B122 A Developmental Validation of a Body Fluid Identification Multiplex Via DNA Methylation Analysis

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Learning Overview: After attending this presentation, attendees will understand the results of a developmental validation of a novel method for the determination of body fluids based on DNA methylation markers and sequencing techniques. The procedure involves a multiplex amplification of tissue specific differentially methylated regions followed by pyrosequencing to determine the identity of the tissue.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing results from the evaluation and developmental validation of a multiplexed approach to body fluid identification which is currently done in serology in several separate tests, expanding the information that can be gleaned from an individual's DNA.

The origin of a DNA extract can be used in criminal investigations to prove criminal intent and is possibly more compelling than simply confirming the presence of DNA. In the case of child abuse from a parent or guardian, it is the type of body fluid present and submitted as evidence that can aid investigators in determining the sequence of events, rather than the DNA's presence in the first place. The McCord DNA research group has located and validated several DNA methylation markers for identifying whether a DNA extract originated from blood, saliva, vaginal epithelia, or semen.[1,2] These loci, known as tissue specific differentially methylated regions (tDMRs) can show hypomethylation in one body fluid type and the opposite in other body fluids. Additionally, DNA methylation has been found to can predict biological age, but the body fluid can influence the accuracy of prediction models.[3,4] More recently, the authors combined the four best markers for these body fluids into a multiplex PCR assay and demonstrated their ability to still accurately identify the four aforementioned body fluids. However, questions remained about the ability for this technique to be implemented in to forensic laboratories.

In particular, the sensitivity of the method, the ability to identify mixtures of body fluids, and the effects of degradation and inhibition needed to be examined. Samples of saliva, blood, vaginal epithelia, and semen were collected, extracted by PCIA, and quantified by Alu primers. The samples then underwent bisulfite conversion to convert unmethylated cytosines to uracil. Samples were then amplified in multiplex and sequenced using a Qiagen Q48 Autoprep® Pyrosequencer (Qiagen, CA). For sensitivity, the multiplex was tested with input DNA from 20ng to 10pg. In the mixture study, the multiplex was tested with 100:0, 75:25, 50:50, 25:75, and 0:100 ratios of two body fluids at a time. For degradation, the samples were exposed to 95°C for 10-25 minutes, and for inhibition, samples were exposed to hematin and humic acid at concentrations of 0.08 mM and 0.24mg/mL, respectively.

The results of the sensitivity study showed the method working reliably with input DNA at 250pg, and with some results attained at DNA inputs all the way down at 50pg. For the mixture study, it was possible to make assumptions about which two body fluids were present, but not the exact ratio. The methylation value for a mixture of two body fluids was simply a value somewhere in between the expected methylation values of each body fluid on its own. For the degradation and inhibition study, the method continued to work for samples that were heated for up to 20 minutes, before negative effects were observed. For inhibition, the hematin and humic acid did not significantly affect the results when added to the sample before bisulfite conversion. However, if the inhibitor was added after bisulfite conversion, the sample would fail to amplify.

The overall results of this study have demonstrated this body fluid identification multiplex is able to accurately identify body fluids at concentrations that are largely like what is found in forensic cases. Additionally, the method works with mixtures of body fluids and withstand significant levels of degradation. Inhibition was a non-issue due to the bisulfite conversion process's action as a secondary cleanup of DNA extracts. This multiplex method to body fluid identification will open the door to more informative epigenetic tests in the near future. For example, age identifying methylation markers would give even more information to investigators.

Reference(s):

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