Bacterial Spore Detection (Anthrax)

RTA Application Note # 05

Our ***Simple SERS Syringe Capillaries*** consist of a metal-doped sol-gel immobilized in a glass capillary that can be optically accessed along its length to generate and collect surface-enhanced Raman spectra (SERS). This allows performing rapid separations or extractions and SERS analyses of chemicals at concentrations as low as 1 part-per-billion (1 ppb = 1 picogram per microliter). A simple two-step procedure is followed, in which a syringe is used to draw the sample solution through the sol-gel, and then the Raman optical probe of our ***Industrial Raman Analyzer*** scans the length of the sol-gel. In the present application, the sol-gel is designed to extract the analyte in less than a second. High quality spectra are collected in less than 1 minute.

Extent-of-exposure assessment: Rapid detection of contaminated surfaces can save lives.

The anxiety resulting from the distribution of anthrax causing endospores by terrorists through the U.S. postal system in October 2001 was exacerbated by the extensive time required for positive identification of the *Bacillus anthracis* spores and the unknown extent of their distribution. The subsequent infection and death of several postal workers and national media employees emphasized the critical need for methods that can rapidly detect such biological warfare agents and that can provide emergency management with data such that the extent-of-attack or the extent-of-contamination can be assessed and decisions made. However, the challenges are formidable considering that the Center for Disease Control (CDC) estimates that inhalation of 10,000 *anthracis* endospores or 100 nanograms will be lethal to 50% of an exposed population (LD_{50}). Although polymerase chain reactions, and immunoassays have been developed to augment or replace the standard laboratory method of growing microorganisms in culture media, the first method still requires hours to perform, while the second method produces an unacceptably high rate of false-positive responses.

Dipicolinic acid extracted from *Bacillus Cereus* spores.

As an alternative method, RTA has developed the ***SporeDestroyer*** solution that breaks apart *B. anthracis* spores in 1 minute at room temperature releasing dipicolinic acid (DPA) and the ***SERS-Active Extractive Capillaries*** to concentrate the DPA and enhance its Raman signal. This allows detecting and quantifying as little as 100 spores in 1 minute using our ***Portable Raman Analyzer***. The system is ideal for First Responders. Using DPA as a signature is a viable approach because only spore forming bacteria contain this chemical (as the calcium salt), and the most common, potentially interfering spores, such as pollen and mold spores, do not. Furthermore, Raman spectra of *bacilli* spores are dominated by DPA peaks and these spectra provide a suitable anthrax signature at the genus level.

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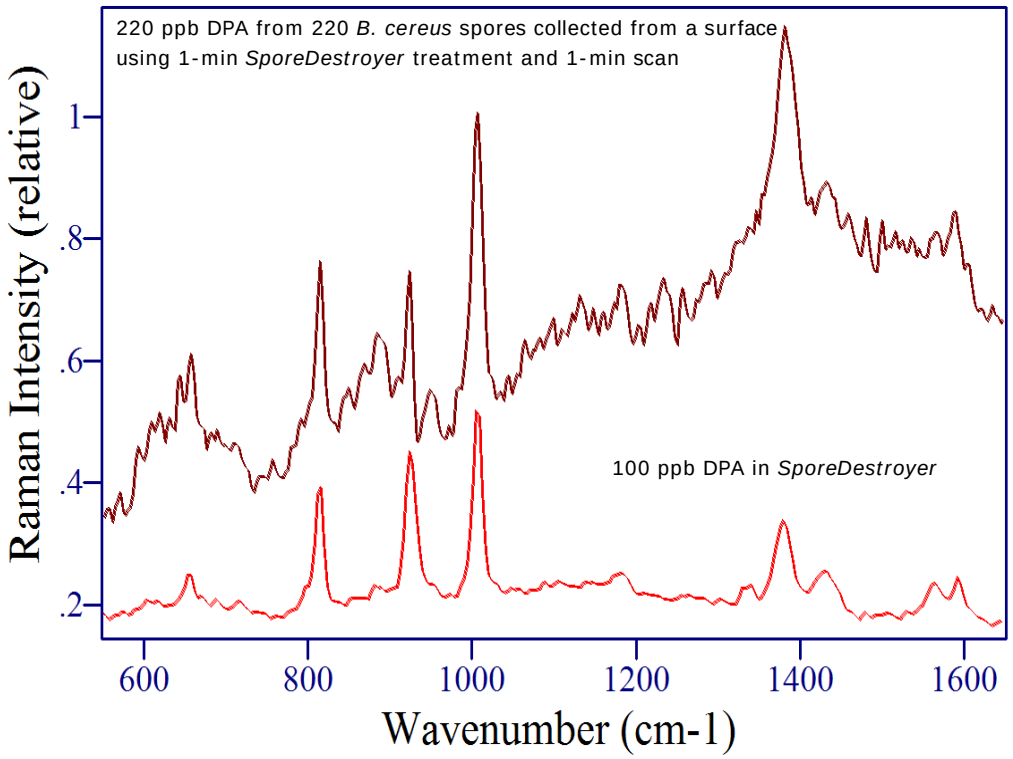
Detection of 220 spores in 2 minutes!

As a simple experiment, 2200 *B. cereus* spores were dried on a surface. To this 10 microliters of RTA's ***SporeDestroyer*** solution were added. After a 1 minute exposure, 2 microliters of this solution were drawn into a ***SERS-Active Extractive Capillary*** and placed on RTA's ***Portable Raman Analyzer***. The spectrum measured in 1 minute using 90 mW of 785 nm laser excitation is shown at right. It is assumed that all of the 10% DPA in each spore was extracted to produce a 220 picogram/microliter DPA solution (220 ppb), well below the LD_{50} set by the CDC. Comparison to an internal SERS reference and a 100 ppb concentration standard confirms this concentration.

SERS of DPA extracted from 220 *B. cereus* spores.



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