B37 The Utilization of Sex Hormone Antibodies for Screening and Separation of Trace Biological Mixtures

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Learning Overview: After attending this presentation, attendees will understand: (1) how to optimize staining of epithelial skin cells with antibodies; (2) how to assess binding efficiency of antibody probes; and (3) if pairing antibodies to different molecular targets enhances the signal intensity or facilitates more distinct staining patterns for male versus female epithelial cells.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the novel application of fluorescently labeled antibodies targeting testosterone, Dihydrotestosterone (DHT), and estradiol molecules to selectively stain epithelial skin cells in trace biological mixtures. Using these antibodies to screen for male epithelial cells is an innovative concept that will benefit forensic biology units tackling touch/trace mixtures. Screening for the presence of male cells and separating them from female cells could eventually be applied to casework samples in order to simplify complex touch/trace mixtures or eliminate these mixtures altogether prior to forensic DNA analysis.

In recent years with the increased sensitivity of DNA analysis instrumentation and Short Tandem Repeat (STR) typing kits, evidence submitted to forensic laboratories for DNA analysis has shifted such that a large proportion consists of “touch” or trace evidence. Moreover, touch or trace DNA mixtures constitute one of the most difficult types of evidence to successfully profile and interpret. If male cells can be differentiated by antibody staining from female cells, this could be exploited to separate out contributors in a touch or trace mixture and increase the success rate of profile interpretation. Testosterone, the primary male sex hormone responsible for producing male phenotypes, is a potential antibody target due to its abundance in males at approximately ten times that of females in blood serum levels and its long-term stability in biological samples.

In this study, skin epithelial cells were used to model touch or trace mixture evidence, which constitutes half of the casework evidence items submitted for DNA analysis. For the optimization of antibody staining, male and female skin epithelial samples from the same donors were incubated and hybridized with fluorescently labeled anti-testosterone, anti-DHT, and anti-estradiol antibody probes separately, each at varying concentrations. Antibody binding efficiency was assessed by analyzing stained single-source male, female, and control epithelial skin cells through flow cytometry to determine if the staining was specific to either cell population compared to the unstained control. The objective was to maximize probe binding to male cell populations and the selective labeling of male and female cells. Once an optimal staining condition was established, it was tested across several individuals from each sex. If an improved signal was observed, as demonstrated by an increased median fluorescence and separation between male and female samples, then the testing moved forward to Fluorescence Activated Cell Sorting (FACS) analysis.

The results for testosterone labeling showed an increased median fluorescence for male cell populations compared to female. Staining with anti-DHT probes resulted in only a slight shift in median fluorescence from unstained to stained cells and showed no separation in median fluorescence between male and female cell populations. The results for estradiol antibody probes showed an increased median fluorescence separation between male and female cell populations. When testosterone and DHT antibodies were used simultaneously, the median fluorescence increased between unstained and stained cell populations. However, there was not a large separation in median fluorescence between male and female cell populations. Overall, these results suggest that antibody probes targeting testosterone and estradiol may be used to differentially label contributor cell populations in touch or trace mixture samples. This can potentially be used to presumptively screen evidence samples for the presence of either cell population or as the basis for separating cell populations based on fluorescence prior to DNA profiling.

DNA Mixtures, Touch/Trace Evidence, Cell Separation