

INVESTIGATING THE INTRAVARIABILITY OF SALIVA METHYLATION MARKERS USING A VARIETY OF ORAL FLUID COLLECTION METHODS

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In conventional forensic casework, DNA from a biological exhibit is extracted to generate an STR profile that will then be compared to a reference database. Nonetheless, it can also be crucial to identify the body fluid that contributed to that genomic material. For instance, the defendant may claim that his DNA recovered from the victim is the result of innocent transfer, while in fact, the actual body fluid could be semen, evidence of sexual assault. To date, multiple DNA methylation markers have been developed to identify blood (peripheral and menstrual), semen, vaginal epithelia, and saliva using different molecular techniques such as SNaPshot and pyrosequencing.

In the literature, for oral fluids, candidate markers have been selected based on either buccal or spit samples. However, saliva is known to be heterogenic in terms of cellular composition and a mix of epithelial, leukocytic, and bacterial cells may be present. The proportion of each would differ depending on the method of oral fluid collection: a buccal swab for example would have more epithelial cells compared to a saliva spit. This disproportionality may affect the percent methylation of markers that are more cell-specific (epithelial v. leukocytic) rather than fluid-specific (saliva).

Hence, it is important to evaluate the intravariability of the oral fluid markers on a variety of saliva sample types. In this study, we examined the informativeness of multiple saliva markers in BCAS4, SLC12A8, SOX2OT, and FAM43A genes, on buccal, spit, lip, tongue, chewing gum, and nasal swabs. The extracted DNA from each sample is bisulfite converted and pyrosequenced to compare the percent methylation values at multiple CpG sites per marker. The results show differences in methylation levels per sample type that would necessitate the need of a universal saliva marker or a combination of multiple markers to account for cell composition differences among forensically common evidences.