## A156 Comparison of an Automated Image Analysis Software Versus Visual Examination to Search for Fluorescently-Stained Spermatozoa in Sexual Assault Cases

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After attending this presentation, attendees will have a better understanding of one image analysis software optimized and validated to automate the search for human spermatozoa stained using a fluorescently-based assay and how it compared to visual examination to count spermatozoa.

This presentation will impact the forensic science community by providing an alternative to visual counting of spermatozoa. A routine approach can be developed to automate the scoring of human spermatozoa in sexual assault exhibits. This can enhance case throughput, increase assay sensitivity, and standardize the search for spermatozoa.

The image analysis software was purchased in the hope of developing an automated method of counting fluorescently stained spermatozoa. The fluorescence-based staining assay was first optimized and validated as a replacement to the current human spermatozoa detection method based on phase contrast microscopy. In this assay, the mouse monoclonal antibody specific for human sperm heads is linked with Alexa 488, which fluoresces in green using a FITC filter. A second dye will appear blue when nucleated cells are present using a DAPI filter.

The development of appropriate classifiers within the software was challenging but essential to teach the system to specifically recognize human spermatozoa. As part of the optimization, different minimum and maximum integration times (exposure times) were tested in order to reduce the background without missing any human spermatozoa.

Optimized classifiers were tested/validated using a diverse sample of slides prepared from mock sexual assault samples containing a limited or a large number of human spermatozoa (fecal swabs, vaginal swabs, all mixed with different semen dilutions in addition to urine, blood, and yeasts for a subset of those swabs, contaminated with lubricants, spermicides, medicated creams, and non-human semen). Automated spermatozoa counts were compared to visual spermatozoa scoring. The performance of the image analysis software was recorded with respect to missed spermatozoa, false positives (rejected counts) and time required for the detection of human spermatozoa in each sample.

An excellent concordance was noted between automated and manual counts. Some human spermatozoa were missed by the image analysis software due to their location at the periphery of a classify field (area of search) or outside the predefined circle for searching. False positives or rejected counts were caused by high DAPI or FITC background in that area and red dots (positive in FITC but negative in DAPI). Most false positives in the image analysis software were quickly rejected by visualizing the gallery (captured cells) on the computer screen. Ambiguous signals/cells were accepted or rejected by visual examination using both the FITC and DAPI filters. Manual scoring of human spermatozoa and the setting up of the image analysis software took the same amount of time. While the image analysis software carries out the automated scoring of human spermatozoa, other tasks can be performed. A major advantage when counting multiple slides is the elimination of eye strain as reviewing galleries shown on the large computer screen is an easy and quick step.

The results of this study indicate that automated scoring of fluorescently-stained human spermatozoa in mock sexual assault exhibits can be carried out reliably and reproducibly using well-developed classifiers for the image analysis software system. The automated scoring of spermatozoa combining the fluorescence-based staining assay and the image analysis software is currently being tested on a large number of sexual assault cases as part of a pilot project within an operational setting.

**Automated, Spermatozoa, Image Analysis Software**

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