B107 Evaluation of Track-Etch Filters for Isolating Sperm DNA in Rape Kits

Carll Ladd, PhD, Eric J. Carita, MS, and Elaine M. Pagliaro, JD, Connecticut Forensic Laboratory, 278 Colony Street, Meriden, CT 06451; Alex Garvin, PhD, Bureco Corporation, 18 Gewerbestrass, Allschwil, CH-4123, Switzerland; Timothy M. Palmbach, JD, University of New Haven, 300 Orange Avenue, West Haven, CT 06516; and Henry C. Lee, PhD, Connecticut Forensic Laboratory, 278 Colony Street, Meriden, CT 06451*

The goal of this presentation is to evaluate the efficacy of track-etch filters as an alternative approach to the standard selective lysis protocol for isolating sperm DNA profiles from sexual assault samples

This presentation will impact the forensic community and/or humanity by providing the forensic community with a tool to evaluate the effectiveness of track-etch filter for the processing of rape kit evidence.

The large number of unprocessed sexual assault cases nationwide constitutes an ongoing concern for the forensic community. Many of these cases have sufficient numbers of sperm to generate DNA profiles that could be used to query the CODIS database and identify rape suspects. The standard method for purifying sperm from these swabs is to first resuspend the sample and to selectively digest the epithelial cells with Proteinase K. The intact sperm are then separated from the contaminating solubilized DNA by centrifugation, careful removal of supernatant, and extensive washing of the sperm pellet, all steps that are difficult to automate. The authors have evaluated a vacuum driven filtration method as an alternative approach for separating sperm from digested epithelial cells that is more easily automated in a 96 well format. First, the sample is digested with Proteinase K for 1 hour at 56°C (in standard DNA extraction buffer). Sperm are collected on 2 micron track-etch filters, while the epithelial cell DNA is collected in the filtrate (vacuum pressure = 300 torr). The filters are then washed, and the sperm DNA is solubilized with a reducing agent and collected in the filtrate. The goal of this project is to optimize and validate a faster, more effective, less-labor intensive, and more cost-effective method to isolated sperm DNA from sexual assault samples to address the backlog of unprocessed biological evidence.

Mock body fluid sample mixtures (5,000-100,000 sperm per swab) were processed by the vacuum filtration method and the standard differential extraction procedure to determine their ability to separate the male profile from the female profile using the Profiler Plus and COfiler STR kits from Applied Biosystems, Inc. Various parameters were tested to optimize the filtration method including efforts to overcome the recurring problem of filter clogging. These efforts included introducing centrifugation and filter washing steps, using 10 micron pre-filters to remove cell debris, prewarming washing reagents, using different reducing agents (BME and DTT, various concentrations), adding additional Proteinase K, and slowly adding the sample to the track-etch filter. Membrane clogging can be overcome by centrifuging (3000 G) the sample through the membrane in lieu of vacuum filtration. However, both approaches are less sensitive than the standard differential method and the centrifugation steps are not easy to automate.

The authors have shown that use of track-etch filter can be effective for identifying the DNA profile of the semen donor from mixed body fluid samples. To date, the efficiency of separation using track-etch filter has been variable and the method is not as sensitive as the standard differential procedure.

This project has been funded by the National Institute of Justice, Grant #2003-IJ-CX-K103

Track-Etch Filters, Sperm DNA Isolation, Differential Extraction

 ** Presenting Author*