A4 Standardization of Spermatozoa Identification in Sexual Assault Cases Using a Fluorescence-Based Assay

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After attending this presentation, attendees will have an understanding of how a fluorescence-based staining assay was optimized and validated at the Royal Canadian Mounted Police (RCMP) using a large number of sexual assault type specimens.

This presentation will impact the forensic science community by enhancing the search and standardizing the identification of spermatozoa in sexual assault exhibits.

The detection of spermatozoa on swabs and suspected seminal stains on fabric provide strong confirmatory evidence in sexual offence cases. Currently, the identification of spermatozoa by the RCMP Evidence Recovery Unit Search Technologists is carried out using phase contrast microscopy. In case of specimens with little spermatozoa, the search may become labor-intensive and time-consuming.

The high specificity of the fluorescence-based staining assay selected in this study lies on the unique mouse monoclonal antibody specific to human sperm heads incorporated in the staining protocol. The validation studies performed by initial users of the fluorescence-based staining assay provided strong positive feedback which prompted the RCMP's evaluation of this kit.

The optimization of the fluorescence-based staining assay involved testing different glass slides and coverslips, finding the optimal amount of 1M DTT pH 8.0 to use on vaginal and fecal slides for optimal staining, finding ways to preserve the fluorescence signals on slides, assessing the possibility of re-staining slides, finding optimal sample slide preparation (comparing a swab extract versus rolling a swab versus using a swab clipping), and performing some spermatozoa integrity tests.

The validation studies performed to evaluate the limitations of the fluorescence-based staining assay included: sensitivity, reproducibility, specificity and robustness using non-human semen, other human body fluids and contaminating yeasts, fibers and dyes, spermicides, sexual lubricants, condoms, medicated creams, and 24-year old semen stains. The practicality, sensitivity, and speed of the fluorescence-based protocol was compared to the current phase contrast protocol and examined in the context of an improved workflow in processing sexual assault cases.

Experiments carried out indicate that the fluorescence-based staining assay is: (1) highly specific and valid (no interference from semen obtained from various animals and absence of positive fluorescence signals in all control slides prepared from swabs with only blood, yeast, urine, vaginal epithelial cells, or fecal material); (2) sensitive and reliable (spermatozoa detected in vaginal swabs soaked in 1:1,000 and 1:10,000 semen dilutions; spermatozoa detected more effectively in real casework samples containing few spermatozoa compared to phase contrast microscopy); (3) fast (one minute versus an average of 10 minutes or more using phase contrast microscopy for <10 spermatozoa); (4) robust (spermatozoa detected from 24-year-old suspected semen stains, no interference from spermicides, lubricants, fluorescent and non-fluorescent condoms and antifungal creams); and, (5) simple to use for the detection of spermatozoa in a variety of sexual assault samples.

The swab clippings used to prepare the slides for spermatozoa identification produced very informative DNA profiles. The fluorescence-based staining assay results were merged with duo female: male DNA quantification ratios and PCR amplification kits profiling outcomes. The following general correlations were found between the fluorescence-based staining assay results and STR male profile outcome: (1) vaginal swabs with 1:25 human semen dilution gave an average of 136 spermatozoa count from swab clippings (N=12), an average female:male DNA ratio of 0.24 and a major male profile using PCR amplification kit for which a match probability could be calculated; (2) vaginal swabs with 1:100 human semen dilution gave an average of 45 spermatozoa count, an average female:male DNA ratio of 1.3, a complete Yfiler profile and mixed profiles using PCR amplification kit with minor male components for which an inclusion probability estimate was calculated at 8-9 loci or a major male profile for which a match probability could be calculated; (3) vaginal swabs with 1:1000 human semen dilution gave an average of 4 spermatozoa count, an average female:male DNA ratio of 15.6, a complete Yfiler profile and mixed profiles with minor male components for which an inclusion probability estimate was calculated at 4, 5, or 8 loci. The optimized fluorescence-based staining assay combined with the use of 6mm circle slides could expedite and standardize the search for spermatozoa in specimens containing a limited number of spermatozoa.

Spermatozoa, Sexual Assault Cases, Fluorescence

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