



### E44 The Application of Eukaryotic Community Succession on Porcine Remains for Postmortem Interval (PMI) Estimation

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After attending this presentation, attendees will understand how changes in the structure of eukaryotic communities found on decomposing remains may aid in the estimation of the PMI.

This presentation will impact the forensic science community by increasing their understanding of methodology currently in place and their awareness of emerging forensic techniques. This presentation will also provide recommendations as to how investigators may better approach crime scenes to preserve and collect evidence for use in necrobiome sequencing. Finally, this presentation will highlight how the application of next generation sequencing may change the way in which postmortem interval is determined by providing a supplemental technique to traditional estimation methods. This presentation will also highlight a novel area of research that provides a useful alternative to traditional PMI estimation techniques by employing next generation sequencing. Furthermore, this study explores the use of eukaryotic communities for such estimations, an area which has not yet been fully explored.

Every cadaver is the host to a complex mixture of prokaryotic and eukaryotic communities, collectively referred to as a necrobiome. Since necrobiomes respond to environmental changes in predictable patterns during the decomposition process, it is possible to use necrobiome succession as a “microbial clock” for PMI estimation.<sup>1</sup> Several recent studies have used bacterial and eukaryotic community succession on murine, porcine, and human remains for PMI estimation; however, these studies either had limited replications or were conducted in a laboratory environment.<sup>2-5</sup>

The main goal of this study was to determine eukaryotic community succession associated with skin of porcine remains for long-term PMI estimation (>1,500 Accumulated Degree Days (ADD) or >60 days) in well-replicated ( $n=6$ ) field conditions. To accomplish this goal, six sets of porcine remains were allowed to decompose in field conditions for 62 days. Samples were collected by swabbing the surface of the skin at the lateral thoracic and lateral abdominal regions every day for the first week, every alternate day for the second week, and once every week after the second week, for a total of 96 samples. Microbial DNA was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) bead-mill extraction process. Sufficient DNA yields for all samples were determined using a Qubit<sup>®</sup> 2.0 Fluorometer.

Hypervariable region V9 of the 18S recombinant DNA (rRNA) gene was amplified according to the Earth Microbiome Project protocol (<http://www.earthmicrobiome.org/protocols-and-standards/18s/>) and amplified products will be sequenced on the Illumina<sup>®</sup> MiSeq<sup>®</sup> FGx platform using a dual-index strategy. Sequence data will then be used to perform taxonomic identification and applied to a statistical model for PMI prediction. It is expected that the changes in eukaryote community structure seen during decomposition on a porcine model may parallel those seen in a human model, given the similarities between the two organisms.

#### Reference(s):

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#### Necrobiome, Postmortem Interval, Next Generation Sequencing