B44 The Development of a Single-Tube Assay for the Simultaneous Detection of Blood, Semen, and Saliva Utilizing DNA Methylation and ScreenClust® High Resolution Melt Software

Ellyn A. Zeidman, BS, Towson, MD 21252; Cynthia B. Zeller, PhD, Towson University, Towson, MD 21252*

Learning Overview: After attending this presentation, attendees will be familiar with methylation-specific Polymerase Chain Reaction (PCR) and High-Resolution Melt analysis (MSP-HRM) and the utility of this method for detection of blood, semen, and saliva, individually and in combination.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by allowing forensic scientists to use a single DNA extract to obtain serological test results as well as a genotype, reducing the overall amount of sample and analyst time required. This multiplex assay will allow quick, straightforward, and simultaneous serological determination of bodily fluids.

Using MSP-HRM analysis, blood, semen, and saliva were differentiated. To identify bodily fluids in this manner, tissue-specific methylation differences are targeted.[1] To do this, extracted DNA samples were bisulfite-treated, causing the unmethylated cytosines in DNA to be converted to uracils. These uracils will then be converted into thymines during PCR, lowering the melting temperature of the resulting amplicon due to the decreased guanine and cytosine content in the DNA. During the bisulfite treatment, methylated cytosines in the sequence will remain the same, thus exhibiting a higher melting temperature.

Previously published studies have found specific regions of DNA that are differentially methylated in forensically relevant bodily fluids.[2-6] Using these previously described primers such as BCAS4, DACT1, DDX4, DPPA5, and ZC3H bodily fluids were able to be distinguished from one another in a multiplex reaction due to the differences in melt temperatures. These melt profiles were then statistically separated using the ScreenClust software, which performs principle component analysis. This software examines the differences in the melt profiles to distinguish the components of a sample.

This research has been able to discriminate between the bodily fluids individually as well as in various combinations. Additionally, this protocol has provided the ability to identify bodily fluids with as little as 2.0 ng of DNA. The study has been shown to work on samples of blood, semen, and saliva from different individuals.

Currently the assay is being performed using mixtures of bodily fluids with differing ratios of DNA extract to help establish a limit of detection. Casework-like samples of mixed bodily fluids are prepared and analyzed using this method. This involves mixtures of bodily fluids themselves as opposed to mixtures of the extracts to determine if the assay remains consistent. This will be conducted as a single-blind study to remove bias during the analytical process. It is anticipated that the results of this analysis will conclude in an assay that is cost-effective, efficient, and reliable, which can be incorporated into the current forensic DNA workflow using instrumentation commonly found in forensic laboratories.

Reference(s):

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Body Fluid Identification, High Resolution Melt Analysis, ScreenClust