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Advancing forensic RNA profiling

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Chapter 4

Prevalence of human cell material: DNA and
RNA profiling of public and private objects
and after activity scenarios

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Abstract

Especially when minute evidentiary traces are analysed, background cell material unrelated to the crime may contribute to detectable levels in the genetic analyses. To gain understanding on the composition of human cell material residing on surfaces contributing to background traces, we performed DNA and mRNA profiling on samplings of various items. Samples were selected by considering events contributing to cell material deposits in exemplary activities (e.g. dragging a person by the trouser ankles), and can be grouped as public objects, private samples, transfer-related samples and washing machine experiments. Results show that high DNA yields do not necessarily relate to an increased number of contributors or to the detection of other cell types than skin. Background cellular material may be found on any type of public or private item. When a major contributor can be deduced in DNA profiles from private items, this can be a different person than the owner of the item. Also when a specific activity is performed and the areas of physical contact are analysed, the “perpetrator” does not necessarily represent the major contributor in the STR profile. Washing machine experiments show that transfer and persistence during laundry is limited for DNA and cell type dependent for RNA. Skin conditions such as the presence of sebum or sweat can promote DNA transfer. Results of this study, which encompasses 549 samples, increase our understanding regarding the prevalence of human cell material in background and activity scenarios.

Introduction

In recent years, the sensitivity of DNA profiling methodologies has increased tremendously and now allows for analysis of minute evidentiary samples previously regarded unsuitable for STR profiling. This increased sensitivity, however, has also led to an increased possibility of profiling DNA that is not related to a crime, but residing in the background. Collecting data regarding the prevalence, transfer, persistence and recovery of human cell material has increasingly gained attention, for instance because of the importance in addressing activity-level questions [1-3]. To name a few studies: human DNA has been analysed in dust, neck and fingernail samplings [1][4-9]; the DNA on hands or touched items was found to often originate from DNA-rich areas like the mouth or nose [10] and the presence of sebaceous glands in skin surfaces [11] or cell-free nucleic acids in sweat was shown to significantly affect transfer of DNA [12]. Others describe the direct and indirect transfer of DNA traces in specific situations or in the washing machine [13-16]. Furthermore, factors influencing the transfer and recovery of DNA have been investigated, like the moisture level of the stain, the type of biological substance, the type of surface, the application of friction and individual shedding state [16-23]. All these studies, however, focussed on inventorying background DNA. Additional knowledge may reside in examining what cell types are present. mRNA profiling can be used for the inference of body fluids as well as organ tissues [1][24-32]. Presumably, skin is the cell type most abundantly present in contact traces, as an individual sheds approximately 5×10^8 cells each day [33]. However, skin cells do not contain much DNA [34], thus minute amounts of body fluids that have higher DNA contents may affect the genetic analyses.

In this study, DNA and mRNA profiling is performed on samplings of various items, which may contribute to or be a vector for the deposition of background cell material. These items are derived from considering the events contributing to human cell material deposits in an exemplary activity, namely dragging a person at the trouser ankles as depicted in Figure 1. The items can be grouped as everyday public objects and private samples. Besides, transfer and persistence of cellular material in the washing machine is investigated to determine possibilities of cell residues on fabrics after washing. Additionally, the effect of sebum and sweat on the transfer of cellular material is assessed.

Materials and methods

Experimental setup and sample collection

A total of 549 samples were analysed for this background study. Samples are divided

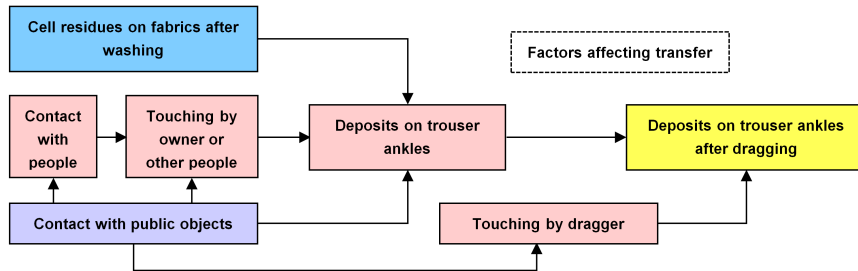


Figure 1. Overview of various factors, which may contribute to background traces found on items prior and post an activity-related scenario (in this example dragging by trouser ankles). In this study, we focussed on analysing public items (purple box), private samples (red), activity-related samples (yellow), factors affecting transfer (white) and washing machine samples (blue).

over four categories, namely public, private, transfer-related and washing machine samples. An overview of the samples and sampling locations is shown in Table 1. All samples except the fingernail samples were collected using tape lifts [35] and areas were stubbed until tapes were saturated. Fingernail samples were collected using a moistened (Nuclease-free water; Ambion) mini-tip swab (MWSCS, MWE Medical Wire). Patches used for the sweat, (non-) sebaceous skin and washing machine experiments were cleared from contaminating nucleic acids by irradiating each side in a CL-1000 UV-CrossLinker at 900 mJ/cm² for 60 minutes. The front and back of the cloths/patches were sampled with an individual tape lift.

The first sample set includes 105 public samples as presented in Table 1, set 1. Public items were sampled at areas most accessible to physical contact. Banknotes and coins were collected from wallets of 13 individuals, thus the last person handling the money is known.

The private sample set consists of 164 samples subdivided in samples collected directly from the volunteers' skin (neck, hands, fingernails; 80 samples in total) or clothes (winter gloves, trouser leg, armpits of shirts; 84 samples in total). Sampling locations for the private samples are described in Table 1, set 2.

The third set consists of 168 transfer-related samples: 94 activity-related samples (grabbing and dragging (Table 1, set 3.1)) and 74 samples to assess the effect of skin condition on transfer (Table 1, set 3.2). In the grabbing scenario, a grabber wore his/her personal winter glove on his/her dominant hand and firmly grabbed the bare arm of a "victim" during 5 seconds during which the "victims" were asked to resist the activity. Both the external surface of the grabber's winter glove and the victim's arm were sampled immediately after the activity. For the dragging scenarios, a "victim" who was sitting in a chair was dragged along a corridor at the trouser ankles or armpits of shirts during one minute. Draggers used their bare hands for the activity without

any instructions regarding hand washing. Immediately after the activity, samples were taken from exterior surfaces of the ankle area of the trousers and the armpit area of the shirt. Couples performing these activity-related scenarios were selected to have a low number of shared alleles (three to eight when considering NGM loci) to maximise the information per donor. The same winter gloves, trousers and shirts had also been sampled prior to the activity and represent the samples described in set 2.2 (Table 1). To allow for new “background” to be deposited, a considerable amount of time was left between the prior-activity sampling and the activity namely at least six hours for the shirts and trousers during which they were worn all the time and at least 5 days for the winter gloves during which they were worn approximately half an hour each day without washing. The skin conditions examined for effect on the transfer of DNA are sebaceous and sweaty skin (Table 1, set 3.2). For five volunteers, the forehead and palm of the hand was stubbed to collect sebaceous and non-sebaceous skin, respectively. Volunteers were asked to wash the sampling locations with water and soap, dry with paper tissues and not touch/use these skin areas for 30 minutes. Non-sweaty and sweaty skin samples were prepared by pulling UV-irradiated cloths prior to and after running activities, respectively. Two samples were collected from UV-irradiated cloths prior to pulling to assess for possible remainders of contaminating. For 16 volunteers, post and prior to running the right and left hand were sampled, resulting in 64 samples.

The last sample set comprises 112 samples generated from washing machine experiments (Table 1, set 4). Sampling areas of 5x5 cm were marked with a permanent marker on denim cloths (15 x 21 cm) prior to UV-irradiation. Ten samples were collected from five UV-treated denims prior to washing to assess for possible remainders of contaminating DNA or RNA. For part one of the washing machine experiments, two volunteers combined four patches of denim with their regular laundry; patch 1 and 2 had two marked “blank” areas. Patch 3 and 4 both had four marked areas; a dried bloodstain (500 μ L), a dried saliva stain (1000 μ L) and two marked “blank” areas. Patch 1 and 2 each resided in a separate washing bag to assess indirect transfer, while patch 3 and 4 were tumbled within the laundry without a washing bag (to study direct transfer). Patch 1 and 3 were air-dried; patch 2 and 4 tumble-dried. Each volunteer performed the experiment twice, resulting in a total of 96 samples. Part two of the washing experiment involved the washing of two “blank” denim patches (alike those used in part one) in individual washing bags, without any laundry. One patch was air-dried; the other tumble-dried. Each volunteer performed the experiment twice, with no other washes in between, giving a total of 16 samples. Both volunteers used a 40 °C wash program and included fabric softener.

All samples used for this study were collected with informed consent of the voluntary donors whose cell material was used.

Table 1. Overview of the samples collected for this study. The number of DNA profiles generated equals the number of samples analysed. RNA analysis was performed in four-fold to allow profile interpretation according to “ $x=n/2$ ” guidelines [36].

Set	Item	# for DNA profiling	# for RNA profiling
1	Public objects	105	420
	Escalator rails train station, rubber surface, $n=6$	6	24
	Stair rails train station, painted metal surface, $n=6$	6	24
	Public toilet door handle, smooth metal surface, $n=6$	6	24
	Public toilet flush button, plastic surface, $n=6$	6	24
	Shopping cart handle, plastic surface, $n=6$	6	24
	Shopping basket handle, plastic surface, $n=6$	6	24
	Library books, laminated carbon, $n=6$	6	24
	Coin money, $n=6$	6	24
	Handle bar indoor, smooth metal surface, $n=6$	6	24
	Banknotes, $n=51$	51	204
2	Private samples	164	656
2.1	Skin	80	320
	Neck samples, front and back, $n=10$ each	20	80
	Hands, right and left, $n=20$ each	40	160
	Fingernails (index, middle, ring finger combined), right and left hand, $n=10$ each	20	80
2.2	Clothing	84	336
	Winter gloves dominant hand, fingers and thumb area, $n=10$ each (5 polyester; 3 leather; 2 wool)	20	80
	Trouser, right and left leg, $n=24$ each (jeans only)	48	192
	Armpits of shirt, right and left, $n=8$ each (regular shirts)	16	64
3	Transfer-related samples	168	0
3.1	Activity-related scenarios	94	0
	Winter gloves after grabbing, finger and thumb area, $n=10$ each	20	0
	Arm victim after grabbing by glove, $n=10$	10	0
	Trouser after dragging, right and left, $n=24$ each	48	0
	Armpits of shirt after dragging, right and left, $n=8$ each	16	0
3.2	Factors affecting DNA transfer	74	0
	Sebaceous vs non-sebaceous skin, forehead and palm of hand, $n=5$ each	10	0
	Sweaty vs non sweaty hands, right and left hand, before and after running, $n=16$ each	64	0
4	Washing machine: transfer and persistence	112	448
4.1	Cloths combined with regular washes, air-dried and tumble-dried, $n=48$ each	96	384
	Areas with blood or saliva stain, $n=16$ each	32	128
	Blank areas on spotted or blank cloths, $n=32$ each	64	256
4.2	Blank cloths, blank washes, air-dried and tumble-dried, $n=8$ each	16	64

DNA/RNA extraction, DNA quantification, ethanol precipitation, reverse transcription

DNA/RNA co-extraction, DNase treatment, DNA quantification, ethanol precipitation and reverse transcription were performed as described in Lindenbergh et al. [24]. Tape lifts were processed entirely and when two tape lifts were derived for a sample, these were extracted together. RNA extracts were ethanol-precipitated prior to reverse transcription when the total DNA yield of a sample was below 1

ng and processed as described in Ref. [25]. DNA extracts were ethanol-precipitated [24] when the DNA concentration of a sample was below 50 pg/ μ L. An amount of DNA extract was taken so that approximately 500 pg DNA resided in the ethanol precipitate. For all transfer-related samples (set 3.1 and 3.2, Table 1), only the DNA fraction was processed after co-isolation.

RNA profiling

Cell type inference was performed using in-house developed multiplexes [24-25] [37]. The multiplexes described in Ref. [24] and Ref. [25] were used for the public objects, neck, winter gloves, armpits of shirt and some trouser ankle samples (Table 1) and allow for the inference of blood, saliva, vaginal mucosa, menstrual secretion, semen and skin; the multiplex described in Ref. 37 was used for hands, fingernails, most trouser and washing machine samples (Table 1) and targets besides the aforementioned body fluids nasal mucosa. Amplification and product detection was performed according to standardized protocols [24]. A serial cDNA input (e.g. 0.2, 1, 4 μ L cDNA) PCR was used to determine the input providing an informative RNA profile. Supplementary PCRs were performed to obtain four informative PCR replicates per sample. When RNA extracts were ethanol-precipitated prior to reverse transcription, the 20 μ L cDNA was divided over four replicate PCRs with 5 μ L input. PCR products were purified [24] prior to detection using a 3130XL Genetic Analyzer (Life Technologies). Amplification products were analysed using POP-7 or POP-4 (Life Technologies) separation matrix using 3 kV, 10 s injection settings. Profile analysis was performed using Genemapper ID-X version 1.1.1 (Life Technologies) with a detection threshold of 150 relative fluorescence units (rfu).

DNA profiling

DNA profiles were generated using the AmpF ℓ STR $^{\circ}$ NGM $^{\text{TM}}$ PCR Amplification Kit (NGM) (Life Technologies) using a maximum of 500 pg DNA. PCR products were separated according to standardized protocols [24] using a 3130XL Genetic Analyzer (Life Technologies) with POP-4 (Life Technologies) separation matrix using 3 kV, 15 s injection settings. Profile analysis was performed using Genemapper ID-X version 1.1.1 (Life Technologies) and a detection threshold of 50 rfus.

Data analyses

Donors with known genotypes were used for all samples (except the public items), so that the percentage of total rfus in the STR profile for each donor or for unknown contributors could be determined. In case of multiple known donors (e.g. activity-related samples), shared alleles were considered as a separate class. Profile

interpretation was performed using the 15 STR loci in the NGM kit (Amelogenin was not included). Maximum allele count [38] was used to determine the minimum number of contributors in a profile. All samples collected after dragging or grabbing experiments were analysed using the LoCIM-tool [39] to deduce the alleles of the most prominent component in the STR profile and determine whether the “perpetrator” or the “victim” represents the major contributor in the DNA profile.

RNA data interpretation was performed according to the “ $x=n/2$ ” rule as described in Ref. [36]. This method compares the number of observed (x) to the number of theoretically possible peaks (n) in all replicates. A cell type is scored “observed” when at least half of the possible peaks are detected ($x \geq n/2$), scored “sporadically observed” when less than half of the possible peaks are detected ($0 < x < n/2$) and scored “not observed” when no peaks are detected ($x=0$). Cell types that are co-expressed with other detected cell types are scored “(sporadically) observed and fits” (e.g. vaginal mucosa when menstrual secretion is detected). “Sporadically observed” signals are generally regarded not reliable; “and fits” scorings are generally regarded not present as such.

Results

Public objects

Public items such as shopping basket handles, stair rails in train stations or money are regularly touched by or localize near multiple people. To gain understanding of the composition of human cell material residing on such surfaces, we investigated a set 105 public items (Table 1).

Details on DNA and RNA profiling results of the analysed public items are shown in Figure 2. DNA yields range from 0.0 to 41.1 ng, the minimum number of contributors lies between 0 to 6 donors and the number of observed cell types between 0 and 3. High DNA yields do not necessarily relate to increased numbers of cell types or contributors. Reliable quantification results (above 0.5 pg/ μ L) were obtained for 91% of the samples. In 17% of the samples a major contributor could be deduced in the STR profile. For the banknotes ($n=51$) and coin money ($n=6$) the last user was known, but this last user was not necessarily the major contributor in the DNA profile: for the 9 samples for which a major was deduced, 5 times this represented the last user. Skin was scored “observed” in 96% of the samples; no cell type was scored “observed” in the remaining 4% of samples. Skin was occasionally (78%) scored “observed” for samples with DNA quantification results below the detection threshold of the quantification system (0.5 pg/ μ L). “Observed” scoring of a cell type other than skin occurred in 35% of the samples and mainly involved saliva and occasionally vaginal mucosa, menstrual secretion or semen (results not shown).

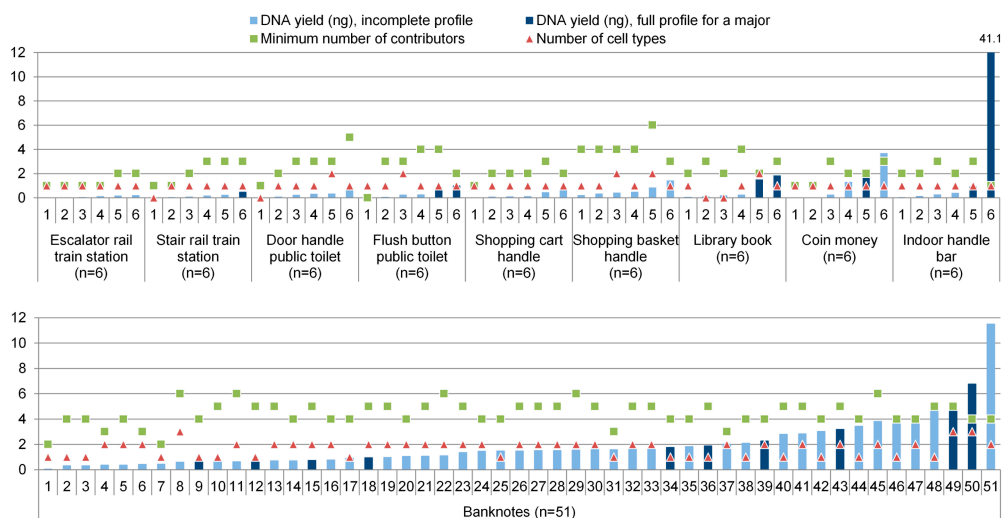


Figure 2. DNA profiling and mRNA cell typing results of 105 public items. Blue bars indicate the DNA yield (ng) for each sample. Light blue bars indicate the samples that produced incomplete STR profiles; dark blue bars represent samples for which a single source full DNA profile or a clear major contributor was observed. Additionally, the minimum number of contributors (green dot) and the number of observed cell types (red dot) are shown. When the DNA yield exceeds 12 ng (the maximum value at the y-axis), the yield is indicated above the bar.

Private samples

With private samples, both cell material of the owner and other people may be present due to everyday activities. We investigated the composition of 164 private samples (Table 1). The first subset (set 2.1, Table 1) comprised samples taken directly from various skin areas for different reasons, namely 1) neck samples for information on background in cases of manual strangulation; 2) hand samples as hands make contact with public object, private items and skin areas (own and non-own) and 3) fingernail dirt samples to gain knowledge on background in e.g. sexual assault or murder cases. Additionally, private objects such as clothing can be involved in criminal acts: gloves can be worn during manual strangulations and a victims' clothing can be touched when a perpetrator relocates the body and such items reside in subset 2.2 (Table 1).

An overview of DNA and RNA profiling results of the analysed private items is shown in Table 2 and details per individual sample are provided in Figures 3A-F. DNA yields vary more between individuals and less for samples from the same person (e.g. right and left hand for one individual tend to give similar yields except for few instances). No obvious differences for donors of different gender or varying fabric types were seen (data not shown). The estimated minimum number of contributors ranged up to five; most often at least two (55% of skin and 42% of clothing samples)

Table 2. Summary of DNA and RNA profiling results after analysing 164 private samples.

Results	Skin			Clothing		
	Neck	Hands	Fingernails	Winter gloves	Trouser ankles	Armpits of shirts
	Front + Back	R+L ^a	3 fingers both hands	Fingers + thumb dominant hand	R+L	R+L
	(n=20) Fig. 3A	(n=40) Fig. 3B	(n=20) Fig. 3C	(n=20), Fig. 3D	(n=48) Fig. 3E	(n=16) Fig. 3F
DNA						
Avg. DNA yield (ng)	0.4	5.9	6.6	0.7	3.2	0.2
Range DNA yield (ng)	0.0-5.0	0.0-67.5	0.3-58.6	0.0-2.5	0.1-29.0	0.0-0.6
Number of contributors	1-3	1-4	1-3	1-4	2-5	1-3
Full profile for sampled donor	67%	88%	25%	80%	83%	56%
Sampled donor is major contributor	100%	93%	100%	100%	60%	100%
Samples with background alleles	83%	88%	80%	90%	100%	100%
Avg. % non-owner r/us	10	9	6	13	20	12
Range % non-owner r/us	0-40	0-66	0-20	0-26	0-62	0-27
Avg. PH ^a ratio donor/background	7.6	8.8	6.0	4.2	4.4	5.3
Range PH ratio donor/background	1.2-19.1	0.5-21.8	1.7-16.2	1.9-8.3	0.8-19.2	1.2-21.5
RNA						
Skin "observed"	100%	100%	100%	95%	100%	100%
No cell type "observed"	0%	0%	0%	5%	0%	0%
Extra cell type "observed" next to skin	17%	18%	5%	15%	8%	19%
DNA yield (ng) samples extra cell type	0.2-5.0	0.2-67.5	58.6	0.4-2.0	2.3-12.9	0.1-0.3

^a R, right; L, left; PH, peak height.

or three (42% of skin and 40% of clothing samples) contributors appear present. Non-owner alleles are observed in the majority of samples, but generally the donor alleles are substantially higher than non-owner alleles. Hence, for most samples a major contributor could be deduced that corresponded to the owner except for some hand and trouser samples for which no major contributor was deduced (Figure 3B+E). For some of these samples the average peak height of donor alleles is higher than non-donor alleles (peak height ratio above 1, Figure 3B+E), but the presence of few rather high background alleles prevents deducing the donor as major contributor in the STR profile. RNA profiling resulted in an "observed" scoring for skin in all but one of the samples. For this sample no cell types were scored "observed". Other cell types than skin are occasionally (14% of the samplings) scored "observed" and these mainly involve blood, saliva or vaginal mucosa. Nasal mucosa is scored "observed" in few samples, which are the samples resulting in a relatively high DNA yield (at least 58.6 ng) [37]. "Sporadic" scorings, which we regard unreliable as described in Ref. [36] were detected in 70% of the samples.

Transfer-related samples

Activity-related scenarios

In crimes with physical contact, DNA can be transferred between the contact areas of "perpetrator" and "victim". We investigated the composition of 94 samples (Table 1) obtained from three different activities that exemplify activities in criminal acts, namely 1) grabbing during which the "perpetrator" wore a winter glove and grabbed the bare

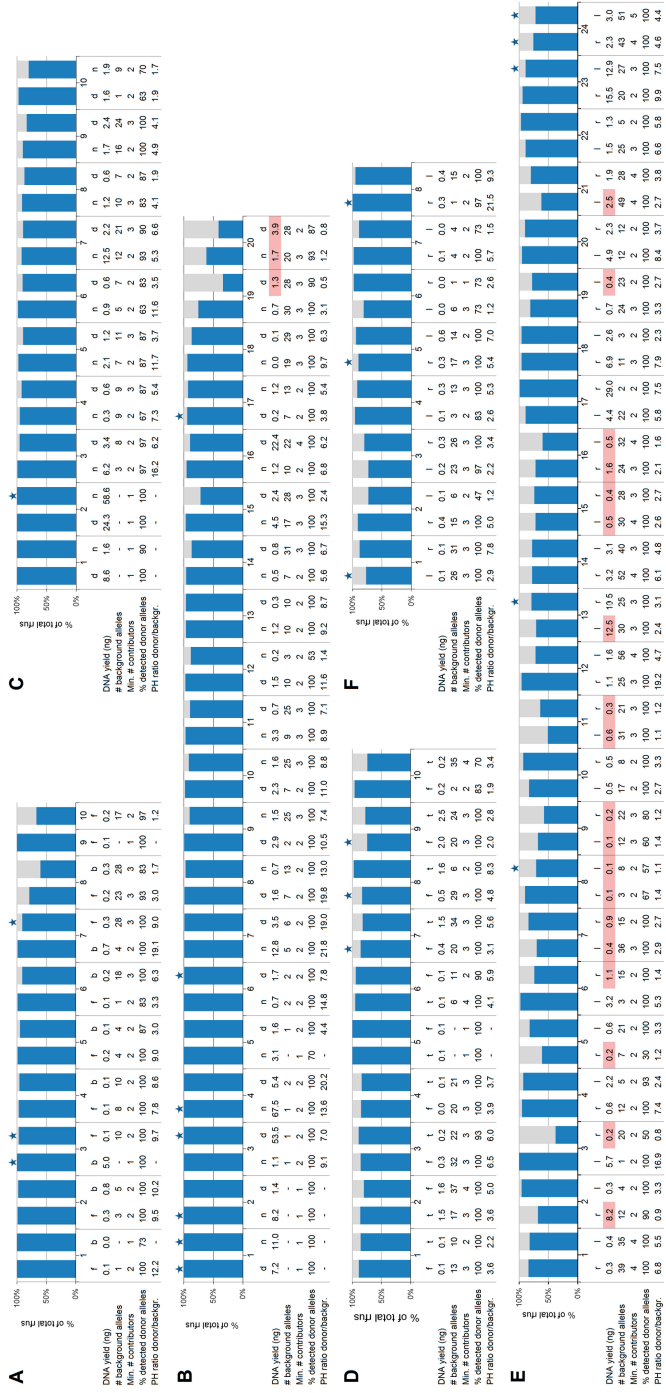


Figure 3. Detailed information for the 164 analysed private samples: A) 20 samples from the front (f) and the back (b) of the neck of ten volunteers. For two samples (9 and 10) results were obtained only for samples collected from the front side of the neck; B) 40 samples from the dominant (d) and non-dominant (n) hand of 20 volunteers; C) 20 fingernail samples from the dominant (d) and non-dominant (n) hand of ten volunteers; D) 20 samples from the finger (f) and thumb (t) area of the winter glove worn on the dominant hand of ten volunteers; E) 48 samples from the right (r) and left (l) armpits of eight volunteers. Indicated per sample are the percentages of total rFus in the STR profile belonging to the donor from the right (r) and left (l) armpits of shirts of eight volunteers. Blue star above the bar indicates samples for which the major contributor deduced from in the STR profile was not the owner. (blue bars) and background (light grey bars). A blue star above the bar indicates samples for which the major contributor deduced from in the STR profile was not the owner. Red markings in the DNA yield section indicate samples for which the major contributor deduced from in the STR profile was not the owner.

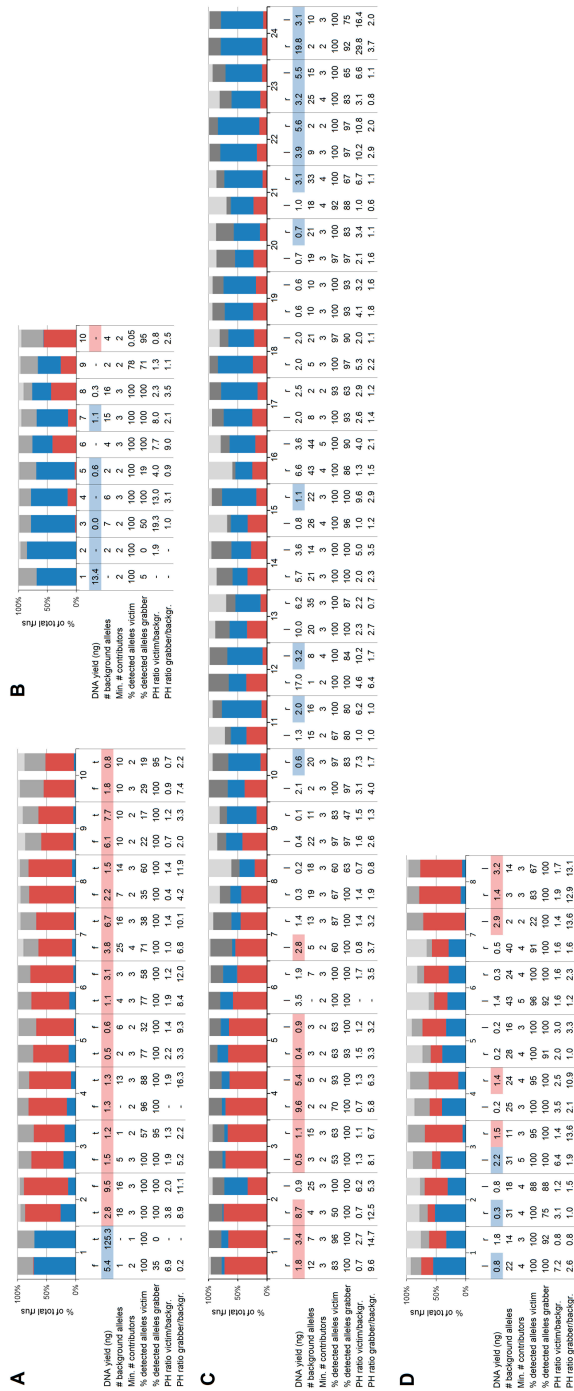


Figure 4. Detailed information for the of 94 analysed activity-related samples: A) 20 samples from the finger (f) and thumb (t) areas of winter gloves worn on the dominant hand of ten “perpetrators” who grabbed the bare arm of a “victim”; B) samples from grabbed arms of ten “victims” after the grabbing activity as described in A; C) 48 samples from the right (r) and left (l) trouser leg ankles of 24 volunteers collected after a dragging activity; D) 16 samples from the right (r) and left (l) armpits of shirts of eight volunteers collected after a dragging activity. Indicated per sample are the percentages of total rfs in the STR profile belonging to the “victim” (blue bars), “perpetrator” (red bars), shared alleles (dark grey bars) and background (light grey bars). Markings in the DNA yield section indicate samples for which the “victim” (blue) or “perpetrator” (red) was deduced as major contributor in the STR profile, absence of colour indicates samples for which no major could be deduced.

arm of the “victim”; 2) dragging of a “victim” by a “perpetrator” at the trousers ankles; 3) dragging of a “victim” by a “perpetrator” at armpits of shirts. The winter gloves, trouser ankles and armpits of shirts that were sampled after these activities represent the same items analysed without an activity in section “Private samples” (this sampling had occurred at least six hours earlier).

An overview of DNA and RNA profiling results is shown in Table 3 and details per individual sample are provided in Figures 4A-D. Alike the public items (section “Private samples”), large variations in DNA yields and number of contributors are observed. For most samples, an increase in DNA yield is observed when comparing yields prior (section “Private samples”) and post activity (Figure 3D-F *versus* Figure 4A,C-D).

Alleles corresponding to both the “grabber” and the “victim” were detected in the majority of the samples. The “perpetrator” was not necessarily the major contributor in the STR profile (Figure 4, Table 3). When no major contributors could be deduced (34% of all samples), this was mainly because of approximately equal peak heights for alleles corresponding to the “victim” and the “perpetrator” and not because of high non-owner signals. For most samples the donor alleles could be distinguished from non-owner alleles as these had generally relatively low peak heights (Table 3).

Table 3. DNA profiling results of 94 activity-related samples.

	Winter gloves Fingers + thumb dominant hand	Grabbed arm	Trousers ankle R+L ^a	Armpits of shirts R+L
Results	(n=20) Fig. 4A	(n=10) Fig. 4B	(n=48) Fig. 4C	(n=16) Fig. 4D
Avg. DNA yield (ng)	9.2	3.1	3.4	1.2
Range DNA yield (ng)	0.5 - 125.3	0.0 - 13.4	0.1 - 19.8	0.2 - 3.2
Higher DNA yield after activity (compared to Table 2)	95%	na ^a	71%	81%
Number of contributors	1 - 4	2 - 3	2 - 5	2 - 5
Samples with background signals	90%	90%	98%	100%
Average % non-owner rfus	4	3	10	15
Range % non-owner rfus	0-13	0-9	0-41	0-37
Avg. % detected alleles grabber	91	64	91	96
Range % detected alleles grabber	0-100	0-100	47-100	75-100
Avg. % detected alleles victim	64	88	90	90
Range % detected alleles victim	17-100	0-100	50-100	22-100
Full grabber profiles	80%	40%	40%	69%
Full victim profiles	25%	80%	56%	50%
Grabber is major contributor in STR profile	90%	10%	21%	31%
Victim is major contributor in STR profile	10%	60%	25%	19%
Samples lacking a major contributor	0%	30%	54%	50%
Avg. PH ^a ratio grabber/background	25.4	2.9	3.2	5.2
Range PH ratio grabber/background	0.2-240.4	0.9-9	0.6-14.7	0.8-13.6
Avg. PH ratio victim/background	5.6	6.5	4.2	2.6
Range PH ratio victim/background	0.4-60.2	0.8-19.3	0.7-29.8	0.8-7.2

^a R, right; L, left; na, not applicable; PH, peak height

Factors affecting DNA transfer

Sebaceous fluid secreted by the sebaceous glands is described to contribute to the amount of DNA available for transfer [11]. To confirm and extend these findings, we assessed the effect of the presence of sebum or sweat on skin surfaces on DNA transfer. In total 74 samples were analysed (Table 1). In addition, two samples were taken from UV-irradiated cloths, which confirmed removal of DNA.

An overview of DNA results is shown in Figure 5A-B. Large variations in DNA yields are observed for these various types of skin samples: yields ranged between 0.1 to 2.4 ng for non-sebaceous samples and between 0.7 to 19.9 ng for sebaceous skin samples. Yields for non-sweaty skin samples ranged between 0.1 and 10.8 ng and those for sweaty skin samples between 0.1 and 191.8 ng. When comparing the yields for the two skin types collected per donor, the sebaceous skin samples always resulted in higher DNA yields (2.6 to 186 times higher) than the non-sebaceous skin samples. The sweaty hands had higher DNA yields in 72% of the comparisons (up to 153.6 times higher).

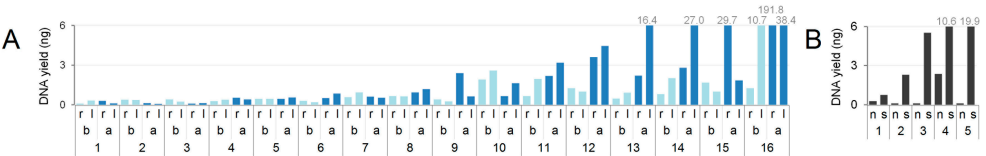


Figure 5. DNA yields (ng) for the various skin samples analysed to assess the effect of the presence of sweat or sebum on DNA transfer: A) 64 sweat and non-sweat samples of the right (r) and left (l) hands of 16 individuals before (b) and after (a) running activities; B) non-sebaceous (n) and sebaceous (s) skin samples of five individuals. Light blue bars indicate DNA yields for samples taken before the activity, dark blue the samples taken after the activity. For samples with a DNA yield above 6 ng (the maximum of the y-axis), the yield is indicated in grey above the bar.

Washing machine: transfer and persistence

Washing machine experiments were performed to assess the transfer and persistence of DNA and RNA during laundry. The denim patches were derived from worn jeans and treated with UV irradiation. Although no DNA resided on these patches, skin mRNA markers were detected in some of the patches (Table 4, analysis of ten samples). It was therefore decided to regard the skin marker results with caution. The experiment had two parts. In part one “blank” patches and patches with controlled inputs of two body fluids were combined with regular laundry to assess indirect (to the “blank” patches) and direct (within a patch on which body fluid stains are deposited) transfer of DNA and RNA, and persistence at the stain location. An overview of the DNA and RNA profiling results is shown in Table 4.

Table 4. Details on DNA and RNA profiling results after analysing 112 washing machine samples. Average yields were determined including samples with concentrations below the detection threshold of the quantification system.

Part			Average recovered DNA yield (ng)	Persistence rate (%) ^a	Cell types "observed"		
					Blood	Saliva	Skin
0	UV-treated patches	Blank areas (n=10)	0.01 (0.00-0.01)	na ^b	0%	0%	60%
1	Blood stain	(n=16)	0.11 (0.03-0.42)	0.001 (0.000-0.003) ^c	0%	0%	94%
	Spotted patches	Saliva stain (n=16)	0.04 (0.02-0.09)	0.001 (0.000-0.002) ^d	0%	0%	81%
		Blank areas (n=32)	0.05 (0.02-0.17)	na	3%	0%	97%
	Blank patches	Blank areas (n=32)	0.07 (0.03-0.22)	na	6%	0%	91%
2	Wash 1	Blank areas (n=8)	0.00 (0.00-0.00)	na	0%	0%	63%
	Wash 2	Blank areas (n=8)	0.00 (0.00-0.00)	na	0%	0%	38%

^aDetermined by comparing the DNA yield recovered at the stain area after washing to the amount of DNA spotted on the patch

^bNot applicable

^cA blood stain is inferred to contain 13,339 ng DNA

^dA saliva stain is inferred to contain 5,550 ng DNA

Persistence rates of blood and saliva on the stained denims were determined by comparing the amount of DNA spotted on the patch (derived from determining the DNA yield in an aliquot of liquid body fluid *i.e.* 10 µL blood or 20 µL saliva) to the DNA yield recovered at the stain area after washing. Average persistence rates of less than 0.001% were determined for both body fluids (Table 4). Since DNA from the patches is recovered by tape lifting, these persistence rates will be an underrepresentation. DNA yields were low for all samplings and ethanol precipitation of the full DNA extract was required to generate profiles with information. The DNA profiles derived for the body fluid stain areas contain on average 19% of the non-shared alleles of the blood donor and 3% of the saliva donor at the respective spot locations. Since in addition some alleles not corresponding to the blood or saliva donor were detected, we cannot be fully sure that all matching alleles derive from the spotted body fluids. Direct transfer of blood and saliva was assessed by sampling "blank" areas on the same cloth. Incomplete profiles with a maximum of 9 alleles were obtained and the origin of the alleles (blood, saliva, washing volunteer or other) was unclear. Indirect transfer, referring to transfer to patches that had not been in contact with the body fluid stains as these patches resided inside a washing bag, resulted in profiles with only very few alleles of unclear origin.

Both the locations where body fluids were spotted and the "blank" areas, were assessed using mRNA profiling. Blood nor saliva was scored "observed" on the stain locations. Blood was however scored "observed" in three of the analysed blank areas that resided either on a spotted or a blank cloth (Table 4). Saliva was not scored "observed" in any of the sampled areas. Skin mRNA was observed in the majority of samples (Table 4), but the origin of these skin signals is unclear, as skin mRNA was not fully removed upon UV irradiation of the patches. Possibly skin signals are added from co-washed laundry or remnants in washing machine, as skin is scored "observed" in

a higher percentage of the patches (Table 4). In the second part of the experiment we assessed whether skin mRNA is removed upon washing by performing multiple and subsequent “blank patch washes”, during which blank patches were washed without the presence of any other laundry. Wash 1 followed a normal laundry load and wash 2 immediately followed this wash 1. Results are shown in Table 4 and again very low DNA yields and only empty or STR profiles with few alleles were obtained. Skin remained detectable using mRNA profiling, although the percentage of positive patches is lower than when regular laundry is present as well (Table 4). For both parts of the experiment the effect of tumble- or air-drying was additionally studied, however, no clear differences were observed when comparing results of air- or tumble-dried samples (data not shown).

Discussion and conclusion

In this study, we assessed the amount and composition of human cell material prevailing on various items. To maximise profiling information, we concentrated DNA and RNA extracts prior to profiling when DNA yields were low. By this approach we observed background cellular material that predominantly gives skin cell typing signals on any type of public or private item although large variations for the DNA yield and number of contributors were seen. Various aspects can have a role such as the function of an item that determines how often, how long and with what intensity an item is touched, the type of substrate from which an item is made, the type of biological substance that was deposited by users, whether or not friction is applied and the individual shedder status of persons touching the items [11-12][16-23]. Results show that high DNA yields do not necessarily relate to an increased number of contributors, or the detection of other cell types than skin. The observation that the detection of skin-specific mRNAs is relatively sensitive when comparing to other cell types or skin-derived DNA [37] and our observation that skin mRNA signals are robust and can even be detected after UV-irradiation, may have a role here as it is possible that a body fluid was deposited of which the DNA is detected and the RNA not. Some body fluids such as nasal mucosa [37] contain more nucleated cells than skin [34]. Results of this study support this, as mainly high DNA yields are obtained for samples with nasal mucosa scored “observed”. The study involved 549 samples that were collected and analysed during a substantial time period. Therefore, three versions of our cell typing multiplex were applied in which the two earliest versions did not carry a marker specific for nasal mucosa. With these multiplexes we occasionally detect vaginal mucosa and menstrual secretion mRNA markers that may originate from the presence of nasal mucosa, as the markers for these body fluids can be co-expressed in nasal mucosa to variable extent [37].

STR profiles were generated to obtain information on whether a major contributor is present and whether this matches the owner in case of a private item. With public objects, 17% of the samples showed a clear major contributor but no relation could be made with type of item or DNA yield. With private objects, 88% of the samples showed a major contributor; 99% of these match the owner (consistent to transfer studied described in Ref. [10]), 1% not (these were all hand samples) and we did not attempt to identify this person and determine the relation to the owner of the private item. Irrespective of the presence of a major contributor, we observed non-owner alleles in 90% of the private samples, confirming the concept that private items carry non-self cell material. Consistent with results described by van Oorschot et al. [40] we found that the DNA yields of samplings vary mainly between individuals and less within samples from the same person. Regarding cell type composition, only few samples contained an additional cell type besides skin and no trends can be derived whether this observation occurs more within or between persons, or for a specific gender.

For the majority of samples collected after activity-related scenarios at least partial DNA profiles for both the victim and perpetrator could be retrieved. In only a portion of the samples a major contributor is deduced and approximately evenly frequent this is the “victim” or the “perpetrator” (29% and 38% of the samples, respectively). Often, no clear major could be deduced (34% of the samples). Since the activities were performed in a controlled setting and because we generally see a similar result for the right and the left sample, we infer that specific aspects of the couples engaged in the activity have a role, such as the type of fabric, number of days that clothing had been worn, shedding features of individuals or personal habits [10][19][21][23][41]. We confirmed [11] that the presence of sebum promotes DNA transfer and habits like regularly touching face or scalp can give an individual a higher DNA transfer rate. We assessed whether the presence of sweat could have a similar effect as sebum, but noticed a less strong effect. As sweat itself contains only limited amounts of DNA [37], moisture may provoke increased DNA yields by releasing more skin cells.

We also studied transfer and persistence during laundry. Generally, low persistence rates are obtained for the saliva and bloodstains that were used, which is consistent with earlier studies describing difficulties in producing full STR profiles of saliva and to a lesser extend blood stains after transfer (not necessarily during laundry) [13][17][23]. The moisture level of a stain may influence the transferability [18][42] and the rehydration of the stains during laundry may increase transfer although one would assume that the washing detergent deteriorates the cell material. No conclusions can be drawn from the detection of skin mRNA after washing, as these markers remained detectable after UV-irradiation of the patches, which indicates a remarkable stability of the skin mRNA markers [37]. Even though only limited cell types and sample volumes were assessed, the overall view is that the transfer and persistence of DNA in a washing machine is very limited, while the persistence of RNA appears cell type dependent.

Even though many other aspects regarding the transfer, persistence and recovery of human cell material remain to be evaluated, results of this study increase insight in the prevalence of human cell material on various sample types and in activity-related scenarios. This study may aid reporting officers in evaluating the strength of evidence at the activity level for instance when using a Bayesian network approach [43-46]. In such an approach, the probabilistic relationships between variables are assessed through a graphical model. These probabilities can be based on expert opinion or – preferably- derived from experimental data such as those presented in this study. For instance when considering a dragging scenario, a probability of 0.21 may be given to the event that the perpetrator occurs as a major in the DNA profile and one of 0.25 that the victim occurs as major when considering the data presented in Figure 4C (in which in ten of the 48 DNA profiles the perpetrator occurs as major and 12 times the victim). Inevitably, experimental designs will not be the exact same as the hypothesized crime scene conditions but information on the occurrences of a certain event and the extent of variation that is obtained, will assist in assigning probabilities. Clearly, this is a challenging task as a large variation is already observed for experiments with straightforward conditions as in this study.

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