Bioinformatics WG Journal Club

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Could Secondary DNA Transfer Falsely Place Someone at the Scene of a Crime?

Cynthia M. Cale B.S.[1,2,*], Madison E. Earll M.S.[2], Krista E. Latham Ph.D.[2] and Gay L. Bush Ph.D.[1]

Author Information:

[1] Strand Analytical Laboratories, Indianapolis, IN

[2] Human Biology Program, University of Indianapolis, Indianapolis, IN

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Disclaimer:

This seminar does not aim to inspire you to commit a crime and frame other people for your felony.

DNA evidence in court – "We need more research on when and how secondary transfer can occur."

- Given the power of modern forensic techniques to pull a DNA profile from a smudge of cells, secondary DNA transfer is no longer a purely theoretical risk. In California in 2013, a man called Lukis Anderson was arrested, held for four months and charged with murder after his DNA was found under the fingernails of a homicide victim.
- Anderson had never met the victim and was severely intoxicated and in hospital when the man was killed. The same paramedics who took Anderson to hospital responded to the murder. Most likely, the paramedics were covered in Anderson's DNA, which they then inadvertently transferred. The charges were dropped.

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.

Questions asked in this context:

- What is the efficiency of the secondary transfer of genetic material to other surfaces / samples?
- What about transfer of a sample in the form of blood, saliva or extracted DNA from surface to surface? How does it depend on the surface characteristics (smooth or absorbent) and form of contact (passive, pressure or friction)
- Contamination & decontamination in a sensitive DNA testing laboratory.

Additional papers included:

- Goray M, Eken E, Mitchell RJ, van Oorschot RA. **Secondary DNA transfer of biological substances under varying test conditions**. *Forensic Sci Int Genet* 2010;**4**:62–7.

- Goray M, Mitchell RJ, van Oorschot RA. **Investigation of secondary DNA transfer of skin cells under controlled test conditions**. Leg Med. 2010;12:117–120.

- Vandewoestyne M, Van Hoofstat D, De Groote S, Van Thuyne N, Haerinck S, et al. (2011) **Sources of DNA Contamination and Decontamination Procedures in the Forensic Laboratory**. J Forensic Res S2:001.

Starting points for the current case-study:

- None of the previous 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.

Forensic Typic Systems & Procedures for Forensic DNA Analysis

- **Restriction Fragment Length Polymorphisms (RFLPs)**
- **AmpliType PM & DQA1 - Sequence Polymorphisms -** 6 loci detected/delineated by hybridization to **allele-specific oligonucleotide (ASO)** probes, a dot-based system.
- **Amplified Fragment Length Polymorphism (AFLP) – VNTRs (**variable number tandem repeat **) detected by PCR amplification.**
- The use of different **fluorescent dyes** allows the **multiplex amplification of STR loci** that have overlapping allele ranges as long as a separate dye is used for each overlapping **STR**.

Short Tandem Repeat Loci based Kits:

- **AmpFℓSTR® Profiler Plus®** PCR Amplification Kit (with fluorescent multicolor dye technology; 8 loci + X-Y; recommended range of input sample DNA is 1.0–2.5 ng; multiplex PCR)
- The **AmpFℓSTR® COfiler®** PCR Amplification Kit *complements the AmpFLSTR® Profiler* Plus® PCR Amplification Kit (two kits together cover 13 loci)
- The **AmpFℓSTR® SGM Plus®** PCR Amplification Kit (10 loci + gender marker) – has the potential of producing a full profile with as little as 25-50 pg of DNA
- later **AmpFℓSTR® kits** include the degenerate unlabeled primer (e.g. AmpFℓSTR® Identifiler PCR Amplification Kit; 13+3 loci in a single tube)

Second Generation Multiplex Plus (SGM Plus), is a DNA profiling system developed by Applied Biosystems.
It is an updated version of Second Generation Multiplex. SGM Plus has been used by the UK National DNA Database since 1998.

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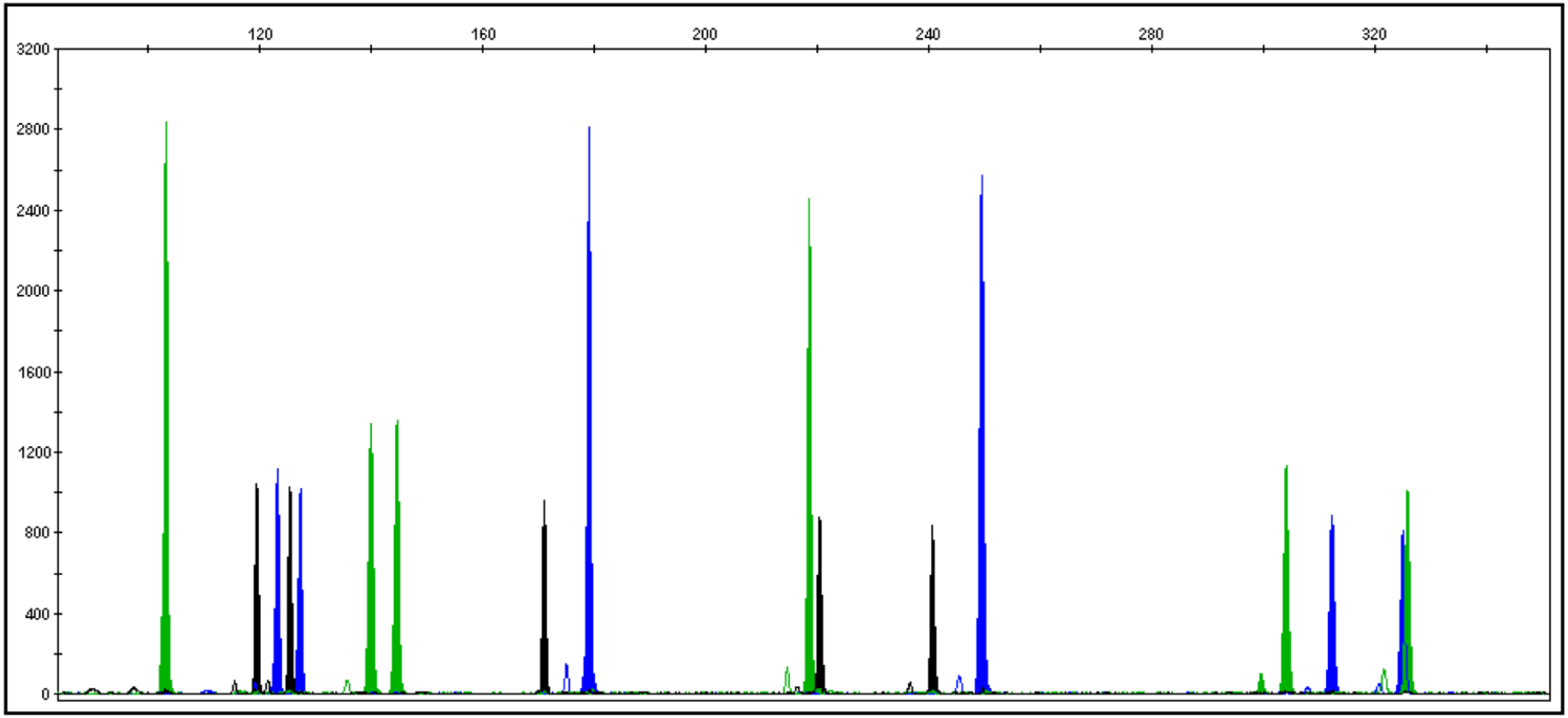
Example SGM Plus profile

SGM Plus profile of subject GT36865 [4]

Locus	Allele values
FGA	22,22
TH01	6,7
VWA	14,16
D2S1338	19,24
D3S1358	17,17
D8S1179	13,14
D16S539	9,13
D18S51	13,16
D19S433	14,15
D21S11	30,30
Amelogenin	XX

Figure 4 AmpF*ℓ*STR® SGM Plus® Kit PCR Amplification Kit results from a 1.2-mm FTA bloodstain disc (25-cycle amplification), analyzed on the Applied Biosystems 3130*xl* Genetic Analyzer



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Today,

- forensic laboratories are in the process of implementing either the PowerPlex® Fusion System (Promega) or the GlobalFiler™ Kit (Applied Biosystems®), both of which amplify **24 loci**. Full profiles can now be expected with as little as 100 pg or less of DNA.

- They include the 13 core STR loci required by the **Combined DNA Index System (CODIS)** and the 12 core **European Standard Set loci** . and Amelogenin. In addition, the male-specific DYS391 locus is included to identify null Y allele results for Amelogenin.

New tests with more markers in development:

SNPforID assay comprising 50 autosomal SNPs:

- amplification of SNPs performed in 2 Multiplex PCRs comprising 21 and 29 primer pairs
- Followed Single-base extension (SBE) using the ABI PRISM® SNaPshot™ kit
- Detection threshold down to 500 pg input DNA [while 100 pg still allowed the detection of 36 out of 50 SNPs (72%), and 25 pg template DNA still led to 19 detectable SNPs (38% success rate)]

Experiment design:

1.5 hours

2 min

M-X

A-L

Can the texture of an object's **surface, rough or smooth**, facilitate the occurrence of secondary DNA transfer ?

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

Samples were evaluated for the following:

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

Profiles were categorized as follows:

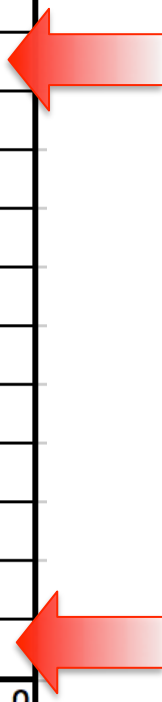
- single source defined as being from a single contributor;
- possible mixture defined as a single source above the analytical threshold with a possible contributor below the threshold;
- two person mixture defined as a profile exhibiting no more than four alleles at any one locus;
- at least two person 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.

Knife	Profile Category	Source(s)	Number of Extraneous Alleles Observed
Smooth-Handled Knives			
A	Single source	Matches primary contributor	0
B	Possible mixture with major component and possible minor component	Major component matches secondary contributor; possible minor component below analytical threshold	0
C	>2 person mixture with major and minor components	Major component unknown DNA profile; minor component consistent with primary and secondary contributors; extraneous DNA detected	17
D	2 person indistinguishable mixture	Consistent with primary and secondary contributors	0
E	>2 person indistinguishable mixture	Consistent with primary and secondary contributors; extraneous DNA detected.	2
F	At least 2 person indistinguishable mixture	Consistent with primary and secondary contributors; extraneous DNA detected	3
G	2 person indistinguishable mixture	Consistent with primary and secondary contributors	0
I	2 person mixture with major and minor components	Major component matches secondary contributor; minor component consistent with primary contributor	0
J	At least 2 person mixture with major and minor components	Major component matches primary contributor; extraneous DNA detected	1
K	Single source	Matches primary contributor	0
L	Possible mixture with major component and possible minor component	Major component matches secondary contributor; possible minor component below analytical threshold	0

Knife	Profile Category	Source(s)	Number of Extraneous Alleles Observed
Rough-Handled Knives			
M	2 person mixture with major and minor components	Major component matches primary contributor; minor component consistent with secondary contributor	0
N	At least 2 person mixture with major and minor components	Major component matches secondary contributor; extraneous DNA detected	2
O	2 person mixture with major and minor components	Major component matches primary contributor; minor component consistent with secondary contributor	0
R	2 person mixture with major and minor components	Major component matches primary contributor; minor component consistent with secondary contributor	0
T	2 person mixture with major and minor components	Major component matches primary contributor; minor component consistent with secondary contributor	0
U	2 person indistinguishable mixture	Consistent with primary and secondary contributors	0
V	Possible mixture with major component and possible minor component	Major component matches primary contributor; possible minor component below analytical threshold	0
W	2 person mixture with major and minor components	Major component matches primary contributor; minor component consistent with secondary contributor	0
X	2 person mixture with major and minor components	Major component matches secondary contributor; minor component consistent with primary contributor	0

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	**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
E	**150**	**0.00514**	0.76	Q	150	0	0
F	**150**	**0.00493**	0.73	**R**	**150**	**0.0076**	1.12
G	**150**	**0.00275**	0.41	S	150	0	0
H	150	0	0	**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
L	**150**	**0.0053**	0.78	**X**	**150**	**0.0121**	1.79
Smooth Cont	150	0	0	Rough Contr	150	0	0
RB 041113	150	0	0	RB 041613	150	0	0



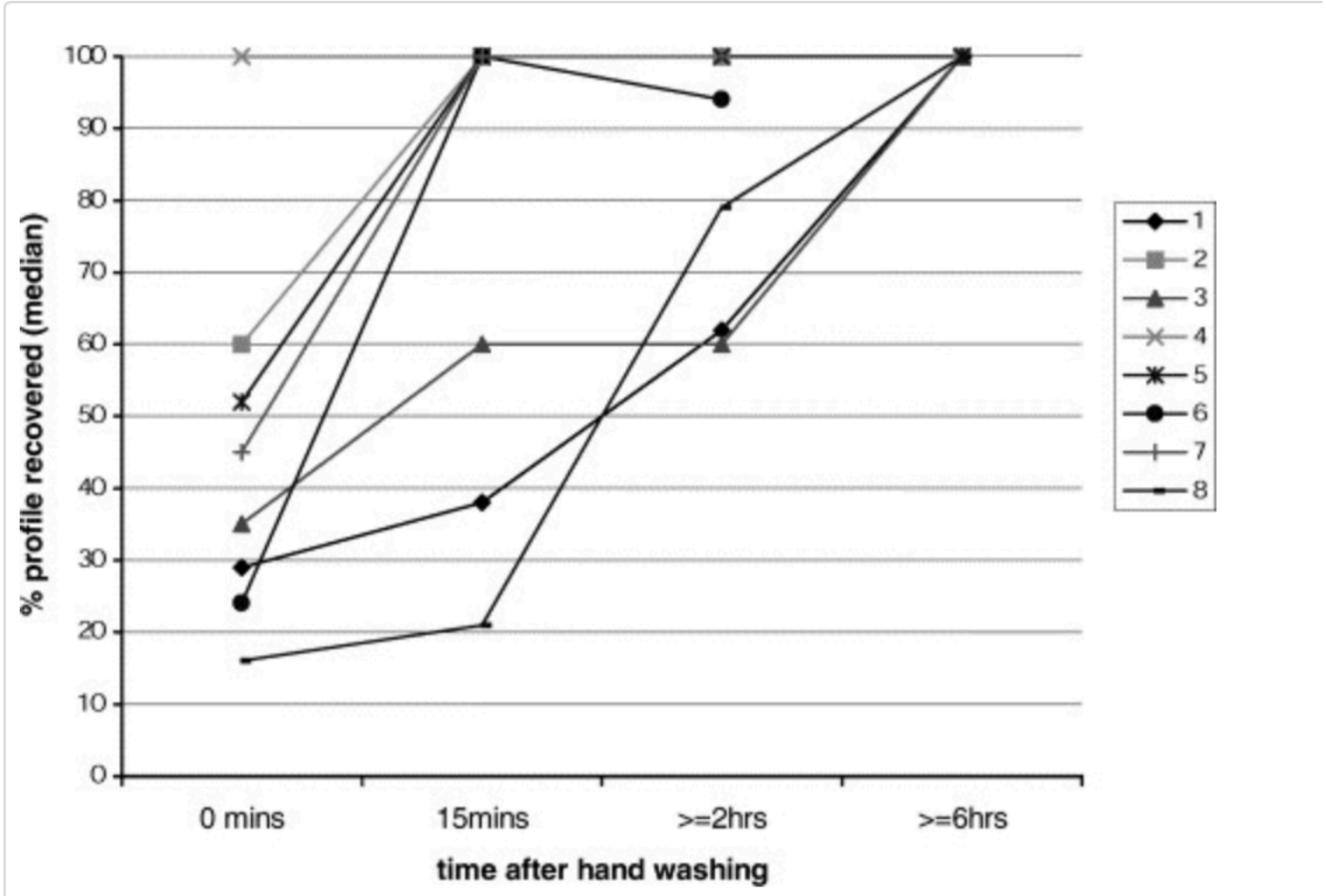


Fig. 1. *Assessment of shedding capabilities of eight different individuals. Showing the percentage subject profile (calculated from the median number of reportable alleles) recovered from an object held for ten seconds after hand washing (A→object) (34-cycle amplification).*

Alex Lowe, Caroline Murray, Jonathan Whitaker, Gillian Tully, Peter Gill

The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces

Forensic Science International, Volume 129, Issue 1, 2002, 25–34; http://dx.doi.org/10.1016/S0379-0738(02)00207-4

Conclusions:

- 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 5 smooth-handled knife samples presence of extraneous DNA complicated the interpretation of the DNA profiles. The profile was compared to the DNA profiles of all participants and laboratory personnel, however the source could not be identified.
- 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.
- 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.
- If these results were presented during a trial as forensic evidence, they would be difficult to dispute.

In summary:

- DNA typing results were obtained from 20 of 24 knife samples.
- The texture of the knife handle had no significant effect.
- **Only 2 profiles were clearly from a single source.**
- **Alleles foreign to the two known contributors** (**source unidentifiable**) were observed **in 5 samples;**
- **Secondary DNA transfer** (i.e., alleles attributable to the individual that did not touch the knife) was detected in **16 instances**.
- In **3** 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 **5 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.

EFFICIENCY OF DEPOSITION AND SECONDARY TRANSFER OF CELLS, BLOOD SALIVA & DNA

Transfer of freshly deposited skin cells:

Transfer rates approximately double from passive contact (average of 5.88%) to pressure (average of 11.05%) and increase further with friction (average of 20.95%), but this order is not observed in all instances.

Table 1.

Mean% transfer (standard deviation) of primary and secondary substrate combinations under passive, pressure and friction contact with freshly deposited touch (skin) cells.

Primary substrate	Secondary substrate					
	Plastic			Cotton		
	Passive	Pressure	Friction	Passive	Pressure	Friction
Plastic	2.7 (6.6)	18.38 (27.2)	29.34 (30.7)	18.46 (19.19)	24.7 (26.1)	14 (18.59)
Cotton	0.28 (0.5)	0.26 (0.43)	7.9 (3.9)	2.07 (2.32)	0.84 (0.72)	32.55 (20.7)

Leg Med (Tokyo). 2010 May;12(3):117-20. doi: 10.1016/j.legalmed.2010.01.003. Epub 2010 Mar 4.

Investigation of secondary DNA transfer of skin cells under controlled test conditions.

Goray M1, Mitchell RJ, van Oorschot RA.

Transfer of dried deposits of skin cells:

Transfer rates depend on the substrate combination and manner of contact. As found with freshly deposited samples, plastic as the primary substrate facilitated greater transfer of skin cells (average of 17.49%) compared to cotton (average of 9.03%) but this difference is insignificant (p = 0.407) and influenced by the type of secondary substrate.

Table 3.

Mean% transfer (standard deviation) of primary and secondary substrate combinations under passive, pressured and friction contact with dried touch (skin) cells.

Primarysubstrate	Secondary substrate					
	Plastic			Cotton		
	Passive	Pressure	Friction	Passive	Pressure	Friction
Plastic	2.09 (5.12)	0.65 (1.3)	39.9 (8.5)	3.63 (3.69)	9.66 (8.62)	49.02 (30.9)
Cotton	0.37 (0.35)	0.33 (0.45)	28.9 (26.8)	1.86 (3.67)	9.75 (4.64)	12.97 (5.7)

Leg Med (Tokyo). 2010 May;12(3):117-20. doi: 10.1016/j.legalmed.2010.01.003. Epub 2010 Mar 4.

Investigation of secondary DNA transfer of skin cells under controlled test conditions.

Goray M1, Mitchell RJ, van Oorschot RA.

% of secondary transfer, wet samples

Table 1.

Mean % transfer (standard deviation) of DNA under experimental primary and secondary substrate combinations and different types of contact (60 s), with wet pure DNA, blood and saliva.

Primary substrate	Biological source	Secondary substrate								
		Plastic			Cotton			Wool		
		Passive	Pressure	Friction	Passive	Pressure	Friction	Passive	Pressure	Friction
Plastic	DNA	–	–	–	98.6 (1.5)	99.9 (0.05)	100 (0.02)	–	–	–
	Blood	48.6 (27.1)	64.1 (7.71)	44.3 (16.6)	98.2 (1.5)	90.2 (8.75)	97 (2.38)	81.5 (6.63)	87.5 (2.41)	88.1 (3.3)
	Saliva	–	–	–	99.4 (0.2)	96.7 (1.24)	99.6 (0.2)	–	–	–
Cotton	DNA	0.005 (0.009)	0.02 (0.01)	0.04 (0.04)	0.02 (0.03)	0.07 (0.05)	0.23 (0.07)	–	–	–
	Blood	0.425 (0.79)	0.28 (0.38)	3.05 (0.77)	0.23 (0.45)	0.98 (0.59)	1.05 (2.1)	0.15 (0.19)	1.7 (1.91)	18.8 (10.7)
	Saliva	0.03 (0.05)	0.11 (0.17)	0.1 (0.07)	0.05 (0.004)	0.58 (0.4)	4.33 (2.45)	–	–	–
Wool	DNA	–	–	–	–	–	–	–	–	–
	Blood	1.63 (0.78)	1.85 (1.74)	2.55 (0.57)	0.23 (0.29)	1.78 (0.79)	15.5 (5.8)	0 (0)	0.2 (0.22)	7.43 (7.45)
	Saliva	–	–	–	–	–	–	–	–	–

Table 3.
Mean % transfer (standard deviation) of DNA under experimental primary and secondary substrate combinations and different types of contact (60 s), with dry pure DNA, blood and saliva.

Primary substrate	Biological source	Secondary substrate								
		Plastic			Cotton			Wool		
		Passive	Pressure	Friction	Passive	Pressure	Friction	Passive	Pressure	Friction
Plastic	DNA	0	0.84 (0.78)	3.75 (1.83)	0.05 (0.01)	0.02 (0.02)	0.25 (0.14)	–	–	–
	Blood	1.45 (2.9)	0.25 (0.5)	44.5 (16.4)	0	3.4 (6.8)	16.1 (10.1)	0.4 (0.47)	0	16.8 (21.7)
	Saliva	0.005 (0.01)	0	0	0.006 (0.01)	0.002 (0.002)	0.27 (0.32)	–	–	–
Cotton	DNA	0	0.004 (0.005)	0.02 (0.03)	0.03 (0.02)	0.06 (0.04)	0.49 (0.47)	–	–	–
	Blood	0	0	0.05 (0.1)	0	0	0	0.08 (0.05)	0	1.43 (1.25)
	Saliva	0	0	0.006 (0.01)	0.01 (0.02)	0	0.57 (0.18)	–	–	–
Wool	DNA	–	–	–	–	–	–	–	–	–
	Blood	0	0.05 (0.01)	1.35 (1.05)	0.05 (0.1)	0.15 (0.1)	1.15 (0.61)	0	0.13 (0.19)	0.5 (0.49)
	Saliva	–	–	–	–	–	–	–	–	–

% of secondary transfer, dry samples

Forensic Research

Vandewoestyne et al. J Forensic Res 2011, S2
http://dx.doi.org/10.4172/2157-7145.S2-001

Sources of DNA Contamination and Decontamination Procedures in the Forensic Laboratory

Mado Vandewoestyne, David Van Hoofstat, Sabine De Groote, Nicky Van Thuyne, Saskia Haerinck, Filip Van Nieuwerburgh, and Dieter Deforce*

Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium

Study part A:

Air sampling was performed in 3 different locations:

- inside a laminar flow cabinet
- on a bench in the pre-PCR laboratory, where mouth masks, gloves and lab coats are worn
- on a desk in an open office shared with 9 people.

Every location was sampled twice.

→ it can be concluded that **air is unlikely to be the source of observed DNA contamination** in the laboratory.

Study part B:

- Laboratory surfaces, tools and equipment, present in a pre-PCR laboratory where mouth masks, hats, gloves and lab coats are worn in order to prevent contamination, were analyzed for the presence of contaminating human DNA.
- 11 samples out of 19 showed at least one contaminating allele with an RFU ≥ 100 while 13 samples had one or more alleles with an RFU ≥ 50. All detected alleles could be attributed to laboratory staff.

Sample preparation

- Samples were taken from 19 different surfaces/equipment using sterile cotton swabs (Greiner Bio-One, Wemmel, Belgium) before any decontamination procedure was applied.
- Subsequently, samples were taken from 8 of the surfaces/equipment that showed the highest numbers of contaminating alleles, before and after decontamination with one of both decontamination procedures.

Surface/equipment	Number of alleles detected	
	RFU≥100	RFU≥50
Drawer of laboratory cupboard (outside surface)	10	17
Laboratory bench	12	19
Centrifuge used for reference samples (outside surface)	1	5
Centrifuge used for evidence samples (outside surface)	1	1
Container with autoclaved tubes (outside surface)	0	1
Pipetholder in laminar flow cabinet	0	1
On/off button laminar flow cabinet	7	9
Container with autoclaved filtertips	0	0
Handle laboratory freezer	7	13
Handle laboratory fridge	2	7
Box containing centrifugal filter devices (outside surface)	1	5
Rack for tubes (empty)	14	18
Vortex	3	7
Electronic pipette	5	10
Outside laminar flow cabinet (front side)	0	0
Inside laminar flow cabinet (bottom)	0	0
Inside pipetting liquid handler with UV lamp (left side)	0	0
Inside pipetting liquid handler with UV lamp (right side)	0	0
Inside pipetting liquid handler with UV lamp (bottom)	0	0

Table 1: Numbers of alleles detected on surfaces and equipment (before decontamination procedure).

Study part C: Decontamination procedures

- **Conventional sodium hypochlorite procedure (NaOCl or bleach):**

A 5% sodium hypochlorite solution was sprayed on the surfaces and equipment to be decontaminated. Subsequently, a 70% ethanol solution was applied over the sodium hypochlorite solution and wiped off with disposable paper towels.

- **DNA ZAPTM procedure:**

DNA *ZAP*TM solution 1 was sprayed on the surfaces and equipment to be decontaminated. DNA *ZAP*TM solution 2 was immediately applied over solution 1. Subsequently, the surfaces and equipment were thoroughly rinsed with distilled water to remove degraded nucleic acids and DNA *ZAP*TM residue.

Surface/equipment	Number of alleles detected			
	Before decontamination		After decontamination	
	RFU≥100	RFU≥50	RFU≥100	RFU≥50
Drawer of laboratory cupboard (outside surface)	8	10	2	6
Laboratory bench	10	15	0	0
On/off button laminar flow cabinet	6	12	0	2
Handle laboratory freezer	1	3	1	1
Handle laboratory fridge	2	4	1	1
Rack for tubes (empty)	5	13	1	5
Vortex	1	5	0	0
Electronic pipette	1	1	1	1

Table 2: Numbers of alleles detected on surfaces and equipment before and after decontamination with sodium hypochlorite.

Surface/equipment	Number of alleles detected			
	Before decontamination		After decontamination	
	RFU≥100	RFU≥50	RFU≥100	RFU≥50
Drawer of laboratory cupboard (outside surface)	3	6	0	0
Laboratory bench	7	9	0	1
On/off button laminar flow cabinet	6	6	0	0
Handle laboratory freezer	8	13	0	0
Handle laboratory fridge	6	8	0	3
Rack for tubes (empty)	10	11	0	0
Vortex	2	9	0	1
Electronic pipette	3	5	0	0

Table 3: Numbers of alleles detected on surfaces and equipment before and after decontamination with DNA *ZAP*™ (Applied Biosystems).

REFERENCE MATERIALS FOR DISCUSSION:

Input DNA amounts in analysis :

- **single cell** based genetic testing **~7 pg**
- modern **forensic testing 25 - 300 pg** (8-24 genetic markers tested)
- whole genome sequencing with Illumina X Ten system ~1 µg (~145 000 cells) => yields roughly 300-400 million paired-end reads to cover the ~ 2.9 $\times 10^9$ bp human genome 30x.

**For next generation sequencing commercial kits can work with as little as 50 ng - 100 ng of input DNA*

#saliva contains ~5–10 ng DNA/µl

New RAININ LiteTouch™ 1.7 ml Microcentrifuge Tubes