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December 15, 2013

Via Electronic Mail
No Hardcopy to Follow

FROM: MARK HERNANDEZ, Principal Investigator

RE: RESULTS SUMMARY FOR STATIC INACTIVATION OF MONODISPURSED PURE CULTURE *Bacillus subtilis UNDER* DEFINED ENVIRONMENTAL CONDITIONS (25C @ 40% RH)

A time series of experiments was performed by immobilizing known quantities of viable *Bacillus subtilis* cells on the filter materials used in SECUREAIRE Air filter units. The filters hosting bacterial cells were exposed to a defined electric field in a pilot-scale system (c.a. 2 ft^2) for 3 hours under static conditions (no flow); these filters were allowed to equilibrate for 3 with the relative humidity of the test laboratory immediately following their inoculation, but prior to their introduction to the pilot units.

Bacillus subtilis was chosen as the candidate for this study because the cells of this bacterium have been used as a model for the environmental behavior of gram positive bacteria in many military and scientific bioaerosol studies. Results from challenges with these cells conservatively approximate disinfection response of bioaerosols containing common pulmonary pathogens. The isolation of bacterial cells is a laborious process, and a detailed protocol is provided in separate document.

EXPERIMENTAL DESIGN: A stock suspension of pure *Bacillus subtilis* cells (American Type Culture Collection # ATCC 14227) was passed through SECUREAIRE filters that were cut into circular, 47 mm diameter coupons for this study. The quantity of cells immobilized on the filter surfaces was confirmed direct microscopic counts of cells eluted from the filter surfaced, using widely accepted methods (Hernandez et al, 1999 and Henningson et al 1997).

Immediately following liquid transfer, filter-immobilized cells were introduced to the electrical field for approximately 3 hr, at 24 ^{o}C, and 40% RH. Following exposure, the filter coupons were submerged in 10 mL sterile phosphate buffered saline using aseptic technique, and the immobilized cells were eluted by gentle shaking for two hours at room temperature. The process was repeated in independent duplicate experiments each with triplicate coupons in the pilot units, as well as otherwise identical coupons that served as controls (no electric field).

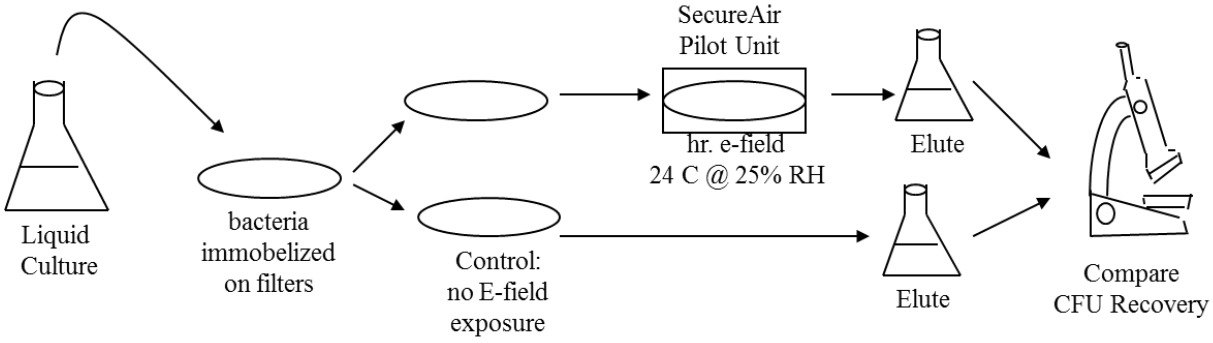

Figure 1. Flow chart highlighting the transfer, exposure and recovery of *Bacillus subtilis* vegetative cells.

RESULTS: All filters were equivalently loaded with greater than 100 million viable *Bacillus subtilis* cells, at a surface loading density greater 10^7 cells/cm^2 on the filter surface — a level great enough to support observations for statistical analysis.

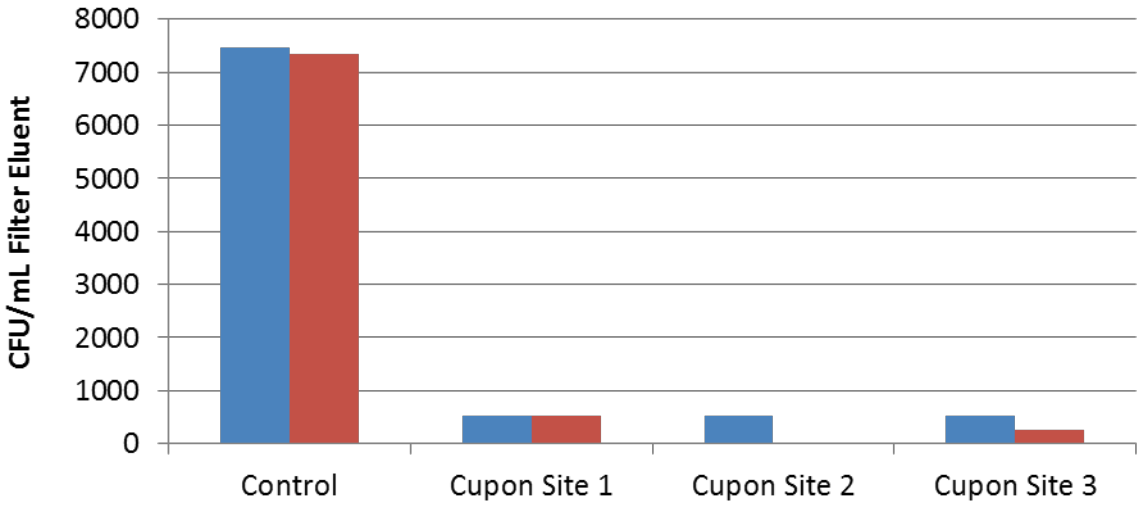

Figure 2. Standard heterotrophic plate counts of *Bacillus subtilis* cells recovered from filters surfaces following 3 hr static electric field exposure at 24 ^{o}C and 40% RH (replicate 1 ■; replicate 2 ■).

DISCUSSION: Exposure to electric field and associated charge under these conditions caused a significant drop in the recovery of *B. subtilis* cells from the filter media coupons installed in these pilot units. As judged by elution and standard plating, exposure in the SECUREAIRE platform reduced total cell numbers between 90% and 99% in three hours. When controlled for aging and desiccation effects, the application of a weak electric field across SECUREAIRE fiber filter media hosting otherwise healthy *Bacillus subtilis* cells appeared to cause damage to these cells such that they could not be could no longer be recognized as microbiological agents by direct microscopic analyses or eluted from filter media.

Henningson, E. W., M. Lundquist, E. Larsson, G. Sandstrom, and M. Forsman, 1997, A comparative study of different methods to determine the total number and the survival ratio of bacteria in aerobiological samples.: Journal Aerosol Science, v. 28, p. 459

Hernandez, M., S. L. Miller, D. W. Landfear, and J. M. Macher, 1999, A Conmbined Fluorochrome Method for Quantitation of Metabolically Active and Inactive Airborne Bacteria: Aerosol Science and Technology, v. 30, p. 145.

Peccia, J., Werth, H., Miller, S. L., and Hernandez., M. 2001 The Effect of Relative Humidity on the UV-induced Induced Inactivation of Airborne Bacteria. *Aerosol Science and Technology* v. 35 p. 728-740.