## H94 Estimating Postmortem Interval (PMI) Using Microbial Succession in Human Cadaver Rib Bones

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**Learning Overview:** After attending this presentation, attendees will understand microbial decomposition of human cadavers, how microbiome technologies can be used to estimate the PMI, and how microbial invasion into human rib bones during decomposition may be developed into a novel form of physical evidence for estimating PMI.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of novel microbiome tools that may be useful in investigations of unattended death scenes, in which PMI is unknown.

In crime scene investigations, estimating the PMI is critical for validating alibis and, in some cases, identifying the deceased. Although there are multiple methods to estimate PMI, there are few tools that are accurate after the first two weeks of decomposition. Therefore, a new tool for estimating PMI on the scale of weeks to months is needed. Microbes are major drivers of decomposition that could serve as evidence of microbial succession in a similar way across bodies during decomposition. Previous research has demonstrated that microbiome data collected from both mouse models and human bodies can be used to create an accurate "microbial clock of death." This study hypothesized that the invasion of microbes into bone is likely a slow process and tracking the succession of microbes into bones after death may provide accurate estimates of PMI for longer timeframes of decomposition.

To test this hypothesis, rib bones from six human bodies were collected, beginning at approximately three months after death. For each body, one rib was collected every three weeks for a total of 48 sampled ribs. Research was conducted at the Southeast Texas Applied Forensic Science Facility, an anthropological research center in Huntsville, TX. After sample collection, samples were shipped to Colorado State University, where each of the rib samples were processed by excising a portion of the bone with a Dremel® drill, cleaning the newly excised piece with bleach and Ultraviolet (UV) irradiation, then pulverizing it into a fine powder. Each of the bone powders were demineralized, then DNA was extracted using the DNEasy® Powersoil Isolation Kit following the Earth Microbiome Project standard protocols. The bacterial microbiome in each sample was then characterized using 16S ribosomal RNA (rRNA) amplicon sequencing on the Illumina® MiSeq® platform at the University of California, San Diego. Data analysis using Quantitative Insights Into Microbial Ecology revealed that similar microbes invade the rib bones during decomposition. This study discovered that PMI could be estimated within approximately one month over a decomposition period of nine months by constructing a random forest regression model using 16S rRNA data. These results are promising because the error of the model (~4 weeks) cannot be more accurate than the sampling frequency (~3 weeks). Therefore, this initial study demonstrates that utilizing the invasion of microbes into bone may be useful for estimating PMI on a longer timescale, and the model may be improved with studies that include more frequent sampling.

**Bone, Microbiome, Postmortem Interval**