B40 Forensic Body Fluid Identification Using Microbiome Signature Attribution Through 18S Recombinant DNA (rDNA) High-Throughput Sequencing

Alyssa Daniels, Henrico, VA 23228; Ines Benaissa, BS, Richmond, VA 23222; Sarah J. Seashols Williams, PhD, Virginia Commonwealth University, Richmond, VA 23284-3079; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA 23284; Denise Wohlfahrt, BS, Virginia Commonwealth University, Richmond, VA 23284; Kathleen D. Brim, BS, Richmond, VA 23221-3013; Eric A. Abshier, MS, Worldwide Counter Threat Solutions, Fredericksburg, VA 22401; Francy S. Nogales, BS, Virginia Commonwealth University, Richmond, VA 23223; Haris Mukic, BS, Virginia Commonwealth University Dept of Forensic Science, Richmond, VA 23284; Angela L. Brand, MS, Richmond, VA 23220*

Learning Overview: After attending this presentation, attendees will have a better understand of eukaryotic communities associated with forensically relevant human body fluids.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by developing an improved method for the accurate identification of body fluids as an alternative to current serological techniques. While the human microbiome has been used for identification purposes in the past, high-throughput sequencing has allowed for a more detailed and rapid analysis of microbial communities.

The conception of the Human Microbiome Project advanced the understanding of bacterial communities in the human body, and previous research has established that unique microbial signatures can help distinguish each body fluid. While these signatures have been developed for the prokaryotic microbiome, the next step is the examination of the eukaryotic microbiome. Eukaryotic signatures could provide a greater specificity and statistical weight when discerning between body fluids. These microbial markers can be implemented to develop a confirmatory assay for body fluid identification that works in tandem with other DNA-based methods in the forensic workflow.

Using an approved Institutional Review Board (IRB) protocol, 100 samples each of urine, feces, saliva, vaginal fluid, menstrual blood, and semen were collected. The semen and urine samples were first collected in a container, then dried onto sterile cotton swabs at room temperature. Saliva, vaginal fluid, menstrual blood, and feces were collected onto cotton swabs and dried at room temperature. DNA was isolated and quantified using DNA extraction methods commonly used by the forensic community. The V9 region of the 18S rDNA was amplified using dual-index strategy as described by Kozich et al.[1] Amplified products were purified, quantified, and pooled in equimolar concentration for paired-end sequencing on the MiSeq® FGx sequencing platform. The sequences will be analyzed using mothur version 1.39.4, and R version 3.4.0.[2,3] Ensemble subspace classification methods will be developed for the identification of body fluid samples in a single test either alone or in combination with bacterial signatures associated with these body fluids.

The 18S rDNA amplification resulted in two amplification products for most of the body fluid samples. Only those fragments of the target size were gel extracted and purified. For the same samples, microbial signatures based on 16S rDNA sequencing showed that except for female intimate samples (menstrual secretions, vaginal secretions, and female urine), the bacterial structure was significantly different between different body fluids. Most probably, an ensemble subspace classification method developed based on combined bacterial and eukaryotic community structure data will be able to differentiate even female intimate samples.

In conclusion, this study will highlight eukaryotic community structure associated with a large number of human biological samples and will develop a method that will be able to identify all forensically relevant biological samples in a single test.

Reference(s):

1. Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and 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).

Microbiome, Eukaryotic, Body Fluid Identification

*Presenting Author