TECHNICAL NOTE

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A Systematic Analysis of Secondary DNA Transfer

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ABSTRACT: The *Nature* letter by R. van Oorschot and M. Jones (1) addressed two topics: the primary transfer of DNA from person to person or to various objects, and the secondary transfer of DNA through an intermediary. Forensic scientists have described the primary transfer of DNA and other biological evidence for many years. However, the authors also reported detecting secondary transfer of DNA from an object to a person's hands, which could adversely affect DNA typing in the forensic context. The prospect of secondary transfer raises questions of interest to both the legal and forensic communities. Therefore, we sought to evaluate parameters potentially leading to secondary DNA transfer. Our data do not support the conclusion that secondary transfer will compromise DNA typing results under typical forensic conditions.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction, secondary transfer, HLA-DQA1, LDLR, GYPA, HBGG, D7S8, GC, D3S1358, vWA, FGA, Amelogenin, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, CSF1PO

The *Nature* letter by R. van Oorschot and M. Jones entitled "DNA Fingerprints from Fingerprints" described primary and secondary DNA transfer from objects touched by hands (1). Forensic scientists have previously reported the primary transfer of DNA and other biological or physical evidence (2–5). However, the authors also asserted that secondary DNA transfer is a realistic possibility which could be problematic for forensic DNA analysis: ". . .in some cases material from which DNA can be retrieved is transferred from object to hand" (secondary transfer). Hence, ". . . genetic profiles from objects handled by several people or from minute blood stains on touched objects may be difficult to interpret." An Associated Press news release (6/19/97) expressed the concern more directly: "if a person can pick up your DNA from a handshake or something you touched, does that mean that person could then leave your DNA on something at a crime scene, falsely suggesting you were there?" If secondary DNA transfer is, in fact, a realistic possibility under field conditions, that may have significant repercussions for DNA testing in criminal investigations.

Given its importance, we have systematically studied the issue of secondary transfer in an attempt to evaluate the findings of van Oorschot and Jones and their relevance to casework. Since the authors' specific methodology was not reported, we were unable to duplicate their experiments exactly. Therefore, we tested a series of conditions where detectable secondary transfer might occur. The study addressed situations that might realistically be encountered in field situations or at crime scenes.

Materials and Methods

Two possible modes of secondary transfer were considered: (1) skin to skin to object (handshaking) and (2) skin to object to skin. For the first scenario, objects were pre-cleaned with 10% bleach and wiped with 95% ethanol. Subsequently, laboratory personnel shook hands for various lengths of time (1", 5", 10", 30", 60"") and then held the pre-cleaned objects for 5 s (Table 1). The handshaking and handling of objects were not static. Palms were rubbed against each other or against the object to promote maximum DNA transfer. The individuals' palms or the handled objects were swabbed with moistened (dH_2O) sterile swabs. For the second mode, coffee mugs (Table 2) were handled per regular usage over 2 h and then handled by a second individual for 10 s. Subsequently, both the mugs and the palm of the second individual were swabbed. Commonly handled objects such as phones, door handles, keyboards, etc. were also tested for evidence of primary transfer (Table 2). DNA was extracted according to standard laboratory protocols (organic extraction/microcon-100 purification) (6) and quantified using the QuantiBlot™ kit (protocol per product insert, Perkin Elmer Applied Biosystems, Foster City, CA). Five nanograms of DNA or up to 20 μL (40% of sample) were amplified using the AmpliType® PM + DQA1 typing kit (Perkin Elmer Applied Biosystems) according to the manufacturer's protocol.

Primary and secondary DNA transfer were also studied using fluorescent STR analysis. First, the samples originally tested for PM and DQA1 (7) were amplified using the 13 STR markers specified by the Combined DNA Index System (CODIS). Subsequently, the secondary transfer experiments were repeated as described above and additional samples (N=3, total N= 6) were tested using the CODIS STR loci. One nanogram of DNA or up to

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TABLE 1—*Handshake results, DNA yields and typing results.*

	Duration of Contact (s)	Average Yield (ng) n=6	Amplification Results
Primary transfer:	1	not detected	negative
	5	1	a
	10	2	a
	30	5	a
	60	4	a
Secondary transfer:	10(s)/5(s)	1	b,c
	30(s)/5(s)	1	b,c

Note: For primary transfer, palms were swabbed after handshaking for the times specified (1–60 s). For the secondary transfer experiments with a person as intermediary, handshaking occurred for various lengths of time (e.g., 10 or 30 s), the door handles were held for 5 s and DNA was recovered from the doors. Scores are as follows:

a. Primary transfer detected with some amplifications – highly dependent on the individual.

b. No secondary transfer detected (no dots > C or S, no peaks >75 RFU).

c. Amplifications predominantly negative (i.e., no dots visible, no peaks above background). Some peaks < 75 RFU and dots < C or S from the second individual were present. Allele and locus dropout were observed.

TABLE 2—*DNA yields and typing results from swabbed objects.*

Object	Average Yield(ng) n = 6	Amplification Results
Primary transfer:		
Door Handles	1.6	a
Telephone Mouthpieces	7	a
Freezer Door Handles	12	a
Briefcase Handles	15	b
Closet Door Handles	1	a
File Cabinet Handles	1	a
Computer Keyboards	5	a
Computer Mouse	6	a
Coffee Cups	6	a
Steering wheels	12	b
Secondary transfer:		
Handled Coffee Cups	6	c
Coffee Cup Handler	5	c,d

Note: Scores are as follows:

a. Object handled by multiple individuals. Mixtures detected. Locus dropout with some amplifications.

b. Object handled by one individual. Primary transfer detected.

c. Secondary transfer experiment with an object as intermediary. Primary transfer from both individuals was observed on the cups. Secondary transfer was not observed (no dots > C or S, no peaks >75 RFU from the second individual).

d. Amplifications predominantly negative (i.e., no dots visible, no peaks above background). Some peaks <75 RFU and dots < C or S from the second individual were present. Allele and locus dropout were observed.

10 µL (20% of extracted sample) was amplified using the AmpF*l*STR^{R} Profiler Plus™ and CO*f*iler™ DNA typing kits. (GeneAmp PCR System 2400 thermal cycler, Perkin Elmer, Norwalk, CT; 25 µL reaction volume, manufacturer's cycling parameters) (8). Amplified products were resolved using the ABI Prism™ 377 DNA Sequencer (Perkin Elmer Applied Biosystems, Foster City, CA) according to manufacturer's protocols (8,9). The peak height threshold for the present study was 75 relative fluorescence units (RFU).

Results and Discussion

In accordance with van Oorschot and Jones, we recovered DNA from palm swabbings, from frequently handled objects such as phones, door handles, and keyboards, and from objects handled under controlled conditions. However, our data do not support several of the key findings reported in the *Nature* letter. On average, we recovered 1–15 ng of human DNA from the tested samples, considerably less than the 2–150 ng reported by van Oorschot and Jones. Most significantly, in no instance was the profile of the second individual detected by AmpliType PM + DQA1.

Although we confirmed that primary transfer of DNA can yield interpretable results, its detection is not guaranteed and is highly dependent upon the individual handling the objects or performing the handshaking. We found that in many instances there was insufficient DNA (or the DNA was too degraded) to generate an interpretable genotype (dots darker than the C or S dot) with both the frequently handled objects as well as the objects cleaned prior to handling. With some of the experimental samples, we obtained amplified product for the smaller but not the larger loci. Therefore, sample degradation appeared to be a significant factor.

Studies have shown that the sensitivity of AmpliType PM + DQA1 is comparable to that of STRs (10,11). (The STR locus HUMTH01 was amplified in the *Nature* letter.) We have generated interpretable results with as little as 100–200 pg of high molecular weight DNA. In addition, the sizes of the PM + DQA1 alleles are similar to those of many STRs including TH01. Nonetheless, to answer the question of sensitivity, all samples were amplified using AmpF*l*STR Profiler Plus + CO*f*iler.

The STR results regarding primary and secondary DNA transfer were identical to those for AmpliType PM + DQA1. Primary transfer was observed in some cases using Profiler Plus and CO*f*iler. As with the dot-based systems, sample degradation in addition to low DNA yield appeared to be significant factors. Locus and allele dropout were particularly problematic for the larger amplicons. With respect to secondary transfer, peaks above background (15–20 RFU) from the second individual were not detected for most STR amplifications. On occasion, minor peaks (below 75 RFU) from the second individual were observed. However, in these instances, allele dropout was routine. The complete secondary profile was never detected, even if the data were analyzed in the 50–75 RFU range. It should also be noted that, generally, amplification would not be attempted on many of the experimental samples we tested since the manufacturer recommends using a minimum of 250 pg (35 cells) of DNA template for PCR. At 125 pg or less, peaks height are close to background; the standard peak height threshold recommended by the manufacturer is 150 RFU (8,11).

Our data indicate that the primary transfer of DNA by handling is possible, but detecting an interpretable genotype is not assured. Secondary transfer was not observed under our experimental conditions. Therefore, our data do not support the inference that the interpretation of DNA profiles from case samples could be compromised by secondary transfer.

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