



## H157 Developing Novel Microbial Community Metrics for Predicting Manner of Death (MOD)

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**Learning Overview:** After attending this presentation, attendees will better understand that the community of microbes after death, or the postmortem microbiome, has further forensic utility than postmortem interval estimation. Attendees will see how beta-dispersion profiles, a novel application of the “Anna Karenina Principle,” have predictive power for MOD determination due to the insight postmortem microbiomes provide of antemortem health conditions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic scientific community by presenting how specific postmortem microbiome community metrics may provide a novel tool for MOD determination.

Microbiomes have an important functional role in a host's life, influencing health, development, and disease susceptibility, among many other aspects of human well-being.<sup>1</sup> Microbes also play an important functional role in decomposition after death.<sup>2</sup> Postmortem microbiomes are known to undergo changes specific to anatomical body sites, and follow successional patterns that track the postmortem interval.<sup>2-4</sup> The postmortem microbiome has potential as a tool for postmortem interval estimation. However, the microbial community has additional applications, due to its ability to reflect antemortem health conditions, such as disease state.<sup>3</sup>

As the antemortem microbiome is consistent with the postmortem microbiome within 24 hours after death, microbial community metrics could potentially reveal associations with MOD.<sup>3</sup> The postmortem microbiome is hypothesized to vary depending on a decedent's antemortem health condition related to lifestyle stressors. Stressors that may include, but are not limited to, heart disease, drug/alcohol abuse, and/or a high anxiety lifestyle indicative of certain manners of death (i.e., homicide). This community structure reflected in the postmortem microbiome is thought to vary with the conceptual context of the Anna Karenina Principle. The Anna Karenina Principle asserts that microbiomes of unhealthy individuals have increased beta-dispersion (i.e., microbial community variability) than those of healthy individuals (less variability).<sup>1</sup> The Anna Karenina Principle has shown shifts in microbial communities in living individuals associated with obesity, infection, and smoking.<sup>1,5</sup> Due to the postmortem microbiome reflection of antemortem conditions, the Anna Karenina Principle provides an important conceptual context for quantifying microbial signatures and developing metrics or profiles associated with MOD determinations.

This study sought to determine how beta-dispersion profiles, or beta-dispersion differences associated with certain MODs or antemortem conditions, could potentially be used to confirm MOD assessments—in other words, answering the question: is a postmortem microbiome profile consistent with an MOD assigned in a death investigation? To determine if postmortem microbiomes could be used to answer such a question, 188 United States routine autopsy cases with samples taken from five anatomical sites to test associations of MOD and antemortem conditions, including heart disease, drug use, and gunshot deaths, were reviewed. Previously published amplicon sequence data were analyzed using QIIME2™ (v.2018.11) and beta-dispersion metrics were assessed in R using the *vegan* library to statistically model microbiomes with different MODs and test if the microbial communities varied with the Anna Karenina Principle.<sup>3</sup>

Applying the Anna Karenina Principle to postmortem microbiome beta dispersion profiles resulted in different degrees of dysbiosis and were associated with different MODs, with predictive accuracy that depended on sample type. Preliminary results showed that beta-dispersion differed among body sites, with rectum communities having the highest beta-dispersion, while eyes had the lowest. Across all body sites, natural deaths and suicides had overall higher beta-dispersion than homicides. Also, cases with cardiovascular disease-related deaths had significantly higher beta-dispersion than gunshot or drug-related deaths, while non-violent deaths had higher beta-dispersion than violent deaths. Overall, increased beta-dispersion is more prevalent in MODs associated with disease states (cardiovascular disease and mental health issues) compared to homicides and drug-related deaths. Previous research has confirmed reduced beta-diversity in violent deaths as well as reduced green space access.<sup>3,6</sup> Therefore, the current results support the hypothesis that antemortem conditions are associated with postmortem microbiome beta-dispersion profiles and that such data could have potential utility in death investigations by providing corroborating, or contradicting, microbial evidence of MOD as determined by a reflection of antemortem lifestyle. However, additional studies are needed to confirm and expand these findings.

### Reference(s):

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### Forensic Microbiology, Postmortem Microbiome, Manner of Death Determination

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