B10 Non-Destructive, Rapid Differentiation of Cell Types Relevant to Sexual Assault Investigations Utilizing Morphological and Autofluorescence Signatures

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Learning Overview: After attending this presentation, attendees will have a better understanding of morphological and/or autofluorescence signatures of cells and their potential for identifying source tissue in sexual assault evidence.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing a novel method of differentiating cell types common to that of sexual assault samples. This can provide more probative information compared to traditional serological analyses and enhance the value of related DNA profiles.

Current methods for confirming the presence of spermatozoa in sexual assault samples can be time-consuming, lack sensitivity, and are not always conclusive; however, this remains the most definitive test for the occurrence of semen. Additionally, male DNA can be deposited without the presence of intact sperm, as may be the case with seminal fluid from vasectomized individuals or sexual activity where seminal fluid is not recovered (e.g., perpetrator wears a condom, digital penetration, penetration without ejaculation, etc.). The ability to detect bodily fluids, including semen, rapidly as well as quantify its presence in a sample could aid in forensic DNA analysis by limiting the amount of time spent performing serological testing as well as screening for probative samples for DNA profiling. Utilizing unique morphological and/or autofluorescence cellular signatures can be a rapid and non-destructive method for cell type differentiation; however, the application in forensic science casework has yet to be implemented. Thus, the goal of this study was to survey these novel signatures in four major cell types associated with sexual assault casework (vaginal, rectal, and penile epidermal cells, and seminal fluid) to develop a method to rapidly analyze these signatures in biological evidence.

For this study, the samples were collected from six males and six females and dried for at least two weeks prior to analysis. Cell populations were analyzed from single-source swabs of vaginal, rectal, and penile cells, as well as swabs of seminal fluid. The samples were extracted from the swabs and analyzed using Imaging Flow Cytometry (IFC) with five different excitation wavelengths and six detector channels ranging between 430nm–780nm, including a brightfield channel. Signatures for each cell type were constructed from ~60 different measurements capturing the size, morphology, and autofluorescence profiles of individual cells (e.g., area, aspect ratio, contrast, intensity, circularity). Finally, linear discriminant analysis was used to develop a quantitative framework for differentiating cell populations and predicting cell type within unknown/blinded samples.

Results showed that semen cell populations could be clearly differentiated from rectal, penile, and vaginal cell populations. Blinded samples of semen that were not included in the original analysis were analyzed and correctly identified as derived from a semen cell population with an accuracy of over 90%. To simulate cases where semen is not expected, this study also compared epithelial cell types (vaginal, rectal, and penile epidermal). Penile epidermal cells were clearly differentiated from rectal and vaginal cells with an accuracy greater than 90%. Interestingly, differentiation was also observed between semen cell populations derived from different donors, providing additional possibilities for how IFC may be applicable in forensic casework. These possibilities include donor differentiation in casework where there are multiple accused individuals or a consensual partner as well as potential for separating cells and developing individual donor DNA profiles from each cell population. The results obtained indicate that each cell type has unique and distinctive cellular signatures that can be detected in a rapid and non-destructive manner. Utilizing these features could aid forensic science casework in identifying the source of DNA in sexual assault evidence and also in predicting DNA yield and/or cellular separation with further research endeavors.

Cell Differentiation, Serology, Sexual Assault Samples

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