



“Bringing a confirmatory molecular serology assay into routine forensic practice will benefit both prosecution and defense, as well as improve public confidence in the quality and accuracy of forensic science and the fair administration of justice.”

—Donald Siegel, NYC OCME

NIJ and The New York City OCME

Validating Confirmatory Body Fluid Identification Assays for Real-World Impact

Problem and Solution Synopses

Identifying a body fluid in a biological sample can provide important contextual information for forensic investigations and for evidence presented in court. Advancing DNA identification technology has enabled forensic science service providers to detect very small amounts of DNA, even from shed epithelial cells. Understanding the source of biological materials can add value to understanding events that may have transpired during a crime. Although forensic laboratories use established presumptive body fluid identification methods, these methods lack sensitivity and specificity (resulting in false positive results) and typically only detect one type of body fluid. Body fluid identification may therefore require several different assays, which consumes time, resources, and sample. To address this need within the forensic community, researchers at the **New York City Office of Chief Medical Examiner (OCME)** have developed and validated a single confirmatory assay to detect human blood, saliva, and semen using high-performance liquid chromatography and multiple-reaction monitoring mass spectrometry. The assay uses a multi-tiered system to confirm each fluid, targeting proteins, protein fragments and peptides. There are three marker proteins from blood, three from semen, and four from saliva; detection of multiple peptides from each protein and four fragment ions from each

peptide increases assay confidence.¹ The validation was performed with diluted and degraded body fluid samples, which were used to prepare dried mock forensic stains for analysis. Non-targeted body fluids (e.g., vaginal fluid) and blood, saliva, and semen from other species were also tested. The assay can also detect and distinguish body fluid mixtures. The OCME successfully validated their body fluid assay and plans to [publish their protocol](#) in the future. This assay has been approved by the ANSI National Accreditation Board and the New York State Commission on Forensic Science and is currently being used in casework at the OCME.

Key Benefits

- Provides reliable and confirmatory results, offering a human-specific assay that can be attested to in the judicial system.
- Tests for three different types of human-specific body fluid in one assay (blood, semen, saliva), saving time, resources, and sample.
- Uses equipment (mass spectrometry) that is often available in forensic laboratories, so it can be easily integrated into existing workflows.
- Detects body fluids at low levels in the nanoliter range.

1. NIJ Forensic Laboratory Needs Technology Working Group (FLN-TWG). (2021, February). *Implementation strategies: LC-MS-based forensic toxicology screening*. <https://forensiccoe.org/fln-twg-lc-ms-based-forensic-toxicology-screening/>

NIJ Research

Support from the National Institute of Justice (NIJ) enabled the New York City OCME to develop and validate proteomic mass spectrometry body fluid assays to support forensic analyses. Initial NIJ support ([2008-DN-BX-K011](#)) enabled the development of a fast and accurate proteomic method for body fluid identification and subsequent assessment of the level of detection, sensitivity, and specificity for blood, semen, and saliva. Current NIJ support ([15PNIJ-21-GG-02712-SLFO](#)) enabled the OCME to validate the body fluid assay for use in casework, which included acquiring several hundred body fluid samples from collaborative efforts in the forensic community.

Bringing Research to Practice

With support from collaborating universities and in-house bioinformatics experts, the New York City OCME was able to build a solid statistical background to effectively validate the assay. The OCME validated this assay and results will be published in the future. Through the development process, the OCME has presented and published multiple resources informing the community about the assay; for example, this technique was discussed in the Forensic Laboratory Needs Technology Working Group's "[Proteomic Mass Spectrometry for Biology Fluid Identification](#)" [🔗](#) paper published in 2021. The body fluid assay was implemented and is currently being used in casework at the OCME.

The Future

The current research that enables the validation of proteomic mass spectrometry body fluid assays to support forensic analyses prioritizes distinguishing blood, saliva, and semen. Future plans include expanding this method to detect menstrual blood and urine.



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