B127 High-Resolution Melt Analysis of DNA Methylation Status as a Novel Method for Human Semen Identification

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After attending this presentation, attendees will understand how DNA methylation patterns differ between cell types and how high-resolution melt curve analysis can be used in conjunction with bisulfite conversion to identify fluids based on their methylation status.

This presentation will impact the forensic science community by introducing a new method for semen identification that is based on DNA methylation and relies on instrumentation that is affordable and currently found in forensic laboratories. The method also offers the potential of being extended to multiple body fluid types.

Identification of the body fluid origin of a biological stain found at a crime scene can shed light on events that may have occurred. Unfortunately, current methods of body fluid identification rely on techniques that are subject to both false positives, through cross-reactivity between multiple fluids, or false negatives, due to protein degradation in the fluid. A recent study shows that it is possible to distinguish body fluids by examining the differentially methylated DNA in the fluid by pyrosequencing.[1] The location of methyl groups on DNA is known to regulate gene expression, and is thus tissue specific. It is also thought to be stable over long periods of time in deposited stains; however, pyrosequencing is a fairly new technique that is time-consuming and uses expensive equipment that is not currently available in most forensic laboratories.

The goal of this study was to develop a cost- and time-effective technique for the identification of body fluids by DNA methylation, using instrumentation typically available in crime laboratories.

Six blood, five saliva, four urine, and four vaginal fluid samples were obtained from 11 volunteers and five semen samples were obtained from different donors in a conical vial by self-collection. All semen samples were then deposited (5uL) on sterile cotton-tipped swabs. Bloodstains from 1998, 2003, and 2010 were also obtained and used to assess the stability of methylation over longer periods of time.

DNA was isolated from each swab using either organic extraction (saliva, urine, and vaginal fluid) or Chelex® 100 extraction (blood and semen) and subjected to bisulfite modification using a Zymo Research EZ DNA Methylation-Gold™ kit, to convert unmethylated cytosines to uracil. The Qiagen® Epitect® High Resolution Melt (HRM) PCR Kit was used for melt curve analysis of amplicons containing known sites of differential methylation.[2,3] Primers used in this kit are proprietary.

All body fluid stains were analyzed in triplicate. Results indicate that semen can be identified using post-amplification melting temperature differences compared to the other fluids used. This shift in melt temperature was found to be statistically significant for all five body fluids using a one-way Analysis of Variance (ANOVA) with a 95% confidence interval ($p=6.34x10^{-5}$), as was expected for genes actively transcribed in tissue samples due to the lack of methyl groups, and thus bisulfite conversion of cytosine residues; however, when semen was removed from the statistical analysis, the remaining body fluids were found not to be statistically different from each other (one-way ANOVA at 95% confidence interval, $p=0.141$). Extraction and analysis of DNA from the 1998 blood case sample resulted in a melting temperature consistent with blood samples from volunteers indicating that the age of the stain does not affect this analysis. The results from case samples 2003 and 2010 yielded insufficient results. This method is also short tandem repeat-typing compatible due to DNA extraction prior to methylation analysis. Although only semen could be identified with this study, there are other potential primer sites that could help identify the other body fluids using the same method. This study does show that body fluid identification is possible for both old and fresh samples using DNA methylation and HRM analysis.

References:

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DNA Methylation, Melt Curve Analysis, Semen Identification

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