B55 New Strategies and Recommendations for Front-End Separation of Compromised Biological Mixtures Using Cellular Fluorescence Profiling and Flow Cytometry

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After attending this presentation, attendees will better understand how cellular properties such as fluorescence, optical scattering, and diversity of surface antigens can be used to analyze and ultimately separate contributor cell populations in aged or degraded biological mixture samples.

This presentation will impact the forensic science community by introducing new methods and technical guidelines for separating biological mixture contributors prior to DNA extraction using flow cytometry. This 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 then isolating fluorescent cells using flow cytometry, can be an effective technique for separating certain types of cell mixtures; however, mixture samples that have been compromised and/or degraded demonstrate a significant decrease in the efficiency of antibody probe binding as well as an overall loss of intact cell targets. This presents an ongoing obstacle for adopting a cell separation workflow for forensic applications. Therefore, the goal of this study was to investigate the molecular dynamics of cellular decomposition in simulated forensic samples and develop new strategies for mitigating biochemical processes that can lead to non-specific probe interactions and subsequent inefficiencies in cell sorting.

To accomplish this, a series of two-person blood mixtures that varied in drying time (between 24 hours and 128 hours), contributor ratio (10:1, 5:1, 3:1, 1:1), and total starting volume of blood (between 20µl and 500µl) was analyzed. This study found that non-specific interactions with the antibody probe were a significant factor in blood mixtures that had been dried for more than 24 hours, such that target cell populations were difficult to identify against non-target populations. This could be mitigated by reducing the antibody-to-cell ratio in the hybridization to <0.1µg per 20,000 cells. Subsequent sorting experiments performed on two-person mixtures with varying contributor ratios demonstrated that the Short Tandem Repeat (STR) profile of the minor contributor was successfully enriched in each sorted cell fraction when compared to the STR profile of the unsorted mixture. For example, in the 10:1, 5:1, and 3:1 mixtures, the ratio of minor contributor increased to ~1:2, ~1:1, and ~1:1 (major:minor), respectively. Although there was no systematic relationship between the degree of profile enrichment and the original contributor ratio, it was observed that the position of the sorting gate had a significant effect on the quality of the resulting STR profile, such that gates designed to capture cells at the tail ends of the fluorescence distribution of the mixture demonstrated the highest enrichment for the target cell population. To test the robustness of this sorting criteria, three different dried blood mixtures were created with 100µl total volume each with a 1:1 contributor ratio and the tails of the fluorescence distribution were sorted in a blind fashion (i.e., without measuring the fluorescence histograms of single source cell populations). The resulting sorted cell fractions showed enrichments of 2:1 and 3:1 for the A02 positive and A02 negative contributor respectively. This was consistent across multiple mixture replicates.

Last, this study conducted a limit of detection test using the above sorting workflow by drying blood mixture samples with 100µl, 50µl, and 20µl total starting volumes and 1:1 contributor ratios for each. Results demonstrated enrichments of the target population of 2:1 to 23:1, suggesting that clear separation can be obtained for blood mixtures that originally contained as little as 20µl of total volume.

Overall, these results suggest that antibody probes coupled to front-end separation with flow cytometry can be used to enrich target cell populations in whole blood mixtures that have been dried and/or compromised. Protocol changes to a traditional cell separation workflow, such as reducing the concentration of antibody probe, targeting cells with different optical properties, and sorting cells representing the "tail ends" of the distribution of fluorescence values for the mixture samples, can be effective strategies for minimizing the effects of cellular decomposition during front-end probe labeling and separation.

Biological Mixtures, Mixture Interpretation, Flow Cytometry