Investigations on vaginal cell/sperm mixtures from microscopical slides

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Introduction

In rape cases the situation sometimes occurs that only a very small number of spermatozoa can be found on the microscopical slide. In some former cases previous analysis was therefore not successful and only the microscopical slides are now available for future analysis.
The availability of the highly sensitive PCR-STR systems has improved the chance for typing such stains.
The aim of this study was to investigate which methods are useful for PCR analysis of microscopical preparations. Three highly sensitive STR systems were selected: HumTH01 (TH01-Edwards et al. 1992), HumVWA (VWA - Kimpton et al. 1992), HumACTBP2 (SE33 - Polymeropoulos et al. 1992).

Previous analyses with dilutions of the cell line K562 gave reliable results with 1 ng template DNA using 30 amplification cycles. Using more cycles the sensitivity could be enhanced to 50 pg template DNA.

Materials and methods

<u>I. Extraction procedure for microscopical slides:</u>

* Removal of the cover glass with xylol followed by 2 ethanol washing steps
* proteinase K lysis performed directly on the slide for 20 minutes at room temperature and then pipetted into a reaction tube. Extraction was continued using differential lysis.
* the whole aliquot of DNA was used for PCR analysis.

<u>II. Micromanipulation and spermatozoa extraction:</u>

The micromanipulation was carried out using a microcapillary with a tip diameter of 10 µm. Before starting the procedure the capillary tube was filled with sterilized aqua bidest to avoid passive uptake into the capillary. Fluid contamination into the tube could be avoided by placing a piece of glass fibre tip in the capillary. At the end of the tube we used a sterile filter tip to avoid contamination with saliva. The preparations were overlayed with a drop of aqua bidest. The fixed sperm were first freed by gently scratching with the capillary tube and than sucked in. Magnification was 320 fold. After blowing the sperms out of the capillary, lysis was carried out in a mixture of proteinase K, PCR buffer and DTT. The total volume was approximately 20 µl.
After inactivation of proteinase K the whole sample was used for direct PCR analysis.

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PCR-protocols, primer sequences and electrophoretical conditions used were according to previous publications (Wiegand et al. 1993a, 1993b) with the exception that the number of cycles was increased. In TH01 a semi-nested PCR was additionally used:
The first amplification was carried out with 30 cycles and the primers published by Edwards et al. 1992. The second amplification with 15 cycles and the primers published by Gill et al. 1992. We called this semi-nested PCR because only the second primer was changed, which annealed 25 bp closer to the repeat sequence.

Results and discussion
In the first part of the study experimental microscopical preparations with defined vaginal-sperm cell numbers were investigated. The detection limit was 150 spermatozoa (Fig. 1).

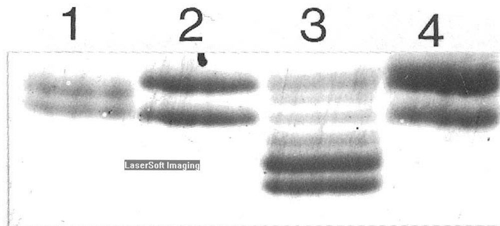

Fig. 1: TH01 amplification (32 cycles) of DNA from a mixture of approx. 150 spermatozoa (lane 2) and 450 vaginal cells (lane 1) extracted from a microscopical silde.
1 = vaginal DNA
2 = sperm DNA
3 = allelic ladder containing 6 alleles
4 = blood DNA pattern which corresponds to the sperm DNA pattern

In the second part of the study micromanipulation was carried out with mixtures containing up to 100 spermatozoa. Amplification with 32 cycles could be successfully carried out using 15 spermatozoa.
To enhance the sensitivity a semi-nested PCR was used. Amplification was successful down to the level of 1 spermatozoa.
The semi-nested PCR enhanced the sensitivity but also led to a higher risk of ladder bands.
Semi-nested PCR was also used to amplify DNA from 25 spermatozoa and 4 spermatozoa from a casework sample. Using 25 spermatozoa the complete heterozygote pattern of the suspect could be obtained, while amplification of 4 spermatozoa led to the detection of only 1 allele (Fig. 2).

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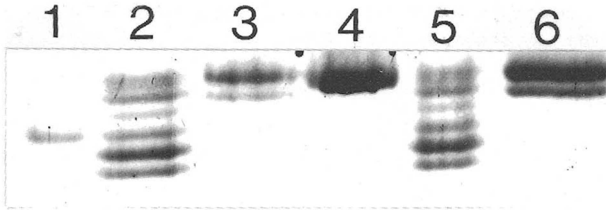

Fig. 2: TH01 amplification (semi-nested PCR) from a casework slide containing a vaginal cell/sperm mixture. Four sperm amplification (lane 4) led to the detection of only 1 allele.
1 = blood DNA pattern of the victim
2,5 = allelic ladder
3 = 25 spermatozoa
4 = 4 spermatozoa
6 = blood DNA pattern of the suspect

The 3 STR systems which were used for sperm DNA investigation showed an improvement in the successful typing using micromanipulation in preparations with very low sperm numbers. THO1 was the most successful STR system and highly sensitive using the semi-nested approach. With VWA a slightly lower sensitivity was found than for THO1 when the cycle number was increased. HumACTBP2 showed also a lower sensitivity and led to the problem of additional bands which may depend on the variable repeat structure of this system. This investigations will now be extended with further STR systems such as HumFES/-FPS and HumF13A1 to test their usefulness for this approach.

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