B82 Antibody-Mediated Separation of Seminal Male/Female Mixtures From Sexual Assault Samples

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Learning Overview: After attending this presentation, attendees will have a better understanding of an alternative method to traditional differential extractions using an antibody-bound, bead-mediated binding mechanism that can be performed in a microcentrifuge tube, or on an integrated sexual assault microdevice.

Impact on the Forensic Science Community: This presentation will impact on the forensic science community by describing a faster and more efficient way to process sexual assault samples that will also reduce the amount of time spent on mixture interpretation.

While efforts have been made to reduce the pervasive backlog of sexual assault kits, the actual laboratory process is still very time-consuming as it often involves a differential lysis step prior to DNA purification, as well as complex mixture interpretation at the end of the forensic DNA workflow. This research explores the use of an antibody-bound, bead-based capture mechanism as an alternative means of cell separation by targeting a relevant cell type (sperm, vaginal, or prostate cells). By using this mechanism for a fractional DNA extraction, cell types could potentially be separated more efficiently than the traditional differential lysis, and various specific cells can be targeted by changing the capture antibody used. In the current study, candidate sperm cell antibodies were first tested via flow cytometry to determine their binding affinity for the cell of interest. Moving forward, antibodies with the highest binding affinity for the target cell type were tested using a microcentrifuge tube-based, antibody-bound, bead capture mechanism. Downstream of separation, samples were analyzed using a traditional forensic DNA workflow, including DNA isolation, human-specific DNA quantification, multiplex STR amplification, and CE-based separation of resulting amplicons. Although sperm-specific PH20 antibody exhibited a binding affinity of 74.2% for sperm cells when tested via flow cytometry, it only captured 23.5% of the total DNA in semen samples using the bead-mediated method. Additionally, sperm-specific antibody AKAP3 bound only 0.167% of gated sperm cells, while 41.1% of the total DNA was retrieved in the bound fraction using the bead-mediated method. However, when these antibodies were tested on semen-vaginal fluid mixture samples using the antibody-bead mediated assay, STR results from the bound fractions showed that the male contributor was present in ratios that were, on average, 10-fold higher than the female. The ≥10:1 and 9.6:1 male to female ratios in the bound fractions for PH20 and AKAP3, respectively, provided for unambiguous single-source male STR profiles, rendering mixture interpretation unnecessary for these samples. Alternatively, cytokeratin-4 (CK4) antibody was used to target vaginal epithelial cells, binding 76.1% of the total DNA in vaginal epithelial cells using the antibody-bead mediated binding method. In the unbound fraction of semen-vaginal fluid mixtures, the male contributed at least 9-fold more DNA than the female to the resulting STR profiles, again providing single-source male STR profiles. This data provides evidence that the CK4 antibody may be a valid antibody for separating the female fraction of a sexual assault sample away from the male, regardless of the male cell type(s) present. Overall, PH20, AKAP3, and CK4 were able to enrich for and isolate clean single-source male profiles from sexual assault mixtures containing semen and vaginal epithelial cells. Future work on this project will include the exploration of additional antibodies for both sperm and non-sperm containing sexual assault evidence, as well as integration onto a microchip-based format. This approach could provide a faster and easier way to separate contributors that could dramatically reduce the amount of back-end mixture interpretation needed for some sexual assault samples.

Differential Extraction, Sexual Assault, Backlog