B182 Streamlining Sperm Cell Detection Via Proximity Ligation Real-Time Polymerase Chain Reaction (PLiRT-PCR) With Forensic DNA Analysis

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After attending this presentation, attendees will gain valuable insight into the research efforts carried out to develop a PLiRT-PCR assay for the confirmatory detection of spermatozoa from sexual assault evidence without the use of a microscope.

This presentation will impact the forensic science community by demonstrating that PLiRT-PCR is a methodology compatible with current DNA Short Tandem Repeat (STR) analysis and is able to detect sperm-specific proteins by using only 2µL of a forensic sample.

Sperm identification plays a critical role in forensic investigation for understanding the circumstances surrounding a crime and determining whether or not a sexual act occurred, yet current methods for sperm detection vary widely in speed, sensitivity, and 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 using the Seminal Acid Phosphatase assay. The next step can be to test for Prostate Specific Antigen (PSA/p30) with commercial 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 and labor intensive, and failure to identify sperm cells by microscopic examination is not conclusive for their absence.

A potential solution to this issue is PLiRT-PCR, which is designed to detect and quantitate the expression of protein markers through an antibody-protein binding reaction followed by PCR. In fact, the assay combines the specificity of an immunological reaction with the sensitivity of quantitative PCR (qPCR). Sperm-specific protein SP10 (ACRV1) has been selected for this purpose. This target is only expressed in the male reproductive tract, specific to sperm cells, and localized inside the acrosome of the spermatozoa. This location protects SP10 from environmental damage until lysis, and thus allows for successful detection with aged forensic samples.

Probes are generated by conjugating polyclonal affinity purified SP10 antibodies to DNA oligos ending either in 3' or in 5'. When probes bind to their sperm target, the DNA strands attached to the antibodies 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 SP10, which is proportional to the amount of 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 presentation discusses the identification, specificity, and the limit of detection of SP10 in: (1) liquid semen; (2) semen elution from cotton swabs; (3) pure body fluids; and, (4) mixed body fluid samples in a 96-well format. Most importantly, these experiments illustrate how PLiRT-PCR can be used to streamline the workflow of sperm confirmation with the generation of a DNA profile of the perpetrator(s) involved in the sexual assault. The assay utilizes a small fraction of the total reaction. Thus, the remaining can then be used for downstream DNA extraction, quantitation, and STR amplification.

In conclusion, this method is robust, quantitative, and more sensitive than the currently used protein-based detection techniques; samples can be processed in parallel on a 96-well plate for high-throughput analysis with minimal sample consumption. In addition, it can overcome the drawbacks associated with the microscopic observation of spermatozoa and can easily be integrated into forensic laboratories as it only requires a thermocycler and a real-time PCR system.

Spermatozoa, Sexual Assault, Proximity Ligation