

**TECHNICAL NOTE****CRIMINALISTICS**

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## Could Secondary DNA Transfer Falsely Place Someone at the Scene of a Crime?\*,†

**ABSTRACT:** The occurrence of secondary DNA transfer has been previously established. However, the transfer of DNA through an intermediary has not been revisited with more sensitive current technologies implemented to increase the likelihood of obtaining results from low-template/low-quality samples. This study evaluated whether this increased sensitivity could lead to the detection of interpretable secondary DNA transfer profiles. After two minutes of hand to hand contact, participants immediately handled assigned knives. Swabbings of the knives with detectable amounts of DNA were amplified with the Identifiler<sup>®</sup> Plus Amplification Kit and injected on a 3130xl. DNA typing results indicated that secondary DNA transfer was detected in 85% of the samples. In five samples, the secondary contributor was either the only contributor or the major contributor identified despite never coming into direct contact with the knife. This study demonstrates the risk of assuming that DNA recovered from an object resulted from direct contact.

**KEYWORDS:** forensic science, criminalistics, DNA analysis, secondary transfer, forensic casework, Identifiler<sup>®</sup> Plus

Locard's Exchange Principle states that a perpetrator of a crime will leave traces of his or her presence at a crime scene and leave with trace evidence from that scene, both of which may be used as forensic evidence in a criminal investigation (1). There have been a plethora of investigations of the primary transfer of DNA from a person to an object or another person and under what conditions primary DNA transfer can and will occur (2). However, it may also be possible that a perpetrator of a crime could bring traces of another individual into a crime scene and deposit these traces via secondary DNA transfer. Secondary transfer occurs when DNA is transferred from one object or person to another via an intermediate object/person. There is a paucity of research projects regarding the continued transfer of DNA (2). Secondary DNA transfer should be a concern for forensic DNA analysts because (i) it could falsely link someone to a crime; (ii) it could introduce extraneous DNA, or foreign DNA, into a forensic sample; and (iii) it could lead analysts and other medicolegal professionals to falsely conclude that DNA left on an object is a result of direct contact.

Secondary DNA transfer was first described in the scientific literature in 1997 (3). During their investigation into whether DNA profiles could be obtained from touched objects, van Oorschot and Jones swabbed vinyl gloves for the presence of wearer DNA. DNA profiles from the wearers were obtained; however, the

authors reported the presence of additional alleles that did not belong to the wearers in two samples. They introduced the idea of secondary DNA transfer as a plausible reason for the presence of those additional alleles and warned DNA analysts to be cautious when handling evidence items because extraneous DNA could be introduced into a sample through both primary transfer and secondary transfer of DNA. The authors subsequently tested the potential for secondary transfer by swabbing the hands of experiment participants before and after a one-minute handshake. One of the four hands tested revealed transfer of DNA from one individual to another. This finding prompted the authors to conclude that secondary DNA transfer was a real possibility and could complicate the interpretation of forensic DNA evidence.

Following the work of van Oorschot and Jones (3), Ladd et al. (4) attempted to disprove the occurrence of secondary DNA transfer. In their analysis, laboratory personnel were instructed to shake hands for varying amounts of time and then handle pre-cleaned objects for five seconds. The participants' hands and the objects were swabbed. Additionally, participants handled coffee mugs for two hours in accordance with regular usage. Afterward, the coffee mugs were handled by a second individual for 10 seconds. The mug and the palm of the second individual were subsequently swabbed. Under these experimental conditions, Ladd et al. did not observe secondary DNA transfer and concluded their data did not support the notion that DNA profiles from case samples could be compromised by secondary transfer (4). Despite stating that secondary DNA transfer was not observed, they did acknowledge the occasional presence of alleles from the secondary individual below their analysis threshold. However, a complete secondary profile was never detected in their samples. The presence of alleles, albeit below the analysis threshold, that were concordant with the profiles of the secondary individuals suggests that secondary DNA transfer may have occurred.

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Since the Ladd et al. (4) investigation into secondary DNA transfer, the technology utilized in forensic laboratories has become more sensitive (5–13). This increase in sensitivity over time has provided additional evidence for the occurrence of secondary DNA transfer. The Ladd et al. (4) investigation utilized the AmpliType PM + DQA1 Amplification Kit (Perkin-Elmer Corp., Norwalk, CT), a dot-based system that did not perform particularly well with degraded samples or low-template samples, as well as the AmpF $\ell$ STR<sup>®</sup> Profiler Plus<sup>®</sup> PCR Amplification Kit (Applied Biosystems<sup>®</sup>, Foster City, CA) and the AmpF $\ell$ STR<sup>®</sup> COfiler<sup>®</sup> PCR Amplification Kit (Applied Biosystems<sup>®</sup>), which together amplified the thirteen core STR loci. Secondary DNA transfer was possibly detected, albeit below the reporting threshold of 75 RFU, with the AmpF $\ell$ STR<sup>®</sup> Profiler Plus<sup>®</sup> PCR Amplification Kit in a 25  $\mu$ L reaction volume following the standard 28 cycle manufacturer's protocol.

In 2002, Lowe et al. (14) detected secondary DNA transfer with the AmpF $\ell$ STR<sup>®</sup> SGM Plus<sup>®</sup> PCR Amplification Kit (Applied Biosystems<sup>®</sup>), which amplified ten STR loci, by increasing the number of amplification cycles from 28 to 34 (5,6). Increasing the number of amplification cycles effectively increased the sensitivity of the kit. At 28 cycles, full profiles would not be expected with < 100 pg using the AmpF $\ell$ STR<sup>®</sup> SGM Plus<sup>®</sup> PCR Amplification Kit; however, 34 cycles had the potential of producing full profiles with as little as 25–50 pg (6). In fact, under both cycling protocols, Lowe et al. obtained a full profile of one individual from an object the person did not touch (14).

In 2010, Goray et al. (15) investigated secondary DNA transfer of biological materials (i.e., pure DNA, saliva, and blood) and detected secondary transfer with the AmpF $\ell$ STR<sup>®</sup> Profiler Plus<sup>®</sup> PCR Amplification Kit following standard protocols; and in 2012, Daly et al. (16) again detected secondary transfer with the AmpF $\ell$ STR<sup>®</sup> SGM Plus<sup>®</sup> PCR Amplification Kit in a 50  $\mu$ L reaction with the standard 28 cycle protocol. Daly et al. concluded from their experiments that profiles obtained from touched objects are more likely to be the result of primary transfer rather than secondary transfer. However, this conclusion was based on assumptions rather than confirmatory results. Daly et al. assumed that the major component of any mixture obtained was from the individual who directly handled the object. In fact, they did not have reference samples from the participants in their experiments to confirm their assumptions (16). None of these additional studies addressed whether the secondary DNA transfer occurred in sufficient amounts to affect interpretation and the final conclusions drawn from a DNA profile.

Based on numerous validation studies, the minimum amount of template DNA required to produce a full profile has decreased (5–13). Full DNA profiles could be expected with a minimum DNA template amount of 300 pg using the AmpF $\ell$ STR<sup>®</sup> Profiler Plus<sup>®</sup> and COfiler<sup>®</sup> PCR Amplification Kits (7) following manufacturer's specifications. The AmpF $\ell$ STR<sup>®</sup> Identifier<sup>®</sup> PCR Amplification Kit (Applied Biosystems<sup>®</sup>) and the PowerPlex<sup>®</sup> 16 System (Promega, Madison, WI) brought the thirteen core STR loci into a single amplification with a few additional loci. Full profiles could be expected with a minimum DNA template amount of 250 pg using either the AmpF $\ell$ STR<sup>®</sup> Identifier<sup>®</sup> PCR Amplification Kit or the PowerPlex<sup>®</sup> 16 System (8,9). These kits were further optimized to overcome inhibition and increase their sensitivity (10,11). With the AmpF $\ell$ STR<sup>®</sup> Identifier<sup>®</sup> Plus PCR Amplification Kit (Applied Biosystems<sup>®</sup>) and the PowerPlex<sup>™</sup> 16 HS (Promega), full profiles could be expected with as little as 125 pg of DNA or even less under standard and extended thermal cycler parameters (10,11).

Today, forensic laboratories are in the process of implementing either the PowerPlex<sup>®</sup> Fusion System (Promega) or the GlobalFiler<sup>™</sup> Kit (Applied Biosystems<sup>®</sup>), both of which amplify 24 loci, including the 13 core STR loci. Full profiles can now be expected with as little as 100 pg or less of DNA (12,13). In addition, as the number of STR loci typed increases from amplification kit to amplification kit, so does the power of discrimination (7–13). The impact of secondary DNA transfer on forensic DNA typing results has not been systematically investigated employing current technology, which has become increasingly more sensitive to improve the likelihood of obtaining interpretable DNA profiles from low-template and low-quality samples.

The aim of this research project was to examine whether the presence of secondary DNA transfer could potentially complicate the interpretation of forensic DNA typing results and ultimately the final conclusion drawn from those results. This study also investigates whether the texture of an object's surface, rough, or smooth may facilitate the occurrence of secondary DNA transfer. Furthermore, the implications of this study on the investigation and prosecution of forensic evidence will be discussed.

## Materials and Methods

Twelve paring knives of the same brand with smooth plastic handles and twelve steak knives of the same brand with textured plastic handles were purchased prepackaged from a retail store and left in a sealed condition until required for the experiment. The smooth-handled knives were designated A through L, and the rough-handled knives were designated M through X. The individuals directly handling the knives were designated the "primary" handlers or contributors. The individuals shaking the hands of the primary handlers were designated potential "secondary" contributors.

After removal from the packages, the knives were cleaned with a 10% bleach solution and exposed to UV light for 30 min (15 min on each side) to remove potential contaminating surface DNA that may have been deposited on the knife surface during manufacturing and packaging. One smooth-handled knife and one rough-handled knife were swabbed after decontamination and prior to the knives being handled by participants. These two samples were designated contamination control samples and were used to evaluate the effectiveness of the quality control measures implemented prior to the knives being handled. The contamination control samples were extracted and quantified along with all other samples collected. Lack of DNA in these samples would be interpreted as meaning that the quality control measures implemented were effective. Further clean technique procedures were followed to minimize the introduction of extraneous DNA such as wearing personal protective equipment during sample collection and using a 10% bleach solution to clean work areas and equipment.

Twelve research participants were paired based on their previously determined STR profiles. Individuals with minimum allele sharing were paired together to maximize detection of mixed DNA profiles from the knife samples and also to verify the origin of any DNA profiles obtained from the knife samples. Prior to the experiment, participants were instructed to lightly rinse their hands with water, dry their hands, and then put on latex gloves. Participants wore gloves for 1.5 hours prior to sample collection in an attempt to reduce foreign DNA present on their hands as well as facilitate the transfer of DNA by potentially increasing sweat and oils on their hands. Immediately upon

removing gloves, participants vigorously shook hands, maintaining constant contact for two minutes to mimic intimate contact. Shaking hands for two minutes does not usually occur under normal circumstances; however, other forms of intimate contact may last longer than two minutes and a two-minute duration of contact was considered a reasonable test parameter. Following the contact period, each participant immediately handled his or her assigned knife for two minutes.

The entire handle surface of each knife was sampled immediately using a wet swabbing technique. Pur-Wraps sterile cotton tipped applicators (Puritan Medical Products Company LLC Guilford, Maine) were moistened with 100  $\mu$ L of sterile phosphate-buffered saline solution (PBS). Swab tips were immediately removed into separate sterile microcentrifuge tubes that were given the same designation as the corresponding knife. The four researchers conducting the experiment each swabbed a set of knives not associated with that researcher to avoid the risk of a false positive for transfer. Each researcher wore a face mask to prevent DNA contamination of the sample. Samples were stored at  $-20^{\circ}\text{C}$  until DNA extraction. In an effort to maintain the same sample collection conditions for each knife type, manipulation of the smooth-handled paring knives and the rough-handled steak knives occurred on separate days using the previously described decontamination and sample collection procedures. The same individuals were partnered for each phase of the experiment to limit the number of variables. Each individual ultimately handled one smooth-handled knife and one rough-handled knife. For example, Individual #1 was the primary handler of smooth-handled knife F and rough-handled knife R while being the secondary contributor of smooth-handled knife E and rough-handled knife Q.

Extraction of the swabs from the smooth-handled and rough-handled knives along with the representative control swabs was performed according to the Qiagen (Hilden, Germany) DNA Purification from Buccal Swabs Spin Protocol. Reagent blanks were used to monitor reagent contamination. The samples were eluted into 150  $\mu$ L buffer AE (Tris-HCL and EDTA).

Quantifiler™ Human DNA Quantification Kit (Applied Biosystems) in conjunction with an Applied Biosystems 7500 Real-Time PCR instrument was used to determine the concentration of human DNA present in each sample. The amount of DNA available for analysis was calculated using the quantitation value obtained from the assay multiplied by the remaining volume of the sample. A statistical comparison of DNA yields obtained from the smooth-handled knives and rough-handled knives was performed using SPSS (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.). After quantification, all samples except for knife sample W were concentrated into 15  $\mu$ L of deionized water using Viamom 500s with Hydrostat® membranes (Vivaproducts, Inc. Littleton, MA) prior to amplification in order to amplify the maximum amount of DNA possible.

Samples with a detectable amount of DNA were amplified with the Identifiler® Plus Amplification Kit (Applied Biosystems) in a 25  $\mu$ L reaction. Except for one knife (knife W), which contained enough DNA to target 1.5 ng, the maximum volume of sample (10  $\mu$ L) was used for amplification. Amplification was performed by PCR on an Applied Biosystems (Forest City, CA) 9700 thermal cycler following manufacturer's specifications. A positive amplification control using 9947A DNA template and a negative amplification control were amplified as well and were used to monitor amplification success and reagent contamination. Amplified product was analyzed in a 10  $\mu$ L reaction

that consisted of 1  $\mu$ L amplified product plus 9  $\mu$ L formamide/GeneScan™ 500 LIZ™ dye size standard (Applied Biosystem) mixture, using capillary electrophoresis on an AB 3130xl (Applied Biosystem) instrument in conjunction with GeneMapper ID (version 3.2.1). Samples were injected for 5 and 10 seconds at 3kv with a spectral generated using the G5 dye set (Applied Biosystem) applied.

Although the possible contributors to these samples were known, the DNA profiles obtained were analyzed and interpreted independently as if they were unknown profiles obtained from casework samples. Data from the 10 second injections were used for interpretation to maximize the number of alleles identified in each sample. The DNA profiles were analyzed using an analytical threshold of 50 RFU and were interpreted with a stochastic threshold of 200 RFU. A peak height ratio of 50% was used to aid in the deconvolution of mixtures. Loci suitable for statistical calculations were identified before any comparisons were made. Statistical comparisons to the known participants' profiles were made only after the knife sample DNA profiles were independently interpreted to avoid bias. Statistical calculations, using a value of 0.03 for  $\theta$ , were conducted as in casework to show the impact the presence of the secondary contributor had on the discriminating potential of the profile. Frequencies published by the FBI for Caucasian, African American, and southwestern Hispanic populations were used for the statistical calculations (17,18).<sup>1</sup> Interpretational parameters utilized were established by internal validation studies conducted at Strand Analytical Laboratories (Indianapolis, IN).

## Results and Discussion

Samples were evaluated for the following: (i) if the DNA profile obtained was from a single contributor or multiple contributors; (ii) if the DNA profile, whether a single source or mixture, was consistent with the individuals associated with the sample; (iii) if foreign alleles not attributed to primary and secondary contributors were identified, could the source of the extraneous DNA be identified; (iv) if secondary DNA transfer (i.e., alleles attributable to the "secondary contributor") was detected; (v) if secondary DNA transfer occurred, could the profile be interpreted and a conclusion drawn regarding the source(s) of the profile; (vi) if the profile was suitable for statistical analysis; and (vii) if the texture of the knife handle facilitated secondary DNA transfer.

Profiles were categorized as follows: single source defined as being from a single contributor; possible mixture defined as a single source above the analytical threshold with a possible contributor below the analytical threshold; two person mixture defined as a profile exhibiting no more than four alleles at any one locus; at least two person mixture defined as a profile exhibiting no more than four alleles at any one locus, but not all alleles consistent with the primary and secondary contributors; greater than two person mixture defined as a profile exhibiting more than four alleles at any one locus and not all alleles consistent with the primary and secondary contributors; an indistinguishable mixture defined as a mixture that could not be deconvoluted using peak height ratios (PHRs); and a major/minor mixture defined as a mixture that could be deconvoluted using PHRs.

<sup>1</sup>Some frequencies were found to be inaccurate. Statistical calculations can be re-evaluated using frequencies published by Hill et al., *Forensic Science International: Genetics* 7 (2013) e82–e83.

TABLE 1—Quantifiler® Human results for knives tested.

Knife	Elution Volume (μL)	DNA Concentration (ng/μL)	DNA Available for Testing (ng)	Knife	Elution Volume (μL)	DNA Concentration (ng/μL)	DNA Available for Testing (ng)
A	150	0.00678	1.00	M	150	0.0063	0.93
B	150	0.00659	0.98	N	150	0.00189	0.28
C	150	0.00353	0.52	O	150	0.00528	0.78
D	150	0.00115	0.17	P	150	0	0.00
E	150	0.00514	0.76	Q	150	0	0.00
F	150	0.00493	0.73	R	150	0.0076	1.12
G	150	0.00275	0.41	S	150	0	0.00
H	150	0	0.00	T	150	0.00702	1.04
I	150	0.00513	0.76	U	150	0.000503	0.07
J	150	0.00956	1.41	V	150	0.00424	0.63
K	150	0.00891	1.32	W	150	0.0338	5.00
L	150	0.0053	0.78	X	150	0.0121	1.79
Smooth Control	150	0	0.00	Rough Control	150	0	0.00
RB 041113	150	0	0.00	RB 041613	150	0	0.00

Values obtained from quantification were reported as determined by the assay (Table 1). However, the values may not be accurate because a majority of the samples quantified below the lowest point of the standard curve (0.023 ng/μL). No human DNA was detected in the contamination control samples or in the reagent blanks supporting the conclusion that the quality control measures implemented to decontaminate the knives were effective and no extraneous DNA was introduced into the samples during extraction. Likewise, four knife samples did not yield human DNA and were not amplified. Given the results reported in previous studies (2,4,14–16), it is clear that the quantity and quality of DNA recovered from touched objects varies. This variation could be due to a number of factors including an individual's propensity to shed DNA (14). The experiment was designed to control sampling variables to the greatest extent possible, but not detecting DNA in some samples is not surprising. A paired-samples *t*-test was conducted using SPSS (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.) to compare the concentration of DNA (ng/μL) determined for the smooth-handled knives and the rough-handled knives. There was no significant difference in concentrations of DNA (ng/μL) from smooth-handled knives ( $M = 0.005$ ,  $SD = 0.003$ ) and rough-handled knives ( $M = 0.007$ ,  $SD = 0.009$ ) conditions;  $t(11) = -0.641$ ,  $p = 0.535$ .

A variety of DNA profiles was obtained during this research project (Table 2). DNA typing results indicated that secondary DNA transfer occurred in 17 of the 20 knife samples (85%) amplified as verified by the presence of alleles consistent with the secondary contributors' DNA profiles. Secondary DNA transfer was not detected in smooth-handled knife samples A and K or in rough-handled knife sample V.

In smooth-handled knife samples C, E, F, and J; and rough-handled knife sample N, alleles from both the primary and secondary contributors were detected; however, the presence of extraneous DNA complicated the interpretation of the DNA profiles. In all five samples, foreign alleles not consistent with either the primary or secondary contributor were identified. For example, a greater than two person mixture with major and minor components was obtained from knife C. The major component was an unknown DNA profile while the minor component was consistent with the DNA profiles of the primary and secondary contributors. The unknown DNA profile was compared to the DNA profiles of all participants and laboratory personnel. The source of the major component could not be identified. As swabings of individual participants' hands were not collected prior to handshaking, it is possible that rinsing hands lightly was not

completely effective at removing all foreign DNA. Likewise, as only a single representative of each knife type was collected, it is possible that the measures implemented to remove background DNA from the knives prior to handling were not as effective as previously thought. Foreign alleles identified were not consistent between samples suggesting that the extraneous DNA was not the result of reagent contamination or cross-contamination, and the foreign alleles were not consistent with the profiles of individuals involved in the experiment, the individuals doing the testing, or other laboratory personnel. Some of the aberrant results could be due to allelic drop-in or elevated stutter products. The presence of the extraneous DNA would render the profiles obtained from knife samples E, F, and J as well as the minor components of knife samples C and N inconclusive for comparison purposes because the number of individuals contributing to the samples could not be determined and most loci exhibited peaks with heights below the stochastic threshold.

Two person mixtures with major and minor components were obtained from rough-handled knife samples M, O, R, T, and W, where the major component was consistent with the DNA profile of the primary handler while the minor component was consistent with the DNA profile of the secondary contributor. However, the secondary contributor was not detected in sufficient quantities to produce a CODIS-suitable profile. The minor components obtained from knife samples M, R, T, and W would most likely be reported as inconclusive for comparison purposes because there were no loci suitable for statistical calculations; the peak heights of the minor alleles were below the stochastic threshold.

Indistinguishable mixtures of both the primary and secondary contributors were obtained from three knife samples. Interpretation of the DNA profiles obtained from smooth-handled knife samples D and G; and rough-handled knife sample U was made more complicated by the presence of DNA from the secondary contributor. Deconvolution of the mixtures obtained from these knife samples into major and minor components was not possible. Interpreting a single-source profile is straightforward, but interpreting a two or greater than two person mixture can be more complicated. In addition, as the number of individuals contributing to a DNA profile increases, the discriminatory power of the DNA profile obtained from that sample may decrease. For example, the DNA profile obtained from knife sample U (Fig. 1) was interpreted as a two person mixture. Only two loci (D8S1179 and D3S1358) were deemed suitable for statistical calculations because the number of alleles identified (four alleles) at these loci met the assumption of a two person mixture.



TABLE 2—Interpretation of the AmpFISTR® Identifier® Plus profiles developed from DNA extracted from the smooth- and rough-handled knives.

Knife	Profile Category	Source(s)	Number of Loci Suitable for Stats	Number of Alleles		Number of Alleles		Number of Extranous Alleles Observed
				Observed/Expected—Contributor	Primary Contributor	Observed/Expected—Contributor	Secondary Contributor	
<b>Smooth-Handled Knives</b>								
A	Single source	Matches primary contributor	12	25/28		0/28		0
B	Possible mixture with major component and possible minor component	Major component matches secondary contributor; possible minor component below analytical threshold	15 major/0 possible minor	0/28		28/28		0
C	>2 person mixture with major and minor components	Major component unknown DNA profile; minor component consistent with primary and secondary contributors; <i>extraneous DNA detected</i>	13 major/0 minor	25*/25		25*/26		17
D	2 person indistinguishable mixture	Consistent with primary and secondary contributors	None	24*/25		26*/26		0
E	>2 person indistinguishable mixture	Consistent with primary and secondary contributors; <i>extraneous DNA detected</i>	None	23*/27		30*/30		2
F	At least 2 person indistinguishable mixture	Consistent with primary and secondary contributors; <i>extraneous DNA detected</i>	None	28*/30		20*/27		3
G	2 person indistinguishable mixture	Consistent with primary and secondary contributors	1	19*/25		25*/27		0
I	2 person mixture with major and minor components	Major component matches secondary contributor; minor component consistent with primary contributor	15 major/1 minor	5*/31		30/30		0
J	At least 2 person mixture with major and minor components	Major component matches primary contributor; <i>extraneous DNA detected</i>	15 major/0 minor	30/30		2†/31		1
K	Single source	Matches primary contributor	15	26/26		0/26		0
L	Possible mixture with major component and possible minor component	Major component matches secondary contributor; possible minor component below analytical threshold	6 major/0 possible minor	0/26		17/26		0
<b>Rough-Handled Knives</b>								
M	2 person mixture with major and minor components	Major component matches primary contributor; minor component consistent with secondary contributor	15 major/0 minor	28/28		1†/28		0
N	At least 2 person mixture with major and minor components	Major component matches secondary contributor; <i>extraneous DNA detected</i>	15 major/0 minor	5†/28		28/28		2
O	2 person mixture with major and minor components	Major component matches primary contributor; minor component consistent with secondary contributor	15 major/2 minor	25/25		7†/26		0
R	2 person mixture with major and minor components	Major component matches primary contributor; minor component consistent with secondary contributor	15 major/0 minor	30/30		10†/27		0
T	2 person mixture with major and minor components	Major component matches primary contributor; minor component consistent with secondary contributor	14 major/0 minor	27/27		2†/25		0
U	2 person indistinguishable mixture	Consistent with primary and secondary contributors	2	17*/31		28*/30		0
V	Possible mixture with major component and possible minor component	Major component matches primary contributor; possible minor component below analytical threshold	15 major/0 possible minor	30/30		0/31		0
W	2 person mixture with major and minor components	Major component matches primary contributor; minor component consistent with secondary contributor	15 major/0 minor	26/26		3†/26		0
X	2 person mixture with major and minor components	Major component matches secondary contributor; minor component consistent with primary contributor	15 major/1 minor	8†/26		26/26		0

\*Number of observed alleles includes shared alleles between primary and secondary contributors when an indistinguishable mixture was identified.

†All minor alleles exhibited peak heights below stochastic threshold.

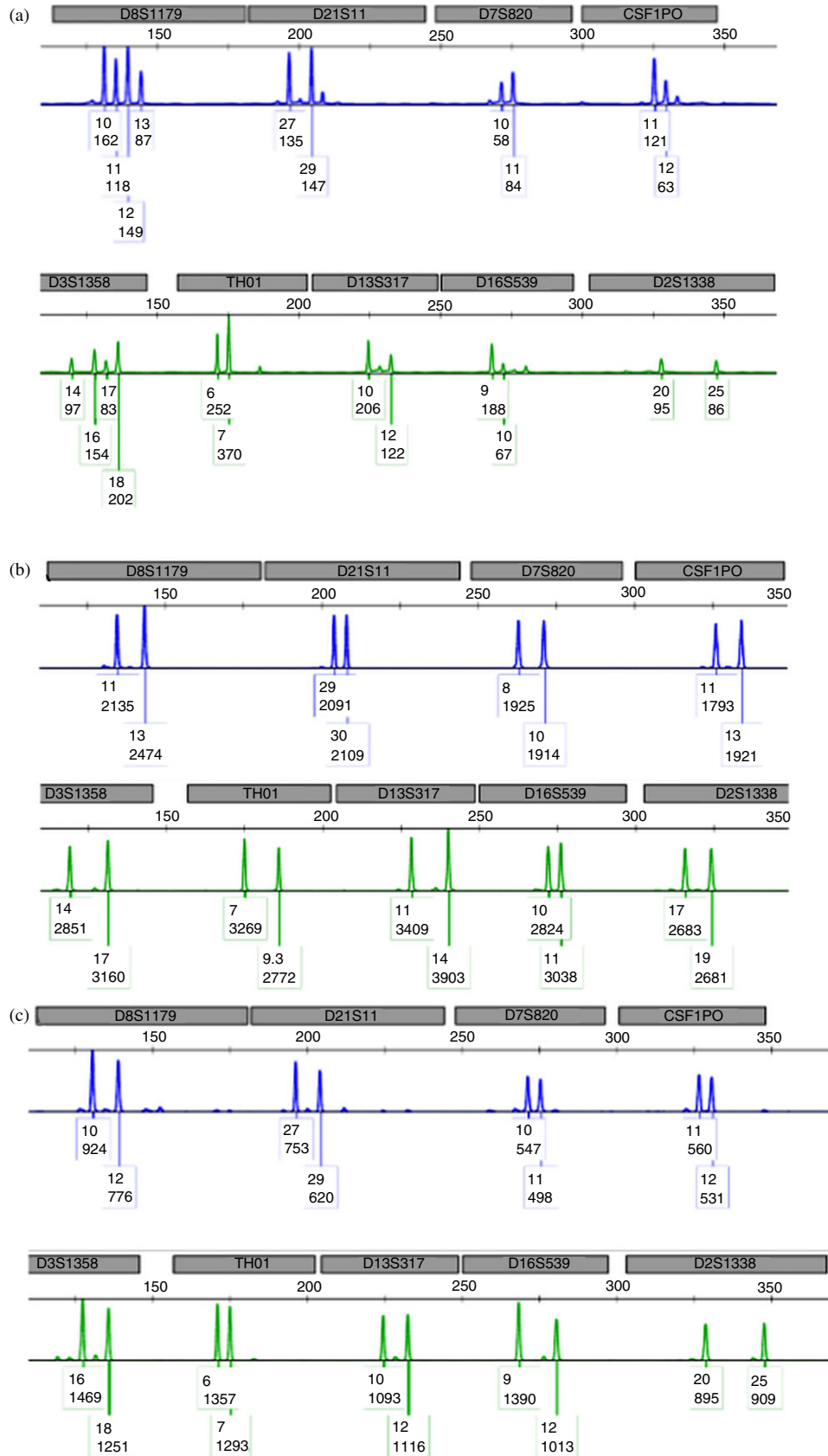


FIG. 1—AmpFISTR® Identifiler® Plus profiles from Knife U, a rough-handle knife (a), and primary (b) and secondary (c) contributors (control profiles). Only FAM & VIC-labeled loci shown for the protection of individual participants' privacy. A total of 0.07 ng was targeted in the 25 μL amplification of knife U and the sample was injected for 10 sec at 3 kv on a 3130xl. Data were analyzed using a 50 RFU analytical threshold and interpreted using a 200 RFU stochastic threshold.

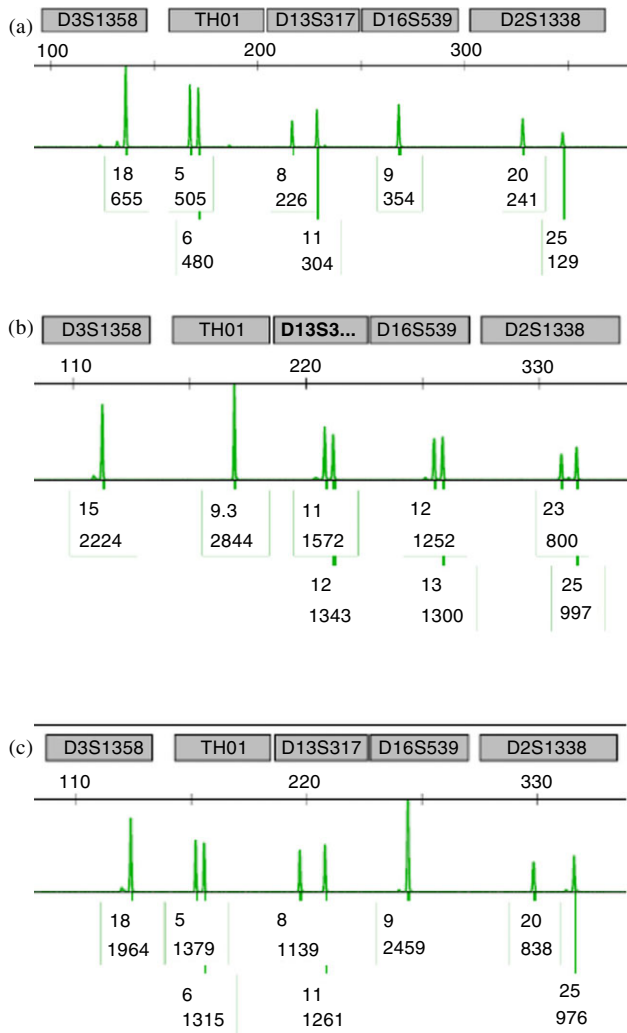


FIG. 2—AmpFISTR® Identifier® Plus profiles from Knife B, a smooth-handle knife (a), and primary (b) and secondary (c) contributors (control profiles). Only VIC-labeled loci shown for the protection of individual participants' privacy. A total of 0.65 ng was targeted in the 25  $\mu$ L amplification of knife B and the sample was injected for 10 sec at 3 kv on a 3130xl. Data were analyzed using a 50 RFU analytical threshold and interpreted using a 200 RFU stochastic threshold.

All other loci exhibited less than four alleles with peak heights below the stochastic threshold, and it is possible that information was missing at the other loci. At some loci, there was clear allelic dropout where peaks were visible below the analytical threshold. Following the same interpretational guidelines, only one locus was suitable for statistical calculations in knife sample G; no loci were suitable for statistical calculations in knife D. In these instances, the presence of DNA from the secondary contributor lowered the discriminatory power of the profiles and possibly rendered them inconclusive for comparison purposes.

The occurrence of secondary DNA transfer was the most pronounced in the DNA profiles obtained from knife samples B, I, L, N, and X, where the secondary contributor was either the only contributor or the major contributor identified despite never coming into direct contact with the knives. For example, the single-source DNA profile identified above the analytical threshold in smooth-handled knife B (Fig. 2) and the major DNA profile obtained from rough-handled knife N matched the DNA profile of the secondary contributor, which happened to be the same

individual. The probability of an unrelated individual selected at random from the population being the source of the DNA profile is approximately 1 in 983 quintillion. (Recalculated using frequencies published by Hill et al., Forensic Science International: Genetics 7 (2013) e82–e83, number would be 1 in 4 sextillion.)

Observing a single-source profile or a major component deduced to a single source together with a discriminating statistical calculation could lead investigators to believe that the source of the DNA profile was the individual who directly handled the object and was the perpetrator of the crime. If these results were presented during a trial as forensic evidence, they would be difficult to dispute. Data obtained from five knife samples (B, I, L, N, and X) suggest that individuals can have their DNA deposited on an item in sufficient quantities to be the only contributor or major contributor identified without ever coming into direct contact with the object. Likewise, it is possible that secondary DNA transfer could result in a discriminating minor profile; however, a discriminating minor profile resulting from secondary DNA transfer was not observed in this sample set. The results obtained from this study illustrate the risk the expert testifying on the DNA results runs by referring to the samples as “touch” DNA or “wearer” DNA. Such terminology implies the source of the DNA profile has to come into direct contact with an object to leave his/her DNA on the object. The demonstrated possibility of secondary DNA transfer could have major ramifications in a forensic investigation; secondary DNA transfer should not be regarded as an event that may only occur under optimal experimental conditions.

In summary, DNA typing results were obtained from 20 of 24 knife samples. The texture of the knife handle did not appear to have a significant effect on the results. Two profiles were clearly from a single source while eighteen were profiles from more than one source. In most instances, the DNA profiles obtained were attributable to the individuals associated with the samples. Alleles foreign to the two known contributors were observed in five samples; the source of these foreign alleles could not be identified. Secondary DNA transfer (i.e., alleles attributable to the individual that did not touch the knife) was detected in 16 instances. In three of the profiles that exhibited secondary DNA transfer, the DNA profile of the secondary contributor was sufficient to affect the interpretation of the results. In five samples, the DNA profile resulting from secondary transfer was suitable for statistical analysis; these profiles had the potential to falsely link an individual to an item of evidence.

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