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Via Electronic Mail
No Hardcopy to Follow

TO: David Black, Kloppenberg Inc.
FROM: Mark Hernandez, PhD, PE, Principal Investigator

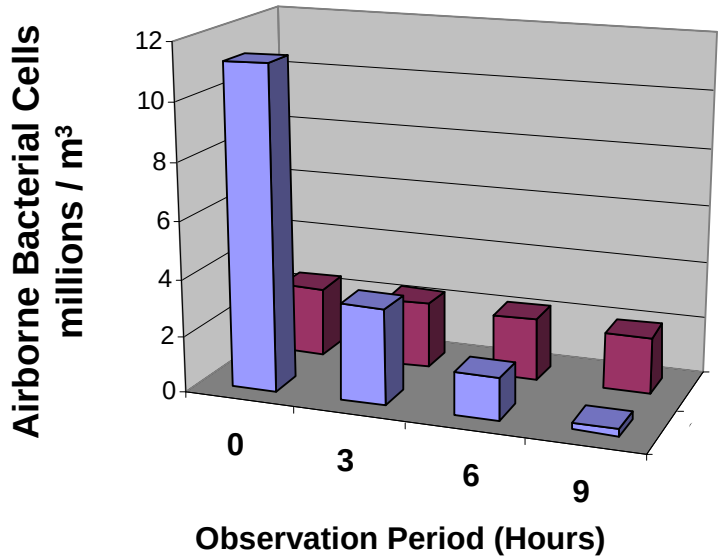
RE: RESULTS SUMMARY FOR MULTIPLE CLEAN AIR DELIVERY RATE (CADR) OBSERVATIONS OF ACCENTS IN WATER FEATURES CHALLENGED WITH MONODISPURSED PURE CULTURE BACTERIAL BIOAEROSOL UNDER DEFINED ENVIRONMENTAL CONDITIONS

OVERVIEW: Replicated experiments were performed by challenging three different ACCENTS IN WATER features, with known quantities of aerosolized *Mycobacterium parafortuitum* cells and *Bacillus subtilis* cells and spores. These cells were harvested fresh and healthy from laboratory broth culture in logarithmic growth phase. The challenge was executed at 30 ± 2 ^{o}C and 50 ± 5% RH.

Operational Challenge: We used *Mycobacterium parafortuitum* for these trials, because they are widely accepted as a surrogates for the environmental behavior and disinfection response of nosocomial diseases agents which cause Tuberculosis. *Bacillus subtilis* was also used for this study because this bacterium has been used as a surrogate for pathogenic bacteria in many bioaerosol studies, and the size response of of *M. parafortuitum,* and *B. subtilis* cells to relative humidity changes is negligible (Peccia, et al, 2001). Results from challenges with these Gram-positive cells are widely-accepted to conservatively represent remove response of resilient and pathogenic bioaerosols (exhaustive reference list available upon request).

EXPERIMENTAL DESIGN and FACILITIES: The University of Colorado has unique pilot (90m^3) 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 transit through a test system. To achieve this control, we installed the ACCENTS WATER FEATURES within our full-scale laboratory chamber, and executed the tests defined by the operational configuration presented in the proposal (delivered summer 2007). One mode was a CADR test which characterizes the airborne microbe removal capability of these features in a recirculation mode representative of performance in a well-mixed room. The interpretation of the equivalent **C**lean **A**ir **D**elivery **R**ates (CADR) observed here can be extended to the operation of a water feature in an ideal environment: Where the CADR reported in units of volume/time (m^3/hour) is representative of how much air a given feature can render free of these bacteria in an hour.

RESULTS SUMMARY to DATE: Total airborne bacterial numbers were directly determined with sensitive biological stains in accordance with established microscopy methods (Hernandez et al, 1999) – For statistical purposes all DIRECT MICROSCOPIC AEROSOL COUNTS are taken as an average of 10 observations from at least three independent trials. **CLEAN AIR DELIVERY RATES** associated with the operation of each waterfall are summarized below for each of the different bacterial cultures tested.



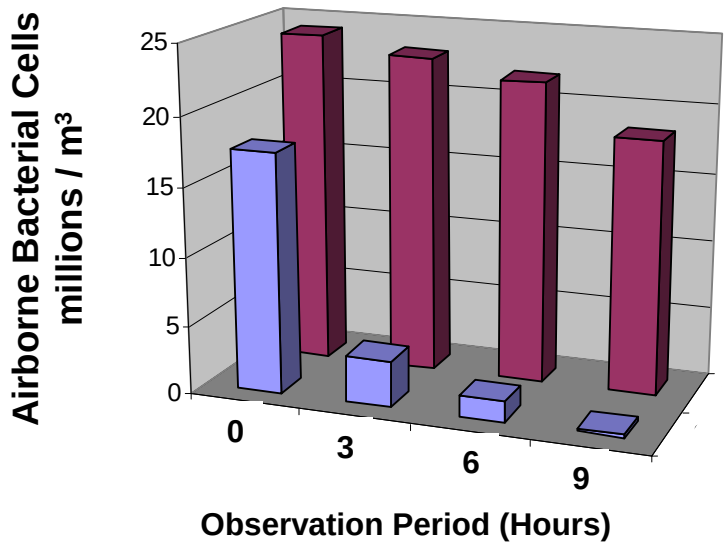
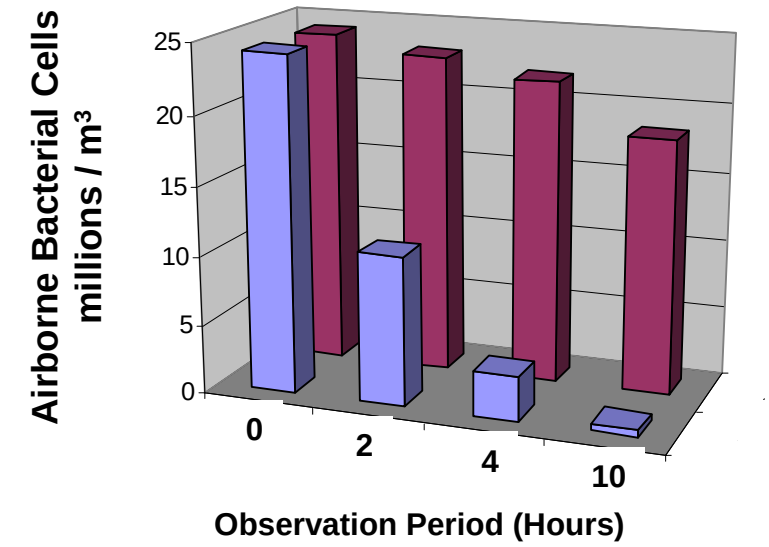


Figure 1. Room air concentration of *Bacillus subtilis* cells in during the operations (■), or disengagement (■) of a 2' x 3' stainless wire mesh ACCENTS water feature in a 90 m^3 bioaerosol chamber operated in a static, well mixed mode. In independent trials, room air was charged with the airborne bacteria

concentration noted at time zero, and monitored for 10 hours.



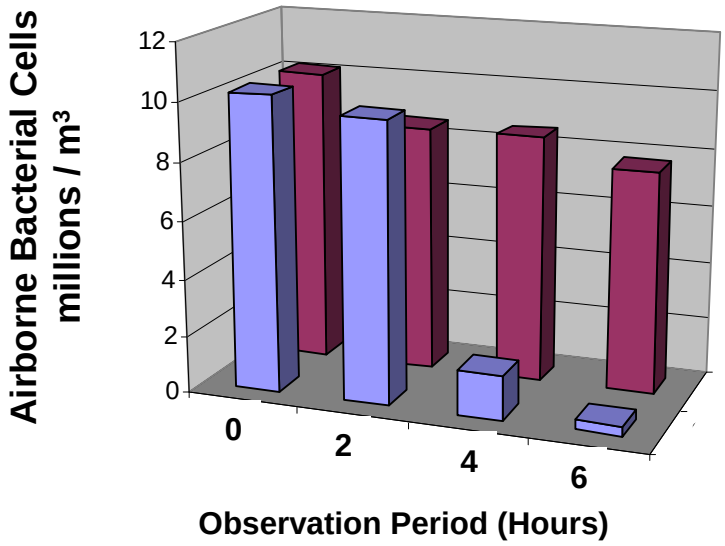


Figure 2. Room air concentration of *Bacillus subtilis* cells (top), and *Mycobacterium parafortuitum* cells (bottom) during the operations (■), or disengagement (■) of a 3' x 7' stainless wire mesh ACCENTS water feature in a 90 m^3 bioaerosol chamber operated in a static, well mixed mode. In independent trials, room air was charged with airborne bacteria concentration at the room air concentration noted at time zero, and monitored for between 6 and 10 hours.

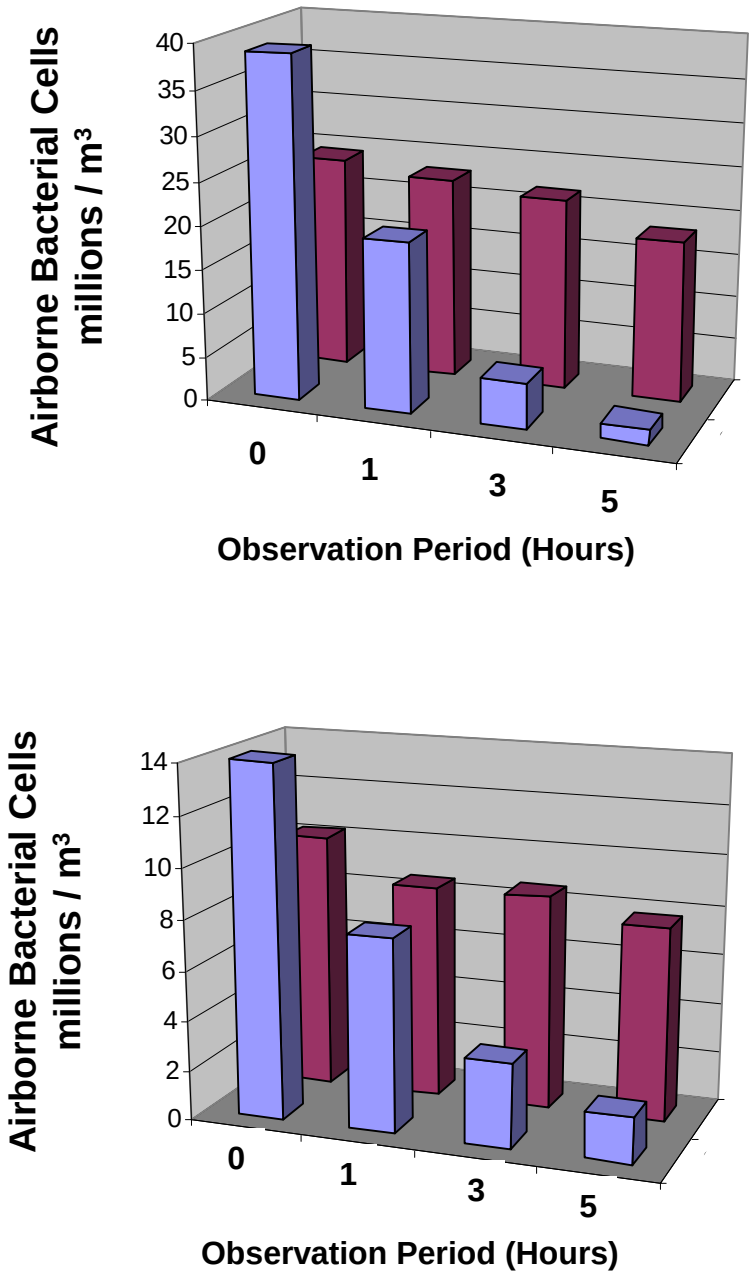
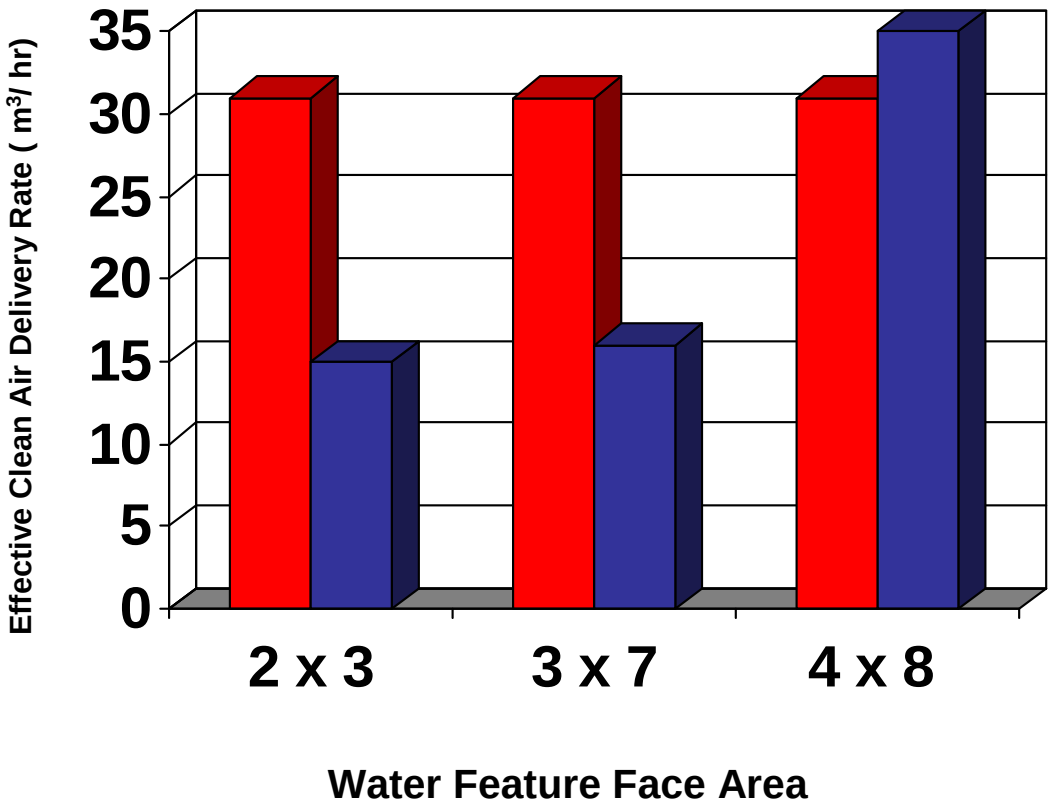


Figure 3. Room air concentration of *Bacillus subtilis* cells (top), and *Mycobacterium parafortuitum* cells (bottom) during the operations (■), or disengagement (■) of a 4' x 8' stainless wire mesh ACCENTS water feature in a 90 m^3 bioaerosol chamber operated in a static, well mixed mode. In independent trials, room air was charged with airborne bacteria concentration at the room air concentration noted at time zero, and monitored for between 6 and 10 hours.



Clean air delivery rate (CADR) observations were replicated and compiled for each of the unit sizes, using the protocols outlined above. DIRECT airborne bacteria counts dropped significantly when the bioaerosol chamber contained an operating water feature, regardless of size. These results suggest that on average, the effective CADR when challenged with airborne *Bacillus subtilis* cells (■) was between 15 m^3/hr and 30 m^3/hr, and approximately 30 m^3/hr when challenged with airborne *Mycobacterium parafortuitum* (■). These CADR averages apply when the water features contained clean distilled water, were operated at their maximum flow rate, and were equilibrated with the air in a 90m^3 room maintained at 30 C.