A86 Proximity Ligation RT-PCR (PLiRT-PCR) for the Forensic Detection of Spermatozoa

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After attending this presentation, attendees will understand the protein detection method of proximity ligation real-time PCR (PLiRT-PCR) and how this assay could be used for the forensic detection of spermatozoa.

This presentation will impact the forensic science community by offering a potential alternative to microscopic identification of spermatozoa. PLiRT-PCR is an inexpensive, sensitive, fast, and amenable to automation technology that could contribute to the reduction of sexual assault backlog. The reproducible sensitivity of this assay could also promote the extension of the time since intercourse collection interval to support the advancements in DNA typing.

Identification of seminal fluid from sexual assault evidence is standard practice in forensic biology laboratories, though procedures for processing these samples may vary. Generally, for the presumptive indication of semen, practitioners use an alternate light source as an enhancement tool followed by testing for Seminal Acid Phosphatase. If positive, the next step may be to test for Prostate Specific Antigen (PSA/p30) with commercially available immunochromatography kits. PSA has been identified at very low levels in other body fluids, thus a positive PSA result may not be considered by all practitioners to be confirmatory for seminal fluid. The only undisputable confirmatory test for the presence of semen is the microscopic observation of spermatozoa.

However, microscopic observation of spermatozoa can be extremely time-consuming. Automated sperm searcher systems decrease the time spent on a single sample; still, these systems can only process samples one at a time. Furthermore, the cost of fluorescent microscopes and automated sperm searching technology is a large financial commitment for a laboratory.

Sexual assault samples are a large contributor to the backlog that many laboratories face. The time, cost, and limited automation capabilities of microscopic observation limit the reduction in this backlog. Forensic laboratories would greatly benefit from the implementation of a faster, cost effective, and amenable to automation method for the identification of spermatozoa. PLiRT-PCR has the potential to be the technology that meets these needs.

PLiRT-PCR is a molecular assay that enables detection and quantitation of a target protein. Using antibody probes that are specific for the target, a representative DNA molecule that can be detected by real-time PCR (RT-PCR) is generated if the antigen is present. The amount of signal from this surrogate amplicon is indicative of the amount of protein in the sample. It is a powerful and highly sensitive assay that combines the specificity of an immunological reaction with the sensitivity of PCR. If a protein only present on spermatozoa could be successfully targeted with PLiRT-PCR, it could serve as a confirmatory assay for the presence of sperm.

Several additional aspects make the PLiRT-PCR assay compatible with forensic laboratories. First, thermocyclers and RT-PCR machines are commonly found in crime laboratories and thus would not be an added cost. Also, PLiRT-PCR minimizes sample consumption as it is sensitive down to femtomolar ranges. Finally, PLiRT-PCR minimizes the chances of sample switching and has the potential to be fully automated and incorporated into a robotic platform because there is only one tube transfer during the whole procedure, which occurs just prior to the final RT-PCR step.

As a proof of concept study, and to evaluate its potential for forensic use, a PLiRT-PCR assay for PSA was developed. Results showed that the PLiRT-PCR assay was able to detect PSA in a 1:5,000,000 dilution of a one year old semen sample. In contrast, 1:10,000 dilutions of this sample yielded negative results with a commonly used forensic immunochromatographic PSA test. Low levels of PSA were also detected in saliva and blood samples with the PLiRT-PCR assay, though this is expected due to the sensitivity of the assay. This presentation will discuss the proof of concept study and the preliminary results obtained with a PLiRT-PCR assay targeting a potential semen specific protein. Sensitivity, specificity, and time necessary for the assay will be addressed. A molecular method for the detection of semen could far surpass the capabilities of microscopic detection in terms of sensitivity, speed, cost, and automation.

Spermatozoa, Sexual Assault, PLiRT-PCR

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