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DETECTION AND ISOLATION OF MALE CELLS IN OLIGO -AND AZOOSPERMIC SEXUAL ASSAULT CASES USING INTERPHASE X-, Y FISH AND LASER MICRODISSECTION

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Each year more then 450 sexual assault cases are submitted to the Netherlands Forensic Institute (NFI) in the Netherlands for investigation of the presence of sperm on the scene of crime evidence. Although indicative tests have been developed for the detection of sperm, tests are dependent upon ejaculation and the sensitivity of the tests is decreasing rapidly after the assault. In addition, acid phosphatase tests and prostate specific antigen tests might result in false positive or false negative interpretations due to the presence of different components in a stains. Moreover, in a lot of cases, the number of spermatozoa are limited (i.e. the semen is oligospermic or azoöspermic), which makes microscopic identification of sperm cells difficult. In sexual assault cases with sperm cells found in a stain and/or vaginal swab, differential extraction will be performed in order to isolate genomic DNA. However, differential extraction is not very sensitive, a high number of mixed DNA profiles is found and differential lysis is mainly problematic when limited number of spermatozoa are found. To overcome these problems, detection and isolation of male cells by Fluorescent In Situ Hybridization (FISH) and isolation of cells with laser microdissection (LMD) has been tested for its use in forensic casework. By labeling the X and Y chromosome, it might be possible to select other male cells originating from the perpetrator from stains with no or limited number of sperm cells present. During sexual assault different types of male cells might be deposited during penetration, ejaculation or from saliva from the assailant. Different probes had been tested in FISH experiments. After FISH detection cells were captured with LMD using the PALM®MicroBeam C HT, robomover Z (Carl Zeiss B.V.) and DNA profiles were generated. Our experiments show that CEP Y satellite III SpectrumOrangeTM DNA probe solution in combination with CEP X α-satellite SpectrumGreenTM DNA probe solution (Vysis) resulted in clear distinct fluorescent signals in male and female cell mixtures. After detection of male and female cells, laser microdissection was used to isolate single nuclei from Poly-Ethylen-Teraphtalate (PET) membrane slides. Using the AmpFlSTR®SGM Plus™ PCR Amplification Kit (Applied Biosystems) complete DNA profiles were obtained from 20-30 unstained, DAPI stained nuclei and/or FISH treated male cells after 34 cycles of PCR.