A181 Molecular Features Observed in Spark- Induced Breakdown Spectroscopy (SIBS) of Biological Warfare Agents

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After attending this presentation, attendees will understand the principles of Spark-Induced Breakdown Spectroscopy (SIBS), the equipment and techniques used, and how SIBS compares to Laser- Induced Breakdown Spectroscopy (LIBS). The aim of this work was to investigate further use of SIBS for bioaerosols as an alternative to the more expensive and well-known LIBS.

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The development of SIBS will impact the forensic community by introducing a unique method for rapidly screening and analyzing biological warfare agents as airborne pathogens. SIBS has the ability to distinguish between atomic spectra of airborne biological particles. These spectra can then be used to differentiate between harmful and harmless biological species, such as *Bacillus anthracis* (Anthrax) vs. *Bacillus thuringiensis* (Bt). Therefore, SIBS can be used as a real-time trigger sensor for biological warfare agents.

SIBS was initially developed as a real-time sensor for toxic heavy metals in aerosols at detection limits of 1 to 10 mg/m^3. SIBS uses a high- energy electrical spark between two electrodes to ionize, vaporize and excite the elements of the sample of interest. The aerosol sample passes through the spark gap containing rod-shaped electrodes, which ablates the sample creating a plasma. Within this plasma, the ablated material dissociates into ionic and atomic species. After plasma cooling, atomic and molecular emission lines of the sample can be observed. The SIBS apparatus is coupled with a spectrometer and an ICCD detector. The more familiar technique LIBS, on the other hand, is very similar, except that it uses a focused laser pulse as the excitation source. When the highly energetic laser is discharged, it ablates a small amount of the sample and creates a plasma.

Previous experiments compared biological warfare simulants Bt, Ragweed Pollen, and Johnson Grass Smut in the spectral region around 380nm. Both atomic and molecular features (CN (B to X), N_2^+, OH) were present in the previous data. However, the size distributions of the simulants are drastically different. Preliminary results concluded that due to the radically different size distribution, it is very difficult to compare processes of the particles.

In this experiment, a sheath flow system was built as a modification to the existing SIBS apparatus to alleviate size distribution complications. The addition of a sheath flow allows for single particle analysis of various biological samples by eliminating accumulation of particles on the electrodes. The sheath flow system also collimates particles for determination of the upper particle size limit for quantitative analysis of aerosols. Biological warfare simulants Bt, ragweed pollen, and johnson grass smut were again compared in the spectral regions around 300, 380, and 442nm. Biological samples of similar size distribution were also compared using the sheath flow system. This presentation will include data acquired in each of the three spectral regions at a variety of delay times. This presentation will also include a comparison of the molecular blood enhancement reagents are typically employed to locate residual blood. Luminol is a very sensitive blood enhancement reagent. Unfortunately, the reagent must be prepared fresh and the crime scene darkened in order to see the short-lived chemiluminescent blue-light emission. Bluestar© is a relatively new product which uses a modified luminol reagent, emitting an intense blue light in the presence of blood. Hemascein™ is a new commercial product emitting a green light when excited with an intense blue light source.

Criminals have also used fire to destroy any incriminating evidence. In these situations, bloodstains may be completely destroyed, difficult to locate in the fire debris, or difficult to recognize due to the charring of surfaces by the intense heat. This study evaluated the effectiveness of luminol, Bluestar©, and Hemascein™ as screening reagents when testing burnt bloodstain samples. The three reagents were tested for their intensity and duration of light emission using a Bio-Rad VersaFluor™ fluorometer. With the Luminol and Bluestar© reagents, the excitation light from the instrument was blocked and only an emission filter of 460 ± 5 nm was used. With Hemascein™, an excitation filter of 460 ± 5 nm and an emission filter of 520 ± 5 nm were used. To determine the features CN (B to X), N_2 and OH sensitivity of detection of each reagent, dilutions of canine blood were

The results of this and other studies demonstrate that biologicalsamples have unique molecular features that behave differently, and this information can be used for more complete sample analysis. SIBS can spectrally distinguish between biological and non-biological samples, as well as distinguish between biological samples within the same species. The low detection limit, sensitivity, and discrimination potential of

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SIBS indicates this system as an alternative to the costly LIBS system.

SIBS, Bioaerosols, Air Sampling

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