H139 Using Culturomics to Investigate the Mouse Thanatomicrobiome

Christi Meyer, Yukon, OK 73099; Scott S. Crupper, PhD, Emporia State University, Emporia, KS 66801*

Learning Overview: The goal of this presentation is to demonstrate how culturomics can complement the metagenomic investigation of the mouse thanatomicrobiome.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating how the isolation and identification of living microbes from the thanatomicrobiome will increase understanding of its culturable bacterial diversity. This knowledge will complement existing molecular data to aid in determining if the thanatomicrobiome can provide an alternative to existing methodologies for Postmortem Interval (PMI) estimation.

Forensic microbiology is a rapidly expanding field that has many broad applications in forensic science. Recent advances in DNA sequencing and computing technologies (e.g., metagenomics) have provided researchers with a molecular toolkit to explore a multitude of diverse forensic questions—for example, investigating microbial changes that occur postmortem using next generation DNA sequencing to yield a profile of the thanatomicrobiome, or microbes found associated with internal organs after death. Despite these technological advances and data obtained, many questions remain regarding how to interpret the results and their direct forensic application.

While the use of metagenomics has revealed a complex and diverse thanatomicrobiome that changes with Postmortem Interval (PMI), all interpretations are based on a small piece of DNA and not the organism itself. Indeed, the recent revival of culture-based microbiology, referred to as culturomics, is gaining momentum in the scientific community. Being able to study a living microbe rather than information from a piece of DNA may provide additional opportunities to understand the relationship between the thanatomicrobiome and PMI estimation.

Previously, this laboratory has been examining the usefulness of a mouse model system to study the thanatomicrobiome. Using an Illumina® MiSeq® platform, DNA sequencing of the V3 hypervariable region of the 16S ribosomal RNA (rRNA) gene indicated that *Clostridium* species dominated as early as seven days postmortem. As we continue to gain a better understanding of the mouse thanatomicrobiome and its usefulness in PMI determination, a culturomics-based approach was initiated to start obtaining information based on the direct isolation of microbes from the mouse thanatomicrobiome.

Mice were sacrificed by CO_2 asphyxiation and placed in sealed containers that allowed air flow for up to seven days. Livers were harvested at PMIs of two and seven days. The harvested livers were homogenized in a saline solution and put through a series of serial dilutions. These dilutions were plated on pre-reduced blood agar plates, placed into an anaerobic chamber, and left to incubate at 37°C until bacterial colonies appeared (about three days). Organisms were isolated and total DNA purified using the ZR Fungal/Bacterial DNA MicroPrep kit. Subsequently, the 16S rRNA gene was amplified by Polymerase Chain Reaction (PCR), followed by DNA sequencing. The basic local alignment search tool (Basic Local Alignment Search Tool [BLAST]) was utilized for bacterial identifications.

The obligate anaerobe *Clostridium* dominated the isolations at both PMIs, followed by the facultative anaerobes *Escherichia* and *Shigella*, which could not be discriminated from each other. Less abundant facultative and aerotolerant anaerobes were also identified. Although a limited number of bacterial isolates were examined, this data supports the *Clostridium* effect dominating the culturable thanatomicrobiome, although no discrete trends were seen. Further examination into the non-*Clostridium* population is needed to ascertain if culturomics can support the metagenomic investigation of the thanatomicrobiome and its usefulness in PMI determination.

Thanatomicrobiome, Culturomics, Postmortem Interval