H124 The Postmortem Microbiome: An Evaluation of 16S Ribosomal RNA (rRNA) Profiles

Gulnaz T. Javan, PhD, Alabama State University, Montgomery, AL 36104; Sheree J. Finley, PhD, Alabama State University, Montgomery, AL 36104; DeEtta Mills, PhD, Florida International University, Miami, FL 33199*

Learning Overview: After attending this presentation, attendees will learn how to use hypervariable regions V1 and V2 of the 16S rRNA gene to determine the postmortem interval.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information on the determination of time of death using amplicon Length Heterogeneity-Polymerase Chain Reaction (LH-PCR) and capillary electrophoresis.

The ability to quantify the microbial diversity within a human postmortem microbiome is fundamental to the elucidation of molecular functions after a human dies. Before the advent of advanced forensic molecular techniques, the study of the functions of microbial communities has been challenging to investigate due to their different scales (e.g., spatial and temporal). However, with increased use of high-throughput DNA sequencing, combined with bioinformatic analyses, these limitations are quickly diminishing. To date, the "gold standard" of DNA analytical approaches involve a multifaceted process of metagenomic sequencing that is moderately costly and time-consuming to perform. One effective method used to screen microbiomes is to generate a community DNA profile using fluorescent-based DNA profiling methods such as amplicon LH-PCR followed by capillary electrophoresis. Due to the natural occurrence of insertions and deletions within 16S rRNA genes, different length amplicons are generated when the DNA is amplified using universal primers. The hypervariable regions, V1 and V2, demonstrate an extensive range of amplicon lengths that represent the minimum microbial diversity. While any one amplicon could represent different nucleotides, thus different microbial species, it enables forensic scientists to efficiently survey the dynamics of a community under differing postmortem growth conditions. This approach is beneficial because it allows for the rapid production of a genetic pattern, or snapshot, of the bacteria present at the time of sampling, which is an important aspect to consider when choosing samples for downstream metagenomic sequencing.

In the present, first-of-its-kind postmortem microbiome study, 32 samples (brain, heart, liver, and spleen) from eight cadavers of various causes of death from criminal cases were investigated. The results reveal two noticeable trends that affect the number of postmortem microbes and conceivably (1) the frequency of proliferation, (2) the time since death, and (3) the cause of death. Therefore, time-since-death rates of proliferation may prove advantageous for forensic science in determining postmortem interval. LH-PCR is a valid, rapid technique that can monitor these dynamics and, therefore, screen postmortem samples that would be of interest for sequencing analyses. Furthermore, this method is a very inexpensive technique to screen samples to compare community patterns over time and between individuals.

16S rRNA, Postmortem Microbiome, Capillary Electrophoresis