B177 Can Detection of Testosterone With Anti-Testosterone Antibody Be Used to Identify Male Cells?

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After attending this presentation, attendees will better understand the potential of targeting testosterone to presumptively identify male cells on evidence items and at crime scenes, as well as its potential use for cell separation techniques prior to DNA profiling.

This presentation will impact the forensic science community by introducing a novel screening method for male cells that has the ability to decrease the time spent examining evidence items in the lab and, thus, have a potentially significant impact on casework backlogs.

Currently, research on fluorescent molecules as a screening tool for sex-specific cells has been primarily limited to sperm cell antigens and to Y-chromosome DNA (e.g., Fluorescent *In Situ* Hybridization (FISH)). Given that testosterone is normally present in most non-sperm cell types of males and is at approximately ten times the level of that found in females, it is a promising candidate probe molecule for identifying male cells. Therefore, the goal of this study is to test for the preferential labeling of male versus female cells using fluorescently-tagged anti-testosterone antibodies. The development of a potential screening tool to identify male cells in a biological mixture is the ultimate goal. Initial tests of several fluorescent reporter molecules with common laboratory alternative light sources showed the green CF514 dye (excitation and emission wavelengths, 516nm and 548nm, respectively) was clearly visible up to a 1:10,000 dilution. Based on these findings, an anti-testosterone antibody conjugated to a CF514 dye was investigated along with a Fluorescein Isothiocyanate (FITC) -tagged anti-testosterone antibody (excitation and emission wavelengths, 495nm and 519nm, respectively).

CF514-linked antibody hybridization was performed with buccal and epithelial skin cells of both male and female origin. Male and female buccal cells displayed no clear differences in fluorescence intensity when viewed with a fluorescence microscope after incubation with the anti-testosterone antibody. Epithelial skin cells were then tested because they are a known target tissue of testosterone action. Pressure was applied when retrieving the skin cells to ensure that not just the most outer layer of keratinized cells was collected. An aliquot of the epithelial cells was removed, the DNA extracted and quantitated, and the presence of DNA detected, suggesting the possible presence of deeper epidermal cells.

Due to this finding, antibody hybridization was performed with the epithelial skin cell samples fixed to glass slides. Slides that were dried after the antibody incubation without a coverslip displayed a visible difference in fluorescence between male and female cells and compared to the negative control; however, slides that were hydrated with water after antibody incubation displayed no noticeable difference in fluorescence between sexes. Antibody specificity testing with purified testosterone and estradiol indicated that CF514-linked and FITC-linked anti-testosterone antibodies preferentially bind to testosterone. Therefore, the difference between dry and hydrated cell labeling may be due to other molecular or optical dynamics, which might appear as fluorescence in the hydrated cell samples.

Testosterone may be successful in identifying and resolving male components in forensic casework; however, testing with a more sensitive instrument, such as a confocal laser scanning microscope, may prove to be more informative. Moreover, samples may need to be incubated with a reaction mix that contains multiple antibodies in order to enhance the fluorescent signal and assessed using the Fluorescence Activated Cell Sorter (FACS).

Anti-Testosterone Antibody, Male Cell Screening, Fluorescence Microscopy

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