B130 An Evaluation of the Stability of High Throughput Sequencing (HTS) of Microbial DNA From Compromised Human Samples

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Learning Overview: After attending this presentation, attendees will have a greater understanding of the effects of the environment and contaminants to the use of High Throughput Sequencing (HTS) of microbial DNA from human samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information and validation concerning the use of microbial signatures in forensic applications.

The human microbiome study showed distinct microbial signature at different body sites that have the potential for development of forensic tools such as postmortem interval estimation method, human individualization, identification of trace evidence and human body fluid identification. Although microbial signature associated with each body fluid is distinct and can be utilized for the identification of body fluids at the crime scene, it is not known how this method will work for forensic evidence samples that are degraded (because of harsh environmental exposure) or contaminated (because of chemical treatments). This study would validate such techniques prior to implementation in the field. Although scientific research using HTS technology is an emerging field based on the cost-effectiveness, there is currently insufficient research regarding microbial signatures of compromised samples from human body fluids. This study aims to address that issue by assessing bacterial concentration, sequencing and classification of microbial taxa from body fluids frequently compromised or tampered at crime scenes in comparison to untreated samples.

To assess the effects of compromised samples through environmental exposure or chemical treatment, a variety of human body fluids were exposed to harsh treatment conditions that evidence samples could be subjected to at the crime scene. Venous blood, saliva, seminal fluid, urine, fecal matter, vaginal and menstrual secretions were collected from female and male volunteers under an approved human subjects research protocol. These were then exposed to prolonged times of 24 and 96 hours in high temperatures, ultraviolet exposure, bleach contamination and detergent exposure. Treated samples and positive controls were extracted using standard DNA isolation protocols and quantified using an optimized qPCR method with universal 16S rDNA primers. Amplification was conducted using the variable region V4 and sequenced on an Illumina® MiSeq FGx™ platform (Illumina®; San Diego, California).

The resulting data was analyzed via MiSeq® SOP Mothur version 1.36.1 and statistical comparison were obtained with analysis of molecular variance (AMOVA). The results from these analyses provide further evidence for the utility of microbial signatures for body fluid identification in forensic casework and assessed the error associated with this method.

Microbiome, Body Fluid ID, Compromised Samples