A136 Raman Spectroscopy as a Tool to Measure Laboratory Production Processes of *Bacillus Cereus* Spores

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After attending this presentation, attendees will understand the significance of the growth medium composition on the physicochemical properties (phenotypes) of bacteria, the forensic issues associated with microbial threat agent characterization, and the use of Raman spectroscopy with multivariate data analysis in forensic identification.

This presentation will impact the forensic community by not only introducing a rapid spectroscopic technique that can assist in the characterization of microbial evidence, but also by demonstrating how experimental repeatability and reproducibility affect the conclusion reached from the analysis. Because an organism's phenotype is influenced by laboratory culturing conditions, knowledge of the growth media signatures may help provide investigative leads and/or exclude a suspect facility during a forensic investigation.

Analytical procedures to determine signatures for production processes have been established using the analysis of stable isotope ratios, carbohydrates, fatty acids, and agar residues. Raman spectroscopy provides an attractive combination of rapid analysis with minimal sample preparation, non-destructive analysis, small required sample sizes, and low detection limits. Raman spectra are composed of energy-scattering bands due to vibrational movements of chemical bonds in nucleic acids, lipids, proteins, carbohydrates, and small molecules. As a result, this technique reflects the overall composition of the cell and, in turn, provides more information than the aforementioned analytical procedures.

Raman has been proven successful in the identification of bacterial species and strains. Changes in Raman spectra have been observed in the same species grown in different culture conditions; however, the repeatability of spore preparation using the same species of bacteria grown under the same culture conditions has not been fully investigated. Successful discrimination of bacterial spore production recipes requires signature bands specific to a species' metabolism of the culture medium; however, it also requires determination of the minimum within-source variability (variation in repeated preparations of bacteria under the same culture conditions) and its comparison to the between-source variability (variation between different spore production recipes).

The purpose of this research is to use Raman spectroscopy to identify reliable signatures due to the culturing conditions of bacterial spores. The research focused on growth of *B. cereus* T-strain (*Bc*T) in five culture media (each with at least three independent preparations): Modified G (MG); Medium Brain Heart Infusion G (MBG); Schaeffer's sporulation broth and agar; and Columbia Agar with Blood (CAB) to determine the repeatability of Raman spectra of *Bc*T in each of these five media.

To estimate the relative within- and between-source variability, Raman spectra were collected from multiple preparations of *Bc*T spores grown in the various media listed above. For each medium, the mean spectra of the spores and the distribution of all individual spectra belonging to the media were used to represent the variation between preparations. The use of multivariate statistics accentuates subtle changes in the Raman spectra which, in turn, provide an indication of the reliability and reproducibility of the bacterial spectra. Principle Components Analysis (PCA) followed by Linear Discriminate Analysis (LDA) with complete leave-one-out cross-validation was used to classify bacteria based on the Raman shifts that account for the most variability between each preparation.

Raman Spectroscopy, Multivariate Statistics, Microbial Forensics

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