B116 The Identification of Forensically Relevant Body Fluids Using Methylation-Specific Polymerase Chain Reaction (PCR) and High-Resolution Melt (HRM) Analysis

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After attending this presentation, attendees will be informed of the benefits of using Methylation-Specific PCR (MSP) and HRM analysis to differentiate between different body fluids.

This presentation will impact the forensic science community by informing attendees of the ability to quickly and easily determine the presence of a specific body fluid prior to completing DNA analysis.

The combination of MSP with HRM analysis is a method that can demonstrate the difference between various forensically relevant body fluids. This type of body fluid identification involves targeting expressed genes and comparing their methylation status to other cell types.[1] DNA extracted from different body fluids undergoes a bisulfite conversion process that converts any unmethylated cytosines to uracil, which ultimately becomes thymines during PCR amplification. Forward and reverse primers are designed to amplify a target region of DNA that contains multiple CpG sites. The methylation status of the cytosines results in an alteration to the Melting Temperature (T_M); methylated cytosines produce a higher T_M than unmethylated cytosines. The primers can then be designed to bind specifically to methylated or unmethylated template strands.

Several studies have previously identified gene sequences that are differentially methylated in several relevant body fluids. The majority of these studies used pyrosequencing to identify changes in the methylation status of semen, blood, and saliva. The goal of this study is to use MSP and HRM analyses to visualize these differences and identify each body fluid. Using those results, two or more primers will be multiplexed to ensure a greater specificity in determining the presence of a forensically relevant body fluid.

Throughout this study, ten known primer sets and several primer sets of new design were studied to enhance body fluid identification. The forward and reverse primers were analyzed during their methylated and unmethylated forms to display the various T_Ms. ZC3H12D and DACT1_F have already been identified and analyzed using HRM analysis.[1,2] The eight remaining primer sets were obtained from studies involving sequencing to determine the differentiation likely to occur among body fluids. These include FGF7, cg06379435_W, BCAS4, cg06379435_S, DACT1_B, USP49, DDX4_B, and B_SPTB_03.[3-6] This study has proven the ability to differentiate semen from other body fluids. Further analysis is being performed to differentiate between blood and saliva, as these body fluids tend to display a similar T_M for many primer sets. The next step for this study is to combine two or more of the primer sets into a multiplex assay which will increase the specificity of the distinct body fluid identification.

Reference(s):

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