B85 Forensic Body Fluid Identification Using Microbiome Signature Attribution Through 16S Ribosomal DNA (rDNA) High-Throughput Sequencing (HTS)

Denise Wohlfahrt, BS, Virginia Commonwealth University, Richmond, VA 23284; Raquel Green, BS, Richmond, VA 23220; Angela L. Brand, BS, Southport, NC 28461; Eric A. Abshier, MS, Worldwide Counter Threat Solutions, Fredericksburg, VA 22401; Elena Martinez Planes, Virginia Commonwealth University, Richmond, VA ; Antonio Tan-Torres, MS, Virginia Commonwealth University, Richmond, VA 23284; J. Brooks, PhD, Virginia Commonwealth University, Richmond, VA ; Sarah J. Seashols Williams, PhD, Virginia Commonwealth University, Richmond, VA 23284-3079; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA 23284*

Learning Overview: After attending this presentation, attendees will understand how microbial communities found in the body may be used to identify various forensically relevant body fluids.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by helping forensic scientists overcome the limitations often encountered when using current serological and molecular methods. While using the microbiome as identifying markers is not new, the emergence of high-throughput sequencing allows for a more rapid and detailed analysis of these communities.

Previous studies such as the Human Microbiome Project suggest that body fluids have a distinct, stable, and distinguishable microbial signature. Identifying these microbial markers within forensically relevant fluids not only has the advantage that previously indistinguishable samples, such as venous and menstrual blood, may be separated, but these markers can also be easily implemented into comprehensive high-throughput sequencing (HTS) panels used in the forensic workflow alongside the typical human phenotypic marker characterization.

Using IRB approved collection methods, 200 urine, 200 blood, 152 feces, 149 saliva, 100 vaginal fluid, 64 menstrual secretion, and 39 semen samples were collected. Briefly, urine and semen were collected into sterile containers and stored at -80°C. Semen was dried onto sterile cotton swabs prior to DNA extraction. Venous blood was collected onto cotton swabs using a finger prick and dried at room temperature. Saliva, vaginal secretions, menstrual secretions, and feces were collected onto cotton swabs and dried at room temperature. DNA Investigator kit with the standard Forensic Casework Sample protocol on the QIAcube was used to extract blood, semen, saliva, vaginal secretions, and menstrual secretions according to manufacturer protocol. QIAamp Power Fecal DNA kit was used to extract DNA from fecal samples, and DNA Micro kit was used to extract urine samples, according to manufacturer protocol. Following extraction, V4 region of the 16S rDNA was sequenced on Miseq FGx sequencing platform following the dual-indexing protocol as described by Kozich et al (2013).[1] Sequences were then analyzed using mothur version 1.39.4, and statistical analysis was performed using R version 3.4.0. [2,3] Random subspace cross validation was performed to test the robustness of the classification accuracy.

Results of this study indicate that the microbial signatures are diverse, unique, and stable within a body fluid, common across most of the population assessed, and distinguishable from other body fluids, except for vaginal and menstrual secretions. These secretions may be distinguished from the other body sites when classified collectively as female intimate samples. Using indicator taxa, feces had a higher relative abundance of *Bacteroides* and *Faecalibacterium*, saliva had a higher relative abundance of *Streptococcus* and *Veillonella*, female intimate samples had a higher relative abundance of *Lactobacillus*, and semen had a higher relative abundance of *Corynebacterium*, when compared to each other. Random subspace classification successfully classified feces at 100%, saliva at 100%, semen at 83.3%, and female intimate samples at 94.6% accuracy, with an overall classification accuracy of 97.6%.

In conclusion, the newly developed microbiome signature-based method can identify all biological fluids except vaginal and menstrual secretions, with strong statistical certainty.

Reference(s):

1. Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform.Applied and Environmental Microbiology,79(17), 5112–5120. http://doi.org/10.1128/AEM.01043-13
2. Schloss, P. D. et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537-7541, doi:10.1128/AEM.01541-09AEM.01541- 09 [pii] (2009).
3. R: A language and environment for statistical computing. (R Foundation for Statistical Computing, http://www.R-project.org, Vienna, Austria., 2011).

Body Fluid ID, 16S rDNA, Microbiome