A NOVEL DIFFERENTIAL PROTOCOL FOR SEXUAL ASSAULT SAMPLES USING A TARGET-SPECIFIC ANTIBODY-BEAD COMPLEX

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The sexual assault kit backlog continues to be a serious challenge in the forensic science community. While efforts have been made to reduce the backlog, the laboratory processing of these samples has remained largely unchanged. Traditionally, this involves a tedious and time-consuming manual differential cell lysis step, as well as complex mixture interpretation analysis on the back-end of the workflow. Our research has explored the use of an antibody-bead complex that targets relevant cell types (sperm or vaginal epithelial) in order to separate the contributors of a sexual assault sample before the cell lysis step, as an alternative to conventional differential lysis. Initially, flow cytometry was used to screen candidate antibodies. Sperm-specific antibodies targeting SPAG8, CRISP2, MOSPD3, PH-20/SPAM-1, and AKAP3, as well as mucosal epithelial-specific CK4 antibody were chosen for testing of the microcentrifuge tube-based bead capture mechanism. Briefly, this mechanism utilizes streptavidin-coated polystyrene beads bound to biotin-tagged antibodies of interest. Once separated into bound and unbound fractions, DNA was liberated using an enzymatic digest, followed by a traditional forensic DNA workflow, including human-specific DNA quantification, multiplex STR amplification, and CE-based separation of resulting amplicons. The best performing antibodies (in both flow cytometry and/or the capture assay) were PH-20/SPAM-1, AKAP3, and CK4. PH-20 and AKAP3 generated average male:female ratios of ~10:1 in the bound fraction when used to separate semen:vaginal mixtures. In addition, CK4 yielded average male:female ratios of ~9:1 in the unbound fraction of the same mixtures. These results demonstrate the ability of this assay to produce enriched male STR profiles, requiring little-to-no mixture interpretation. This data also provides evidence that the CK4 antibody may be a valid antibody for separating the female (epithelial cell) 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. In the future, this assay could be easily integrated onto a microdevice platform, allowing for an automated method and faster, more efficient processing of sexual assault evidence.