B2 The Development and Evaluation of Real-Time Polymerase Chain Reaction (PCR) Methods for DNA Methylation-Based Identification of Semen and Blood

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After attending this presentation, attendees will understand how the methylation status of the semen-specific unmethylated DNA region and the blood-specific methylated DNA region are analyzed using a real-time PCR technology. Attendees will also understand the usefulness of these methods for semen and blood identification.

This presentation will impact the forensic science community by demonstrating methods that can accurately and sensitively identify semen and blood from various samples, including highly aged body fluid stains. In addition, these methods can be readily established in forensic laboratories and are simple to perform.

The identification of body fluids provides important evidence in forensic investigation to prove the existence of criminal events, such as murder, injury, and sexual assault. Recently, DNA regions that are specifically methylated or unmethylated in different types of body fluid have been reported as novel markers for body fluid identification. The stable nature of DNA offers an advantage for forensic use because forensic samples have often been exposed to severe conditions before laboratory analysis.

Previous preliminary work reported the development of a MethyLight-based-method for analyzing the —C— phosphate—G— (CpG) sites in the DACT1 gene, which was previously reported as a semen-specific unmethylated region.[1,2] MethyLight is a method for quantitating the methylation ratio of a targeted region using a real-time PCR device and a pair of TaqMan® probes that are designed for methylated or unmethylated status.

In this presentation, further evaluations of the method are described to enable detection of the DACT1 region for semen identification. A MethyLight-based method was also developed and evaluated for detecting the methylation ratio of cg06379435 and its neighboring CpGs, which were previously reported to be specifically methylated in blood. To set the threshold methylation ratios for semen or blood identification, DNA from various body fluid samples (blood, semen, saliva, and vaginal fluid), which were bisulfite-converted using an EpiTect Bisulfite Kit, were analyzed by the two methods, each using a pair of TaqMan® probes, the EpiTect MethyLight PCR Kit and the Smart Cycler II system. The results revealed almost exclusive non-methylation of the DACT1 region in semen and high methylation levels of the cg06379435 region in blood, which is consistent with previous studies. Based on these data, the threshold methylation ratios were set for semen and blood identification. For semen identification, another threshold ratio to detect semen mixed with other tissues could be set near the average methylation ratio of DACT1 in non-semen samples, because the DACT1 region was almost fully methylated in non-semen samples. Use of these thresholds can accurately identify semen or blood from other samples, including highly aged (29-year-old) semen, blood stains, and mixture samples containing a smaller amount (20%) of semen DNA. The sensitivity of these methods was evaluated by analyzing pooled samples of semen and blood DNA and demonstrated the necessary amounts of DNA for bisulfite conversion to be 1ng for both methods.

In conclusion, these methods can accurately and sensitively identify semen and blood. Because a real-time PCR device is common in forensic laboratories, this method can easily be introduced without the purchase of further equipment. Moreover, because a real-time PCR-based method requires no substantial work beyond preparing the

PCR reaction mixture, simple and quick analysis may be realized with a reduced risk of contamination. Therefore, these methods could be better suited to forensic work compared with other methods of methylation analysis, such as bisulfite sequencing methods.

Reference(s):

1. Watanabe K et al., Development of a real-time PCR-based method for analyzing semen-specific unmethylated DNA regions and methylation status in aged body fluid stains. *J Forensic Sci.* 2016; 61(S1): S208–S212.
2. Eads CA et al., MethyLight: a high-throughput assay to measure DNA methylation. *Nucleic Acids Res.* 2000; 28(8): e32.

Body Fluid Identification, DNA Methylation, Real-Time PCR