B87 The Impact of Environmental Exposure and Chemical Contaminants on Microbial Signature Associated With Forensically Relevant Human Biological Samples

Denise Wohlfahrt, BS, Virginia Commonwealth University, Richmond, VA 23284; Francy S. Nogales, BS, Virginia Commonwealth University, Richmond, VA 23223; Raquel Green, BS, Virginia Commonwealth University, Richmond, VA 23220; Najai Bradley, Richmond, VA 23220; Kathleen D. Brim, BS, Richmond, VA 23221-3013; James P. Brooks, PhD, Virginia Commonwealth University, Richmond, VA 23284; 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 have a better understanding of the microbial DNA associated with various human biological samples and how contamination and environmental changes can impact them.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by increasing understanding in how handling, transport, and storage conditions can alter these microbial signatures, specifically in forensic applications.

Studies such as the Human Microbiome Project have shown that human biological samples can be successfully identified based on their distinct microbial signatures. Identifying these microbial markers not only has the benefit of an additional identification method, but may also be used to supplement current methods to distinguish incomprehensive samples, samples with low human DNA content, as well as having the potential to be implemented into next generation sequencing panels for easy implementation into the forensic workflow.[1] Even though the microbial signatures of body fluids are found to be distinct and stable, the experimental samples often do not compare to those found at the crime scene. Here, environmental exposure or contamination through chemicals or mishandling can often cause severe degradation of forensic evidence.

Venous blood, saliva, semen, urine, feces, vaginal fluid, and menstrual blood were collected from female and male volunteers using Institutional Review Board (IRB) -approved collection methods. This study was designed to investigate how environmental or chemical changes influence the microbial signatures in body fluids. To assess these effects, body fluid samples were exposed to elevated temperatures at various exposure times, detergent, bleach, and Ultraviolet (UV) light. DNA was extracted from the treated samples using DNA Investigator kit with the standard Forensic Casework Sample protocol on the QIAcube for blood, semen, saliva, vaginal secretions, and menstrual secretions according to manufacturer's protocol. QIAamp® Power Fecal DNA kit was used to extract DNA from treated fecal samples, and DNA Micro kit was used to extract treated urine samples, according to the manufacturer's protocols. These treated samples were compared to positive controls (untreated samples) to evaluate changes in bacterial DNA concentration, sequencing read variations, as well as identifying how the microbial taxa would vary between treatments.

Immediately after extraction, the V4 region of 16S rDNA was sequenced on MiSeq® FGx sequencing platform following the dual-indexing protocol as described by Kozich et al.[2] Sequences were then analyzed using mothur version 1.39.5, and statistical analysis was performed using R version 3.5.0.[3,4]

At the phylum level, similar patterns of relative abundance of bacterial phyla were seen between treatments. Major variations of these phyla were seen in the bleach-treated samples in all body fluids. At the genus level, major variations in the relative abundance were seen in the UV-treated blood samples and fecal samples treated with extended periods of elevated temperatures. Menstrual blood and vaginal fluid were least impacted by the treatments, while semen and urine showed the most variation when compared to the positive controls.

In conclusion, the microbial signature-based body fluid identification method is robust and reliable with common environmental extremities and contaminants. Findings from this study will help in minimizing errors associated with the accuracy of the microbial signature-based method for body fluid identification.

Reference(s):

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2. Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. (2013). *Appl. Environ. Microbiol.* doi:10.1128/AEM.01043-13.
3. Schloss, P.D. et al. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–41 (2009).
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Body Fluid ID, Microbiome, Compromised Samples

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*Presenting Author