B7 The Identification of Biological Fluids Based on DNA Methylation Differences Using High Resolution Melt Curve Analysis

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After attending this presentation, attendees will understand how high resolution melt curve analysis may be used to distinguish between biological fluids using DNA methylation patterns that differ between cell types.

This presentation will impact the forensic science community by providing a novel method of potentially distinguishing menstrual blood and peripheral blood, as well as differentiating deposited vaginal fluid, saliva, and semen stains. In addition, methylation analysis uses the same DNA extraction protocol as Short Tandem Repeat (STR) typing, making these two methods compatible for side-by-side analysis.

Identifying the origin of the biological fluid is important in crime scene reconstruction and provides information to link evidence to the crime. Traditional methods of identifying body fluids rely on proteins for identification and are initially presumptive in nature. Presumptive tests may not be reliable due in part to cross reactivity with other biological fluids, resulting in false positives. In addition, false negatives may result due to degradation of protein antigens by heat and humidity. Once a stain presumptively tests positive for a biological fluid, an additional confirmatory test is necessary in order for the evidence to have legal and scientific standing in a court of law. Due to time and budgetary constraints as well as insufficient amounts of sample, confirmatory assays are not always able to be performed by forensic laboratories.[1] DNA methylation markers and pyrosequencing have recently been evaluated as an alternate method for body fluid identification. While these studies were able to discriminate the specific body fluid, pyrosequencing is expensive and uses equipment that is not available in most forensic laboratories.[2]

This study is a continuation of previous research that differentiated semen from other body fluids using DACT1 primers.[3] In this study, menstrual blood, blood, vaginal fluid, saliva, urine, sweat, and semen samples were obtained from volunteers by self-collection. An organic extraction was used to isolate DNA from each swab prior to subjecting the DNA to bisulfite conversion, which converts unmethylated cytosines to uracil, using a Zymo Research EZ DNA-Methylation-Lightning™ kit. High resolution melt curve analysis was carried out to analyze the melting temperature of the amplicons containing known sites of differential DNA methylation patterns using the QIAGEN® EpiTect™ High Resolution Melt Polymerase Chain Reaction (PCR) Kit. Primers used in this study are either proprietary or previously published.

Results from this study indicate that the HOX-B6 primer distinguished four out of ten menstrual blood samples from circulating blood samples. The primer also distinguished five out of ten vaginal fluid samples from other biological fluids (including blood, saliva, urine, and sweat). Using the SOX2OT primer, seven out of ten saliva samples were differentiated from other biological fluids (including menstrual blood, blood, vaginal fluid, urine, and sweat). This indicates that when samples are analyzed in tandem using the HOX-B6, SOX2OT, and DACT1 primers, menstrual blood, vaginal fluid, saliva, and semen may be differentiated from other body fluids.

Reference(s):

1. Li R. Forensic serology. In: Kobilinsky L, editor. *Forensic Chemistry Handbook.* New Jersey:John Wiley & Sons, 2012;269-290.
2. Park J.L., Kwon O.H., Kim J.H., Yoo H.S., Lee H.C., Woo K.M., Kim S.Y., Lee S.H., Kim Y.S. Identification of body fluid-specific DNA methylation markers for use in forensic science. *Forensic Sci Int Genet* 2014;13:147-153.
3. Deppen C. High-resolution melt curve analysis of DNA methylation status as a novel method for human semen identification. *Proceedings of the American Academy of Forensic Sciences*, 67th Annual Scientific Meeting; Orlando, FL. 2014.

Forensic Science, DNA Methylation, Biological Fluids

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