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Short Communication

Touch DNA sampling with SceneSafe Fast™ minitapes

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ABSTRACT

To achieve optimal results in the forensic analysis of trace DNA, choosing the right collection technique is crucial. Three common approaches are currently well-established for DNA retrieval from items of clothing, notably cutting, swabbing and tape-lifting. The latter two are non-destructive and therefore preferable on items of value. Even though the most recently established technique of DNA retrieval by adhesive tapes is widely used since quite some years now, little information has been published so far on how well it performs compared to other methods. Even more important, when it comes to choosing the right DNA extraction method for forensic lifting-tapes, the available information one can rely on as a forensic geneticist is quite scarce.

In our study we compared the two widely used, commercially available and automation suitable magnetic bead-based extraction methods "iPrep Forensic Kit" and "PrepFiler *Express* BTA™ Kit" to conventional organic solvent extraction. The results demonstrate that DNA extraction from standardized saliva samples applied to SceneSafe Fast™ minitapes is most efficient with phenol–chloroform. We also provide evidence that SceneSafe Fast™ minitapes perform better than wet cotton swabs in the sampling of touch DNA from cotton fabric. Applying the tape only once in every spot on the tissue is thereby sufficient for a considerably better collection performance of the tapes compared to swabbing.

1. Introduction

Tape-lifting has become more and more popular in recent years for the collection of minute amounts of touch DNA, especially from items of clothing. A couple of published studies already describe the sampling potential of tape-lifting in the forensic context [1–6]. However, to our knowledge, only two recent studies provide systematic comparisons of different direct extraction methods for sticky tapes [7,8]. Given the more complicated chemical nature and the less convenient size of adhesive minitapes compared to swab heads, extraction protocols for minitapes need to be thoroughly checked for efficiency by validation studies. Even though most labs that are processing forensic lifting tapes probably undertook some internal validation to optimize their extraction procedures, these studies are not publically available. To be able to assess the full potential of tape-lifting we need to make sure that we use the most efficient extraction protocol. This step is crucial to avoid an underestimation of the sampling potential of tapes compared to the well-established sampling method of swabbing.

Using adhesive tapes for DNA collection and subsequently swabbing off the collected material from them is tedious and may result in a loss of DNA due to incomplete retrieval from the tapes. Therefore, a direct extraction from the collection tapes is favorable. In the present study we compare three different DNA extraction protocols – two magnetic bead-based methods and a phenol-chloroform protocol – routinely used for case work in our accredited forensic laboratory. As a sampling tape we chose the SceneSafe Fast™ minitape. Due to its easy handling it has gained in popularity among several Swiss police corps in recent years and it has been already characterized in some other studies [1,2,4,6–8]. After selection of the most suitable of the tested extraction methods, we compared the touch DNA sampling performance of SceneSafe Fast™ minitapes to the one of cotton swabs. We thereby focused on a tightly woven cotton fabric as an exemplary substrate for touch DNA sampling, because most common clothing items such as t-shirts, sweatshirts or jeans as well as most bedsheets are made of tightly woven cotton fibers.

2. Material and methods

2.1. Extraction protocols

SceneSafe Fast™ minitapes (SceneSafe, UK) are certified DNA-free. They consist of a small handle and a 19 × 25 mm adhesive zone. For all extractions, the adhesive portions of the tapes were cut in 6–8 pieces with sterile disposable scalpels. Swabs for the comparison in sampling efficiency were extracted with the AutoMateExpress™ device and the

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PrepFiler *Express*™ Kit (both Thermo Fisher, US), representing our standard procedure for touch DNA swabs. Efficiency of the swab extraction method has been checked for standardized amounts of blood, saliva and semen by previous lab internal validation studies and showed to be comparable to extraction with phenol-chloroform [9].

2.1.1. Phenol-chloroform extraction

Tapes were incubated in 500 µl extraction buffer (0.01M Tris, 0.01M EDTA, 0.1 M NaCl, 0.039 M DTT, 2% SDS; all Merck, Germany) with 10 µl Proteinase K (20 mg/ml, Merck, Germany) for at least two hours at 56 °C. After this first incubation step, 5 µl of fresh Proteinase K (20mg/ml) were added, followed by a second incubation step for at least two hours at 56 °C. The pieces of adhesive tape were transferred to a spin basket (Thermo Fisher, US) to collect the liquid from the fragments by centrifugation. 800 µl of Phenol:Chloroform:Isoamyl Alcohol 25:24:1 (Sigma-Aldrich, US) were added to the buffer for extraction. The aqueous phase was then cleaned in Vivacon® 2 ETO columns (Vivaproducts, Inc., US) by centrifugation at 2000*g*, adding 1 × 1 ml and 2 × 2 ml of distilled water to the sample. We also employed this protocol for the 6 swabs with touch DNA that were used to compare the extraction efficiency of organic solvent to the one of PrepFiler *Express*™.

2.1.2. iPrep forensic kit

1ml ChargeSwitch® Lysis Buffer L13 (Thermo Fisher, US), 10 µl Proteinase K (20 mg/ml) and 50 µl 1 M DTT were added to the tape fragments. They were shook on a Precellys®24 homogenizer (Bertin instruments, France) 2 × 30 s at 5900 rpm followed by incubation at 56 °C for at least two hours. After this first incubation step, 10 µl of fresh Proteinase K (20 mg/ml) were added and the sample was again incubated for at least two hours at 56 °C. The pieces of tape were then transferred to a spin basket to collect the liquid from them by centrifugation. DNA was extracted from the lysate with the iPrep™ Purification Instrument (Thermo Fisher, US) using the iPrep™ Forensic Card and the iPrep™ ChargeSwitch® Forensic Kit with elution in 75 µl TE.

2.1.3. PrepFiler Express BTA™ Kit

Tapes were incubated in PrepFiler LySep™ columns (Thermo Fisher, US) on a thermoshaker in 220 µl PrepFiler *Express* BTA™ lysis buffer (Thermo Fisher, US) supplemented with 3 µl 1M DTT and 7 µl Proteinase K (20 mg/ml) for 40 min at 56 °C and 750 rpm. After centrifugation, DNA from the lysate was extracted on the AutoMate Express™ Forensic DNA Extraction System (Thermo Fisher, US) using the PrepFiler *Express*™ and PrepFiler *Express* BTA™ protocol card.

2.2. Comparison of extraction methods

Saliva of a male collaborator was mixed 1:1 with 154 mM NaCl. 30 µl of this solution were applied on a minitape and dried overnight at room temperature. Tapes were cut into pieces for extraction and processed as described above. DNA quantification was done by Real-Time-PCR (qPCR) using the Quantifiler® HP Kit from Life Technologies on a 7500 RT PCR System (Thermo Fisher, US).

2.3. Touch DNA sampling

A cotton bedsheet was cut into 10 × 15 cm pieces. The pieces were washed separately in a conventional household washing machine and air dried. For the controlled application of trace DNA, a male donor first rubbed his hands against each other to more evenly distribute potential loose cellular material. He then held two corners of a 10 × 15 cm tissue with two fingers of one hand and moved the other hand once over the tissue applying medium pressure (Fig. 1). At every time point both hands were sampled on two separate pieces of bedsheet and the sampling method – swab or tape – was alternated every time for the left and the right hand. Between two samplings, the donor carried out some office work for about 30 min. Both hands were sampled 6 times at 3 different days. So, every day the left hand and the right hand were sampled three times each by either SceneSafe Fast™ minitape or Prionics cotton swab (cardboard evidence collection kit; Thermo Fisher, US) respectively. The tape was applied once in every position on the piece of tissue, resulting in a total number of 30 contacts to cover the entire 10 × 15 cm piece of fabric with a 1.9 × 2.5 cm tape. Swabbing was performed with one swab moistened with ultrapure water, as routinely done in the lab. On every sampling day, two untouched pieces of fabric were sampled as negative controls, one by tape and one by swab. To check for background contamination, DNA profiles were established by multiplex-PCR using the AmpFlSTR® NGM Select™ Kit (Thermo Fisher, US). For amplification of samples with a DNA concentration lower than 50 pg/µl, we used the maximum volume of 10 µl, and for higher concentrated samples, we used 0.5 ng of DNA per reaction.

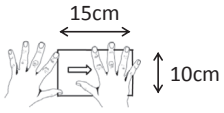

Fig. 1. Schematic illustration of touch DNA sample preparation. While two fingers of one hand hold the tissue sample, the other hand is slid over it.

3. Results

3.1. Comparison of extraction methods

To compare the DNA extraction efficiency of different methods, it is crucial to start with the same amount of cells in every sample. Since standardization of touch DNA amounts is virtually impossible, we chose to perform the comparison of extraction methods with samples of diluted saliva, directly applied on the tapes. The major DNA bearing components in this cell suspension should be buccal epithelial cells and white blood cells. Tapes were all prepared in parallel with the saliva solution, cut in pieces and then extracted with 3 different methods. The results are shown in Fig. 2. The phenol-chloroform protocol appears to be twice as efficient for extraction as the two bead-based methods. Numeric mean values for the series of four experiments are 71.6 ng ± 3.7 ng (standard deviation) for PrepFiler extraction, 74.8ng ± 5.9ng for iPrep extraction and 146.6 ng ± 17.1 ng for phenol-chloroform, respectively.

3.2. Swab sampling vs minitape sampling

Given the clear results from the comparison of extraction methods, all SceneSafe Fast™ minitapes were extracted with phenol-chloroform for this part of the study.

Fig. 3 shows the summarized results from the sampling comparison. The boxplot (Fig. 3a) demonstrates the range of collected DNA amounts for the 18 sample pairs. We collected between 0.28 ng and 3.33 ng of

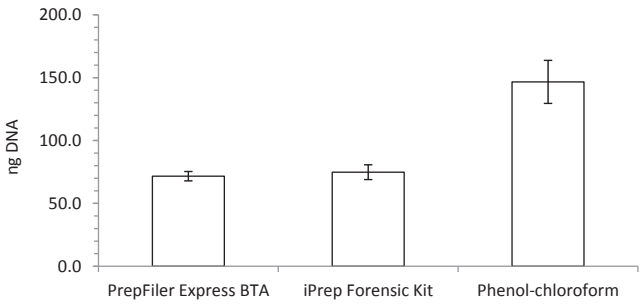

Fig. 2. Comparison of the DNA extraction efficiency of standardized dried saliva samples from SceneSafe™ Fast minitapes. The scale indicates the total amount of DNA recovered. Error bars indicate standard deviations (n = 4).