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Fast and simple DNA extraction from saliva or sperm cells obtained from the skin or isolated from swabs

N. Wurmb-Schwark *, V. Mályusz, H. Fremdt, C. Koch, T. Schwark, M. Oehmichen, E. Simeoni

Institute of Legal Medicine, Christian-Albrechts-University of Kiel, 24105 Kiel, Germany

Abstract. The forensic scientist often has to cope with problematic samples from the crime scene due to their size and thus the amount of extractable DNA. The retrieval of DNA from swabs taken from the surface of the skin, for example in cases of strangulation, can be especially difficult. We systematically investigated swabs taken from the skin (to obtain a genetic profile from the victim and also from a possible offender) and from sperm cells containing swabs using two extraction kits: the Invisorb Forensic and the Spin Swab kit (both Invitek, Germany). DNA quality and quantity were tested on ethidium bromide containing agarose gels and in a highly sensitive Duplex-PCR which amplifies fragments specific for mitochondrial and nuclear DNA. Absolute quantification was done using real time PCR. Samples which were positive in the Duplex-PCR were also employed to genetic fingerprinting using the Powerplex ES and the AmpF*l*STRIdentifiler™ kit. Our study shows that the easy-to-use Spin Swab kit is very suitable for DNA isolation from swabs taken from the skin and also from sperm cells. Retrieval of cells from the skin with swabs modified in extraction buffer, not in distilled water, led to a significant higher DNA yield. © 2005 Published by Elsevier B.V.

Keywords: DNA extraction; Swab; Saliva; Genetic fingerprinting

1. Introduction

Extraction of DNA is a crucial step in forensic genetic analysis. The DNA has to be of sufficient quality concerning the fragment length, the overall amount and the presence of inhibiting substances. For a forensic routine application the handling and the time necessary for extraction are also important. There are many different DNA extraction kits for different tissues commercially available which usually lead to acceptable results. However, sometimes DNA extraction is still challenging, for instance when dealing with

* Corresponding author. Tel.: +49 431 597 3633; fax: +49 431 597 3612.
E-mail address: nvonwurmb@rechtsmedizin.uni-kiel.de (N. Wurmb-Schwark).

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abrasions from the skin to detect DNA from a possible perpetrator, e.g. in cases of strangulation or sexual assault. The aim was to find an easy to use extraction method that allows DNA typing from the skin (e.g. bite marks) using cotton swabs.

2. Material and methods

2.1. Samples and DNA isolation

Blood from lab people was used for positive controls, DNA was extracted using the Nucleo Spin Blood Quick pure kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. Sperm cells were taken from the lab's positive control (sperm on cotton cloth). The traces were employed to DNA extraction using the Invisorb Forensic and the PSP Spin Swab kits (both Invitek, Germany) with slight modifications.

2.2. Creation of artificial traces

Artificial traces were produced using 20 μl saliva from a male donor that was applied to female lab worker's skin (5 cm^2). After drying the traces were removed with swabs moistened in water or in extraction buffer specific for the particular kit.

Sperm cells (in NaCl) and saliva (20 μl) were applied on swabs (Copan innovation, Sarstedt, Germany) and dried overnight.

2.3. DNA analysis and detection

DNA was quantified using real time PCR according to [1]. To detect slightest amounts of DNA, a Duplex-PCR was performed which is sensitive down to 10 pg [2]. 1 μl of each sample was employed to multiplex PCRs using the AmpF*l*STRIdentifiler™ (Applied Biosystems, Weiterstadt, Germany) and the Powerplex ES kits (Promega, Mannheim, Germany). DNA fragments were analyzed on an ABIPrism310, using the corresponding 310 Gene Scan 3.1.2 software. Peaks below 50 relative fluorescent units (rfu) were not analyzed in this study.

3. Results and discussion

3.1. DNA extraction from saliva and sperm on swabs

In comparison with the Invisorb Forensic kit, the PSP Spin Swab kit led to a significantly higher DNA yield (average 3.5 ng/μl saliva). Incubation overnight led to an even higher DNA yield that exceeded that mentioned by the manufacturer. DNA from sperm cells was easily extractable using the Spin Swab kit; incubation overnight also increased the DNA yield considerably.

DNA was fully typable after the recommended extraction procedure (30 min incubation) as well as incubation over night, showing that reliable STR typing from different sources is possible even in a very short processing time. This can be especially valuable in criminal case work to exclude or include a suspect.

3.2. DNA yield and STR typing from skin abrasions

The recovery of DNA from saliva using swabs moistened with extraction buffer greatly improved the DNA yield compared to the common moistening with distilled water even when the double swab technique is used [3].

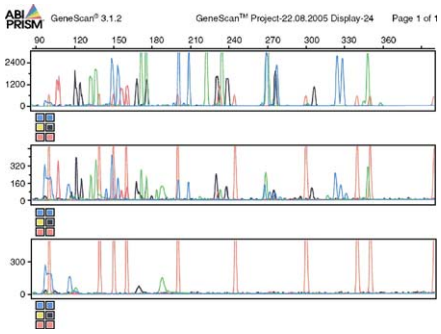

Fig. 1. STR-typing using the Amp/FSTR Identifiler kit. 1 μl of supernatant after Spin Swab extraction was employed to the multiplex PCR according to the manufacturer's instructions. The first panel shows the female positive control from the "victim", the second panel presents STR typing after wiping 5 × 5 cm of the skin with a swab moistened in extraction buffer. The third panel shows the negative PCR control.

About 2 ng DNA could be retrieved from the skin using bidest and 8 ng when swabs were moistened in extraction buffer. The STR profile of the "victim" was almost fully detectable with both kits after the use of the extraction buffer (Fig. 1), while bidest led to lower reproducibility and a smaller number of detectable STR loci.

3.3. DNA retrieval from saliva on skin

Using the Spin Swab kit, sufficient amounts of DNA could be retrieved from the skin according to real time PCR results. Again, using extraction buffer led to much better results in DNA quantity and thus higher retrieval than described elsewhere [4]. The male profile could be obtained using the Identifiler and Powerplex kits without disturbing alleles from the victim. Those were totally inhibited.

Taken together, the modified Spin Swab extraction seems to be an easy to use method for DNA retrieval from the skin of possible victim or perpetrators. The use of cotton swabs moistened in extraction buffer greatly improves the DNA yield and is useful in cases where only little DNA is expected. Saliva stains, e.g. from bite marks, sperm traces or epithelial cells after strong person to person contact can be reproducibly analysed and typed for STR comparisons.

References

[1] N. von Wurmb-Schwark, et al., Anthropol. Anz. 63 (1) (2005) 1–12.
[2] N. von Wurmb-Schwark, et al., Leg. Med. (Tokyo) 6/2 (2004) 80–88.
[3] D. Sweet, et al., J. Forensic Sci. 42 (1997) 320–322.
[4] D. Sweet, et al., J. Forensic Sci. 42 (1997) 447–451.