

A Suggested Procedure for Sampling "Suspect White Powders" Where Law Enforcement Agencies Require a "Credible Threat" Before Responding

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Immediately following the bioterror associated anthrax cases in September, there were an unprecedented number of reports of "suspicious white powdery substances" nationwide. Some of these reports were hoaxes. Others were honest cases of mistaken identity. A very few involved anthrax spores.

An incident on our campus caused the temporary closing of a portion of a dormitory building and disrupted the activities of a large number of students, faculty, and staff. When campus officials contacted the FBI for assistance in evaluating the suspect material they were advised that the bureau was so overwhelmed by reports of suspicious substances that they were unable to respond effectively unless additional circumstances suggested a credible biohazard threat. The material in our incident eventually proved to be laundry detergent. Faced with the potential for major disruption of campus activity by similar incidents, a group of administrators, campus police officers and Environmental Health and Safety (E.H.S.) staff sought to develop an in-house procedure to examine suspect materials for the possibility of anthrax contamination.

After considering and rejecting several high tech, expensive, approaches to anthrax identification, phase contrast light microscopy was identified as the simplest, most reliable, and economical method. The high refractive index of bacterial endospores, and their characteristic size and morphology, facili-

tate identification by an experienced observer (Figure 1). Phase contrast microscopy will also demonstrate the presence of other microbial forms such as vegetative bacterial cells, fungal hyphae and spores, and protozoans. It may contribute to characterization of dispersant materials (*i.e.*, bentonite) mixed with the spores.

Phase microscopy has several obvious limitations as well. The first is the restriction to cases where there is a macroscopic bulk of suspect material in which the endospores constitute a reasonable portion of the total, say 1% or more by volume. Therefore it could not, for example, be used to monitor ventilation systems for trace contamination.

Secondly, phase microscopy alone cannot distinguish *Bacillus anthracis* endospores from those produced by many other species of *Bacillus* and *Clostridium*. We assumed that the presence of any bacterial endospores would elevate a "suspicious powder" to the status of credible threat and should serve to elicit a vigorous and timely response from higher authorities.

A third limitation is that viral pathogens are undetectable.

We designed a simple sample kit consisting of two polypropylene micro centrifuge tubes with a nominal capacity of 2 mL. The tubes have threaded caps and neoprene O-Rings for positive sealing (Sarstedt 72-693). One of the sample tubes ("analysis tube"; see Figure 2) contains 200 microliters of 10% aqueous glycerol with a trace of basic fuchsin dye. The other tube ("reference tube") is left empty. Inasmuch as the sample material must be suspended in an aqueous fluid for microscopy, we decided to complete this step in the field, as part of the sampling itself, to minimize potential aerosolization of the sample. The glycerol in the sample suspension fluid will further minimize aerosolization in the event of spillage and evaporation. The dye enhances visualization of small droplets. Neither the dye nor the glycerol interferes with microscopy. The suspect material is gathered, and samples placed into both the reference and the analysis tubes.

The two small sample tubes are then placed in a larger, 15 mL capacity polypropylene screw-capped tube (Falcon 352097)

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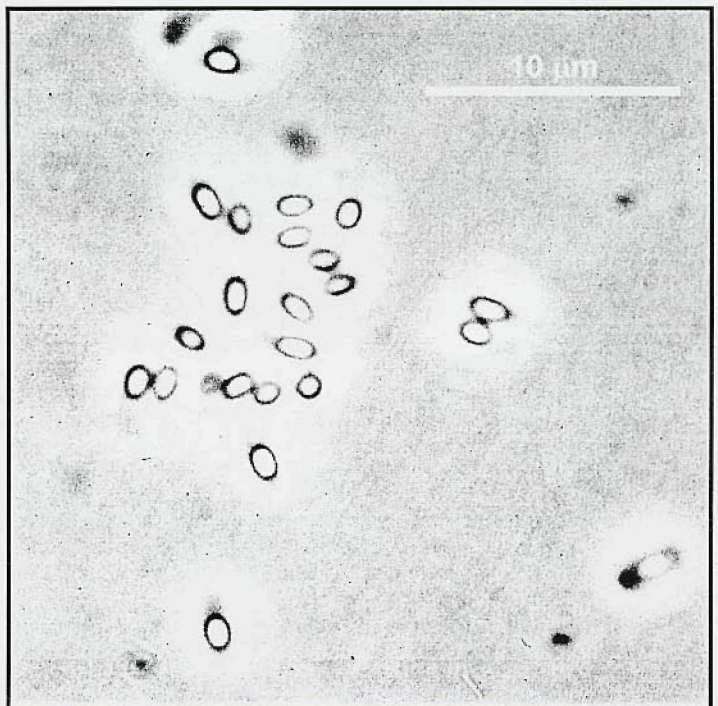


Figure 1. Phase contrast micrograph of *B. anthracis* spores. Note that they look much like any other bacterial spores, and cannot be identified as *B. anthracis* by microscopic imaging alone.

as secondary containment. This in turn is placed in a biohazard bag for transport.

Collection and analysis of suspect materials is not a casual activity and is done with serious consideration of potential risks. Samples are collected by trained campus E.H.S. personnel with appropriate personal protection gear as an integral part of their response plan.

In the laboratory, the containment tube is immersed in hypochlorite solution for 30 minutes to effect surface decontamination. The containment tube is opened and filled with hypochlorite solution for 30 minutes to decontaminate the exterior of the two sample tubes.

A small volume of the sample suspension is transferred to a microscope slide with coverslip and the coverslip is sealed to the slide. Following visual observation, the specimen is documented by photomicrography.

The reference sample tube remains unopened in the secondary containment tube and is retained for further analysis.

This analysis is conducted in a facility with practices and equipment consistent with Biosafety Level 2*. Sample handling is done in a certified Type II biosafety cabinet and all sample-exposed materials are immediately autoclaved. ■

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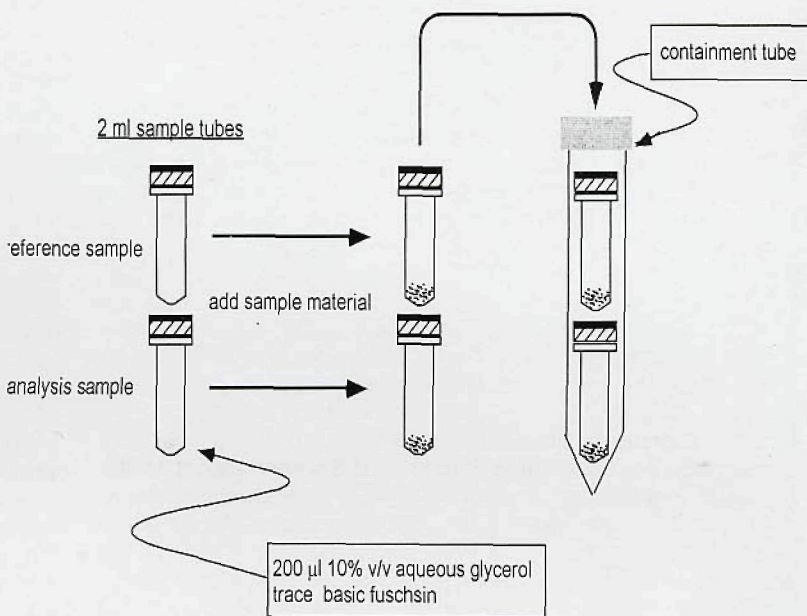


Figure 2. Sampling procedure to determine if a "suspect white powder" contains bacterial spores and therefore is a "credible threat".

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