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SHORT COMMUNICATION

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# Potential DNA mixtures introduced through kissing

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**Abstract** The use of saliva samples is an alternative to blood samples when a large number of control samples are to be compared by DNA investigations. The most convenient and safe method is by using cotton wool swabs. In this investigation the average DNA content of saliva samples taken by three different sampling techniques (i. e. cotton wool swab, filter paper, liquid saliva) was compared. In addition the possibility of a DNA mixture of saliva samples after intensive kissing was investigated by taking samples from voluntary pairs. Mixed STR patterns were found in five samples but restricted to the first sampling after kissing within max. 60 s.

**Key words** Saliva samples · DNA mixture · Kissing · STR · mtDNA

## Introduction

In major crime cases it is sometimes necessary to compare the DNA typing from hundreds of control samples in order to identify a crucial sample. Since it is easier to collect saliva samples than blood samples (i. e. no phlebotomy and taking can be supervised by non-medical personnel) this has become the method of choice especially in such cases. However, the possibility to introduce a mixture of saliva samples is theoretically much higher than with blood samples because blood samples are taken from a closed system. Additionally, it is known that spermatozoa can be detected in swabs taken from the oral cavity up to 8 h after a sexual assault (Willott and Crosse 1986). This paper presents preliminary results of experiments to detect saliva mixtures after kissing using STR systems and mtDNA. The samples were taken by the methods generally in use in Germany to obtain a comparison with practical case work.

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## Materials and methods

Saliva samples from ten volunteers were taken by three different methods: by rubbing the buccal mucosa with a cotton swab (sterile cotton swab; Engelbrecht Medizin- und Labortechnik GmbH, Germany), by pressing a filter paper (S&S filter paper circles; Schleicher & Schell, Germany) against the mucosa and by spitting into a plastic test tube. The average DNA contents of the cotton wool swab, a 0.25 $cm^2$ piece of the filter paper and 50 µl liquid saliva were compared. DNA was extracted by the Chelex method (Walsh et al. 1991) resulting in a final volume of 200 µl and the DNA content was estimated by the slot-blot technique (Waye et al. 1989).

A total of five male and female pairs participated in the experiments and saliva samples were initially taken from every person for comparative purposes using a cotton wool swab. Each pair then kissed intensively for at least 2 min to simulate a maximum mouth to mouth carry-over of saliva. Saliva samples were taken from both partners as soon as possible after kissing (max 60 s) and at 5, 10, 30, and 60 min intervals during which they abstained from eating and drinking. The samples were taken with a cotton swab without touching the mucosa but only taking liquid saliva from the oral cavity. DNA typing was carried out for the three autosomal STR-systems ACTBP2 (Warne et al. 1991), FGA (Mills et al. 1992) and VWA (Kimpton et al. 1992) using 5 µl extracts (DNA amount varied between 0.1 and 9 ng) and methods described previously (Möller et al. 1994, 1995; Rolf et al. 1997). The saliva samples from the female partner were additionally investigated for the male specific Y-chromosomal marker DYS 390 (Kayser et al. 1997) and for two pairs mitochondrial DNA was amplified and sequenced in the HV1 region (Pfeiffer et al. submitted).

## Results and discussion

The DNA content of the samples differed widely not only between the techniques, but also between the individuals (Figs. 1, 2).

The liquid saliva samples showed the highest DNA content with a maximum of 1200 ng DNA from 50 µl saliva. About 30 µl saliva had been taken off with the cotton wool swabs (estimated by the weight difference before and after sampling). Even if the values are extrapolated to 50 µl saliva the DNA content of the cotton wool swabs remained lower than that of liquid saliva. Therefore it seems that the number of epithelial cells rubbed off with the cotton wool swabs was not substantial.

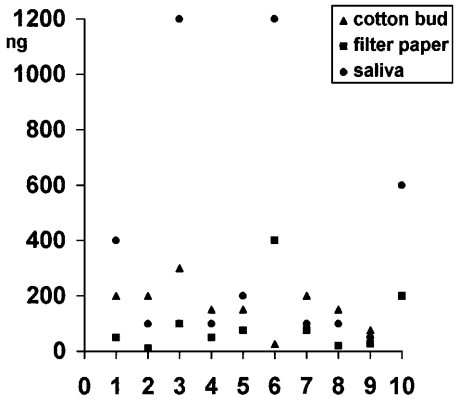


**Fig. 1** DNA-content in ng from different test persons

| Technique | Averqage DNA-content | min. – max. (ng/200 µl) |
|---|---|---|
| Cotton swab (complete) | 165 ng | 25– 300 |
| Filter paper (0.25 $cm^2$) | 100 ng | 9– 400 |
| Saliva (50 µl) | 405 ng | 50–1200 |

**Fig. 2** DNA-content of saliva samples collected with different techniques

Mixed DNA patterns were detected in five samples (three from females, two from males) taken max. 60 s. after kissing but all other samples showed only the DNA alleles from the respective donor. Y-chromosomal markers in female samples were found only in combination with a mixed pattern in the autosomal STR-systems. The fact that contaminating alleles were detected in more systems in the samples from females might indicate that more saliva was transferred by the male to the female (three systems in every sample by a female; one system in both samples by the male persons).

In one sample where no indication of a mixture could be detected by the STR systems, mtDNA sequencing also showed no signs of a mixture. Furthermore in two other samples where a mixture of nuclear DNA patterns was detected, no indications of a mixture were found in the mtDNA sequences. This phenomenon could be explained by a very low ratio between the two different components in the mixture. However, in one sample where a mixture was detected only in the FGA system, a very clear mixture (approx. 1 : 1) of mtDNA was detected at the appropriate sites in the sequence.

This experiment shows that a contamination of saliva samples by intensive kissing is possible but restricted to a very short time period immediately following a kiss. In none of the cases could a complete reversal of the DNA pattern be found. When present, mixtures were always obvious so that a contamination could easily be recognised in case work. Therefore, if a mixed DNA pattern is detected in a known saliva sample that was taken for comparative purposes the results must be regarded as inconclusive and a new sample should be taken under controlled conditions. If the act of kissing is considered in isolation as a source of erroneous results, there does not seem to be a serious risk that a DNA pattern will be falsely interpreted. There seems to be no correlation between the concentration of nuclear and mitochondrial DNA in mixtures of saliva samples. The interpretation of mixtures using mtDNA was not possible when the differences between the concentrations of the component DNAs were too great. Therefore in forensic casework STR typing should be the preferred method for the differentiation of components of a mixed sample. The mtDNA sequence analysis causes problems with the interpretation of peak heights in a sequence pattern specially in cases with low ratios of the mixture components.

## References

Kayser M, Caglià A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herrmann S, Herzog B, Hidding M, Honda K, Jobling M, Krawczak M, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Weichhold G, de Knijff P, Roewer L (1997) Evaluation of Y-chromosomal STRs: a multicenter study. Int J Legal Med 110:125–133

Kimpton C, Walton A, Gill P (1992) A further tetranucleotide repeat polymorphism in the vWF gene. Hum Mol Genet 1:287

Mills KA, Even D, Murray JC (1992) Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). Hum Mol Genet 1:779

Möller A, Wiegand P, Grüschow CP, Seuchter SA, Baur MP, Brinkmann B (1994) Population data and forensic efficiency values for the STR systems HumVWA, HumMBP and HumFABP. Int J Legal Med 106:183–189

Möller A, Schürenkamp M, Brinkmann B (1995) Evaluation of an ACTBP2 ladder composed of 26 sequenced alleles. Int J Legal Med 108:75–78

Pfeiffer H, Steighner R, Fisher R, Yoon C-L, Mörnstad H (1998) Mitochondrial DNA extraction and typing from isolated dentin – experimental evaluation in a Korean population. Int J Legal Med (in press)

Rolf B, Waterkamp K, Hühne J (1997) Allele frequency data for the FGA locus in eight populations. Int J Legal Med (in press)

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 4:506–513

Warne D, Watkins C, Bodfish P, Nyberg K, Spurr NK (1991) Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene 2 (ACTBP2) detected using the polymerase chain reaction. Nucleic Acids Res 19:6980

Waye JS, Presley LA, Budowle B, Shutler GS, Fourney RM (1989) A simple and sensitive method for quantifying human genomic DNA in forensic specimen extracts. Biotechniques 7:852–855

Willott GM, Crosse MA (1986) The detection of spermatozoa in the mouth. J Forensic Sci Soc 26:125–128