DEVELOPMENT AND VALIDATION OF A SEMEN-SPECIFIC DNA-BASED METHYLATION ASSAY

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Identifying the source tissue of biological material found at crime scenes can be very informative in a number of cases. In sexual assault cases, in particular, identifying the presence of semen with a confirmatory test can be very helpful in reconstructing some aspects of the crime. Despite their usefulness, current visual, catalytic, enzymatic, and immunologic tests for presumptive and confirmatory tissue identification are applicable only to a subset of samples, can suffer limitations such as lack of specificity and sensitivity, and are substantially impacted by environmental insults. Moreover these assays are incompatible and thus cannot be multiplexed or run under similar analytical conditions. Thus they are less amenable to automation and consume sample unnecessarily. In addition their results are operator-dependent. A better approach would be to use a DNA-based assay due to the inherent stability of the molecule and its well-established use for human identity testing. Tissue-specific methylation patterns of DNA are known to exist in nature. The SemenIdentifier assay (Nucleix, Tel- Aviv, Israel) capitalizes on tissue specific methylation patterns to determine if a DNA sample is derived from semen. DNA samples are subjected to digestion by a methylation-sensitive restriction endonuclease followed by multiplex amplification of specific genomic targets with fluorescent-labeled primers, capillary electrophoresis of amplification products, and automatic signal analysis by dedicated software. Results to date are semen specific and unique among confirmatory assays yield a probability of the likelihood that the sample originated from semen. The single tube assay was designed for easy integration by forensic laboratories as the assay utilizes the same platforms as current forensic STR profiling. The system is fully automatable, provides operator-independent results, and allows combining tissue identification with profiling in a single procedure. Our validation study follows the relevant criteria recommended by SWGDAM. These include limits of detection precision, accuracy, tissue mixtures, and the effect of inhibitors and sample degradation on the assay. Electrophoretic artifacts have been identified and remedied. Although still limited in sample size, no methylation pattern differences were detected among population groups. Based on our results, SemenIdentifier provides law enforcement agencies a robust and reliable tool for determining the presence of semen from forensic biological evidence. The same platform can be used to develop other tissue specific assays.