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Investigation into "Normal" Background DNA on Adult Necks: Implications for DNA Profiling of Manual Strangulation Victims

ABSTRACT: Others have investigated the role that DNA profiling could play as a method for identifying the perpetrator of manual strangulation. These studies have demonstrated that it is possible to collect offender DNA from the skin surface of a victim following physical contact. It is not known whether nonself biological material is normally present on the skin surface due to adventitious transfer occurring during innocent everyday interactions. To test the hypothesis that detectable amounts of nonself DNA are normally present on the skin surface of healthy adult individuals due to the adventitious transfer of DNA occurring during normal day-to-day social interactions, we designed an experiment in three phases. Phase 1 was used to deduce which DNA collection, extraction, and amplification methods were suited to investigating this question. During phase 2, the neck surface of 24 healthy adult volunteers was swabbed. DNA was extracted using the QIAamp DNA mini kit and amplified using the SGM Plus PCR amplification kit, using 28 PCR cycles. The work carried out during phase 3 involved a simulated assault to investigate primary and secondary transfer of DNA during physical contact. It was found that 23% of neck areas swabbed during phase 2 of this investigation showed nondonor alleles in the resulting DNA profile, with 5% of areas showing six or more nondonor alleles. The results of phase 3 showed that primary, secondary, and zero transfer of victim and/or offender DNA could be observed after physical contact and that alleles from an unknown source could still be detected in this more controlled experiment. The data presented in this paper demonstrate that DNA profiles generated after swabbing the skin surface of healthy adults can include components of an unknown source, present due to adventitious transfer. These components, if present in large quantities, have the potential to interfere with DNA profile interpretation of swabs taken for the investigation of physical assault by DNA profiling.

KEYWORDS: forensic science, DNA, strangulation, manual, normal, offender, victim

Wiegand and Kleiber (1) and Rutty (2) investigated the role that DNA profiling could play as a method for identifying the perpetrator of manual strangulation. Both these original studies demonstrated that it is possible to collect the offender DNA from the skin surface of a victim following physical contact. Both experiments were however carried out under controlled conditions, with sampled areas being specifically washed before initiation of each transfer experiment. The possibility that a DNA profile could arise from a source other than the two individuals involved in each experiment was discussed by Rutty. He proposed that adventitious DNA transfer occurred prior to or after the experimental transfer experiment resulting in the deposition and possible transfer of third party DNA onto the sampled areas of both offender and victim.

The existence of secondary transfer is perhaps the most controversial and least understood area of forensic DNA profiling. The potential problem was first reported by van Oorschot and Jones (3). They described that substantial DNA transfer could occur during initial contact, and that objects handled by numerous individuals produced mixed DNA profiles, with the most prevalent DNA profile not always arising from the last individual to handle each particular object. It was quickly realized that persistence of DNA sources from numerous individuals on inanimate objects could potentially hinder DNA profiling of trace evidence, such as fingerprints, by resulting in DNA mixtures, including components of innocent third parties (4). Although secondary transfer was not observed during the investigations of Ladd et al., it was again observed that DNA deposition on animate and inanimate objects appeared to be dependent upon

the individual tested. In an attempt to address these conflicting results, Lowe et al. initiated a new series of laboratory controlled experiments, resulting in the concept of shedder status (4,5). The shedder status of an individual accounts for the difference in an individual's ability to deposit their own DNA onto an object that both van Oorschot and Ladd had previously commented upon.

We hypothesize that detectable levels of nonself DNA are normally present on the skin surface of healthy adult individuals due to the adventitious transfer of DNA that occurs during normal day-to-day social interactions. However, to date, the background level of nonself DNA present on adult neck or finger pad surfaces has not been investigated. A study was undertaken to investigate whether background levels of nonself DNA can be detected using the standard DNA collection and STR profiling methods currently practiced within the U.K. The potential implications that high levels of nonself background DNA could have on forensic investigation of physical neck assault are discussed.

Materials and Methods

Local ethical permission was granted for the collection of biological material from nonvulnerable, adult volunteers (LREC: 6940). Prior to swabbing, each volunteer was asked to complete a questionnaire, providing details of the immediate history of the swabbed area including time since washing the area and possible sources of adventitious DNA transfer onto the swabbed area. In phases 2 and 3, the epithelial shedder status of each volunteer was determined by the method described by Lowe et al. (5). A buccal swab was collected from each volunteer for production of a reference DNA profile. Finally, as manual strangulation involves the potential application of finger pads to both the front and back of the neck, both anatomical areas were considered in this study.

Phase 1

During phase 1, the method of DNA recovery was assessed by investigation of different methods of swabbing surfaces. Sweet et al. recommends that a double swabbing method, involving swabbing the area of interest with a moist swab then dry swab, should be employed to maximize collection of saliva from bite marks (6). Double swabbing was compared to single swabbing for collection of control DNA from a nonabsorbent surface, namely a section of laminated wooden flooring to determine whether any advantage is conveyed by double swabbing. Following these tests, two methods of swabbing the adult neck surface were compared. The first method was designed to maximize the area of collection for mapping the background levels of self and nonself DNA present. The second method was designed to focus on a smaller area, similar to that which might be left by the finger tips of the offender in a manual strangulation situation.

To establish whether any advantage was conveyed by employing a double swabbing technique, two rows of known amounts (5, 10, 20, and 50 ng) of control DNA were deposited onto a nonabsorbent sterile surface, and were allowed to air dry overnight. On the following morning, *c.* 15 h after DNA deposition, the top row of DNA deposits was swabbed using a single moistened sterile cotton swab head, using a circular motion. The second row was swabbed using the same method, followed by a second swabbing using a dry sterile cotton swab, following the technique of Sweet et al. (6). This procedure was repeated to generate 16 swabs for comparison.

To resolve whether a brushing or twirling motion of swabbing the neck surface was most suitable, three volunteers had their necks swabbed on two nonconsecutive days: brush swabbing on day 1 and point swabbing on the day 2. Swabbing of skin surfaces was performed by moistening the cotton swab with sterile distilled water, then rubbing the swab head over the area of interest using either a brushing or twirling motion. The neck was divided into five areas (A–E) for brush swabbing and 10 areas for point swabbing (A–J) (Figs. 1*a* and 1*b*).

To determine the inter- and intra-personal variation in DNA recovery and the effect of personal washing habits and product usage on the neck surface, three female and two male single volunteer adults were then recruited and asked to record all neck washing, use of products (for example perfume or moisturizer), clothing around the neck, items worn around the neck, and possible sources of adventitious DNA transfer throughout the DNA sampling period. Neck areas A–E were swabbed on five nonconsecutive occasions for volunteers 1, 3, and 5. Neck areas A–E were swabbed on three non-consecutive occasions for volunteers 2 and 4, generating 120 swabs for analysis.

Phase 2

Sixteen female and eight male volunteers including single, married, and individuals with partners, were recruited to determine the levels of self and nonself DNA on their neck skin. The volunteers were asked to assume a normal routine prior to sampling and again were asked to provide a brief history of activities related to the neck surface prior to sample collection. DNA profiles were analyzed for the number of donor and nondonor alleles present. Samples were collected from five areas of the neck (A–E) using a single brush swabbing technique. Each volunteer was asked not to perform any unusual or different activities that would result in the addition or removal of biological material from their neck surface. DNA profiles were analyzed for the number of donor and nondonor alleles present. Donor alleles were scored out of a maximum of 22 components by comparison to a reference DNA profile
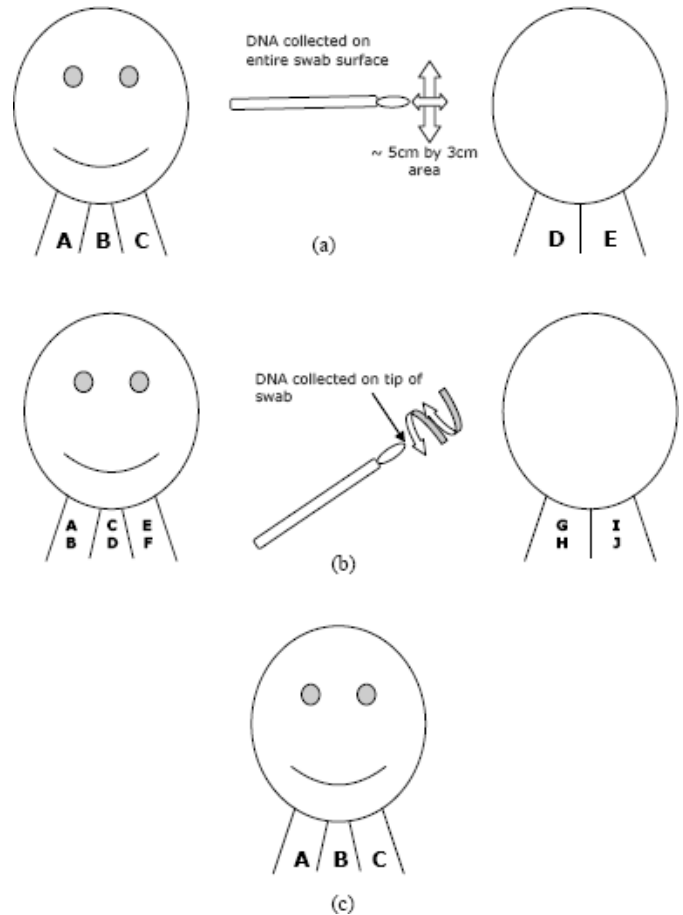

FIG. 1—*(a) and (b) DNA collection methods; brush swabbing and point swabbing. (c) Neck areas used in phase 3. For volunteers in active relationships, saliva was transferred to areas A and C. For simulation of manual strangulation, contact was made in area A.*

produced by amplification of buccal cell DNA. DNA profiles were generated using the SGM Plus PCR amplification kit (Applied Biosystems, Foster City, CA); this kit co-amplifies 10 autosomal STRs and the Amelogenin marker. Using this system, a maximum of 22 components consisting of two heterozygous alleles, or one homozygous allele scored twice, could be scored for each volunteer. A homozygous allele was scored as 1 if the peak height was below 150 RFU and as 2 if the peak height was above 150 RFU, *and* the peak height of both alleles at known heterozygous loci within the same DNA profile were equally amplified. Nondonor components were recorded as any peak with a peak height greater than or equal to 50 RFU that was not present in the reference profile of the volunteer. Nondonor alleles in (n − 1) stutter positions were only assigned if the peak height (RFU) was greater than 15% of the associated allele.

The shedder status of each volunteer was also determined using a method adapted from Lowe et al. (5). Each volunteer was asked to wash their hands and refrain from using gloves for 15 min. They were then asked to tightly grip a sterile, DNA-free 25 mL Universal tube for 30 sec using their dominant hand. After the grip, the tube was immediately swabbed to collect any DNA containing material transferred during this contact. The swabs were then extracted using the QIAamp DNA mini kit (Qiagen, West Sussex, U.K.) and amplified using the SGM Plus PCR amplification kit, for 34 PCR cycles, as described below.

Phase 3

Phase 3 was designed to directly investigate the transfer of DNA from the finger pads to the surface of the neck. Ten individuals were selected based on their relationship status, five classified themselves as single at the time of the experiment and five were married or in long-term relationships and were living with their partner. Sampling took place over a 2-day period. On day 1, the finger pads of the first and second fingers on both right and left hands were swabbed to assess the level of nonself DNA present due to normal daily activities. At this time, the five volunteers in active relationships were asked to allow their partner to deposit saliva on two areas of the neck (areas A and C, Fig. 1*c*) by licking. A questionnaire detailing the time of saliva deposition and washing history of the neck surface following saliva deposition was completed for each volunteer. The five single volunteers were asked to avoid any situation that may result in nonself DNA being transferred to their neck surface. On the second day, the volunteers were divided into pairs containing one single individual and one individual in an active relationship. The shedder status of each individual was taken into account when the pairings were made. Three good–poor, 1 poor–poor, and 1 good–good shedder pairings were assigned. To simulate manual strangulation, the model devised by Wiegand and Kleiber (1) and verified by Rutty (2) was employed. This involved the "offender" placing the finger pads of the first and second fingers of their dominant hand onto the neck surface in area A, away from the carotid sheath, of the "victim" and applying force for a period of 1 min. The contact was made on the same area as saliva deposition to investigate whether secondary transfer or DNA profile replacement would occur in this situation. The finger pads of the first and second fingers of both hands and neck areas A, B, and C were swabbed immediately after the contact was made.

DNA Extraction

During phase 1, DNA was recovered from swabs using a modified Chelex extraction technique (7). Each whole swab head was placed into a sterile 1.5-mL eppendorf tube. Five-hundred microliter sterile UP H_2O was added to each eppendorf tube. The mixture was vortexed and incubated at room temperature for 30 min, with occasional vortexing during this incubation period. After this incubation, a "piggy-backing" step was performed by transferring each swab head into a new sterile 0.5-mL eppendorf tube with a hole punched into the bottom. The 0.5-mL tubes were placed into 2.0-mL sterile screw cap tubes and were vortexed at $16,000 \times g$ for 1 min. The liquid recovered from each swab head was transferred back into the original 1.5-mL eppendorf tube. This total recovered liquid was then centrifuged for 3 min at $16,000 \times g$ to pellet any biological material present in the sample. All but *c.* 50 μL of the supernatant was then removed from the 1.5-mL eppendorf tube and discarded. Hundred microliter 5% (w/v) Chelex solution was then added to the 1.5-mL eppendorf tube. The tube was vortexed to resuspend the pellet before incubation for 20 min at 56°C, with occasional vortexing. The sample was then incubated for 8 min at 100°C before centrifugation at $16,000 \times g$. Approximately 125 μL of the DNA-containing supernatant was then transferred into a new sterile 1.5-mL eppendorf tube for immediate investigation or storage at −20°C.

For phases 2 and 3, DNA was recovered from swab heads by using QIAamp DNA mini kit following the swab extraction protocol. A piggy-backing stage was added to the manufacturer's protocol, as described above, to ensure that a minimum amount of liquid was lost when the swab head was discarded. DNA was eluted in 100-μL Buffer AE.

DNA Quantification

Single stranded DNA, recovered after Chelex extraction, was quantified using OliGreen ssDNA Quantitation Reagent (Molecular Probes, Eugene, OR) according to manufacturer's protocol. Double stranded DNA, recovered after QIAamp DNA extraction was quantified using PicoGreen dsDNA Quantitation Reagent (Molecular Probes) according to manufacturer's protocol.

DNA Profiling

Profiling of extracted DNA was carried out using the AmpF*l*STR SGM Plus PCR amplification kit (Applied Biosystems) in a final reaction volume of 25 μL (8). A total of 1 ng template DNA was added to each reaction. Twenty-eight PCR cycles were used for the amplification of all samples, with the exception of shedder status samples, for which 34 PCR cycles were used. PCR products were separated and visualized on an ABI PRISM 377 DNA sequencer (Applied Biosystems) at a run temperature of 50°C for 2.5 h. Fragment sizing was carried out using GeneScan software version 2.1 (Applied Biosystems) The light smoothing option was selected and size calling was performed using the local Southern Method. Allele designation was carried out using Genotyper software version 3.7 by running the Kazam macro supplied with the DNA profiling kit.

Results

Phase 1

Single Versus Double Swabbing—A full SGM Plus DNA profile was amplified for 15 of 16 swabs processed during the single versus double swabbing test using 34 cycles of PCR (9). For one swab, used to recover 5 ng control DNA by the double swabbing method, no SGM Plus amplification was achieved despite repeated PCR attempts. The reason for this amplification failure is unknown, and the second attempt to recover 5 ng DNA by this method was successful. Negative extraction control, consisting of an unused swab, processed in parallel with samples, and negative PCR control, consisting of sterile water were negative for amplified DNA and a positive PCR control (007 control DNA; Applied Biosystems) amplified correctly, indicating that the PCR reaction was performing as expected, with no evidence of reaction failure or inhibition. There was no significant difference observed between the DNA profiling results for single or double swabbing methods. The single swab method was therefore chosen for use in this project to reduce the number of manipulations required during the DNA extraction procedure, which also reduces the opportunity for laboratory-based contamination to enter the reaction.

Brush Swabbing Versus Point Swabbing—A significant difference was observed both between swabbing techniques and individuals with brush swabbing producing the larger yield of DNA ($p < 0.001$). The results of DNA profiling, expressed as a percentage of the donor profile observed from each neck surface swab are show in Figs. 2*a* and 2*b*. As a consequence of these results, single application brush swabbing was adopted for phases 2 and 3.

Intra- and Interpersonal Differences—It was found that there was no significant difference in DNA profile recovery between each of the five areas of the neck ($p > 0.1$), no significant
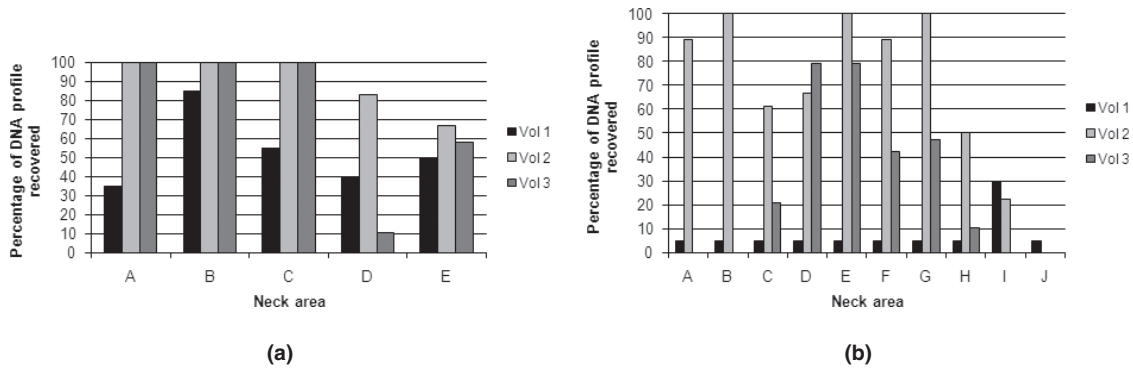
FIG. 2—*Bar charts illustrating (a) results of the percentage of volunteers' DNA profile recovered following brush swabbing of adult neck surfaces. Areas A–E correspond to the five neck areas in Figs. 1a and b. (b) The results of the percentage of volunteers' DNA profile recovered following point swabbed adult neck surfaces. Areas A–J correspond to the five neck areas defined in Fig. 1c.*
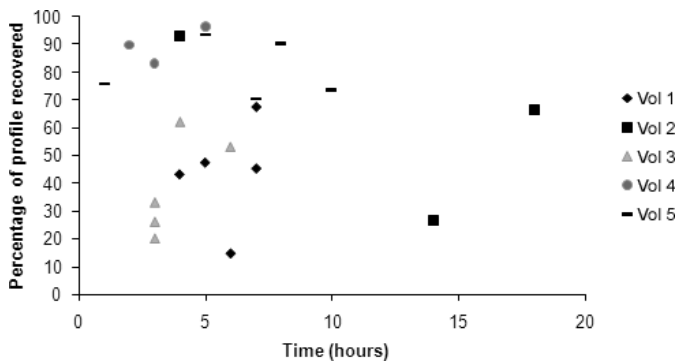

FIG. 3—*Scatter plot to demonstrate the relationship between the percentages of SGM Plus DNA profile observed during phase 1 of this investigation and the time since the neck surface was last intentionally washed.*

difference in DNA profile recovery between male and female necks ($p > 0.5$), and no correlation between time since last washing neck and DNA profile recovery ($r = -0.06014$) (Fig. 3).

Phase 2

DNA profiling using a standard 28 cycle protocol revealed non-self DNA was present on the neck surface of 14 of 24 volunteers. The number of nonself DNA components ranged from 1 to 14 alleles, with the most nonself DNA being detected on the neck surface of volunteers who were married or lived with partners. Seven of 16 nonsingle individuals showed more than six nonself alleles on at least one area of the neck. This observation was not however statistically significant ($p > 0.1$). There was also no significant difference in the level of nondonor allele detection among the five neck areas (A–E) ($p > 0.1$). The results of DNA profiling for all neck swabs collected during phase 2 of this investigation are summarized in Table 1.

Phase 3

The results of DNA profiling during phase 3 of this investigation demonstrated all levels of DNA transfer that have previously been reported in the forensic literature. DNA components consistent with arising from the volunteers' partners were observed on three of five neck surfaces that were intentionally licked. The absence of partners' DNA on two neck surfaces, despite saliva being deposited could potentially be explained by

the existence of an "oral shedder status," which similarly to epithelial shedder status could explain the deposition of differing amounts of DNA-containing material by different individuals. This possibility is currently being investigated in our laboratory. Where partners' DNA was observed, DNA profile components consistent with arising from them were also observed on the finger pads of two participants, demonstrating that secondary transfer of DNA-containing material can occur in this situation. It is interesting to note that when this secondary transfer of material was observed, high levels of primary transfer were also observed on the finger pads with one full and one almost full minor victim profile being observed. It is also interesting to note that the pattern of primary DNA transfer between the offender and victim pairings did not correspond to the epithelial shedder status of the participants, as determined by the method adapted from Lowe et al. (5). Of the two good–poor shedder pairings, transfer of good shedders DNA was not observed in either direction, to or from the neck to the fingers. Additionally, an almost complete profile was obtained from the finger pads of a poor shedder after contact with the neck surface of a second poor shedder. These results are in agreement with those of Phipps and Petricevic (10) and provide additional evidence that the determination of epithelial shedder status may not be as straight forward as previously thought. The DNA profiling results of all neck and finger swabs collected during phase 3 of this investigation are shown in Tables 2–6. These tables include reference profiles for each individual who participated in the transfer experiments and the partner of the individual in an active relationship who was asked to deposit saliva onto the neck surface of their partner prior to simulated assault.

Discussion

The collection of biological evidence from the skin surface for the identification of the offender of manual strangulation is not a new concept (1,2). The experiments presented in this paper however were designed to add to the current level of understanding of primary and secondary transfer of DNA by conducting experiments under less controlled, but more forensically relevant conditions. It adds to the current literature concerning DNA identification of the perpetrators of manual strangulation by providing information pertaining to the background levels of nonself DNA that can be collected from the front and back neck surfaces and the effect of gender, skin products, day to day interactions, and intentional application of finger pad force on the ability to detect it.

TABLE 1—*Results of DNA profiling carried out during phase 2 of this investigation are expressed as the number of donor, followed by the number of nondonor alleles detected on each area swabbed.*

					Neck Area				
ID	Sex	Marital Status	Shedder Status	Time Since Wash (h)	A	B	C	D	E
1	F	M	G	5.5	22, 1	22, 2	22, 0	21, 0	22, 0
2	F	S	P	4	12, 0	14, 0	14, 0	16, 0	16, 0
3	M	M	G	5.5	22, 0	22, 0	22, 0	22, 0	13, 0
4	M	P	P	5	22, 0	22, 3	19, 0	14, 1	0, 0
5	F	S	G	3.5	22, 0	14, 0	8, 0	5, 0	5, 0
6	M	M	P	13	10, 1	6, 0	2, 0	6, 0	13, 0
7	F	P	P	6	15, 0	22, 0	5, 0	3, 0	16, 0
8	F	M	P	7	22, 7	17, 0	10, 0	5, 0	2, 0
9	F	M	P	7	14, 3	22, 0	22, 13	9, 9	5, 0
10	M	S	P	6	22, 0	22, 0	22, 0	22, 0	22, 0
11	M	P	P	6	15, 14	22, 0	22, 0	12, 0	10, 3
12	F	S	P	?	14, 0	19, 0	6, 0	11, 5	2, 0
13	F	S	G	5.5	20, 0	20, 0	13, 0	2, 0	20, 0
14	M	M	P	72	22, 0	22, 0	8, 0	19, 3	3, 0
15	F	S	G	8.5	20, 2	17, 0	22, 0	14, 4	18, 0
16	F	M	P	8	14, 0	21, 4	21, 5	12, 0	16, 0
17	F	S	P	8	0, 0	1, 0	16, 0	2, 0	6, 0
18	F	M	P	8.5	21, 0	21, 0	19, 0	14, 0	4, 0
19	F	S	P	5	8, 1	0, 0	8, 2	6, 0	1, 0
20	F	M	P	8	22, 0	19, 0	22, 0	22, 0	17, 0
21	F	M	P	9	22, 0	22, 0	22, 0	13, 0	13, 0
22	M	M	P	75	3, 0	22, 0	22, 13	21, 1	22, 0
23	F	M	P	33	21, 5	21, 2	22, 0	22, 0	22, 0
24	M	M	P	10	22, 2	22, 6	17, 2	22, 1	22, 1

M, male; F, female; S, single; P, partner; M, married; G, good; P, poor; ?, answer not provided.

Each DNA profile was scored out of a possible 20 self alleles of the 10 STR loci included in the SGM Plus kit plus X and Y chromosome markers. A homozygote loci was scored as 2, if the peak height exceeded 150 RFU and was larger than heterozygote peaks in the same profile.

DNA profiling results are reported as number of self alleles detected, number of nonself alleles detected.

TABLE 2—*Results of SGM Plus DNA profiling of swabs taken from the "victim's" neck (areas A–C) and the "offender's" left (L) and right (R) hands after simulated assault during phase 3 of this investigation.*

Marker	D3	vWA	D16	D2	AMEL	D8	D21	D18	D19	THO1	FGA
PAIR 1 (P1 = Good shedder, S1 = Poor shedder)											
Reference Profiles											
P1	16, 18	17, 18	11, 11	16, 24	X, X	13, 14	30, 30	15, 16	14, 14	9.3, 10	22, 25
P1 partner	15, 15	16, 18	8, 11	16, 20	X, Y	10, 12	29, 30	16, 19	14, 14	6, 8	21, 23
S1	15, 15	14, 17	11, 12	19, 23	X, Y	13, 14	28, 32.2	11, 17	14, 15.2	7, 9	22, 23
Victim = P1 Offender = S1											
P											
A	16, 18	17, 18	11, 11	16, 24	X, X	13, 14	30, 30	15, 16	14, 14	F, F	22, F
B	16, F	17, 18	11, F	16, 24	F, F	13, F	30, F	F, F	14, F	F, F	F, F
C	16, 18	17, 18	11, 11	16, 24	X, X	13, 14	30, 30	15, 16	14, 14	F, F	F, F
S											
L	15, F	14, 17	11, F	F, F	X, F	13, F	28, F	F, F	14, F	F, F	F, F
R	15, 15	14, 17	11, 12	19, 23	X, Y	13, 14	28, 32.2	11, 17	14, 15.2	7, 9	F, F
Victim = S1 Offender = P1											
S											
A	14*, 15, 18	14, 16*, 18*	11, 12	19, 23	X, Y	12*, 13, 14	28, 32.2	11, 17	14, 15.2	7, 9	22, 23
B	15, 15	14, 17	11, 12	19, 23	X, Y	13, 14	28, 32.2	11, 17	14, 15.2	7, 9	22, 23
C	15, 15	14, 17	11, 12	19, 23	X, Y	13, 14	28, 32.2	11, 17	14, 15.2	7, 9	22, 23
P											
L	16, 18	17, 18	11, 11	16, 24	X, F	13, 14	30, F	F, F	14, F	F, F	F, F
R	F, F	F, F	F, F	F, F	F, F	F, F	F, F	F, F	F, F	F, F	F, F

F, amplification failure.

The left hand was used to make contact during the assault.

*Alleles of an unknown source.

Based on the publication of Sweet et al. (6), skin swabbing for nonself DNA is usually undertaken utilizing a double swabbing technique. The results of single and double swabbing experiments carried out in this investigation did not reveal any advantage in the use of two swabs for sample collection. These results are supported by the work of Maguire et al., who presented a method for DNA recovery from the skin surface of children's faces by use of a single swabbing technique (11). The use of a single swab also requires a fewer number of manipulations during DNA extraction, reducing the chances of laboratory-introduced contamination or DNA loss during this process; this method was therefore used throughout this project.

TABLE 3—*Results of SGM Plus DNA profiling of swabs taken from the "victim's" neck (areas A–C) and the "offender's" left (L) and right (R) hands after simulated assault during phase 3 of this investigation.*

Marker	D3	vWA	D16	D2	AMEL	D8	D21	D18	D19	THO1	FGA
PAIR 2 (P2 = Poor shedder, S1 = Good shedder)											
Reference Profiles											
P2	16, 18	15, 19	9, 12	19, 20	X, Y	10, 13	28, 29	14, 17	13, 13	9, 9.3	23, 25
P2 partner	16, 18	16, 16	11, 12	19, 20	X, X	8, 12	29, 31	16, 20	14, 14	9.3, 9.3	23, 24
S2	15, 17	17, 18	11, 12	17, 24	X, X	11, 13	28, 28	15, 16	13, 15	9.3, 9.3	20, 24
Victim = P2 Offender = S2											
P											
A	16, 18	15, 19	9, 12	19, 20	X, Y	10, 13	28, 29	14, 17	13, 13	9, 9.3	23, 25
B	16, 18	15, 19	9, 12	19, 20	X, Y	10, 13	28, 29	14, 17	13, 13	9, 9.3	23, 25
C	16, 18	15, 19	9, 12	19, 20	X, Y	10, 13	28, 29	14, 17	13, 13	9, 9.3	23, 25
S											
L	F, 17	17, 18	11, 12	17, F	X, X	11, 13	28, F	15, F	13, F	9.3, F	20, F
R	F, 17	F, F	F, 12	17, F	X, F	11, F	F, F	F, F	F, F	9.3, F	F, F
Victim = S2 Offender = P2											
S											
A	15, 17	15, 17, 18	11, 12	17, 24	X, X	11, 13	28, 28	15, 16	13, 15	9.3, 9.3	20, 24
B	15, 17	17, 18	11, 12	17, 24	X, X	11, 13	28, 28	15, 16	13, 15	9.3, 9.3	20, 24
C	15, 17	17, 18	11, 12	17, 24	X, X	11, 13, 14*	28, 28	15, 16	13, 15	9.3, 9.3	20, 24
P											
L	F, F	F, F	9, F	19, F	X, F	F, 10	F, F	F, F	13, F	F, F	F, F
R	F, F	15, F	F, F	F, 20	X, F	F, 10	F, F	F, F	13, F	F, F	F, F

F, amplification failure.
The left hand was used to make contact during the assault.
*Alleles of an unknown source.

TABLE 4—*Results of SGM Plus DNA profiling of swabs taken from the "victim's" neck (areas A–C) and the "offender's" left (L) and right (R) hands after simulated assault during phase 3 of this investigation.*

Marker	D3	vWA	D16	D2	AMEL	D8	D21	D18	D19	THO1	FGA
PAIR 3 (P3 = Good shedder, S1 = Good shedder)											
Reference Profiles											
P3	14, 18	17, 18	11, 12	20, 24	X, Y	14, 16	27, 30	16, 18	14, 16	8, 9.3	21, 24
P3 partner	15, 16	15, 18	9, 11	19, 26	X, X	8, 13	29, 30	12, 17	14, 15	6, 9.3	22, 23
S3	16, 17	15, 17	10, 12	17, 17	X, X	12, 14	28, 28	15, 16	14, 14	6, 7	22, 25
Victim = P3 Offender = S3											
P											
A	14, 15, 16, 18	15, 17, 18	9, 11, 12	19, 20, 24, 26	X, X, Y	8, 13, 14, 16	27, 29, 30	12, 16, 17, 18	14, 15, 16	6, 8, 9.3	21, 22, 23, 24
B	14, 15, 16, 18	15, 17, 18	9, 11, 12	19, 20, 24, 26	X, Y	8, 13, 14, 16	27, 29, 30	12, 16, 17, 18	14, 15, 16	6, 8, 9.3	21, 22, 23, 24
C	14, 15, 16, 18	15, 17, 18	9, 11, 12	19, 20, 24, 26	X, Y	8, 13, 14, 16	27, 29, 30	12, 16, 17, 18	14, 15, 16	6, 8, 9.3	21, 22, 23, 24
S											
L	14, 15, 16, 17, 18	15, 17, 18	11, 12	19, 20, 24	X, Y	14, 16	27, 30	15, 16, 18	14, 16	6, 8, 9.3	21, 24
R	F, F	F, F	F, F	F, F	X, F	F, F	F, F	F, F	F, F	F, F	F, F
Victim = S3 Offender = P3											
S											
A	14, 16, 17, 18	17, 18	10, 11, 12	20, 24	X, Y	12, 14, 16	27, 28, 30	16, 18	14, 16	7, 8, 9.3	21, 24
B	16, 17	15, 17	10, 12	F, F	X, X	12, F	28, F	15, F	14, F	6, F	F, F
C	16, 17	15, 17	10, 12	17, F	X, X	12, 14	28, F	F, 16	14, F	6, 7	F, F
P											
L	14, 16, 17, 18	15, 16*, 17, 18	10, 11, 12	17, 20, 24	X, Y	12, 14, 16	27, 28, 30	15, 16, 18	14, 16	6, 7, 8, 9.3	21, 22, 24, 25
R	14, 16, 17, 18	15, 17, 18	11, 12	20, 24	X, Y	14, 16	27, 29*, 30	16, 18	14, 16	8, 9.3	21, 24

F, amplification failure.
The left hand was used to make contact during the assault.
*Alleles of an unknown source.

During phase 1, DNA was recovered following the Chelex method of DNA extraction. Nonself DNA was not detected in any sample. Although this may have been a true result, it was suspected that excess dilution, loss of DNA eluate during collection, or potential carryover of PCR inhibiting Chelex particles may be influencing the results. This observation led to the replacement of extraction technique with the QIAamp DNA mini kit for following phases and the observation of nonself alleles in the resulting profiles. The results obtained during phase 2 demonstrated that nonself DNA was present on the neck surface of adult volunteers at a level

TABLE 5—*Results of SGM Plus DNA profiling of swabs taken from the "victim's" neck (areas A–C) and the "offender's" left (L) and right (R) hands after simulated assault during phase 3 of this investigation.*

Marker	D3	vWA	D16	D2	AMEL	D8	D21	D18	D19	THO1	FGA
PAIR 4 (P4 = Poor shedder, S4 = Good shedder)											
Reference Profiles											
P4	14, 17	14, 16	11, 14	20, 24	X, X	11, 13	30, 31	12, 18	13, 14	7, 9.3	22.2, 24
P4 partner	16, 17	16, 18	9, 11	17, 24	X, Y	9, 15	28, 31.2	13, 22	13, 13	7, 9	22, 23
S4	15, 18	16, 18	12, 14	19, 20	X, X	12, 14	30, 31.2	16, 17	13, 14	7, 8	21, 23
Victim = P4 Offender = S4											
P											
A	14, 16, 17	14, 16, 18	9, 11, 14	17, 20, 24	X, X, Y	9, 11, 13, 15	28, 30, 31.2	13, 18, 22	13, 14	7, 9, 9.3	22, 22.2, 24
B	14, 16 17	14, 16	11, 12, 14	17, 20, 24	X, X, Y	8, 11, 12*, 13	29, 30, 31	12, 18	12, 13, 14	7, 9.3	22.2, 24
C	14, 15, 16, 17	14, 16, 18	9, 11, 14	17, 20, 24	X, X, Y	9, 11, 12*, 13, 15	28, 30, 31	12, 18, 22	13, 14	7, 9.3	22.2, 24
S											
L	16*, 17	14, 16, 17*	11, 12	19, 22	X, Y	8, 13, 16	29, 29	13, 15	13, 14	9, 9	23, 24
R	15, 16*, 17	14, 16	9, 10, 11, 12	17, 19, 22	X, Y	8, 12*, 13, 16	28, 29	F, 15	13, 14, 15*	7, 9, 9.3	23, F
Victim = S4 Offender = P4											
S											
A	17, 17	14, 16	11, 12	19, 22	X, Y	8, 16	29, 29	13, 15	13, 14	9, 9	23, 24
B	17, F	F, F	F, F	F, F	X, Y	F, F	F, F	F, F	F, F	F, F	F, F
C	17, 17	14, 16	11, 12	19, 22	X, Y	8, 16	29, 29	13, 15	13, 14	9, 9	23, 24
P											
L	14, 15*, 16, 17	14, 16	11, 14	20, 24	X, X, Y	8, 11, 12*, 13	28, 29, 30, 31	12, 18	13, 14	7, 9.3	22.2, 24
R	14, 15*, 16, 17	14, 15*, 16, 17*, 18	11, 12	17	X, X, Y	11, 12*, 13	30, 31	F, F	F, 14	F, 9.3	F, F

F, amplification failure.
The left hand was used to make contact during the assault.
*Alleles of an unknown source.

TABLE 6—*Results of SGM Plus DNA profiling of swabs taken from the "victim's" neck (areas A–C) and the "offender's" left (L) and right (R) hands after simulated assault during phase 3 of this investigation.*

Marker	D3	vWA	D16	D2	AMEL	D8	D21	D18	D19	THO1	FGA
PAIR 5 (P5 = Poor shedder, S5 = Poor shedder)											
Reference Profiles											
P5	15, 18	16, 18	12, 14	19, 20	X, X	12, 14	30, 31.2	16, 17	13, 14	7, 8	21, 23
P5 partner	17, 17	17, 18	9, 10	23, 24	X, Y	13, 13	29, 30	12, 17	12, 14	6, 9	21, 23
S5	15, 16	14, 16	12,12	17, 21	X, X	10, 16	30, 32.2	16, 17	12, 13	7, 9.3	21, 23
Victim = P5 Offender = S5											
P											
A	15, 17, 18	16, 17, 18	9, 10, 12, 14	19, 20, 24	X, X, Y	12, 13, 14	29, 30, 31.2	12, 16, 17	12, 13, 14	7, 8	21, 23
B	15, 18	16, 18	12, 14	F, F	F, F	12, F	F, F	F, F	F, F	F, F	F, F
C	15, 17, 18	16, 17, 18	9, 10, 12, 14	19, 20, 23, 24	X, Y	12, 13, 14	29, 30, 31.2	12, 16, 17	12, 13, 14	6, 7, 8, 9	21, 23
S											
L	15, 16, 17, 18	14, 16, 17, 18	9, 12, 14	17, 19, 20, 21	X, X, Y	8*, 10, 12, 13, 14, 16	29, 30, 31.2, 32.2	16, 17	12, 13, 14, 14.2*	7, 8, 9.3	21, 23
R	14*, 15, 16, 17, 18	14, 15*, 16, 17	11, 12	17, 21 23	X, X, Y	8*, 10, 11*, 12, 13, 16	28*, 29*, 30, 32.2	16, 17	12, 13, 14, 14.2*, 15*	7, 8, 9.3	21, 23
Victim = S5 Offender = P5											
S											
A	15, 16	14, 16, 17*	12, F	17, F	X, X, Y	10, 12, 16	F, F	F, F	12, 13	F, F	F, F
B	15, 16	14, 16	12, F	17, 21	X, X, Y	10, 12, 16	30, 32.2	16, 17	12, 13	7, 9.3	F, 23
C	F, 16	F, F	F, F	F, F	X, F	F, F	F, F	F, F	F, F	F, F	F, F
P											
L	15, 16, 18	16, 17, 18	12, F	19, 20	X, X, Y	12, 13, 14, 16	30, 31.2	16, 17	13, 14	7, 8	21, F
R	15, 18	16, 18	12, 14	19, 20	X, X	12, 14	30, 31.2	16, 17	13, 14	7, F	21, F

F, amplification failure.
The left hand was used to make contact during the assault.
*Alleles of an unknown source.

that could be detected using standard collection, amplification, and analysis methods as used for routine forensic casework within the U.K.

Phase 2 identified that background levels of nonself DNA could be detected on 23% of swabs from 24 adult volunteers under standard, 28 cycle, DNA profiling conditions. None of the unknown

alleles was observed in negative control samples, indicating that they were collected from the neck surface and were not present due to contaminants introduced during laboratory processing. Unlike larger (visible) stain samples in which presumptive testing for blood or semen may be carried out prior to DNA profiling, during trace DNA investigations, the source of any foreign biological material collected from a victim or crime scene surface cannot be determined (5,12). Equally, it is not possible to determine exactly when or by what mechanism the foreign material was deposited onto the surface being sampled. It has previously been hypothesized that secondary and, potentially, tertiary transfer of DNA, from frequently handled objects, to the sampled site could be a possible mechanism for these findings (2). Under this hypothesis, nonself DNA is unknowingly deposited onto the neck surface via finger pads. This mode of adventitious transfer is likely to have contributed to the findings of this report.

It has also been demonstrated that DNA-containing material can be deposited onto surfaces without physical contact being made. In 2003, Rutty et al. carried out a series of experiments to determine the extent to which a crime scene could be contaminated by crime scene investigators (13). It was hypothesized that DNA contamination could arise from orally projected saliva particles or could be due to the sloughing of epithelial cells around the area that the face mask was in contact with the face. To distinguish the contribution of orally projected biological material from shed epithelial cells, Port et al. undertook a series of simplified follow-up experiments to investigate orally projected biological material only. The results of these experiments showed that DNA containing biological material could be detected on the surface in front of an individual after only 30 sec of talking (14).

Secondary transfer and orally projected DNA may both contribute to the background levels of nonself DNA that were detected on the neck surface during phase 2. In addition to secondary transfer from handled objects and orally projected sources of DNA, it was noted that higher levels of nonself DNA were detected on the neck surface of individuals who were married or living with a partner at the time of sampling. Although it was theorized that partners and/or family members may be contributing to the nondonor DNA observed on the neck surface of married individuals, no attempt to deduce the source of observed nondonor DNA was undertaken during phase 2 of this investigation.

The source of nondonor DNA was instead considered during the phase 3, whereby only single individuals and individuals with live-in partners, but no children living with them, were invited to take part. Analysis of DNA profiling results showed that after primary transfer of saliva, by licking the neck surface, allowed for amplification of a full DNA profile in two of five cases, a partial profile in one case and no nondonor DNA was observed in the remaining two samples. Saliva was chosen as a source of purposely transferred nonself DNA due to its apparently ubiquitous presence on the bite and suck marks of sexual assault victims (6,15,16), licked stamps and envelopes (17,18), and even partially eaten food stuffs (19). The absence of DNA derived from the saliva of the partners of volunteers was not expected but could be due to the use of standard rather than low copy number PCR conditions. An alternative explanation could be the existence of an oral shedder status, which similarly to the recognized epithelial shedder status, may result in different levels of DNA being deposited on surfaces from oral sources (work in progress). The results obtained during phase 3 produced a mixture of results, demonstrating all levels of DNA transfer that have previously been observed and reported in forensic literature, i.e., zero, primary, and secondary transfer. This work also increases support for the theory that the shedder status of an

individual greatly influences the amount of DNA deposited by different individuals.

The importance of this work does not however reside in the confirmation of previously published works; it serves to highlight the complications that can be encountered during DNA profile interpretation due to the presence of alleles of an unknown source unconnected to the physical contact. To assess the potential implications of normally present extraneous sources of DNA, the experiments undertaken in this study were designed to minimize artificial transfer to or from the neck surface of participants. During the analysis of actual forensic casework, following good scientific practice, mixture interpretation is completed without prior knowledge of reference profile and is usually aided by computational expert systems (20). Under such guidelines, once a mixture is detected in any given DNA profile, every allele observed must be taken into account during interpretation. The presence of alleles unassociated with the event under investigation will add unnecessary and time-consuming complications to the interpretation. Under a worst case scenario, this could lead to false intelligence being passed to the investigative authority and aid the evasion of the actual perpetrator.

As this is a purely theoretical research project, DNA profiles analyzed during phase 3, mixture analysis was carried out with *a priori* knowledge of reference profiles, including the reference profiles of partners of individuals taking part. Despite the fact that all phase 3 participants were requested to avoid physical contact with any persons other than their usual partners for the duration of the sampling time, between one and nine allele(s) of an unknown source were observed on five of 30 neck areas and seven of 20 finger pads sampled, accounting for 24% of all samples collected. These results support the hypothesis that detectable levels of nonself DNA are normally present on the neck surface of the adult neck and finger pad surface.

Summary

The presence of background levels of nonself DNA on the skin surface has, at the time of writing, not been investigated or reported in the forensic literature. The results presented in this article demonstrate that nonself DNA can be recovered and amplified from the skin surface of the neck and finger pads of adult volunteers using standard techniques, equivalent to those used within British forensic casework. During the investigation of serious crime, such as assault, rape, or murder, DNA may be collected from the skin surface over areas in which physical contact has taken place in an attempt to identify the perpetrator. In such circumstances, every allele observed in a resulting DNA profile must be scrutinized and accounted for. Once a mixed DNA profile has been observed and analyzed blind, reference profiles from the sample donor and all persons who are known to have had contact with the donor in the period leading up to the investigated incident will be compared to the mixture in an attempt to elucidate the potential sources of observed nondonor contributors. DNA profile components that cannot be assigned to known sources will be considered as potentially originating from the unknown perpetrator. The work presented here demonstrates that such unknown DNA profile components may originate from innocent sources, unconnected to the event being forensically investigated, and may therefore provide false intelligence that may hinder the investigation. This article is intended to highlight this possibility and open the arena to further investigations into the background levels of nondonor DNA that may be present on surfaces, both animate and inanimate, that may be swabbed for trace DNA during forensic investigation.

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