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ORIGINAL ARTICLE

# An evaluation of the transfer of saliva-derived DNA

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**Abstract** Studies of DNA transfer have focused largely on the transfer of sloughed off epithelial cells from individuals' hands. This research examines primary, secondary, and tertiary transfer events involving DNA originating from saliva, a commonly encountered body fluid. More routine human behaviors were simulated to evaluate transfer, and the effects of drying time, moisture, and surface composition were investigated. The results agree with previous findings which indicate that the presence of moisture, as well as a smooth nonporous surface as the primary substrate, increases the efficiency of transfer. Previous transfer studies have found that the last individual to come into contact with an item is usually the major contributor to the resulting DNA mixture, unless conditions are simulated in which a "good shedder" serves as the primary depositor and a poor shedder serves as the secondary depositor. The results of this study indicate that when saliva is the source of the transferred DNA, the primary depositor is often the major contributor. These findings suggest that shedder status is less relevant with regard to touch DNA samples in a forensic setting and emphasize the need for caution when analyzing such samples.

**Keywords** Primary transfer · Secondary transfer · Tertiary transfer · Saliva · STR typing · Shedder · Moisture

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## Introduction

Validated forensic DNA analysis techniques are capable of providing reliable genetic information from biological evidence that can be used to associate or exclude individuals as potential contributors of samples collected at crime scenes [1–4]. These methods offer both high discrimination power and a high sensitivity of detection. In 1997, van Oorschot and Jones [5] found that objects handled by single individuals yielded profiles consistent with those of the handlers, while objects handled by multiple individuals produced a DNA mixture. They also suggested that a handshake between two individuals for as little as 1 min was sufficient to transfer DNA between the individuals. Since then, "touch DNA" analysis, the examination of DNA transferred through contact, has become a subject of interest in the field of forensic genetics. Subsequent studies investigated primary transfer, i.e., events wherein DNA is directly transferred from an individual to an object or another individual [6–8]. Barbaro et al. [9] analyzed DNA originating from residual sweat and epithelial cells left on pens and compared it with DNA obtained from semen and saliva stains; the results allowed for correct source attribution. Other studies [10–12] have found that certain individuals, termed "good shedders," appear to have a greater propensity for depositing DNA when touching an object, as measured by complete genetic profiles; others, described as "poor shedders," do not leave behind as much DNA. Djuric et al. [13] also noted that transfer from an individual to another individual follows a pattern similar to that of transfer from an individual to an object, and that the DNA obtained is often a mixture consistent with the profiles of both individuals. Touch DNA analysis has since been employed for the investigation of a wide variety of commonly touched sample types, including bullet casings, documents, and even bedding [14–17]. The principles associated with this form of DNA analysis have

direct bearing on the interpretation of forensic profiles and the relevance of such information even for single-source samples. Therefore, it is necessary to understand the potential impact of DNA transfer on a given sample.

While the aforementioned studies mainly addressed primary transfer, other studies have investigated secondary transfer, a variation of DNA transfer in which the original source individual does not make direct contact with the final recipient individual or object. Instead, DNA is transferred through an intermediary vector. Secondary transfer was noted by van Oorschot and Jones [5] in their original study, where they mentioned that the genetic profiles of handlers of an item were sometimes observed in profiles obtained from the hands of subsequent holders of the item. Additional studies confirmed these findings and documented the occurrence of secondary DNA transfer [18–21]. Studies of secondary transfer involving human vectors often indicated that, under normal conditions, the majority of detectable DNA on the final object generally originates from the vector [18, 22, 23]. Profiles originating from the primary individual have only been observed as the dominant profiles in secondary transfer studies conducted under arranged conditions in which the primary individual was a good shedder and the vector was a poor shedder [10, 18]. Furthermore, most secondary transfer studies have been limited in that they have focused mainly on DNA deposited through skin epithelial cells sloughed off during contact with individuals' hands.

Skin cells are expected to slough off and transfer through contact. However, skin cell transfer studies are somewhat contrived and likely do not approximate real-world activities. Skin cells may not be the primary source of transfer DNA in a number of scenarios, and other sources richer in DNA may be transferred routinely. Saliva, for example, contains substantial amounts of DNA, well above what may be considered trace levels [24–26]. However, there have been very few studies of DNA transfer with regard to saliva, even though it is a body fluid commonly encountered and transferred between individuals and/or objects. For instance, it is not uncommon for a person to lick his or her thumb while turning the pages of a book or for an individual to hold a pen in his or her mouth while studying or reading. In the latter example, the deposition of saliva-derived DNA on the pen is a primary transfer event. If the pen is later handed to a second person, the transmission of the first individual's DNA to the surface of the second individual's hand constitutes a secondary transfer event.

The study described herein was conducted under the hypothesis that saliva, which is rich in epithelial cells, may be a more prevalent source of genetic material during transfer events than the epithelial cells deposited from a hand. Thus, the transfer of saliva-derived DNA could often result in higher levels of detectable genetic material than have been observed with previous hand contact transfer studies. Furthermore, the genetic profile of the primary individual, i.e., the source, may be more prevalent in such cases of secondary transfer due to the presence of saliva-derived epithelial cells.

## Materials and methods

### Subjects

Following the University of North Texas Health Science Center IRB approval, four individuals (two males and two females) were used for this study. One male subject was paired with one female subject in a manner that allowed for the fewest shared alleles between the genetic profiles of the individuals within each pair to obtain maximum value of mixture data interpretation.

### Extraction, quantification, amplification, capillary electrophoresis, and data analysis

DNA was collected from each donor using the Fitzco® CEP Swab™ Cell Collection System (Fitzco Inc., Spring Park, MN) and extracted from the swabs using the Qiagen® QIAamp® DNA Mini (Qiagen Inc., Valencia, CA) extraction procedure for buccal swabs. The quantity of extracted DNA was determined using the Applied Biosystems® Quantifiler™ Human DNA Quantification Kit (Life Technologies, Carlsbad, CA) on an Applied Biosystems® 7500 Real-Time PCR System (Life Technologies), according to the manufacturer's protocol. Amplification was performed using the Applied Biosystems® AmpFlSTR® Identifiler® Plus PCR Amplification Kit (Life Technologies) on an Applied Biosystems® GeneAmp® PCR System 9700 thermal cycler (Life Technologies), according to the manufacturer's recommendations. Capillary electrophoresis was then performed on an Applied Biosystems® 3130*xl* Genetic Analyzer (Life Technologies) using POP-4™ polymer (Life Technologies) and analyzed using Applied Biosystems® GeneMapper® ID v3.2 software (Life Technologies), according to the manufacturer's recommended protocol. In cases where the standard 28 cycles of PCR did not yield interpretable results, samples were re-amplified in duplicate using 34 cycles. Alleles were only called if they appeared in both replicates and had peak heights ≥50 relative fluorescent units (RFU).

### Experimental design

This study was divided into three sets of experiments to examine the different types of DNA transfer: primary, secondary, and tertiary. At the beginning of all trials, subjects washed their hands with soap and water and dried them. In

trials where thumbs were moistened with saliva, the subjects extended their tongues and ran their thumbs down their tongues once. Deposition of saliva on pen surfaces was accomplished by having the subjects hold the back (non-capped) ends of pens (that had been exposed previously to UV irradiation) in their mouths for 30 s. For some experiments, DNA was transferred via contact with plastic conical tubes. In these tests, subjects were required to grip UV-treated 50 mL conical tubes with moderate pressure for 15 s. In certain trials, the presence of sweat was simulated by spraying the subjects' thumbs or palms once with DNAse/RNAse-free distilled water from an atomizer. In all trials, DNA collection was performed using the double swab technique [27]. Unless otherwise noted, drying times of 5 and 30 min were employed. Tests were conducted in duplicate for each of these drying times. Specific descriptions of the individual experiment conditions are listed in Table 1.

### Data analysis

DNA quantification values were used to determine the amount of DNA loss due to transfer steps, as well as to gauge the general efficiency of the PCR. The efficiencies of the transfer events themselves were determined by calculating the percentages of alleles in each individual's profile that were observed following transfer. The proportions of DNA contribution by each individual in dual-subject trials were obtained by comparing the peak heights of alleles at loci that displayed at least one allele unique to one of the individuals in the test pair to the total contribution of those alleles. A locus containing only one unique allele from individual A, for example, was counted as having 100 % contribution from individual A. A locus containing a 750-RFU allele peak unique to individual A and a 250-RFU allele peak unique to individual B was counted as having 75 % contribution from individual A and 25 % contribution from individual B. Contribution percentages were then averaged for each individual across all loci to obtain the final values.

## Results and discussion

A number of studies have focused on the transfer of DNA between clean hands and/or objects. The study herein contributes to our understanding of primary and secondary transfer when another source relatively richer in DNA is involved in the transfer. Saliva is a common biological material that is routinely transferred among individuals. To address the potential impact of saliva on the interpretation of DNA typing results associated with transfer, primary and secondary transfer experiments were conducted that approximated more typical human behavior, but still in a controlled manner (e.g., male and female subjects were required to lick their thumbs or hold pens in their mouths to simulate common habits). An additional set of experiments addressed tertiary transfer events, where DNA deposited from an individual to an object or individual is then transferred to another object or individual. The genetic data collected from the experiments were analyzed to assess the amount of DNA transferred, the relative decrease in the levels of genetic material that occurred as the number of vectors increased, and the proportions of DNA that were contributed by the primary and vector individuals.

### Primary transfer trial results

The quantity of saliva-derived DNA obtained from the licked bare thumbs of the subjects was compared with that obtained from the subjects' gloved thumbs to roughly estimate DNA transferred in the single saliva deposition event. The DNA yield from the bare palms of the subjects also was quantified to develop a general baseline of native DNA levels for the individuals involved in the studies.

DNA quantification results for the primary transfer experiments, such as those shown for subject 001 (Fig. 1), revealed that, in many instances, slightly more DNA was yielded from gloved thumbs than bare thumbs. It is likely that this observation was due to the smoother, less porous surface of the glove allowing for more efficient collection of DNA via the swabbing technique than from the rougher, ridged thumb surface. The findings are consistent with those described by Goray et al. [19], who demonstrated that smooth, nonporous surfaces, such as plastic, yielded higher quantities of recovered transferred DNA than rougher, porous surfaces, such as cotton and wool. The effects of a smoother surface composition, in addition to the greater surface area, also explain why DNA was sometimes obtained in larger quantities from pens held in the mouths of subjects as opposed to the subjects' thumbs (Fig. 1). In addition, substantial variation in DNA yield is observed from one replicate to another in each trial, indicating, as expected, that deposited DNA varies widely from one instance to the next of deposition by licking. The variation in DNA deposition and the effects of the different surface areas on the DNA yield made the estimation of saliva-derived DNA quantity in a single saliva deposition event problematic. Thus, quantifying loss through transfer was difficult, and inferences made from this part of the study must be recognized as providing trends and general conclusions.

Amplification at 28 cycles of the DNA from the primary transfer trial samples yielded full genetic profiles in almost every case (Table 2). The notable exception to these results was the group of trial 3 samples (i.e., swabbing of bare palms), which generally yielded no genetic profiles. The DNA from the trial 3 samples was amplified using 34

**Table 1** Transfer experiments

| Trial no. | Transfer type | Transfer steps | Procedure |
|---|---|---|---|
| 1 | Primary | M→BTh | Thumbs were licked and swabbed after each drying period; 16 samples (4 per subject) |
| 2 | Primary | M→GTh | Similar to trial 1, except that subjects wore gloves before washing; 16 samples (4 per subject) |
| 3 | (Reference) | Pa | Bare palms were swabbed after washing, to collect DNA; 16 samples (4 per subject) |
| 4 | Primary | M→Pe | Saliva was deposited on pens, which were swabbed after each drying period; 16 samples (4 per subject) |
| 5 | Secondary | M→BTh→Tu | Similar to trial 1, except that subjects grasped sterilized plastic conical tubes after each drying time; tubes then were swabbed; 16 samples (4 per subject) |
| 6 | Secondary | M→Pe→Pa | Similar to trial 4, except that subjects were required to pass the pens to their designated partners after each drying time; pens were gripped like tubes; the partners' palms then were swabbed; 16 samples (8 per pair) |
| 7 | Secondary | M→GTh→Tu | Similar to trial 2, except that subjects gripped plastic conical tubes after each drying time; 16 samples (4 per subject) |
| 8 | Secondary | M→Pe→MstPa | Similar to trial 6, except that recipient subjects' palms were moistened before they grasped the plastic tubes, to simulate sweat; palms then were swabbed; 16 samples (8 per pair) |
| 9 | Secondary | M→MstBTh→Tu | Similar to trial 5, except that subjects' thumbs were moistened after each drying time, in order to imitate sweat; 16 samples (4 per subject) |
| 10 | Tertiary | M→BTh→Tu→Pa | Similar to trial 5, except that after grasping the plastic tubes, subjects passed them to their partners; partners grasped the tubes, and their palms then were swabbed; roles of each pair then were reversed; 5-min drying time only; 8 samples (4 per pair) |
| 11 | Tertiary | M→BTh→Tu→MstPa | Similar to trial 10, except that recipient partners' palms were moistened prior to gripping the tubes, to imitate sweat; roles of each pair then were reversed; 5-min drying time only; 8 samples (4 per pair) |
| 12 | Tertiary | M→Pe→Pa→Tu | Similar to trial 6, except that recipient partners each grasped plastic tubes after gripping the pens; tubes then were swabbed; roles of each pair then were reversed; 5-min drying time only; 8 samples (4 per pair) |
| 13 | Tertiary | M→MstBTh→Tu→Pa | Similar to trial 9, except that initial subjects' thumbs were moistened prior to grasping the tubes, to simulate sweat; recipient partners' palms then were swabbed; roles of each pair then were reversed; 5-min drying time only; 8 samples (4 per pair) |
| 14 | Tertiary | M→Pe→MstPa→Tu | Similar to trial 12, except that recipient subjects' palms were moistened prior to them grasping the pens, in order to imitate sweat ; tubes then were swabbed; roles of each pair then were reversed; 5-min drying time only; 8 samples (4 per pair) |
| 15 | Tertiary | M→GTh→Tu→Pa | Similar to trial 10, except that each initial subject wore a latex glove after washing; after gripping the tubes, recipient partners' palms were swabbed; roles of each pair then were reversed; 5-min drying time only; 8 samples (4 per pair) |
| 16 | Tertiary | M→Gth→Tu→MstPa | Similar trial 15, except that recipient subjects' palms were moistened prior to grasping the tubes, to simulate sweat; recipient partners' palms then were swabbed; roles of each pair then were reversed; 5-min drying time only; 8 samples (4 per pair) |

For each trial, the type of transfer, shorthand notation, and procedural notes are listed. Trial 3 was used as a reference point for the amount of DNA on a bare palm and thus did not represent a transfer event

*M* mouth, *BTh* bare thumb, *GTh* gloved thumb, *Pa* palm, *Pe* pen, *Tu* tube, *MstPa* moistened palm, *MstBTh* moistened bare thumb

PCR cycles and reanalyzed. Even with increased sensitivity of detection, these samples only yielded genetic profiles showing up to 22.2 % of the expected alleles, with one exception that showed 53.8 % of the alleles (data not shown). These results indicated that the sloughed off epithelial cells on the subjects' palms were not sources of abundant DNA.

## Secondary transfer trial results

The quantities of recovered DNA were compared with those assessed in the primary transfer experiments to provide a rough estimate of the proportion of DNA lost during the steps of the transfer process. The percentages of obtainable profiles for trials 5 and 9 (transfer of saliva on bare thumbs to plastic tubes and transfer of saliva on moistened bare thumbs to plastic tubes, respectively), as well as trials 6 and 8 (transfer of saliva on pen surfaces to palms and transfer of saliva on pen surfaces to moistened palms, respectively), were compared to assess the effects of simulated sweat (i.e., moistened hands) on secondary DNA transfer. In trials that involved two subjects (trials 6 and 8), the DNA profiles were compared with the subjects' reference profiles to assess the relative ratios of primary and secondary

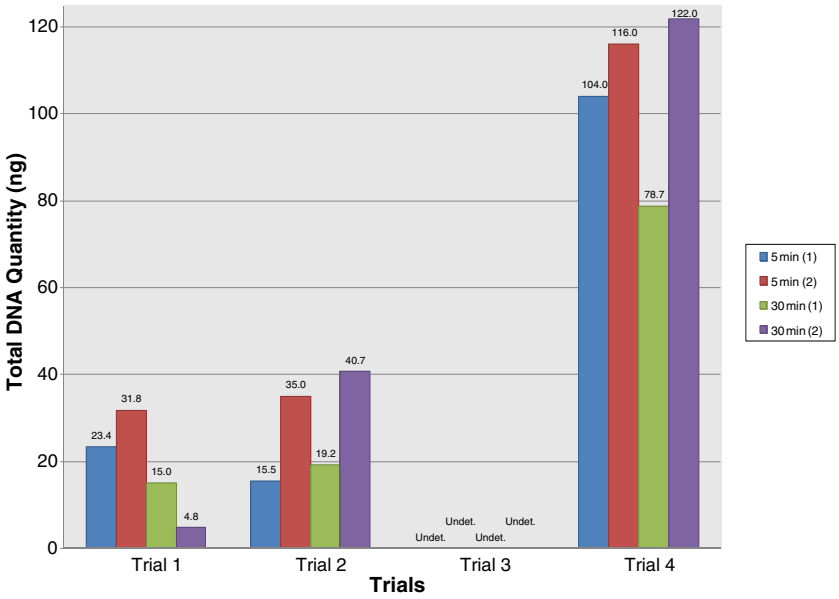


**Fig. 1** Primary transfer trial DNA quantities—subject 001. The total quantity of collected DNA is shown for each of the primary transfer experiments involving subject 001. Drying time replicates are displayed without averaging to illustrate the degree of variation from one saliva deposition event to the next. Times reflect duration of drying after the transfer event. Trial 1 was the primary transfer of saliva to bare thumbs, while trial 2 represented the primary transfer of saliva to gloved thumbs. Trial 3 was the swabbing of bare palms; Trial 4 was the primary transfer of saliva to pen surfaces

contributor DNA, based on the peak height ratios observed in the electropherograms.

Quantification results from the secondary transfer trials wherein moisture was absent indicated that a single transfer event can lead to dramatically reduced yield of DNA from a dry source, which is consistent with the findings of Goray et al. [19]. The trial 6 samples, however, yielded sufficient interpretable DNA quantity values to provide a rough estimate of the decrease in recoverable DNA (Table 3). DNA quantity estimates for each subject's samples in this trial were averaged, and these averages were compared. The mean percentage of DNA loss due to a single transfer step was 81.2 %, indicating that DNA loss was substantial. Further estimation of DNA loss due to transfer events was not possible because most of the quantity estimates for the various secondary transfer trial samples (i.e., non-moistened transfers) corresponding to previously quantified primary transfer trial samples were undeterminable (Table 3).

Amplification of 10 μL of extract at 28 PCR cycles was insufficient to yield full genetic profiles from samples from trials 5–8 (Table 4). In fact, 90.6 % of the single-subject samples from these trials amplified at 28 PCR cycles yielded profiles that contained less than half of the expected alleles. Of these, 65.5 % failed to show even a single allele. The samples were re-amplified at 34 PCR cycles, and more complete genetic profiles were thus obtained (Table 4). Samples from trials 8 and 9 were amplified only at 34 cycles, forgoing the 28-cycle amplification, as the previous

**Table 2** Primary transfer trial profile completion percentages (28 PCR cycles)

| | | Trial 1 (%) | Trial 2 (%) | Trial 3 (%) | Trial 4 (%) |
|---|---|---|---|---|---|
| Subject 001 | 5 min (1) | 100.0 | 100.0 | 0.0 | 100.0 |
| | 5 min (2) | 100.0 | 100.0 | 0.0 | 100.0 |
| | 30 min (1) | 100.0 | 100.0 | 0.0 | 100.0 |
| | 30 min (2) | 100.0 | 100.0 | 0.0 | 100.0 |
| Subject 002 | 5 min (1) | 100.0 | 100.0 | 11.5 | 100.0 |
| | 5 min (2) | 100.0 | 100.0 | 0.0 | 100.0 |
| | 30 min (1) | 100.0 | 100.0 | 0.0 | 100.0 |
| | 30 min (2) | 100.0 | 100.0 | 0.0 | 100.0 |
| Subject 003 | 5 min (1) | 100.0 | 100.0 | 0.0 | 100.0 |
| | 5 min (2) | 100.0 | 100.0 | 0.0 | 100.0 |
| | 30 min (1) | 100.0 | 100.0 | 0.0 | 100.0 |
| | 30 min (2) | 100.0 | 100.0 | 0.0 | 100.0 |
| Subject 004 | 5 min (1) | 100.0 | 100.0 | 0.0 | 96.7 |
| | 5 min (2) | 100.0 | 100.0 | 0.0 | 100.0 |
| | 30 min (1) | 100.0 | 100.0 | 0.0 | 30.0 |
| | 30 min (2) | 100.0 | 100.0 | 3.3 | 56.7 |

The percentages of observed alleles of each subject's full genetic profile following 28 cycles of PCR are listed for each drying time replicate in each experiment. Times reflect duration of drying after the transfer event. Trial 1 was the primary transfer of saliva to bare thumbs, while trial 2 represented the primary transfer of saliva to gloved thumbs; Trial 3 was the swabbing of bare palms; Trial 4 was the primary transfer of saliva to pen surfaces

**Table 3** Secondary transfer trial DNA quantities

The total quantities of DNA (in nanograms) collected for each of the replicates in each secondary transfer trial are listed. Values of "–" represent no detectable DNA by the quantification assay. Times reflect duration of drying after the primary transfer event. Trial 5 was the transfer of saliva on bare thumbs to plastic tubes; Trial 6 represented the transfer of saliva on pen surfaces to palms; Trial 7 was the transfer of saliva on gloved thumbs to plastic tubes; Trial 8 was the transfer of saliva on pen surfaces to moistened palms; Trial 9 represented the transfer of saliva on moistened bare thumbs to plastic tubes

| | | Trial 5 | Trial 6 | Trial 7 | Trial 8 | Trial 9 |
|---|---|---|---|---|---|---|
| Subject 001 | 5 min (1) | – | – | – | 0.75 | 1.33 |
| | 5 min (2) | – | 1.66 | – | 1.66 | 0.29 |
| | 30 min (1) | – | 3.73 | – | 4.86 | - |
| | 30 min (2) | 0.42 | – | – | 2.90 | 0.51 |
| Subject 002 | 5 min (1) | – | 1.38 | – | 3.32 | 0.50 |
| | 5 min (2) | – | 5.34 | – | 3.58 | 0.54 |
| | 30 min (1) | – | – | – | 3.53 | 0.27 |
| | 30 min (2) | – | 3.86 | – | 0.85 | 0.76 |
| Subject 003 | 5 min (1) | – | 2.96 | 0.86 | 4.82 | 3.11 |
| | 5 min (2) | 0.40 | 5.06 | – | 1.65 | 2.94 |
| | 30 min (1) | 0.78 | 0.43 | – | 1.35 | 3.85 |
| | 30 min (2) | 2.55 | 2.44 | – | – | 2.16 |
| Subject 004 | 5 min (1) | – | 0.84 | – | – | 0.27 |
| | 5 min (2) | 1.15 | 5.14 | – | – | 0.54 |
| | 30 min (1) | 1.58 | – | – | – | – |
| | 30 min (2) | 0.85 | 0.60 | – | – | 0.58 |

trials had demonstrated that the recoverable DNA from such samples could be considered "low-copy DNA" and required more amplification cycles to yield detectable results [28, 29]. Based on the results, subsequent tertiary trial samples were amplified only at 34 cycles, as well.

In the cases of trials 6 and 8, both of which involved two subjects, the peak heights of the observed alleles were compared to attempt to determine the percentage of each subject's contribution to the DNA mixture. Any allele unique to one of the subjects in the pair was considered for these calculations. The percentages of major and minor allele contribution were averaged for each replicate in each trial. In all but one of the replicates in trial 6 that yielded unique alleles, the original depositor (the subject that held the pen in his/her mouth) was definitively shown to be the major contributor of the DNA (Fig. 2). Similarly, the original depositor was shown to be the major DNA contributor in all but two replicates of trial 8 (data not shown). These results are not surprising since the DNA quantity estimates from the trial 3 samples indicated that very little DNA was present on subjects' clean palms. Thus, the transfer of DNA-rich saliva to a pen and then onto a subject's palm would likely result in a DNA mixture that is predominantly from the saliva.

The effects of simulated sweat on the transfer process were investigated in trials 8 and 9. To do so, the percentages of obtainable profiles for trials 5 and 9 were compared. In all but two of the replicates in these trials, the samples from trial 9 yielded profiles that displayed more of the expected alleles than those yielded by the trial 5 samples (data not shown). Of these 14 samples, ten yielded profiles that displayed an additional 25 % or more of the expected alleles from the depositing subject. These results suggest that moist surfaces facilitate DNA transfer more efficiently than dry ones, which is consistent with the results obtained by Goray et al. [19]. A comparison of the percentages of obtainable profiles for trials 6 and 8 was not as informative because the samples from both trials yielded fairly complete genetic profiles from the primary contributors. The percentages of obtainable profiles from the secondary contributor (the receiving subject) were not significantly different. These results also are consistent with the results obtained in previous trials that indicated that there was not a relatively substantial quantity of DNA on a clean palm initially.

## Tertiary transfer trial results

DNA typing data obtained from this third set of experiments were used to evaluate the effects of tertiary transfer of DNA. The majority of the quantities of recovered DNA were not sufficient to be detected by the quantification assay. Thus, it was not feasible to estimate the amount of DNA lost during the third step of the transfer process. The fact that the quantities were mostly undetectable did indicate that a very large portion of the recoverable DNA is lost during the tertiary transfer process, which is consistent with the previous findings of over 80 % DNA loss in a single transfer event. Each tertiary transfer trial had one or more corresponding versions involving the use of simulated sweat. The percentages of obtainable profiles for these trials were compared to assess the effects of moisture on tertiary DNA transfer.

Overall, the percentages of subject profiles that were observed following the tertiary transfer events were much

**Table 4** Secondary transfer trial profile completion percentages —trials 5 and 7

| | | Trial 5 | | Trial 7 | |
|---|---|---|---|---|---|
| | | 28 Cycles (%) | 34 Cycles (%) | 28 Cycles (%) | 34 Cycles (%) |
| Subject 001 | 5 min (1) | 0.0 | 0.0 | 0.0 | 0.0 |
| | 5 min (2) | 0.0 | 0.0 | 0.0 | 0.0 |
| | 30 min (1) | 0.0 | **14.8** | 0.0 | 0.0 |
| | 30 min (2) | 3.7 | **37.0** | 0.0 | **3.7** |
| Subject 002 | 5 min (1) | 0.0 | **38.5** | 0.0 | 0.0 |
| | 5 min (2) | 7.7 | **42.3** | 0.0 | 0.0 |
| | 30 min (1) | 3.8 | 0.0 | 0.0 | **11.5** |
| | 30 min (2) | 3.8 | 7.7 | 0.0 | 0.0 |
| Subject 003 | 5 min (1) | 31.0 | **86.2** | 3.4 | 0.0 |
| | 5 min (2) | 41.4 | **93.1** | 0.0 | **3.4** |
| | 30 min (1) | 31.0 | **65.5** | 0.0 | **17.2** |
| | 30 min (2) | 100.0 | 89.7 | 0.0 | **3.4** |
| Subject 004 | 5 min (1) | 43.3 | **66.7** | 0.0 | 0.0 |
| | 5 min (2) | 30.0 | **56.7** | 0.0 | 0.0 |
| | 30 min (1) | 56.7 | **96.7** | 0.0 | 0.0 |
| | 30 min (2) | 60.0 | **93.3** | 0.0 | 0.0 |

The percentages of alleles of each subject's genetic profile detected via capillary electrophoresis following 28 and 34 cycles of PCR are listed for each replicate in each trial. Instances in which amplification at 34 cycles of PCR allowed for the detection of a greater percentage of alleles are displayed in bold. Times reflect duration of drying after the primary transfer event. Trial 5 was the transfer of saliva on bare thumbs to plastic tubes; Trial 7 represented the transfer of saliva on gloved thumbs to plastic tubes

lower than those recovered after the secondary transfer events. In fact, 87.5 % of the profiles observed after tertiary transfer displayed less than half of the expected alleles (data not shown). These findings were consistent with the concept that tertiary transfer substantially diminishes the amount of recoverable DNA.

The recovered profile percentages for tertiary transfer trials involving subject 003 (Fig. 3), for example, demonstrate the effects of imitated sweat (moisture) on the tertiary transfer process. When the percentages of primary subjects' profiles yielded by trial 10 (transfer of saliva on bare thumbs to plastic tubes and then to palms) were compared with those yielded by trial 11 (transfer of saliva on bare thumbs to plastic tubes and then to moistened palms), it was evident that greater portions of the primary subjects' DNA profiles were transferred when the

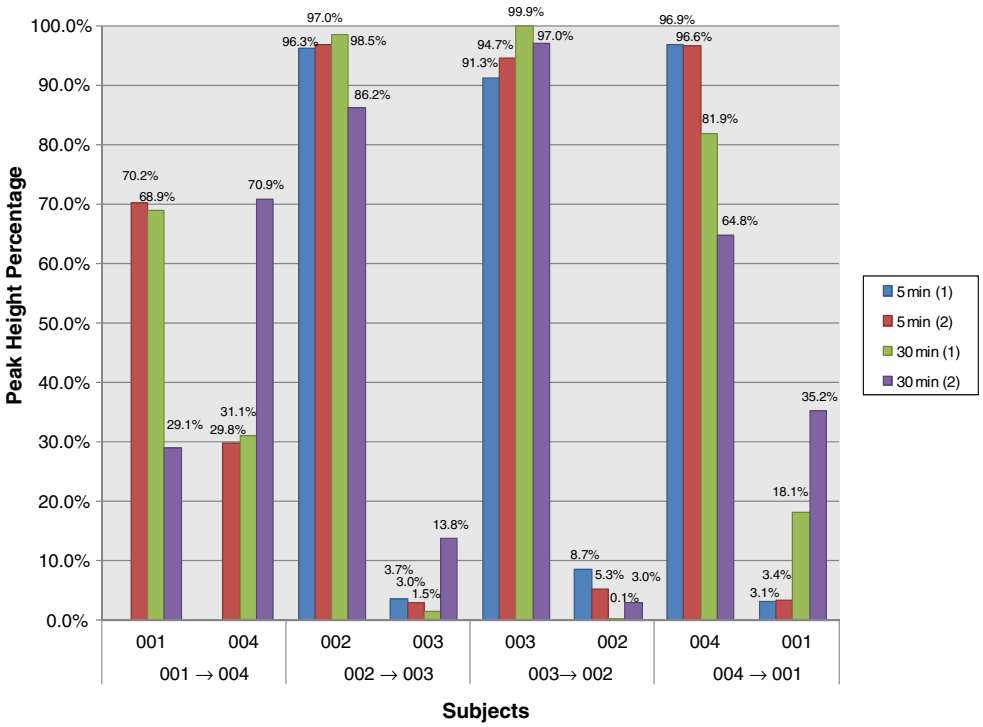


**Fig. 2** Major/minor contributor percentages (peak height)—trial 6 (34 PCR cycles). The calculated percentages of contribution to the DNA mixture by each individual's unique alleles, based on peak height data, are displayed for each replicate. Times reflect duration of drying after the primary transfer event. The order of transfer for each pair of subjects in the study is shown on the *X* axis. Trial 6 represented the secondary transfer of saliva on pen surfaces to palms

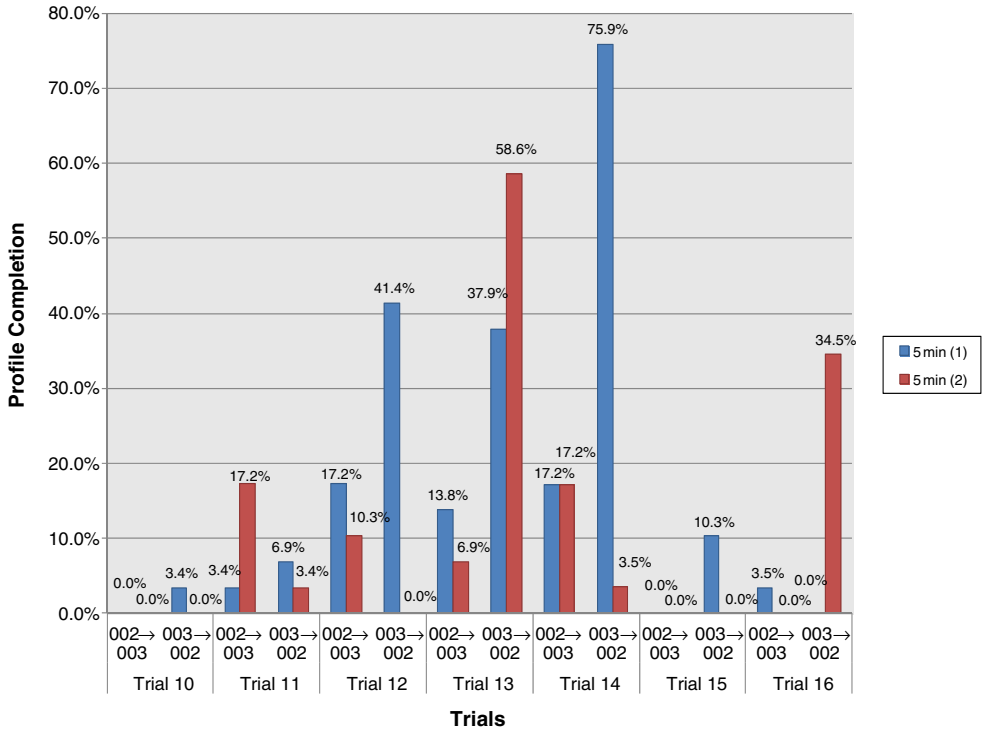


**Fig. 3** Tertiary transfer trial profile percentage—subject 003. The percentages of alleles in subject 003's genetic profile are displayed for each replicate in each trial. The order of transfer used in each trial is shown on the *X* axis. Times reflect duration of drying after the primary transfer event. Trial 10 was the transfer of saliva on bare thumbs to plastic tubes and then to palms; Trial 11 represented the transfer of saliva on bare thumbs to plastic tubes and then to moistened palms; Trial 12 was the transfer of saliva on pen surfaces to palms and then to plastic tubes; Trial 13 was the transfer of saliva on moistened bare thumbs to plastic tubes and then to palms; Trial 14 was the transfer of saliva on pen surfaces to moistened palms and then to plastic tubes; Trial 15 was the transfer of saliva on gloved thumbs to plastic tubes and then to palms; Trial 16 represented the transfer of saliva on gloved thumbs to plastic tubes and then to moistened palms

recipient partners' palms were moistened. This observation was consistent with the secondary transfer results and the findings of Goray et al. [19]. It should be noted that higher proportions of the primary subjects' DNA were transferred to the recipients' palms when the primary subjects' thumbs were moistened prior to gripping the tubes. A comparison of the profile percentages for trials 12 and 14 (transfer of saliva on pen surfaces to palms, and then to plastic tubes, and transfer of saliva on pen surfaces to moistened palms, and then to plastic tubes, respectively) and trials 15 and 16 (transfer of saliva on gloved thumbs to plastic tubes, and then to palms, and transfer of saliva on gloved thumbs to plastic tubes, and then to moistened palms, respectively) showed the same general trend of more efficient transfer when moisture was present.

As with the dual-subject secondary transfer trial results, the peak heights of the observed alleles in these tertiary transfer trials were compared to attempt to determine the percentage of each subject's contribution to the DNA mixture. Generally, the secondary depositors (the subjects whose palms were swabbed) were shown to be the major contributors of the DNA mixtures in trials where moisture was absent. This was the case in all of the replicates in trials 10 and 15 based on unique alleles. These results agree with previously published observations of DNA transfer [18, 22, 23] and differ from the results of the secondary transfer portion of this study, where the primary contributor of the DNA was shown to contribute the majority of the DNA in the resulting mixture. However, these findings should be expected, as the additional transfer step involved in the tertiary transfer process likely diminished the amount of DNA deposited by the initial contributor. Two applicable replicates of trial 11, though, revealed that the primary contributor's DNA was the major component of the mixture (Table 5). These results were consistent with our earlier findings; that is, the presence of moisture at a subsequent transfer step increases the likelihood of transferring DNA deposited by a primary contributor during tertiary transfer. The results of other trials involving the presence of moisture generally indicated that the primary depositor was the major contributor of DNA, as well. For instance, all of the replicates in trial 14 indicated that the primary contributors' DNA made up the majority of the mixtures. These results are consistent with the earlier findings of this study, indicating that greater smooth surface area increases efficiency of

**Table 5** Major/minor contributor percentages (peak height)—trials 10–16 (34 PCR cycles)

| | | Primary depositor | | | | | | Secondary depositor | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 min (1) | | | 5 min (2) | | | 5 min (1) | | | 5 min (2) | | |
| Trial 10 | 001→004 | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] | 100.0 % | (1) | [468 RFU] | 100.0 % | (2) | [513 RFU] |
| | 002→003 | – | – | – | – | – | – | – | – | – | – | – | – |
| | 003→002 | – | – | – | 0.0 % | (−) | [−] | – | – | – | 100.0 % | (1) | [213 RFU] |
| | 004→001 | – | – | – | 0.0 % | (−) | [−] | – | – | – | 100.0 % | (5) | [456 RFU] |
| Trial 11 | 001→004 | 10.9 % | (1) | [344 RFU] | 50.0 % | (1) | [334 RFU] | 89.1 % | (5) | [572 RFU] | 50.0 % | (1) | [435 RFU] |
| | 002→003 | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] | 100.0 % | (1) | [982 RFU] | 100 | (4) | [764 RFU] |
| | 003→002 | 100.0 % | (1) | **[413 RFU]** | – | – | – | 0.0 % | (−) | [−] | – | – | – |
| | 004→001 | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] | 100.0 % | (7) | [1,441 RFU] | 100.0 % | (9) | [999 RFU] |
| Trial 12 | 001→004 | 100.0 % | (2) | **[676 RFU]** | 100.0 % | (9) | **[1,099 RFU]** | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] |
| | 002→003 | 100.0 % | (9) | **[990 RFU]** | 100.0 % | (2) | **[630 RFU]** | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] |
| | 003→002 | 100.0 % | (6) | **[1,002 RFU]** | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] | 100.0 % | (1) | [462 RFU] |
| | 004→001 | 100.0 % | (7) | **[947 RFU]** | 100.0 % | (8) | **[3,371 RFU]** | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] |
| Trial 13 | 001→004 | 100.0 % | (2) | **[1,241 RFU]** | 100.0 % | (3) | **[828 RFU]** | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] |
| | 002→003 | 81.3 % | (2) | **[857 RFU]** | 33.3 % | (1) | [869 RFU] | 18.7 % | (1) | [289 RFU] | 66.6 % | (2) | [471 RFU] |
| | 003→002 | 87.5 % | (7) | **[620 RFU]** | 100.0 % | (8) | **[1,037 RFU]** | 12.5 % | (1) | [650 RFU] | 0.0 % | (−) | [−] |
| | 004→001 | – | – | – | 0.0 % | (−) | [−] | – | – | – | 100.0 % | (8) | [596 RFU] |
| Trial 14 | 001→004 | 100.0 % | (6) | **[742 RFU]** | 100.0 % | (7) | **[3,169 RFU]** | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] |
| | 002→003 | 100.0 % | (6) | **[900 RFU]** | 100.0 % | (9) | **[902 RFU]** | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] |
| | 003→002 | 100.0 % | (13) | **[1,370 RFU]** | – | – | – | 0.0 % | (−) | [−] | – | – | – |
| | 004→001 | 100.0 % | (1) | **[356 RFU]** | 100.0 % | (2) | **[523 RFU]** | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] |
| Trial 15 | 001→004 | – | – | – | – | – | – | – | – | – | – | – | – |
| | 002→003 | – | – | – | – | – | – | – | – | – | – | – | – |
| | 003→002 | 0.0 % | (−) | [−] | – | – | – | 100.0 % | (1) | [289 RFU] | – | – | – |
| | 004→001 | 0.0 % | (−) | [−] | 0.0 % | (−) | [−] | 100.0 % | (7) | [1,348 RFU] | 100.0 % | (7) | [1,012 RFU] |
| Trial 16 | 001→004 | – | – | – | – | – | – | – | – | – | – | – | – |
| | 002→003 | 0.0 % | (−) | [−] | – | – | – | 100.0 % | (1) | [662 RFU] | – | – | – |
| | 003→002 | – | – | – | 0.0 % | (−) | [−] | – | – | – | 100.0 % | (11) | [2,265 RFU] |
| | 004→001 | 0.0 % | (−) | [−] | – | – | – | 100.0 % | (2) | [660 RFU] | – | – | – |

The calculated percentages of contribution to the DNA mixture by each individual's unique alleles, based on peak height data, are listed for each replicate in each trial. The numbers of unique alleles observed are listed in parentheses. The average peak heights are listed in brackets. The orders of transfer for each pair of subjects in the trials are indicated. Instances in which the primary depositor was shown to be the major contributor of DNA to the mixture are displayed in bold. Times reflect duration of drying after the primary transfer event. Trial 10 was the transfer of saliva on bare thumbs to plastic tubes and then to palms; Trial 11 represented the transfer of saliva on bare thumbs to plastic tubes and then to moistened palms; Trial 12 was the transfer of saliva on pen surfaces to palms and then to plastic tubes; Trial 13 was the transfer of saliva on moistened bare thumbs to plastic tubes and then to palms; Trial 14 was the transfer of saliva on pen surfaces to moistened palms and then to plastic tubes; Trial 15 was the transfer of saliva on gloved thumbs to plastic tubes and then to palms; Trial 16 represented the transfer of saliva on gloved thumbs to plastic tubes, and then to moistened palms

transfer. Similar results were obtained from trial 13, where all but two applicable replicates showed that the primary contributors were the major sources of the DNA in the mixtures. The results of trial 16, however, showed that the secondary depositor was the predominant contributor. It should be noted that the results of trial 12, wherein moisture was absent, still revealed the primary depositor to be the main contributor. This may be due to the characteristics of the pens' surfaces and variation in the amount of DNA deposited, as noted above. Lastly, the number of unique alleles observed in the tertiary study was a small percentage of the total possible alleles between the pairs of individuals, and therefore, the number of alleles displayed in Table 5 should not be misconstrued. The data were only the unique alleles and not the total alleles observed. Moreover, the method used in the study to increase sensitivity of detection employed only additional PCR cycles. Sensitivity can be enhanced further, for example, by reduced PCR volumes and post-PCR clean-up. With increased sensitivity of detection methods, more alleles will likely be detected, but still following the trends observed in this study.

## Conclusions

Previous studies on DNA transfer events have focused primarily on the transfer of DNA found in sloughed off epithelial cells from individuals' palms. In these cases, the major contributor to the resulting DNA mixture often was shown to be the last person to come in contact with the tested object. The data herein support that there is notable loss of DNA with each transfer event. In addition, the results of this study indicate that when saliva was the original source of the transferred DNA, the initial contributor's genetic material can comprise the majority of the resulting mixture. The presence of moisture during the transfer event as well as the texture and surface area of the object(s) to which the DNA was transferred were factors contributing to this phenomenon. Also, the results of the tertiary transfer trials indicated that the presence of moisture during the initial deposition of DNA from the primary source played a more substantial role in the transfer of this DNA than moisture present during subsequent transfer steps. Given that saliva is a likely source of transferred DNA, all individuals can essentially be considered good shedders. This concept, coupled with the inherent uncertainty as to the means of DNA deposition in forensic samples, negates the relevance of shedder status consideration in low-copy number forensic analysis. Caution should be exercised when inferring that the major component of a touched DNA sample was derived from the last person to come in contact with the item. This study involved only four individuals and yet constituted a substantial amount of work. While the trends are likely to hold with an increased number of individuals being studied, additional studies are advocated. Studies of DNA transfer events using other commonly encountered sources of genetic material also would benefit the field of forensic genetics.

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