## B27 Separation of Spermatozoa and Epithelial Cell Mixtures by Laser Microdissection for Forensic DNA Analysis

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After attending this presentation, attendees will learn a method for the selective separation of sperm and epithelial cell mixtures in sexual assault evidence using Laser Microdissection such that the retrieved cells can be individually typed for Short Tandem Repeat (STR) analysis.

This presentation will offer the forensic community a new method for cell separation in challenging sexual assault casework samples.

The goal of this presentation is to present to the forensic community a method for the selective separation of sperm and epithelial cell mixtures in sexual assault evidence using Laser Microdissection such that the retrieved cells can be individually typed for Short Tandem Repeat (STR) analysis.

PCR and STR analysis has become a valuable tool in identifying the source of biological stains particularly in the investigation of sexual assault crimes. Difficulties in analysis arise primarily in the interpretation of mixed genotypes when cell separation of multiple donors is incomplete or when only a small number of target cells are available in a mixed sample following the application of traditional preferential lysis procedures.

A typical rape evidence swab may comprise of sperm cells from the assailant and vaginal epithelial cells from the victim. The differential extraction has been the most commonly used method to separate sperm cells from epithelial cells in sexual assault evidence. It can preferentially lyse epithelial cells and/or blood leukocytes cells releasing the DNA, which can then be removed from the sperm cell pellet. Although this method can generally provide two cellular fractions, one comprising of sperm cell DNA and the other of epithelial cell DNA, the separation is not always complete, and there may be carryover from one fraction to another making eventual genotype interpretation and further statistical analysis challenging. This challenge is frequently encountered in cases where the ratio of vaginal epithelial cells to sperm cells is large or there are very few numbers of sperm.

Laser Microdissection technology (LMD) has emerged as a method to capture single cells or a group of cells of interest from heterogeneous tissue. This technology is typically employed on histological tissue cryosections to collect specimens for further DNA, RNA or protein analysis. The purpose of this research is to use LMD on biological smears to identify both stained and unstained sperm and epithelial cells while selectively dissecting and recovering the cells of interest for forensic DNA analysis.

Slide smears comprising of sperm and oral epithelial cell mixtures were prepared on glass foiled PEN microscope slides. Smears were processed both stained (Hematoxylin & Eosin or Nuclear Fast Red & Picroindicocarmine) and unstained.

Automated laser microdissection was performed on the Leica AS LMD System. The instrument comprises of a Leica upright microscope with a motorized stage and a single-handed xyz control. Coupled with a camera and computer software, target cells can be visually identified and selected on the computer screen for microdissection. The microscope is integrated with a UV laser of 337µ which performs the laser ablation of the plastic PEN film around the cell(s) of interest. The cut material is then deposited automatically into one of the designated PCR tubes. An inspection mode allows the confirmation and image documentation of the targeted cells in the PCR tube before further molecular analysis.

The Laser Microdissection method presented physically dissects target cells without the contamination of adjacent foreign cells in a mixture then collects the target cells for direct DNA isolation. This bypasses the multi-step process of a preferential lysis procedure, which can reduce yield through loss by liquid transfers and premature lysis of target cells. Unsuccessful analysis of sexual assault evidence due to low numbers of sperm cells or a large epithelial/sperm cell ratio could instead be subjected to LMD to provide effective cell separation and maximal cell yield.

**Laser Microdissection, Forensic DNA Analysis, Sexual Assault Evidence**

 ** Presenting Author*