## B106 Separation of Compromised Blood Mixtures Using Fluorescence-Activated Cell Sorting (FACS) for Single-Source Short Tandem Repeat (STR) Profiling

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After attending this presentation, attendees will better understand how FACS coupled with Human Leukocyte Antigen (HLA) labeling may be used to resolve complex blood mixtures that have been degraded or compromised.

This presentation will impact the forensic science community by introducing a new method for separating contributors prior to DNA extraction, which can improve the efficacy of mixture interpretation in DNA caseworking units.

Previous studies have demonstrated that hybridizing cell mixtures with fluorescently labeled antibody probes and FACS can be an effective technique for separating certain types of cell mixtures. These include sperm and epithelial cell mixtures, where each cell can be easily differentiated based on their biochemical composition and morphological properties. Mixtures containing only one cell type have proven more difficult to resolve using this approach, especially when the mixture has been compromised and/or degraded. Sorting the cells from such samples has proven to be a challenge due to the complex chemical and physical changes associated with cellular decomposition, which lead to non-specific fluorescence and significant sample loss. The objective of this study was to develop new methods for labeling and sorting cells in compromised mixture samples and subsequently test them against samples approximating those encountered in forensic casework.

To accomplish this, a series of two- and three-person whole blood mixtures was created in which the total mixture volume ranged from 100µl-500µl, and each contributor was present in equal volume ratio. Mixtures were dried for 12 hours and then hybridized to antibody probes that targeted the HLA-A*02 allele. While flow cytometry analysis showed that dried samples experienced some cell loss, intact, immunoreactive white blood cells could be recovered from all samples (~300-13,000 cells). More importantly, when comparing flow cytometry results of one contributor's dry blood sample against their fresh whole blood sample, the dried blood sample showed similar binding specificity to the HLA-A*02 probe, such that A*02 positive donor(s) could still be resolved from negative donor(s) in mixture samples. Differences in the fluorescence intensity of HLA positive and negative contributor cell populations were subsequently used to define sorting criteria for isolating each mixture fraction. STR profiling was then performed to determine the sorting efficiency and assess the utility of this technique for forensic casework.

STR results from seven different mixtures showed successful isolation of both HLA-A*02 positive and negative contributors from each mixture. Profiles from the positive contributors were identical to their presorted, single-source profiles across 16 different loci. Allelic contributions from non-target contributor(s) was rarely observed, and, when present, was easily resolved from contributor alleles (10:1 to 50:1 contributor to non-contributor ratio). The sorted cell fraction from the HLA negative contributor showed similar results with STR profiles showing at least a ten-fold enrichment in contributor-specific alleles compared to the presorted mixture profile. Overall, these results suggest that HLA antibodies can be used to differentially label cell populations in a compromised blood mixture and, when coupled to FACS, cells from different contributors can be physically separated from a mixture to generate single-source STR profiles.

**FACS, Human Leukocyte Antigen, Mixture Interpretation**