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Via Electronic Mail Hardcopy to Follow

TO: Freeman Swank, Sceptor Industries
FROM: Mark Hernandez, PhD, PE, Principal Investigator

RE: SUMMARY OF COLLECTION EFFICIENCY and RETENTION OF MONODISPURSED PURE CULTURE AEROSOLIZED *Aspergillus fumigatus* spores, vegetative *Bacillus subtilis* cells, and latex heads UNDER DEFINED ENVIRONMENTAL CONDITIONS (25C @ 40% RH)

OVERVIEW: Experiments were performed by challenging two OMNI 3000 aerosol sampling devices, fitted with custom glass impingers, with pure culture stocks of airborne microorganisms and latex spheres. A consecutive series of 5 identical collection assessments was performed by independently aerosolizing known quantities of each of the following agents: *Aspergillus fumigatus* spores (mean aerodynamic diameter (MAD): 2.5 μ m), vegetative *Bacillus subtilis* cells (MAD: 1.25 μ m), and two types of fluorescein labeled latex beads (mean aerodynamic diameters of 0.5 μ m and 2.5 μ m). The challenges were executed at 25 °C and 40 % RH.

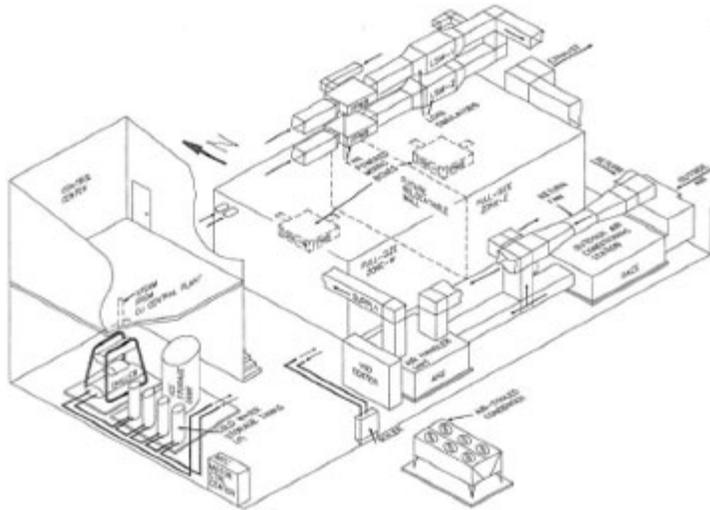
All agents were aerosolized into a 90m³ room containing the OMNI 3000 units, the recovery of which was directly compared with widely accepted reference samplers (SKC biosamplers, SKC Inc., Eighty Four, PA). *Aspergillus fumigatus* was chosen as a candidate for this study because the spores of this microorganism have been used as a surrogate for pathogenic fungi in many bioaerosol studies, and the size response of *Aspergillus* spores to relative humidity changes is negligible (Reponen et al., 1996). Results from challenges with these spores are widely-accepted to conservatively approximate collection behavior of bioaerosols containing a wide variety of molds. This analogy applies to *Bacillus* spp. cells as an environmental surrogate for many pathogenic Gram-positive bacteria. We used *B. subtilis* for these trials, which is widely accepted as a surrogate for the environmental behavior of the biological weapons agent *B. anthracis*. Results from challenges with these Gram-positive cells are widely accepted to conservatively represent the collection behavior of resilient pathogenic bioaerosols (references available upon request). All cultures used in this study were supplied by the American Type Culture Collection (ATCC) (Manassas, VA), and latex spheres used in this study were provided by Polysciences Inc, Warrington, PA.

Microbiological Stocks: Bacteria were grown on Soybean-Casein Digest Agar (SCDA) at 37°C, and *Aspergillus* spores were grown on Malt Dextrose Agar (MEA) at 20°C; all media was supplied by Difco Laboratories (Detroit, MI). Just before aerosolization, bacteria cells were removed from agar plate surfaces by aseptic scraping with a sterile glass rod, and were suspended in a 15 mM phosphate buffer solution (PBS; 10 mM sodium phosphate buffer; 5 mM NaCl; pH 7.2). Just before aerosolization, *A. fumigatus* spores were removed from plate surfaces by aseptic shaking with 3-mm glass beads (Fisher Sci., Pittsburgh, PA), and were suspended in sterile water with addition of 0.1% Tween 80 (Sigma, St. Louis, MO).

EXPERIMENTAL DESIGN and FACILITIES: The University of Colorado has unique pilot (90m³) bioaerosol facilities with the capability to support live bioaerosol challenges of full-scale disinfection equipment. This facility is designed to generate bioaerosols in conditions representative of many aerosol environments, but allow stringent control of environmental factors (temperature and humidity) that bioaerosols experience prior to, and during their collection. To achieve this control, we installed two MINI 3000 within our full-scale laboratory chamber, and executed 5 consecutive collection tests to assess variability. The collection tests were executed in a simple, well-mixed configuration, which characterizes the ability of the equipment to collect airborne microorganisms in a sustained exposure.

Technical facilities description. A schematic diagram of the bioaerosol laboratory is shown below. Two 90-m³ chambers are housed inside the laboratory; one of these rooms was used as a test chamber to challenge the OMNI 3000 with bioaerosol. The floor-to-ceiling height is 2.4 m (8 ft) and contains 37 m² (400 ft²) of clear floor area. The room is capable of maintaining a stable temperature in the range 50-95°F and an RH in the range 10% to 95%. The room has insulated walls, a raised floor, plenum ceiling, one door and no windows. The test room is equipped with a computer controlled ventilation system that delivers a minimum of 2 ACH and a maximum of 8 ACH of high-efficiency-particulate-air (HEPA) filtered outside air through two circular diffusers located in the ceiling. The equipment tested is placed in the test chamber and surrounded by bacterial bioaerosol much as it would be in any field setting.

Test chamber environmental



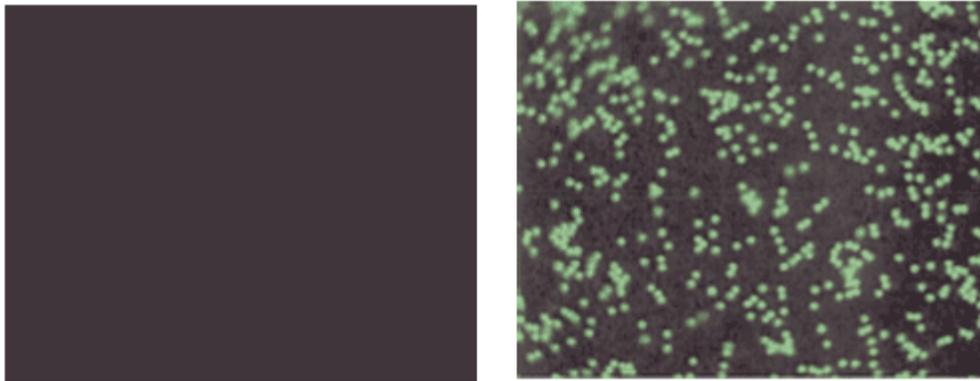
and bioaerosol control. Test chambers were maintained at the designated temperature and relative humidity for experimental preparation – in this case c.a. 25 C and 40% RH. Prior to each experiment, the test-chambers were purged with particle-free air. The test chamber was then sealed and maintained in the pre-determined constant temperature and humidity condition for at least 3 room air exchanges to achieve physical steady-state conditions. Bacteria and fungi were then aerosolized into the room at a constant rate until a uniform airborne microorganism concentration of near 10⁶ / m³ was attained; thus, steady-state was achieved for both physical and biological conditions — these conditions force bioaerosols to equilibrate with the room air prior to their induction into the OMNI 3000 systems. The same approach was used for the polystyrene latex beads, but higher concentrations were achieved. The chamber was always maintained under slight negative pressure with respect to the surrounding laboratory.

Experimental approach for determining collection efficiency. During this study, we modified the experimental designs used successfully in previous full-scale room air UV disinfection studies, to assess the effectiveness of the stand-alone OMNH units. Active bacteria stocks (> 90% culturable/total) were prepared and aerosolized into the chamber using a jet-type Collision nebulizer according to widely accepted bioaerosol dispersion methods (Hernandez et al, 1999); latex beads were aerosolized using a common plastic medical nebulizer provided by Sceptor. Direct microscopic examination of bacteria suspended in the nebulizer reservoir were used to confirm the numbers, size distribution, and dispersed state of cells/spores/beads during their aerosolization. Air samples collected from the air in the full-scale chamber by liquid impingers and filters, were taken at the exact same time the OMNH units were operated in 5 sequential trials. The experimental methodology was a static test — a technique based on the generation of a discrete # of particles to attain a peak concentration, which slowly decays during the experiments. The room air was always maintained in a well-mixed state (the mixing regime of the room air has been previously verified using SF6 tracer studies (Xu et al, 2002)). Prior to air sample collection, biological buffers were added to the contents of the SKC sampler collection reservoirs to prepare the captured microorganisms for staining and microscopy. Direct microscopic counts of airborne microorganisms recovered by the SKC reference samplers, filters and OMNI were performed by skilled technicians, which were "blind" to the sample origins. By using microscopy with sensitive biological stains we directly determine the recovery of the OMNI with respect to the reference SKC sampler when normalized to the volume of air sampled and estimated.

Bioaerosol Collection. We modified the experimental designs used successfully in previous full-scale room air disinfection studies, to assess the collection efficiency of the OMNI units. In this and previous studies, a time series of airborne bacteria, spores and spheres were collected from the bulk test-chamber air using well-characterized swirling-type liquid impingers (Willeke et al. 1998) and filters (10 min collection runs) juxtaposed to OMNH collection (5 min collection runs). Control valves connected to flow meters routed bioaerosol-laden air to impingers, each of which was wrapped with opaque material to shield any collected airborne bacteria from light.

Sampler location and operations. SKC liquid impinger biosamplers were centrally placed within chamber surrounding the unit tested as well as in the influent and effluent chambers of the OMNH units. Bioaerosol was collected for 10 min. at 12 L min⁻¹ with a high-flow sampling pump regulated with rotameters. The impingers concentrated the organisms in 15 ml of sterile, phosphate buffered saline; previous studies showed minimal damage to the organisms during impinger operation (Willeke et al, 1998). Both blanks and spiked samplers were included in all experiments for quality control. The ventilation system was operated such that the room air (and suspended bioaerosol) in a completely mixed condition throughout the experiments. These generation and collection systems have been well tested and are widely accepted in the scientific aerosol literature for bioaerosol studies. During the time frame of observation, removal of airborne bacteria by deposition was negligible.

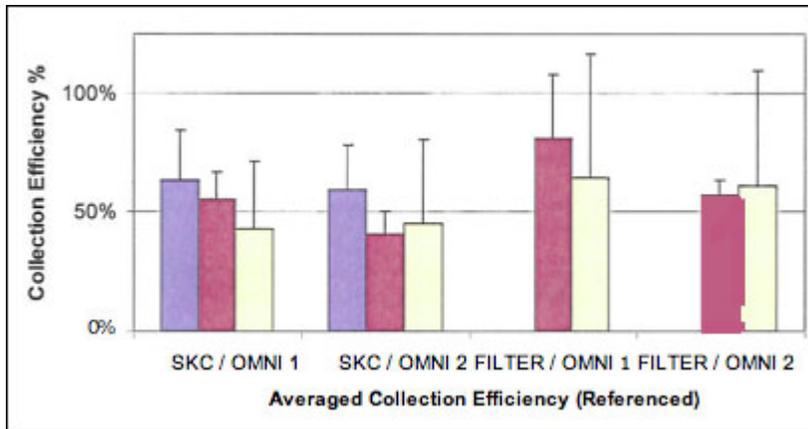
Retention Observations: To assess gross retention, the glass collection devices in both OMNI 3000 units were directly "spiked" with known quantities of *B. subtilis* cells and *Aspergillus* spores from liquid suspension using a calibrated transfer pipette (c.a. 10⁶ cells), while the units were operating, in a clean room. The OMNI units were then allowed to operate for either 3 or 60 minutes, and the quantity of microorganisms was assessed at the end of the time period with respect to the quantity originally introduced. The units were cleaned and the process repeated.



Microscope photographs (x1000) of microorganisms and latex spheres typically recovered from the OMNI devices following aerosol challenges. Left *Aspergillus* spores stained with the DNA intercalating agent DAPI (mean aerodynamic diameter c.a. 2.5 µm) ; Right 2 µm diameter polystyrene latex spheres labeled with fluorescein. These preparations were typical of that used were direct microscopic counting of OMNI and reference retention.

Bioaerosol Analysis. Within 1 hour after collection, OMNI-, filter- and impinger-recovered aerosol samples were be diluted for microscopy. All sample preparation was performed under low intensity, indirect, fluorescent light. The bioaerosol quantitation practices used herein have accepted in the bioaerosol literature to determine total airborne bacterial numbers using sensitive biological stains in accordance with established microscopy methods (Hernandez et al, 1999). The bacteria collected from air samples were incubated with the DNA intercalating agents DAPI or SYTO-9 under the appropriate conditions, and some of the sampler reservoir contents passed through 0.2-µm (pore diameter) polycarbonate membrane filters. The membrane filters were subsequently mounted on microscope slides and stained bacteria and spores identified and counted under UV illumination (x 1100) using widely accepted methods (see reference list).

RESULTS: All sampling runs were challenged with greater than 10⁶ cells (latex spheres) /m³ —a level high enough to support observations for statistical analysis. Shown below is a summary of the collection efficiency of two OMNI 3000 units.



Averaged collection efficiency of two OMNI 3000 bioaerosol samplers with respect to SKC liquid impingers and polycarbonate filters operated at the same time, Averaged collection efficiency for *Aspergillus fumigatus* spores (e), 0.5 um PSL beads (e), and 2.0 um PSL beads (), corresponds to bar height. Error bars represent pooled standard deviation (n 5).

Averaged retention of two OMNI 3000 bioaerosol samplers spiked with c.a. 106 cells/spores incorporating the after 60 minutes of operation. Steady state retention is defined here as the difference between direct microscopic count after 3 minutes and 60 minutes of operation. Averaged retention for *Aspergillus fumigatus* spores (N), and *Bacillus subtilis* cells (0), corresponds to bar height. Error bar represents propagated error of difference between 3 and 60 minutes of operation using pooled standard deviations (n = 3).

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