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Research paper "Total Human DNA Sampling" – Forensic DNA profiles from large areas

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ABSTRACT

Employed for the first time in 1986, DNA profiling is nowadays established as one of the most widely used forensic techniques worldwide. However, until today, no efficient sampling technique existed to collect DNA from human skin cells from a large area, not to say from the floor of an entire room. This has been extremely unfortunate, as there is enormous forensic potential in these DNA traces from the ground to provide clues as to who has been present at a particular location, i.e. at the crime scene. By desquamation, humans loose several millions of skin cells per day, everywhere they stand, sit or walk; and they can do little about it. We developed a fast and simple method by which we can make use of all those lost skin cells. We use a vacuum cleaner equipped with a specialized filter cartridge to sample the ground. Fragmentation of the filter membrane and subsequent parallel processing of the filter fragments, using a modified Chelex® 100 extraction protocol, significantly reduce the complexity of the dust mixture. In this way, a large number of interpretable major contributor DNA profiles can be generated from individuals who have been present on the sampled surface. Overall, at least 38 % of the generated DNA profiles from all sampled test areas fulfilled the criteria for submission of single major contributor profiles to the Swiss DNA database. As demonstrated through a mock crime scene scenario simulating an indoor stabbing event, the perpetrator's DNA could be found on the floor even after a very short stay in the room of less than one minute. Furthermore, already the first application of the method at a real crime scene led to relevant case information for the police. Given its large investigative potential, we recommend Total Human DNA Sampling as a helpful complemental forensic tool to conventional DNA trace collection in major crimes.

1. Introduction

Humans shed their outer skin layer completely once every two to four weeks, thereby losing about 200 million to 1 billion cells every day [1–3]. This results in an estimated quantity of 30–90 milligrams of skin flakes lost every hour [4]. Those shed skin flakes are a major constituent of household dust. Weschler et al. collected and analyzed the dust from 500 bedrooms and 151 childcare facilities. Components characteristic of skin flakes were found in 97 % of the dust samples that were collected [4]. If we assume an average shedding of 500 million cells a day, this corresponds to more than 300'000 cells a minute. To establish a DNA profile from a crime scene sample, 5–10 cells can already be sufficient. The skin cells that fall off our bodies everywhere we go therefore bear an enormous potential to establish connections between places and individuals. Collecting dust at a crime scene and analyzing it for human DNA can lead to new and so far completely unexplored investigative leads in forensics.

DNA sampling today is principally focused on what people might

have touched in a certain environment. In Switzerland, more than 80 % of the analyzed DNA traces are so called "contact" or "touch" DNA traces [5]. They are collected because of an assumed physical contact between an item related to a crime and a potential suspect. For criminals, leaving "touch" DNA traces can easily be prevented by wearing gloves. However, it is much more difficult to prevent the loss of skin cells just through shedding effectively. If we are able to analyze specifically the human DNA in skin flakes, it becomes almost impossible for criminals to leave no traces behind. Swabbing or collection by adhesive tapes are the current state of the art for collecting DNA traces [6,7]. However, those well-established methods are entirely inappropriate for gathering biological material from a large area.

For the first time, we present here a technique that is suitable for the DNA sampling from the floor of an entire room. The method is based on a vacuum cleaner and a forensic filter cartridge, conceived for the collection of fibers. The idea to use vacuum to sample for DNA at the crime scene is not new [8,9]. However, just like swabs or tapes, the existing methods are not suitable to efficiently sample DNA from larger

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surfaces.

2. Materials and methods

2.1. Sampling locations

The first part of the study, namely the development and testing of our method, was performed at the Forensic Molecular Biology unit of our institute. DNA profiles from all collaborators were readily available for quality control purposes. In addition, access to the department is restricted to authorized personnel, therefore limiting the potential contributors to mainly individuals with existing reference profiles.

For the second part, the simulation of a real-life incident, we tested the developed method on a mock crime scene in a private residence. The perpetrator (who has never been in the residence before) and the resident owner gently pushed each other around for a couple of seconds. This melee was followed by a simulated stabbing movement by the perpetrator and the victim sinking to the ground. Immediately after the stabbing and a subsequent simulated looting of the pockets of the victim, the perpetrator left the residence. The resident playing the victim got up again and stepped aside and all traces around and underneath the "victim" were collected. The whole action was kept on video, in order to record the underlying time frame. The profiles obtained from the traces were compared to the ones from the perpetrator, victim, visitors and residents to evaluate if the perpetrator's DNA profile could be discovered, despite the very short stay in the apartment and the assumed presence of excess background DNA in the room.

Table 1 lists area sizes and surface materials of the different sampled locations. We sampled Carpet 1 twice, with a four-week interval, to assess possible differences over time. We also sampled Carpet 2, which is located near an aisle where more people walk by, in order to observe potential changes in mixture profile pattern between the two carpets. The "Crossing Offices" location is a crossing of two aisles leading to five different offices. We aimed to determine whether the collected traces could be connected to the individuals working in those offices. The aisles are cleaned almost daily by professional cleaning staff. The lab room was sampled because it is accessed mostly by laboratory staff. It is therefore suitable to investigate whether traces from other members of the department, e.g. administrative staff, not frequently entering this room, could be found. In addition, the cleaning staff does not have access to this room and it is cleaned once a week by the lab staff.

The floor at the mock crime scene was a tile floor made of fine stoneware in the living room of a family house with four permanent residents. It had been cleaned for the last time five days before the sampling. Three visitors stayed in the house where the scene was set up for three days and left two days before sampling.

2.2. Dust collection

We used a GAS18V-1 vacuum cleaner from Bosch (Gerlingen, GER), equipped with a custom-made tube adapter and a filter unit 6197E from Sirchie Acquisition Co LLC (Youngsville, NC), all purchased from coloprint GmbH (Hilden, GER) (Fig. 1). Prior to sampling, the device was wiped with a paper towel (moistened with 70 % ethanol) and carefully assembled on a cleaned surface close to the sampled area. After the sampling, the filter cartridges were sealed with the enclosed caps and transferred to a plastic bag for transportation to the lab for further processing. At the mock and at the real crime scene, the sampling collaborator wore a disposable coverall during the whole sampling procedure.

2.3. DNA extraction from filter cartridges

To collect human skin cells and other trace evidence such as hairs and fibers, we opened the seal and accessed the filter by opening the filter cartridge on one side. Hairs were carefully removed using a flamed tweezer and transferred to a separate petri dish for potential subsequent DNA analysis. Hairs were not processed further for this project. To prevent any traces from falling off when removing the filter from the cartridge, we first moistened it with 200 μL ultrapure water. After opening the other side of the filter unit, we carefully removed the filter membrane from its support and transferred it onto a petri dish for further processing. The material sampled with the vacuum cleaner tends to accumulate in the middle of the filter membrane. Therefore the membrane was cut in a "Spider web pattern" with a scalpel into pieces of variable sizes, with the smallest pieces located in the middle. The filter was cut into 46-50 fragments. After cutting, the fragments were transferred one by one into 2 ml tubes for DNA extraction. We added 1000 µL of 20 % Chelex® 100 resin (Bio-Rad Laboratories, Hercules, CA, USA) to each of the 45-50 tubes. The samples were then put on a Precellys® 24 homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) for two cycles of 30 s at 5900 rpm and subsequently incubated at 100 $^\circ$ C for 10 min to facilitate DNA release.

2.4. Contamination monitoring

To control that the filter cartridges, which are currently not produced certified DNA free, are not contaminated by human DNA from the production line, we cut three new filters into 48 fragments and



Fig. 1. Bosch GAS18V-1 vacuum cleaner and Sirchie 6197E filter unit used for Total Human DNA Sampling.

Table 1

	Carpet 1		Carpet 2	Crossing offices	PCR-Lab	Mock scene
	Sampling 1	Sampling 2				
Area	4.5 m ²	4.5 m ²	4.5 m ²	3.5 m ²	12 m ²	4.4 m ²
Surface	Carpet	Carpet	Carpet	Linoleum	Linoleum	Tile
Fragments	46	50	48	48	48	46
Profiles	46	50	48	27	37	39
Total DNA	42.3 μg	97.5 μg	23 µg	0.3 µg	2 µg	1.8 µg
DBS profiles	29 (63 %)	34 (68 %)	23 (48 %)	18 (38 %)	26 (54 %)	24 (52 %)
References	29 (100 %)	33 (97 %)	20 (87 %)	17 (94 %)	24 (92 %)	24 (100 %)
					Perpetrator	4 (17 %)
					Residents	13 (54 %)
					Visitors	7 (29 %)

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processed them as described above, resulting in 144 filter fragments for negative control.

Additionally, to assess the potential to carry DNA over from one crime scene to another by the use of the vacuum cleaner, we set up the following monitoring experiment: A filter cartridge was loaded with dust by sampling one of the carpets described above. The dirty cartridge was removed and replaced by a new one. Samples were then taken with the vacuum cleaner for 90 s each from three acrylic glass plates measuring 65×65 cm. The plates were divided into four equal quadrants (32.5 \times 32.5 cm). After using the vacuum cleaner on the plates, we swabbed each of the four quadrants on each of the three plates with viscose swabs (Sarstedt, Nümbrecht, Germany), resulting in 12 contamination control samples per sampling day. On two days, the sampling was effected with dry swabs (n = 24) and on four days with pre-moistened swabs (n = 48). Acrylic glass plates were cleaned with 0.4 % bleach before the sampling. The plates were always controlled for the absence of human DNA by swabbing each of the 12 quadrants before applying the vacuum cleaner, resulting in another 72 pre-trial controls.

DNA from swabs was extracted utilizing the PrepFiler Express[™] kit on an AutoMate Express[™] extraction system (both Thermo Fisher, Waltham, MA, USA) as described previously [5]. All 288 control samples were monitored for the presence of human DNA by qPCR as described below.

2.5. DNA analysis

DNA quantification was performed using the Quantifiler® HP qPCR Kit from Thermo Fisher (Waltham, MA, USA) on an ABI 7500 Real-Time PCR System and HID Real-Time PCR Analysis Software v1.2 (Thermo Fisher, Waltham, MA, USA). For DNA profiling, the AmpFlSTR® NGM SelectTM multiplex kit (Thermo Fisher, Waltham, MA, USA) was used with a maximum of 0.5 ng input DNA per reaction. Samples with DNA concentrations below 50 pg/µL were amplified using the maximum sample volume of 10 µL, while those below 20 pg/µL were amplified with 32 instead of 30 PCR cycles, according to lab internal standard operating procedures [10]. Capillary electrophoresis was conducted on a 3500 xl genetic analyzer (Thermo Fisher, Waltham, MA, USA) and the Genemapper ID-X v1.6 software (Thermo Fisher, Waltham, MA, USA) was used for signal interpretation with an analytical threshold at 100rfu.

2.6. Data analysis

Microsoft® Excel® 2016 (Microsoft Corporation, Redmond, WA, USA) and RStudio v2022.02.3 [11] were utilized for calculations and graph creation. DNA profiles established from the filter fragments were evaluated for contribution from reference individuals through probabilistic genotyping, using STRmix[™] [12]. We used the database search function of STRmixTM to calculate likelihood ratios (LRs) for the potential contribution of all collaborators from the department to all trace profiles from the in-house test areas and for the potential contribution of all collaborators, residents and visitors to traces at the mock crime scene. An LR (likelihood ratio) threshold of 1'000 was applied to assume a real contribution. STRmixTM calculations based on the database file containing all the reference profiles were done for all filter fragment profiles that displayed signals above the analytical threshold in at least 10 out of 16 analyzed loci. LR calculations where done using the Swiss reference population [13] with a FST value of 0.01 to correct for average co-ancestry at the population level. The number of contributors for probabilistic genotyping was estimated by the maximum allele count method with default stutter filters switched on in Genemapper ID-X v1.6. We are aware of the fact that this method tends to underestimate the real number of contributors, especially for higher order mixtures [14].

2.7. Criteria for submission to the Swiss national DNA database

The profiles generated from our study were categorized as either DBS (DataBase Suitable) or non-DBS profiles. In Switzerland, the CODIS (Combined DNA Index System) software is used for the national DNA database. The Swiss government has defined the 16 loci included in the AmpFlSTR® NGMSelect/NGMDetect and PowerPlex® ESI17/ESX17 kits as the core loci for the database [15]. For a database entry in the Swiss national database, a minimum of 6 unambiguously typed loci are required for single or major contributor profiles, and 8 loci for two-person mixtures. The sex locus Amelogenin can be entered but is not searched for in CODIS. For this study, we only counted single and one-person-major contributor profiles and did not consider potential two-person mixtures fulfilling the database criteria. A profile was considered DBS as a single major contributor profile if a ratio of at least 3:1 for major to minor component was reached and if the respective loci displayed a heterozygote peak balance of at least 60 % [16]. Profiles that did not meet these criteria were considered non-DBS. All results are based on single amplifications. We would expect slightly lower rates of DBS profiles with duplicates.

3. Results

Sampling time was dependent on the size and texture of the sampled area and ranged between two and eight minutes. The filter processing prior to DNA extraction took about 1.5–2 h. DNA profiles could be established from the majority of filter fragments across all sampling locations. The sampled material tends to concentrate in the middle of the filter. However, we did not detect an obvious pattern among the filter fragments: major contributor DBS profiles could be detected on filter fragments in the center as well as on the periphery of the filter membrane.

3.1. DNA profiles from the department

The fraction of DBS profiles ranged between 38 % and 68 % for the various in-house test sites. Depending on the sampling location, between 87 % and 100 % of the single major DBS profiles could be attributed to the collaborators working in the department. The total DNA amounts collected by the filter cartridges varied largely, between 0.3 μ g sampled from the aisle cleaned daily and 97.5 μ g from one of the carpets (Table 1). Patterns of DNA mixture contributions also varied between sampling locations (Fig. 2). The complexity of the detected mixture profiles, as measured by the estimated numbers of contributors, varied as well among sites and roughly correlated with the recovered DNA amounts (Table 2).

3.2. DNA profiles from the mock crime scene

The simulated scuffle between the perpetrator and the victim lasted for 14 s, including the simulated stabbing movement. After the resident playing the victim laid down to the ground, the perpetrator simulated a looting, searching the pockets of the victim for 20 s. The perpetrator then left the house and the victim stood up and stepped aside. The subsequent Total Human DNA Sampling of the area below and around the victim took around 4 min. From the 46 filter fragments, 24 single major DBS profiles could be established. All of them could be attributed to known individuals. Four of them (17 %) matched the profile of the perpetrator, 7 (29 %) could be attributed to the visitors and 13 (54 %) were from the permanent residents, including the victim (Table 1). No single major contributor profiles from unknown individuals could be detected. It should be noted that genetic relationships exist among the residents and between some of the residents and some of the visitors. This could potentially lead to a slightly higher number of inclusions, because two related individuals might both fit the partial minor contribution of a mixture with LRs above 1'000, even though only one of

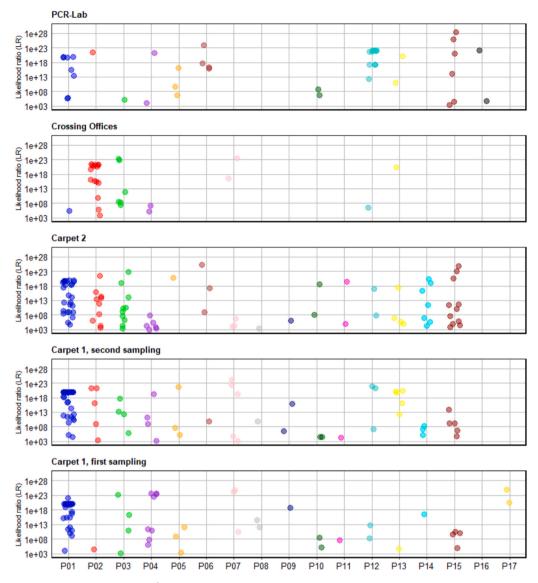


Fig. 2. Likelihood ratios exceeding a value of 1'000 for known reference persons (P01 to P17) at the different sampling locations in the department.

Table 2 Distribution of profiles from the different filter fragments according to minimum number of contributors (NoC), as determined by maximum allele count.

		Number of contributors				
		2	3	4	5	
Carpet 1	Sampling 1	49 %	27 %	18 %	6 %	
	Sampling 2	24 %	46 %	24 %	6 %	
Carpet 2		2 %	8 %	46 %	44 %	
Crossing Offices		74 %	26 %	0	0	
PCR-Lab		30 %	30 %	24 %	16 %	
Mock Scene		36 %	54 %	10 %	0	

them might be the real contributor. We can see that the profile complexity at the mock crime scene tends to be lower than in the department, reflecting the smaller number of individuals frequenting the sampled area (Table 2).

Neither the profile of the person who sampled the mock crime scene, nor the profile of any other collaborator from the department could be detected at the private apartment. All LR values for the contributions detected through probabilistic genotyping are displayed in Fig. 3.

3.3. Contamination controls

None of the 144 negative control samples from three blank filter units contained human DNA, as measured by qPCR. In addition, all 72 samples taken from sampled acrylic glass plates, to control for potential DNA transfer from scene to scene, were negative for human DNA, as well as the 72 pre-trial controls. According to the manufacturer, the detection limit of the qPCR assay is at < 1 pg/µL human DNA.

4. Discussion

Sampling the floor of an entire room will frequently lead to very complex mixtures of biological material from numerous contributors. By fragmenting the filter membrane prior to DNA extraction, we are able to reduce this complexity and end up, not only with DNA profiles that could be evaluated through probabilistic genotyping, but even with a very high percentage of database suitable DNA profiles from single major contributors.

We observed that the detected major contributor profiles thereby correlate well with the regularity of the presence of the different individuals on the test surfaces. Here are some examples:

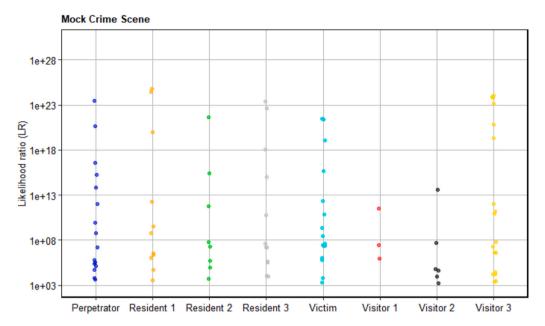


Fig. 3. Likelihood ratios exceeding a value of 1'000 for known reference persons at the mock crime scene.

- (1) During the sampling at the department, the DNA profile of collaborator P01 was detected frequently on Carpet 1 and on Carpet 2 (see Fig. 2). This observation is consistent with the fact that P01 is known to be the sole person to take a break regularly on a chair located on Carpet 1, which in turn is located right next to Carpet 2.
- (2) The absence of P07, P08, P11 and P14 from the sample collected in the PCR lab correlates well with the fact that those individuals are rarely present in the lab room, since they are mainly working in the office. The same applies to P02 and P03 whose DNA could only be detected once in the PCR-Lab.
- (3) The profile of P17 was found twice in the first sampling of Carpet 1, despite the fact that this collaborator has left the department already two months before the sampling took place. Consequently, this person's profile was not detected anymore in the second sampling from Carpet 1. The absence of this individual's DNA in the second sampling suggests that the composition of the DNA mixture on Carpet 1 changed in function of the continued use of this area during the 4 weeks between the two sampling events. This time was however sufficient to accumulate a similarly large DNA quantity than the one that has been collected through the first sampling.
- (4) Carpet 2 is located close to the aisle and as expected showed more complex DNA mixtures than Carpet 1. There were also more major contributor profiles on Carpet 2 that could not be attributed to the staff, what is in line with the fact that this sampling location is closest to the entrance and from all sampled areas, it is the one with most foot traffic from individuals not working in the department. The high percentage of DBS DNA profiles from Carpet 2, an area with frequent foot traffic, a high potential to trap cellular material due to the texture of the floor and consequently a large total DNA amount recovered of almost 100 μg, demonstrates the potential to identify individuals even from samples containing a lot of dust.
- (5) The sampling of the aisles crossing between offices revealed a lower DNA quantity compared to other sampled areas, likely due to the daily cleaning compared to the PCR room cleaned only once a week and the lower potential to trap cellular material than on carpets. Taking into account the different sizes of the sampled areas, we detected about half the DNA than collected in the PCR room. The sampling was done in front of the office of P02, whose

profile was by far the most frequent one in this sample. In addition, we know from previous experience in the lab that PO2 is a rather good DNA shedder, whereas P08, who just occupies the next office down the aisle from the sampling location and whose profile could not be detected at all, is a rather poor DNA shedder. The frequent presence of the DNA of P03 on the aisle crossing is also consistent with the fact that this person's office is also located close to the sampling location.

Whether or not a crime-relevant major contributor profile can be established from a floor sampling is, of course, subject to stochastic processes. One must be lucky that a relevant skin flake, lost by the potential suspect, contributes the largest amount of DNA to the mixture of skin debris on at least one of the filter fragments. Ignoring different shedder status, it will be more probable to find someone's major DNA profile the more time that person spent in a certain location, as we have seen from the in-house sampling at the department.

However, the results of the mock crime scene sampling indicate that even very short exposure times e.g., less than a minute, can permit the deposition of sufficient DNA amounts detectable through Total Human DNA Sampling. This holds even true when a substantial amount of background DNA is present. From the mock scene, we recovered five times more DNA per area than from the daily-cleaned aisles in the office tract and we detected the DNA profiles of all four residents and of all three visitors who were present until two days prior to sampling. No other major contributor profiles from people previously present in the apartment was detected, suggesting that their DNA had been removed through the weekly cleaning routine. However, among the 24 major contributor profiles from the collaborator playing the perpetrator in the simulated stabbing and looting scenario.

It is noteworthy that at the mock crime scene we did neither detect the profile of the sampling operator nor of any other individual from the department, where the vacuum cleaner had been used several times before, also on very DNA-enriched areas such as the two carpets. This suggests that with careful handling of the device and by wearing full protective clothing while sampling, a contamination of the scene can be efficiently avoided. The results from the contamination monitoring confirmed this observation. We were unable to detect even spurious amounts of human DNA on any of the 18 acrylic glass plates sampled by conventional swabbing, after the use of the vacuum cleaner that has previously been used on the DNA rich carpets in the department.

The sampled material is trapped in the filter unit that holds back particles down to the size of $3-10 \ \mu\text{m}$, according to the manufacturer. This is less than the size of a single human skin cell [17]. In case minute amounts of subcellular, DNA-bearing material nevertheless pass the filter unit to the interior of the vacuum cleaner, they should be retained by the HEPA filter, cleaning the air of particles larger than 0.3 μ m before it leaves the dust container. We therefore estimate the overall potential for contamination from crime scene to crime scene as extremely low. In addition, even if a very small quantity of DNA would be transferred from one site to another through the sampling device, the corresponding profile would most likely not be detected, given the DNA richness of most of the collected traces.

Despite the large extraction volume of 1 ml, we obtained many subsamples with high DNA concentrations. The concentration means for the different filter fragments ranged between 2.3 ng/µL for the sampling from Carpet 1 (2nd sampling) and 0.008 ng/µL for the sampling from the "Crossing Offices" location. No profiles could be established from subsamples containing less than a total amount of 1 ng of DNA, corresponding to a concentration of 0.001 ng/µL. Subsamples with low DNA concentrations were not enriched by e.g., vacuum centrifugation or Vivacon® concentration. Such an enrichment, by reducing the sample volume from 1 ml to e.g., 50 µL, would most likely lead to even more DBS profiles. By visualizing also very small DNA amounts, we will also increase the potential to detect DNA that had been transferred to the scene without the respective person having been actually present on site, what is however, not a new issue of the technique presented herein [18].

Through an initial collaboration with our local police, we had the opportunity to test the Total Human DNA Sampling method in a real criminal case. We sampled the floor area around a dresser whose drawers were searched by the perpetrator during a burglary. We were able to establish a single major contributor profile of an unknown individual, not living in the apartment. In the same case, a cigarette butt had been collected from the public street, about 60 m away from the house. The DNA profile from the unknown individual collected in front of the dresser matched the DNA profile from the cigarette butt, which in turn resulted in a hit in the DNA database. This observation enabled us to establish a direct link between the suspect and the crime scene. While it may be easy to find a plausible reason for leaving a cigarette butt in a public space, it gets markedly more challenging for suspects to explain the presence of their DNA in a privately owned house, subjected to a recent break-in. Importantly, conventional DNA sampling based on assumed contact fell short in establishing a connection between the suspect and the crime scene, further highlighting the large investigative potential offered by the Total Human DNA Sampling method.

DNA profiles from floor samples bear an enormous forensic potential, but they are less contextualized than samples collected based on assumed contact. We will inevitably create many profiles unrelated to the crime. However, this limitation is not unique to our method but rather also applies to standard DNA sampling. In a study by Tièche et al. on targeted DNA sampling in burglary investigations, around 75 % of all established profiles were found to be from residents, highlighting the need for reference profiles from individuals authorized to be present at the scene in a more general way [16].

Analyzing up to 50 filter fragments by multiplex PCR causes significant costs. Moreover, the need to establish reference profiles from all individuals with access to the crime scene further increases the costs. Therefore, we expect our method to be used mainly in the investigation of serious crimes. Total Human DNA Sampling can be viewed as a complementary tool to the current standard DNA sampling procedures of the police. Compared to the costs for the DNA analysis, the costs for the filter unit are low and the sampling is very simple and fast. We expect an ETO-treated DNA-free version of the filter to be soon available from coloprint GmbH in Germany for an estimated price of about $60 \in$. However, as demonstrated by the three blank filters we analyzed for this study, even without ETO treatment, the filter cartridges do not seem to

carry substantial amounts of contaminating human DNA. Therefore, we strongly recommend taking floor samples from relevant areas in all major crimes. The filter cartridges can be sealed, stored, and analyzed in a second phase of the investigation, in case classical DNA samples, based on assumed physical contact, do not lead to promising investigative leads, because e.g., the perpetrator wore gloves. In our study, we focused on floor sampling. However, the method could also be useful for DNA sampling from furniture such as sofas or from garments. Furthermore, the filter collects in parallel other traces like fibers or hairs, which could also be used for the investigation.

5. Conclusion

We presented here the first efficient method to sample human DNA from larger areas. Total Human DNA Sampling can collect shed skin cells from an entire room, without major risks of contamination. We recommend this fast and simple method as an additional standard procedure for the crime scene investigation in major crimes.

Ethics statement

All samples from reference persons were collected using written informed consent. The study does not fall under the scope of the Swiss Human Research Act. Accordingly, the responsible cantonal ethics committee issued a declaration of non-responsibility.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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