B71 High-Throughput Spermatozoa Detection Using the Proximity Ligation Real-Time PCR (PLiRT-PCR) Method

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After attending this presentation, attendees will be informed of how the PLiRT-PCR method has been improved for high throughput spermatozoa detection in forensic applications.

This presentation will impact the forensic science community by demonstrating that the PLiRT-PCR method can confirm the presence of spermatozoa from large quantities of samples simultaneously in a streamlined process, demonstrating potential to replace the time-intensive microscopic detection method currently in use.

In the processing of sexual assault evidence, mass media often places emphasis on obtaining a suspect DNA profile; however, equally important but rarely acknowledged is the need for confirmatory identification of spermatozoa, which is often a critical step in determining whether a sexual act occurred or not.

Currently, the only universally accepted method to confirm the presence of spermatozoa is to visualize it microscopically; however, the method has low efficiency as each sample has to be examined individually. Automated fluorescence-based microscopic detection can decrease the time spent per sample, but still processes one sample at a time and requires expensive equipment. Therefore, an alternate method that is capable of processing multiple samples at a time with high accuracy would be a strong candidate for replacing the microscopic method.

The PLiRT-PCR assay combines the specificity of an immunological reaction with the sensitivity of PCR to detect sperm-specific proteins in samples. Specific antibody probes bind simultaneously to target proteins to achieve proximity ligation of conjugated oligonucleotides and the resulting DNA product is detected via real-time PCR. The amount of signal corresponds to the amount of ligated product, which in turn indicates the amount of protein in the sample. The assay only requires a real-time PCR instrument, which is commonly used in forensic laboratories.

The assay has been tested using antibodies targeting two sperm-specific proteins, SP-10 and CRISP-2. Both targets were selected for their localization inside the acrosome of the spermatozoa, which protects them from environmental damage until lysis, increasing the chance of successful detection even with aged samples.

Previously, the assay reported high variability between reactions running aliquots from the same sample. This not only affected the reproducibility of results but also significantly diminished the sensitivity of the assay, as the threshold value for spermatozoa detection is based on three times the standard deviation of Cycle threshold (Ct) values from a set of five No Protein Controls (NPCs). Through protocol and reagent optimization, variability issues have been reduced significantly, with NPCs standard deviations often reaching as low as 0.5 Ct. Testing of both antibodies after optimization places their limit of detection at a 1:100 semen dilution for SP-10 and a 1:1000 semen dilution for CRISP-2, which are conservatively equivalent to 600 and 60 spermatozoa in the assay, respectively. High throughput experiments were also carried out with the Applied Biosystems® 7500 Real-Time PCR System using samples eluted from cotton swabs to simulate actual forensic evidence. In this set-up, three semen swab sample eluates from 2012, 2013, and 2014, as well as a positive control of a 1:100 semen dilution, were run in duplicate along with a set of eight NPCs, in which semen swabs prepared in 2012 and 2014 were successfully detected by the assay.

While these results show promise, several issues have been found. For instance, the probe development process requires further improvement, particularly in the antibody biotinylation step. Also, the new SP-10 antibodies exhibited much less sensitivity than before (reported to be at a 1:5000 semen dilution), whereas CRISP-2 antibodies were found to cross-react with CRISP-3, a protein expressed in salivary glands. As assay development continues, other antibodies that target similar semen-specific proteins will be tested in search of one that provides good balance between sensitivity and specificity. This presentation will discuss the experiments conducted using the optimized PLiRT-PCR protocol, covering the setup, procedure, and results from high throughput runs. The difficulties and potential for further development will also be discussed.

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