



B7 Direct Amplification of Sperm Using Laser Microdissection (LMD) and Promega's® PowerPlex® Fusion 6C Amplification Kit

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After attending this presentation, attendees will be able to adapt this method to help streamline Short Tandem Repeat (STR) analysis of sperm from sexual assault casework samples when equipped with a laser microscope.

This presentation will impact the forensic science community by illustrating that sperm can be directly amplified using a Polymerase Chain Reaction (PCR) kit that captures the 20 Combined DNA Index System (CODIS) -core STR loci now accepted for use at National DNA Index System (NDIS).

Sexual assault cases comprise approximately 45% of all DNA casework and account for a significant amount of casework DNA extractions. The major problem encountered with traditional differential extraction is incomplete separation of male and female DNA, especially when epithelial cells greatly exceed the number of sperm, resulting in a mixture. This can produce difficult DNA profile interpretations, statistical calculations, and ultimately, explanations of the results to a jury. Additionally, the traditional differential extraction method is laborious and time consuming. To overcome these problems, the Arizona Department of Public Safety (AZDPS) developed a direct amplification protocol for sperm isolated via LMD.

LMD microscopy replaces the challenges of traditional sexual assault casework methodologies. It provides the ability to simultaneously identify, physically separate, count, and collect cells from a microscope slide for further DNA analysis. Using LMD with a direct amplification expedites DNA analysis of sex assault evidence by offering the following advantages: (1) efficient physical separation of sperm and epithelial cells; (2) greater precision in locating and collecting small amounts of sperm cells in the presence of large epithelial cell populations; (3) elimination of the traditional differential extraction technique; (4) elimination of traditional quantification; (5) reduction in analyst handling and sample manipulation; and, (6) reduction in time spent on analytical interpretations, all while maintaining the ability to obtain full DNA profiles.

LMD with the Leica™ LMD6500 Microsystem integrates microscope and laser functions. In a user-friendly fashion, images are captured by a camera and combined with image analysis software to allow a large sample image to be displayed on a computer screen. This simplifies the ability to locate and dissect cells into separate tubes. Slides are mounted upside down onto the microscope stage for capture of dissected cells via gravity into PCR tubes located in a substage tube holder.

The AZDPS current direct amplification protocol for sperm isolated via LMD was optimized for use with PowerPlex® Fusion 6C STR amplification kit. This evaluation included testing of the previously validated LMD protocol, as well as experimental variations in the pre-amplification lysis solution, amplification buffer, and sperm lysate input volume. Because of LMD implementation, the laboratory workflow was streamlined and the processing time for producing CODIS-eligible DNA profiles for sperm positive sexual assault casework was significantly decreased.

Laser Microdissection (LMD), Direct Amplification, Sperm