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Comparison of stubbing and the double swab method for collecting offender epithelial material from a victim's skin

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ABSTRACT

After manual strangulation, epithelial cells originating from the offender can often be found on the skin of the victim. In order to obtain a conclusive DNA profile, it is important to secure as many epithelial cells from the offender and as few epithelial cells from the victim as possible. In this study, two methods for securing offender DNA were compared: the double swab method and an adapted tape-lifting method, so-called stubbing. 50 male volunteers were asked to simulate manual strangulation on the forearm of a female volunteer. After securing the epithelial material, DNA profiles were generated. The contribution of both donors to the samples was determined from the number of detected alleles, specific for each donor, and the average peak height of the donor-specific alleles. For the offender, in all cases except one, partial or full profiles were obtained and no difference between the double swab and the stubbing method was observed. For the victim, fewer alleles were detected by means of double swab than by means of stubbing. In conclusion, the double swab method performs slightly better than the stubbing method. However, from a practical point of view, the stubbing method may be preferred over the double swab technique.

1. Introduction

In cases of manual strangulation, strong physical contact occurs between offender and victim. Often, the offender leaves epithelial cells of himself on the victim's skin during this process. After securing these cells from the victim's skin, a DNA profile may be generated [1,2] containing genetic information of the offender.

The double swab technique [3] is a common method for securing epithelial cellular material from objects [4,5]. This method first applies a single, wet swab onto the surface of interest in order to loosen the cells, followed by a dry swab to secure the loosened cells. Apart from the double swab method, several studies describe tape-lifting as a method for securing epithelial cells from objects [6–8]. The tape-lifting method consists of a tape to which the epithelial cells adhere. It is mostly carried out by placing the tape over the surface of interest by a gloved finger, pressing it onto the surface and subsequently lifting it. Some publications report on securing epithelial material from the skin, instead of from objects, by means of tape-lifting [9–11]. However, these publications do not consider securing epithelial material of an offender from the victim's skin. Instead, they focus on collecting cell material from the person that is examined.

In a previous study performed at the Netherlands Forensic Institute, the tape-lifting method was adapted to create more distance between object and researcher (data not published). This so-called stubbing method is based on SEM (scanning electron microscope) stubs, for which a double-sided adhesive DNA-free tape was stuck to a SEM holder. In this way, contamination risks are reduced due to a greater distance between user and object, and pressure can be applied more evenly. The stubbing method was directly compared to the double swab method, which has been tested before for securing offender epithelial cells from a victim's skin [12]. Fifty independent samples were obtained for the stubbing as well as the double swab method by simulating manual strangulation using volunteers with known DNA profiles. To our knowledge, this is the first time that the stubbing method is investigated for securing epithelial cells from the skin of a victim and, in addition, both double swab and stubbing results are compared for such a large population.

2. Materials and methods

2.1. Experimental set-up

50 couples, each consisting of one man and one woman, were matched based on their DNA profiles. The DNA profiles of each couple had a maximum of eight overlapping alleles out of a total of 30 alleles. Volunteers were asked to not wash their hands 2 h prior to the experiment. Before simulating strangulation, the man

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rubbed his hands for 5 s to loosen epithelial cells. Then, the 'offender' (in our study always male) placed his dominant hand on the forearm of the victim (in our study always female) and applied pressure for 5 s. Subsequently, the man rubbed his hand once around the forearm of the woman by moving it 180° around the longitudinal axis of the forearm and moving it back. This process was repeated once. Subsequently, the contact area of approximately 20 cm^2 was marked and was divided vertically in two parts. One of the two areas was stubbed and on the other part the double swab technique was performed. These sampling areas were interchanged per couple to minimize influence of differences in applied pressure by the fingers and the palm of the hand. Experiments were performed at random times and days.

For both the double swab technique [3,5] and the stubbing procedure minimal force was used during sampling to secure as few epithelial cells of the victim as possible.

Swabs were rolled over the surface of the skin in horizontal lines at an angle of about 5–10°, while rotated around their longitudinal axis. Subsequent to sampling, swabs were air-dried for at least 4 and at most 24 h.

The stubbing procedure was performed by gently placing a stub at the skin and removing it. This process was repeated to cover the complete sampling area. Each stub was placed at the skin approximately 20 times before it was saturated. When the stub did not adhere to the skin anymore, a second stub was used. The stubs were stored in a dark room awaiting further processing. Hairs were removed by means of tweezers. All samples were stored for a maximum of seven days before DNA extraction was performed.

2.2. Swabs and stubs

The first swab (Invasive sterile Eurotubo Collection swab, Deltalab, Spain) was moistened with sterile, distilled water (water for injections 10 ml, B. Braun, Netherlands) and rolled over the surface of the skin as described before. The first, wet swab was followed by a second, dry swab. The two swabs were then pooled for DNA-extraction.

Stubs were produced from double-sided tape (12 mm × 33 m, Pritt, Netherlands). This tape was attached to a 12.7 mm^2 sized aluminum short pin stub holder (G301F, Agar Scientific, UK). The containers for the 12.7 mm^2 stub holders were SEM stubs (G3626, Agar Scientific, UK) and could easily and securely be reclosed. In Fig. 1 the stub is shown. Prior to usage, tape and tubes were cleared from DNA contamination by irradiation with 254 nm UV light in a CL-1000 UV CrossLinker (UVP Inc., UK) at 0.9 J/cm^2 during 60 min for each side of the tape. When a set of stubs was produced, a test was performed to check for complete decontamination. All stubs were used within 3 months after production date.

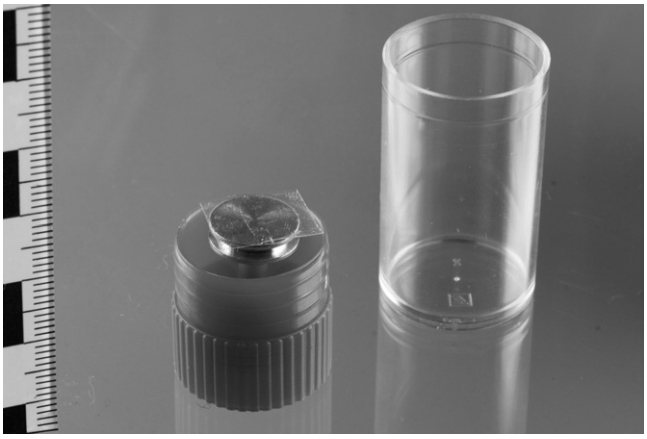

Fig. 1. Photograph of the modified SEM stub. It consists of a plastic container and holder, which contains an aluminum short pin stub holder to which the tape was attached. The holder is 2.5 cm high and ensures distance between investigator and trace.

2.3. DNA extraction, DNA amplification and STR profiling

DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Benelux B.V., Venlo, Netherlands) according to the manufacturer's instructions with a final elution volume of 100 μl. Since all samples contained minimal amounts of DNA, a standard maximum input of 5 μl DNA extract was used for amplification of the samples, using the AmpFℓSTR® Identifiler® PCR Amplification Kit (Applied Biosystems (AB), Nieuwerkerk ad IJssel, Netherlands) in a GeneAmp® 9700 PCR System (AB). In this way, 15 short tandem repeat (STR) loci and the amelogenin locus were simultaneously amplified.

DNA fragments were separated and detected by capillary electrophoresis on the 3130xl ABI PRISM® Genetic Analyzer™ (AB) according to the manufacturer's instructions.

2.4. Profile analysis and interpretation

DNA profiles were analyzed using Genemapper®ID v3.2.1 software (AB). The stubbing and double swab method were compared regarding the number of detected alleles and the average peak heights. The interpretation of the results was done according to the manufacturer's instructions. An exception was made for the results obtained using average peak heights. In this case, the detection threshold was reduced to 20 relative fluorescence units (RFU) instead of the 50 RFU instructed by the manufacturer. The threshold was decreased in order to obtain a satisfactory number of detected alleles to make reliable calculations.

Whenever both donors shared the same allele on one locus this was counted as an 'overlapping allele' resulting in a 'detected' for both donors. In case of a homozygous allele for one of the contributors, the allele was counted as two alleles.

The results shown in this study are based on the 15 short tandem repeats of the AmpFℓSTR® Identifiler® PCR Amplification Kit, the amelogenin locus was excluded.

3. Results

The optimal method for securing offender epithelial cells from the skin of a victim should satisfy the following conditions. (1) It should secure as much offender DNA as possible. (2) The ratio offender DNA:victim DNA should be as high as possible to minimize the disturbing influence of the victim's DNA. In case of manual strangulation, securing epithelial cells of the victim can hardly be avoided. Consequently, peaks corresponding to the victim's DNA will also be visible in a DNA profile. In addition, contamination of third party DNA may be present on the arms of the victim or the hands of the offender. A high offender DNA:victim DNA ratio decreases the complexity of interpretation of the DNA profile.

To establish which method secures most offender DNA, the number of detected alleles of the offender and the victim in the DNA profile were determined. Evidently, the optimal method should result in full offender DNA profiles. The allele distribution of the obtained DNA profiles is presented in Fig. 2A. It shows for what fraction of the 50 offenders no profile, a partial profile of 1–9, 10–19 or 20–29 alleles or a full DNA profile (30 alleles) was obtained. Both methods show roughly the same distribution. Small differences were observed in the number of full profiles (for stubbing 8 full DNA profiles were obtained versus for the double swab method only 5). Overall, for stubbing, 49 out of 50 DNA