B105 Proximity Ligation Real-Time Polymerase Chain Reaction (PLiRT-PCR): A Protein-Based Confirmatory Method for Processing Sexual Assault Kit Samples for Semen and Sperm Cells

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After attending this presentation, attendees will possess valuable insight on the research efforts performed to integrate a potential confirmatory detection method of semen and spermatozoa to sexual assault casework workflow.

This presentation will impact the forensic science community by demonstrating that PLiRT-PCR can be easily integrated into forensic laboratories, as it requires only a thermocycler and a real-time PCR system as well as utilizing a small fraction of the total reaction.

Sexual violence affects millions of Americans. According to the United States Department of Justice's National Crime Victimization Survey (NCVS), each year there is an average of 288,820 victims of rape and sexual assault ages 12 or older. Every two minutes, a victim is being sexually assaulted. Therefore, processing sexual assault evidence is time sensitive. Rapidly confirming semen, and generating the assailant's DNA profile, is crucial for solving crimes and bringing closure to the victims and their families.

A large number of sexual assault kits submitted to forensic laboratories have yet to be analyzed. The current procedures of sexual assault workflow are presumptive serological testing, which include microscopic sperm slide searches, followed by differential extraction methods. This workflow is labor intensive, time consuming, and significantly affects case backlog.

This study has developed an alternative detection method with the potential for replacing microscopic sperm confirmation and enabling the processing of multiple samples at a time on a 96-well plate. In as few as three hours, the assay confirms whether a sample contains semen or sperm cells simultaneously and assists analysts in deciding whether to proceed to differential extraction, therefore helping to reduce backlogged cases.

The PLiRT-PCR assay begins with a small cutting from the evidence (e.g., a fifth of a swab) that is placed in lysis buffer to break open the sperm cells, thus saving the rest of the evidence for the downstream analysis pipeline: differential extraction, quantification, and Short Tandem Repeat (STR) analysis. The lysis step is followed by a binding reaction of specific antibodies against proteins of interest. The antibodies are coupled to short DNA strands, forming so-called pairs of proximity probes. Upon simultaneous detection and proximal binding of the probes to their respective target proteins, the two attached DNA strands are brought in close proximity and are hybridized to a connector oligonucleotide complementary to the oligonucleotide ends of the assay probe pair. Then, the DNA strands undergo an enzymatic ligation reaction, forming a new amplifiable DNA product that can be amplified and measured in a highly quantitative manner by quantitative Polymerase Chain Reaction (qPCR).

This presentation discusses the application of PLiRT-PCR for the identification of semen and sperm proteins from blind samples provided from Bode Cellmark by using only 2µL of the sample. The assay supports both swab samples and other substrates that have different dilution amounts of body fluids spotted on them. Once semen and/or sperm was determined, differential extraction, quantification, and STR typing using Applied Biosystems®

Quantifiler® Trio, GlobalFiler™, and Yfiler® Plus kits were performed. Samples that tested positive with the PLiRT-PCR assays displayed a positive male quantification result with the Y-screen assay, Quantifiler® Trio, further validating the sensitivity of this assay.

This study demonstrates that PLiRT-PCR can be a powerful, robust, and quantitative confirmatory technique the DNA analyst can use to assess whether evidence from sexual assault kits contain semen or sperm cells. Without adding additional resources, the assay utilizes common instruments in the laboratory. This method is more sensitive than the currently used protein-based detection techniques and assists in deciding which sample to process with the differential extraction methods.

PLiRT-PCR, Sexual Assault, Spermatozoa