9]. Note that in the past 2 years, the majority of labs have moved to the much more sensitive systems, hence these differences are to be expected. There are limitations of the quantitation method. The amplification with the PowerPlex® ESX 17 (Promega) is more sensitive than the quantification with the Quantifiler® Duo Kit (Applied Biosystems). On several occasions, no DNA was measured during quantitation, but alleles could be detected after PCR. Quantification is less efficient at low levels due to stochastic effects.

Lehmann et al. [10] conducted a transfer series where touch DNA was deposited on glass or cotton and transferred onto new substrates with six transfers in each series. They only observed a full profile on the first substrate and partial profiles on the 2nd to 5th substrate when the substrate was glass. In the present study a full profile was observed all the way to the third substrate. Since the techniques used in the Lehmann et al. study [10] are in the range of the same sensitivity as used here, the reason for the increased transfer rates in our study is most likely due to the type of substrates and the manner of contact. In this study both pressure and friction was used during the transfers and these are known to facilitate transfer [3,6], while only pressure was used in Lehmann et al. [10].

While the observations of secondary transfer made by Farmen et al. [8] were from low template analysis using enhanced 34 amplifications cycles, the observations made here were all from routine analysis using the manufacturers recommendations (30 amplification cycles). Lowe et al. [7] also observed secondary transfer to some extent during standard analysis, but in much lower numbers than the present study, with secondary transfer of high class profiles in more than 50% of the cases and transfer of 10 donor alleles or more in 90% of the cases. This indicates that there is an increased probability of observing secondary transfer from standard analysis.

We have also observed that foreign DNA present on a person's hand can be transferred onto a new object and follow the transfer chain to the third object. This was only a partial profile but gives support to the fact that under some conditions DNA can be transferred up to four times. This supports Gill's assertion of "sticky DNA" [1] where multiple transfer events can be demonstrated to occur. It would be normal to expect that mixtures of both donors would be observed if there are inter-donor transfers, whereas "investigator-mediated" transfers do not reveal mixtures involving the investigator since he/she is wearing protective clothing that prevents this kind of cross contamination. This is why this kind of substrate 2 → substrate 3 transfer is especially dangerous – it cannot be inferred by the presence of a mixture.

As pointed out in the introduction, the background for this study was based on a case (miscarriage of justice of Amanda Knox and Meredith Kercher) [1] where tertiary transfer with a door handle as a vector of transfer was a possible explanation of the presence of a DNA profile on an evidence item[2]. In this study one occasion of a high quality DNA-profile transferred from the door handle (s1) to the gloves (s2) and further onto the fabric (s3) was observed. There were also instances of a partial profile being transferred. We used a metal door handle in this study but have observed that less DNA was transferred to metal than to the other material surfaces, therefore one could expect more successful transfers if the door handle was of a different material (such as wood). Nevertheless, we have shown that under the conditions of transfer described in this study this could be a possible route of DNA transfer. The effectiveness of gloves as a vector of transfer is also supported in the recent paper by Szkuta et al. [16].

From the logistic regression prediction model there is correlation between the amount of DNA found on the first substrate and the possibility of achieving a profile of a high or low quality class on the second and third substrates. But it is also obvious that factors other than the initial DNA concentration are influencing the transfer probability of a profile. The surface of the materials used in the transfer events will have some impact on transfer rates. We have demonstrated that under controlled conditions the probabilities that describe transfer are subject to a large number of variables that are difficult to control, even under strict experimental regimes. In real casework, this uncertainty places serious challenges on the utility of Bayes nets solutions that may seek to inform models with a small number of probabilities [17] since many variables will be unknown.

Good shedder donors were used to make the initial DNA depositions, these persons were previously known to frequently deposit full profiles to touched objects. Since we have seen that the

[2] In this case the defendant attempted to open a locked door, without success. Investigators subsequently obtained access and it is possible that DNA from the door handled was transferred to the evidence. The investigator contact was simulated for 30 s, and intensely in this example.

transfer probability is dependent on the amount of DNA deposited during the initial contact it is expected that the probability of this type of transfer taking place will vary between good and poor shedders. However, it is very unlikely that in a real case the shedder status of a defendant will be known. Therefore we were more concerned with the question – is it reasonable that secondary or tertiary transfer may occur via gloves, or is it impossible? Our work clearly demonstrates the former to be true.

5. Conclusions

As new and more sensitive techniques are starting to become a part of common practice in the field of forensic DNA analysis, great care to prevent transfer and contamination should be taken during handling and examination of evidence. It is not possible to state with certainty that low levels of DNA could not be the outcome of secondary or subsequent transfer.

Acknowledgements

PG and TE have received funding support from the European Q3 Union Seventh Framework Programme (FP7/2007-2013) and Euroforgen-NOE, under grant agreement no. 285487.

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