B95 Application of Laser Microdissection to Expedite Forensic Sexual Assault Casework

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After attending this presentation, attendees will learn that laser microdissection may be an alternative method to traditional sexual assault sample analysis.

Since DNA evidence has proven itself to be a powerful tool in the courtroom and CODIS has shown that recidivism is a serious threat to the public, demands from local communities for time-sensitive testing in forensic DNA laboratories has become commonplace. Therefore, a good forensic DNA section must reexamine traditional testing procedures to make efficiency a primary goal. This presentation will impact the forensic community and/or humanity by giving the audience an alternative tool that has the potential to improve on, and make more efficient, traditional sexual assault sample analysis.

Whenever possible, a good forensic DNA section must reexamine traditional testing procedures to make efficiency a primary goal. In order to reorganize the DNA section's analysis of samples, the North Louisiana Criminalistics Laboratory (NLCL) identified bottleneck areas in the DNA analysis procedure. Because at least 45% of all DNA cases at the NLCL involve sex crimes, one clear hindrance is the differential extraction of sexual assault samples. Examination of sexual assault evidence (i.e., locating, identifying, and the differentially separating sperm from epithelial cells) results in an extraction process that is time consuming and often imperfect. Laser microdissection (LMD) may be used as an alternative to this process to expedite and improve the separation of sperm and epithelial DNA.

The NLCL chose to validate the Leica™ AS LMD Microsystem to help process sexual assault samples. By using the LMD microsystem, the analyst has the potential to concurrently identify, separate, and quantify sperm and epithelial cells in about an hour. As a result, examination to data analysis can be shortened to less than two days. The overall savings in time will result in decreased sexual assault casework turnaround. Also, LMD analysis will free up analysts' time, allowing much needed time for analysis of other casework, QA/QC, research, and especially keeping up with paperwork and maintenance duties that come with finalizing cases. Overall, successful validation of LMD should help fulfill the demand for time-sensitivity needed in DNA casework at the NLCL.

Principal research for DNA typing from sperm cut via LMD has included: (1) physical separation and counting of sperm and epithelial cells from polyethylene (PEN) slides, (2) use of techniques for pre-amplification sperm lysis, (3) investigation of both reduced volume PCR amplification (RVPCR) and PCR with increased cycle numbers to increase sensitivity, (4) excision of sample spot from the PEN slide for STR analysis in the absence of sperm, and (5) troubleshooting of DNA results. The simultaneous identification and quantification of sperm, and their separation from epithelial cells via the LMD microsystem has been successful. Focus has shifted to sperm cell analysis or detection of male DNA post-LMD, all the while maintaining reduced analysis times.

Pre-amplification treatment of sperm collected via LMD with Proteinase K and DTT, coupled with RVPCR, has provided the best DNA typing results. In the absence of sperm, the sample spot has been successfully excised from the PEN slide and analyzed by traditional methods, showing comparable yield. Since increased sensitivity is desired in order to dissect as few cells as possible, low copy number (LCN) interpretation has to be considered in DNA typing results. Additionally, yield has been shown to be affected by static charge produced by the laser. DNA electropherograms recently have exhibited higher than baseline peaks, showing a need to address possible contamination issues that may come into play with the LMD microsystem in LCN samples.

Laser Microdissection (LMD), Differential, Reduced Volume PCR (RVPCR)

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