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DEVELOPMENT OF AN AEROSOL SYSTEM FOR CREATING UNIFORM SAMPLES OF DEPOSITED BACTERIA. *PAUL BARON, Cherie Estill, Terri Schnorr, National Institute for Occupational Safety and Health, Cincinnati, OH John Wright, Greg Dahlstrom, Jeremy Beard, Daryl Ward, Dugway Proving Ground, Dugway, UT Wayne Sanderson, University of Iowa, Iowa City, IA*

In the aftermath of the anthrax incidents in October 2001, it was apparent that techniques for sampling surfaces for biological agents had not been validated. Several techniques for biological particle removal from surfaces existed but gave varying and uncertain sampling efficiencies, especially at low surface loadings. A project was initiated to develop a system for producing sets of samples having targeted surface concentrations of biological agent-containing particles. Particles aerosolized from a dry powder were to be allowed to settle onto surfaces to simulate the results of the anthrax incidents. A 4' x 4' x 8' test chamber was constructed of static dissipative plastic. Particles were aerosolized using a modified Small Scale Powder Disperser (TSI, Inc.), size selected to less than 5 micrometers using an impactor, and deionized by mixing with air from a bipolar ion source. The aerosol was initially dispersed into the chamber at relatively high air concentrations and monitored using a TSI Aerodynamic Particle Sizer (APS, TSI, Inc.). The aerosol in the chamber was stirred using several fans and the particle concentration in the chamber allowed to decay using stirred settling and dilution (HEPA filter and pump). When the desired air concentration was reached, the sampling surfaces were uncovered and exposed to the (stirred) settling particles. A sample handling and covering system was designed to allow sample manipulation through several glove ports on one side of the chamber as well as sample collection from individual surfaces after exposure. After the desired fraction of particles had settled on the surfaces, the chamber air was flushed clean with HEPA filtered air. Subsequently, the chamber was opened and the surfaces were sampled or removed for evaluation. The APS provided the particle concentration in the chamber, allowing estimation of the number of particles deposited on the surfaces. Four types of surface samples were exposed: agar plates (8), silicon wafers (8), stainless steel rectangles (9), and carpet rectangles (9). The agar plates allowed determination of the colony-forming-unit (CFU) surface concentration. The silicon wafers were evaluated using a light scattering system (Surfscan, KLA-Tencor Inc.) to test for surface deposit uniformity. The stainless steel and carpet surfaces were used to evaluate surface wipe and vacuum sampling techniques. An initial test of the chamber using B. atrophaeus var. globigii (BG) indicated agar surface sample CFU variability of 15% relative standard deviation. Further improvements are planned to reduce this to about 5% and to ensure proper containment of potentially harmful bacteria. Analysis of the samples will be performed by culture techniques and polymerase chain reaction amplification. Tests will be performed with several biological warfare agent simulants. These measurements will allow the estimation of sensitivity, precision, and bias of the surface sampling and analytical methods.

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THE EFFECT OF FILTER MATERIAL ON THE BIOAEROSOL COLLECTION EFFICIENCY: EXPERIMENTAL STUDY UTILIZING BG SPORES AS BACILLUS ANTHRACIS SIMULANT. *NANCY CLARK BURTON, Atin Adhikari, Sergey Grinshpun, and Tiina Reponen, Center for Health-Related Aerosol Studies, Department of Environmental Health, University of Cincinnati, Cincinnati, OH, USA*

Recent bioterrorism events have initiated new interest in developing environmental sampling methods to assess the potential for human exposure to biological aerosol agents. Sampling performed during the clean-up process in the anthrax-contaminated buildings in 2001 utilized a combination of direct agar impaction, wipe, and filter collectors to determine B. anthracis endospore levels. In this study, a laboratory evaluation was conducted to examine extraction efficiency and culturability for Bacillus subtilis var. niger (BG) endospores as a surrogate for B. anthracis. BG spores were aerosolized using a six-jet Collison nebulizer. Four types of filters were tested: mixed cellulose ester (MCE) filters with a pore size of 3 µm, polytetrafluoroethylene (PTFE) filters with pore sizes of 1 and 3 µm, and gelatin filters with a pore size of 3 µm. All filters had porosities of 60% to 80%. Button Inhalable Aerosol samplers, operating at a flow rate of 4 L/min, were used to collect endospores for three sampling periods: 15 minutes, one hour, and four hours. Physical collection efficiency (PCE) was determined by measuring the concentration of BG spores upstream and downstream of the filter with an optical particle counter (OPC). Two extraction methods were tested: vortexing/ultrasound agitation and vortexing/shaker agitation. Culturable count was performed by cultivating the extracted suspension on trypticase soy agar, and total count was conducted by using acridine staining in conjunction with an epifluorescence microscope. Microscopic analysis of the gelatin filter extraction fluid revealed the presence of bacteria, other than B. subtilis, which were also in the media blanks. These species, however, were not identified in the other filter samples. No contamination was found on the culturable plates indicating that the bacteria were rendered non-viable during the gamma sterilization process. The MCE, 1 µm PTFE, and gelatin filters had PCEs of 94% or more. The 3 µm PTFE filter showed inconsistent PCEs between filters (64 ± 32% for eight filters) and was not used for the rest of the experiments. The relative culturability (culturable count/total count) using the vortex/ultrasonic extraction method for the MCE, 1 µm PTFE, and gelatin filters ranged from 72 to 130%; 93 to 100%; and 100 to 126%, respectively. The corresponding values when using the vortex/shaker extraction method ranged from 24 to 88%; 59 to 130%; and 72 to 100%. The differences for relative culturability using the two extraction methods were not statistically significant for the three filters using ANOVA. Extraction efficiencies for the MCE and 1 µm PTFE filters were 66 to 162% and 77 to 153%, respectively. These results showed that MCE and 1 µm PTFE had the best performance among the tested filters for the collection and cultivation of B. subtilis endospores and, therefore, should be similarly effective for B. anthracis samples.