A70 A Comparison of a Dual Human/Male Quantitative PCR System and Serological Methods for Screening Sexual Assault Samples

Heather B. Shacker, BSc, 725 West Cary Street, Apartment 418, Richmond, VA 23220; and Joanna Jackowski, MSc, Carlee Kantautas, BSc, Alison A. Morris, MSFS, and Jonathan S. Millman, PhD, Centre of Forensic Sciences, 4th Floor Biology Section, 25 Grosvenor Street, Toronto, ON M7A 2G8, CANADA*

After attending this presentation, attendees will appreciate the potential for the use of dual human/male quantitation as a sensitive and discriminatory analysis method for detection of seminal fluid on internal sexual assault samples based on a comparison of its sensitivity to microscopic sperm search methods.

This presentation will impact the forensic science community by providing a comparison of a DNA based and a microscopic screening method for the analysis of internal sexual assault swabs; and by providing insight into how seminal fluid may be identified based on quantitation data using Dual Human/Male Quantitative following differential extraction.

Vaginal swabs from sexual assault kits are typically screened using serological and microscopic methods to identify chemical and cellular components of semen. This research compares these established methods with an alternate screening approach using a DNA quantitation system that detects both male and female DNA in a single reaction. A DNA based detection method for internal sexual assault swabs could allow for detection of small quantities of male DNA within a largely female sample. Seminal fluid from three donors, one vasectomized and two non- vasectomized, was collected and applied to vaginal swabs in varying dilutions. Acid phosphatase, p30 testing, and microscopic sperm searches were performed on extracted whole swabs to determine the sensitivity of these approaches. Identically prepared swabs were concurrently subjected to differential extractions and quantified using a real-time PCR instrument with a dual human/male quantitative PCR system.

Both microscopic sperm searches and quantitation of male DNA using the PCR system reproducibly detected semen/male DNA when as few as 500 sperm cells were applied to vaginal swabs. Where dilutions targeting 50 sperm cells were applied to vaginal swabs, the dual human/male PCR system detected male DNA at measured concentrations of less than 1 pg/mL in sperm fractions in 4 of 6 samples, each with a volume of 25 mL. In these samples no spermatozoa were observed microscopically, demonstrating the enhanced sensitivity of the quantitative PCR system. Where possible, STR analysis demonstrated that the DNA detected was attributable to the semen donor.

No sperm were detected microscopically with an azoospermic sample, however male DNA was detected with the PCR system to a dilution of 1:12 800, well below the sensitivity of the serological chemical screening techniques investigated. As expected there was no fractionation of male DNA into the sperm fraction with any of the azoospermic samples.

In order to determine the parameters under which semen can be differentiated from other male body fluids, mixtures of male blood or saliva with vaginal swabs were also subjected to differential extraction and quantitation with the quantitative PCR system. These studies demonstrated that by evaluating the results of male DNA fractionation and quantitation it is possible in most cases to differentiate these body fluids from semen based on: i) absolute male DNA quantity in the sperm fraction; ii) the enrichment of total male DNA in the sperm fraction; and iii) the enrichment of male vs. autosomal DNA in the sperm fraction.

This research demonstrates that both microscopic sperm searches and DNA quantitation using a dual human/male quantitative PCR system are comparable in terms of sensitivity for screening vaginal swabs for the presence of spermatozoa. The quantitative PCR system is more sensitive than other serological techniques, even when liquid semen is directly applied to vaginal swabs, thereby making this technique better for the detection of azoospermic semen. It is also possible to define parameters based on DNA quantitation results that provide strong support for the presence of semen over other male bodily fluids.

Not only does the use of a dual human/male quantitative PCR system provide a sensitive and robust screening tool for internal sexual assault kit swabs, but it also provides information regarding the quantity of male DNA that can further be used to determine the most appropriate analysis technique (autosomal vs. Y-STR) for that sample. This study demonstrates that that this technique could replace the current serological methods in use, including acid phosphatase, p30 and microscopic sperm search techniques, with consequent improvement in processing efficiency.

Sexual Assault, Dual Human/Male Quantitative, Sperm Search

Copyright 2010 by the AAFS. Unless stated otherwise, noncommercial *photocopying* of editorial published in this periodical is permitted by AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by AAFS. ** Presenting Author*