SEMEN AND SPERM CELL DETECTION VIA PROXIMITY LIGATION REAL-TIME PCR

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Semen and sperm identification play a critical role in forensic investigation for understanding the circumstances surrounding a crime and determining whether a sexual act occurred or not. Yet current methods for sperm detection vary widely in speed, sensitivity, specificity, and can sometimes lack the ability to confirm that the test results are conclusive.

Presently, laboratories routinely use an alternate light source as an enhancement tool followed by presumptive testing of semen targeting Seminal Acid Phosphatase. The next step can be to test for Prostate Specific Antigen (PSA/p30) with commercially immunochromatography strips. However, the only undisputable confirmatory test for the presence of semen is the microscopic observation of spermatozoa. This process can be extremely time consuming, labor intensive, and failure to identify semen by microscopic examination is not conclusive for its absence.

Here, we demonstrate the development of a confirmatory method employing the Proximity Ligation Real Time PCR (PLiRT-PCR) for the identification of semen and sperm from sexual assault evidence. Three protein targets have been selected for this purpose: sperm-specific protein SP10 (ACRV1); cysteine-rich secretor protein 2 (CRISP-2); and prostate-specific antigen (PSA). SP10 and CRISP-2 are proteins specifically expressed in the male reproductive tract and localized inside the acrosome of spermatozoa. PSA was selected for the forensic identification of semen from azoospermic or vasectomized perpetrators.

Probes are generated by conjugating polyclonal affinity purified antibodies, specific to the target proteins, to DNA oligos ending either in 3' or in 5'. When each probe binds to its specfic target, the DNA strands come into close proximity and bind to a complementary connecting oligo added to the solution. These oligos are then ligated forming a new amplifiable DNA strand that can then be detected by Taqman® real-time PCR. The quantity of the amplified DNA corresponds to the amount of protein target, which is proportional to the amount of semen and/or sperm cells in the sample. Results are determined based on a cycle threshold (C_T) value derived from three times the standard deviation from the 'no protein control' (NPCs) C_T average.

This study discusses the identification, specificity, and the limit of detection of SP-10, Crisp2, and PSA markers in (1) liquid semen, (2) semen elution from cotton swabs, (3) pure body fluids, (4) and mixed body fluid samples. Also, the data show that the PLiRT-PCR assays are able to process multiple mock evidence samples in parallel in a 96-well format, allowing high-throughput analysis. These results demonstrate the potential for PLiRT-PCR as a confirmatory test for semen. This approach is sensitive, quantitative, high throughput, minimizes sample consumption, overcomes the drawbacks associated with the microscopic observation of spermatozoa, and has the ability to get integrated into forensic laboratories as the technology only requires a thermocycler and a real-time PCR system.