A71 Forensic Mixture Analysis: Pre-Emptive Separation of Whole Cells With Flow Cytometry and MHC Class I Allele Tagging

Lee M. Dean, BS, 6365 Elko Rd, Sandston, VA 23150; Jamie Sturgill, PhD, 1101 East Marshall St., Richmond, VA 23298; Sarah J. Seashols, MS, Virginia Commonwealth Univ, Dept of Forensic Science, PO Box 843079, Richmond, VA 23284-3079; and Christopher J. Ehrhardt, PhD, Virginia Commonwealth Univ, Dept of Forensic Science, 1015 Floyd Ave, Rm 2015, Richmond, VA 23284*

The goal of this presentation is to introduce a novel technique for the separation of complex biological mixtures of samples from multiple individuals using flow cytometry. Attendees will become familiar with Major Histocompatability Complex (MHC) Class I glycoproteins, the nature of antibody staining for cell isolation, as well as the basic principles of flow cytometry and how they will aid in the separation of complex mixtures.

This presentation will impact the forensic science community by providing an alternative approach to complex mixture analysis, one that physically separates an individual's cells from a mixture before STR amplification and analysis. This has the potential to produce multiple single source STR profiles from an evidentiary sample composed of two or more individuals in cases such as rape.

The MHC is an extensively studied region of genes that play a key role in the human immune system. It is divided into three major subgroups denoted by class I, II, and III, and has been found to be the most polymorphic region of genes yet to be identified. The forensic potential of this region lies in the multitude of alleles observed in the human population. MHC class I genes, which are the focus of this study, are referred to as the human leukocyte antigen (HLA) and are denoted by the classical HLA-A, HLA-B, and HLA-C; but may also include non-classical HLA-E, HLA-F, and HLA-G. Further, each HLA is defined by multiple subtypes, each with a unique allele grouping (e.g., HLA-A*02). MHC Class I molecules were chosen as the region of interest due to their expression on all nucleated cells. This is in contrast to more heavily researched alleles in the MHC region such as HLA-DQα, a class II molecule that lacks expression in semen. This presentation aims to describe how polymorphisms within the MHC Class I region can be used to separate individuals from an evidentiary sample using flow cytometry, a technique widely embraced by both the medical and research communities.

For this research, the goal was to take advantage of these MHC features to develop a new forensic technique that utilizes fluorescently tagged HLA antibodies specific for allele subgroups to differentiate individual contributions to a biologic mixture. Antibodies for the classical HLA alleles (ABC) were used to tag blood, semen, and epithelial cells from several different donors. Results show that the levels of MHC expression vary among all biological fluids tested. Blood, with the highest expression, resulted in 81% of all cells being separated from negative controls. Semen, with the lowest expression, produced 8.1% positive selection. It was found that flow cytometry is a fast, economical, and efficient way to separate whole cells from a mixture. It has also been shown that the prevalence of HLA molecules produces strong immuno-staining characteristics that are clearly distinguishable from controls. Initial experiments indicate that flow cytometry is a viable option for forensic mixture separation and could be quickly integrated into a forensic laboratory due to the availability of flow cytometers in nearly all major hospitals and research facilities. Physical separation of cells prior to cell lysis offers numerous advantages over trying to decipher complex electropherograms prior to SNP and STR analysis. Initial research into this technique could provide an alternative for the analysis of common biological samples.

Flow Cytometry, Biological Mixture, MHC Class I

 ** Presenting Author*