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May 29, 2008

Government Scientific Source Inc.
ATTN: Steve Sellentin
12351 Sunrise Valley Drive
Reston, VA 20191

Dear Mr. Steve Sellentin:

RTI is pleased to submit this letter report to Government Scientific Source on our project to test the efficacy of microbe inactivation by the Genesis Air 2002B-MP Unit and the Genesis-assembled in-duct tripartite photo-catalytic oxidation (PCO) unit (Philips Lamps). The 2002B-MP test unit was supplied by Genesis Air and tested as supplied with the integrated fan operated at the lowest (330 cubic feet per minute (CFM)) setting. The in-duct unit was installed in RTI's test rig, which is compliant with ASHRAE 52.2 specifications with modifications for biological sampling, and tested at a flowrate of 1800 CFM.

The testing program included five organisms: one fungus, one virus, one bacterial spore and two vegetative bacteria.

*Bacillus atrophaeus (Bg)* was selected as a simulant for *Bacillus anthracis*. *Bg* is generally recognized by the scientific and testing communities as one of the simulants for *B. anthracis* in numerous biowarfare agent testing scenarios. Accordingly, *Bg* has value as a historically used simulant and permits comparison to past testing and studies.

*Staphylococcus epidermidis (Se)* Because of the growing impact of nosocomial infections and the expressed desire of Government Scientific Source to address this concern, testing of a bacterium closely related to those commonly causing hospital-acquired infections was included. *Se* is an appropriate choice since coagulase-negative staphylococci, mostly strains of *Se*, represent the most frequent causes of nosocomial sepsis and are the most prominent organisms responsible for infections of implanted medical devices. *Se* is a common gram-positive human shedding organism, but can be a pyogenic (fever-causing and pus-forming) pathogen. Numerous *Se* infections have been documented in deep tissues and represent a significant risk in immune-compromised individuals. *Se* is also closely related to *Staphylococcus aureus*, the species responsible for the Methicillin-resistant *Staphylococcus aureus* (MRSA) infections that have garnered increased attention recently. *Se* can also be viewed more broadly as a representative of vegetative bacteria which are generally more susceptible to neutralization than bacterial spores.

MS2 virus Inclusion of a virus simulant, MS2 - an *E. coli* bacteriophage, was important because it will help fill a gap that exists from previous studies of this technology. Although the MS2 virus roughly approximates the aerosol-related physical characteristics of human viruses, individual virus particles of health-threatening agents may be somewhat smaller or larger than MS2. However, since human viruses would likely be encountered as polydispersed aerosols, exact size of individual viruses is not highly crucial. A distribution of virus-containing particle sizes

representative of those in the respirable range generated by sneezing and coughing were used for this testing.

*Aspergillus versicolor (Av)* The capability to address problems created by indoor mold growth was included in this investigation. *Av,* the representative fungus, is frequently reported as a causative agent of hypersensitivity pneumonitis It is also a very common contaminant of indoor building materials including gypsum board as well as carpet and other flooring and has been isolated from a number of problem buildings. Thus it is an appropriate representative for testing the effectiveness of the Genesis Air units in neutralizing mold spores.

*Mycobacterium parafortuitum* (*Mp*) The desire to address the susceptibility of the microbe associated with Tuberculosis motivated the selection of *Mp* for testing as a *Mycobacterium tuberculosis* simulant. *Mp* is another representative of the same genus and has physical characteristics similar to *Mycobacterium tuberculosis*.

*Escherichia coli* (*E. coli*) This gram-negative bacterium is an established source of endotoxin and thus was an appropriate test organism for the endotoxin analysis that Government Scientific Source requested. This bacterium was used only for the single pass in-duct portion of the testing, in which it was aerosolized and introduced into the test duct. The quantity of endotoxin in air samples collected upstream and downstream of the PCO device was assayed to determine whether operation of the test unit resulted in increased or decreased detectable endotoxin activity in treated air when challenged with an endotoxin producing bioaerosol.

*Cladosporium sphaerospermum* (*Cs*) This fungus was selected for the airborne mycotoxin testing that Government Scientific Source requested because of the combination of two attributes. It forms spores small enough to be aerosolized effectively and it produces strong mycotoxin activity. These fungal spores were used only for the single pass in-duct portion of the testing, in which it was aerosolized and introduced into the test duct. The level of mycotoxin activity in air samples collected upstream and downstream of the PCO device was assayed to determine whether operation of the test unit resulted in increased or decreased detectable mycotoxin activity in treated air when challenged with a mycotoxin-producing fungal bioaerosol.

## TEST METHOD

### Chamber Air Cleaner Test

The test for each organism included a natural decay measurement and an air cleaner decay measurement. Both measurements are performed after filling the chamber with challenge bioaerosol. The natural decay is defined as the decay of the test bioaerosols in the chamber with the air cleaner off. The air cleaner decay measurement is defined as the decay while the air cleaner is running.

The test method followed has been described in depth in Foarde et al. (1999). As an overview, the paper describes a test method to determine a Clean Air Delivery Rate (CADR) - type measurement for a device when challenged with microbiological aerosols. The method is a modification of the Association of Home Appliance Manufacturers (AHAM) Standard AC-1, "Standard Method for Measuring Performance of Portable Household Electric Cord-Connected Room Aircleaner" which determines the CADR for three different particulate matter challenges

(smoke, dust, and pollen). The ability to extend the AHAM method to microbial aerosols follows the tradition of the AHAM test of using realistic particle challenges, and allows a means to compare and evaluate different brands of room air cleaning devices regarding characteristics significant to product use. This is a useful approach for evaluating a wide range of devices.

**Test Chamber and Bioaerosol Sampling:**
The Dynamic Microbiological Test Chamber (DMTC) was used for the air cleaner tests (see photos in appendix). The DMTC is a room-sized environmental chamber contained within the microbiological aerosol test facility, a cleanroom (nominally Class 1,000). The chamber is a 2.44 x 2.44 x 3.05 m (18.16 $m^3$ or 640 $ft^{3)}$ cube. The walls and containment ceiling are 10-cm thick prefabricated panels with a stainless steel interior layer. The floor of the chamber was custom constructed of 12-gauge stainless steel with welded seams and insulation underneath between the support members. Floor seams were polished and the coved corners were sealed. The ceiling-mounted mixing fan consists of a two-blade aluminum casting 61 cm in diameter attached to a shaft extending from above the containment ceiling through a sealed bearing. To reduce the number of difficult- to-decontaminate interior features, no electrical outlets were installed inside the chamber. A 5 cm penetration is cut in one wall and finished to allow extension cord access through rubber stoppers.

Temperature and humidity control were provided by a separate external air handler (AHU). The AHU also controls the steam humidifier which adds water to the chamber air while the HVAC system removes some water and controls the air temperature. Airflow through the system is monitored by an airflow station and controlled by a blower speed controller with the AHU. Air cleaning of the chamber is attained through the use of a HEPA (High Efficiency Particulate Air) filter installed on the discharge side of the AHU. It contains both an ASHRAE 30% prefilter and a HEPA filter.

Figure 1 shows an artist's rendition of the DMTC configured for air cleaner testing. The Genesis Air 2002B-MP Unit was positioned near the center of the chamber and operated at the minimum fan speed. According to Genesis Air, this results in a flow rate of approximately 330 CFM.

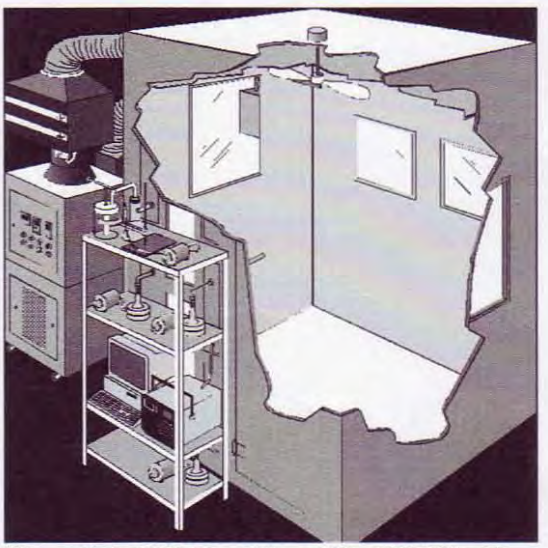


**Figure 1 Artist's Rendition of the DMTC with sampling instrumentation.**

The challenge bioaerosol suspensions were aerosolized using a Collison modified MRE-type six-jet nebulizer (BGI, Waltham, MA). An Erlenmeyer flask, placed in line between the Collison nebulizer and the chamber, was used as a mixing and drying chamber for the test aerosol. It was positioned at the upper left hand corner of the dynamic chamber sampling wall.

Extractive sampling of the bioaerosols was accomplished using ports placed in sampling panels located in one wall of the chamber (see Figure 1). Three sampling ports were used to collect triplicate simultaneous samples. Port A was positioned near the center of the chamber wall,

1.52m above the floor of the chamber and 1.0 m from the front wall. Port B was 1.6 m above the floor but was 0.25 m from the front wall of the chamber. The third port, C, was directly below Port A, but 0.65 m above the floor of the chamber. Stainless steel 1.27 cm diameter piping extending .76 m into the middle of the chamber was used for sample lines. The dimensions of the sample lines were chosen to minimize particle losses during sampling.

Sampling of *Se, Bg, Mp* and *Av* was accomplished using one-stage viable bioaerosol samplers (see Appendix photograph 2) loaded with Petri dishes containing growth media. The one-stage sampler is a multiple-jet impactor. After sampling, the Petri dishes were removed from the sampler and incubated overnight at 37 deg. C for *S and Be*, several days at 37 deg. C for *Mp* and several days at 23 deg. C for *Av*. CFUs (colony forming units) were then enumerated and their identity confirmed.

Sampling of the MS2 virus particles was accomplished using all-glass impingers (see Appendix photograph 3). After sampling, the impinger fluid was diluted (if necessary) and analyzed for viable viruses present. Quantitation of MS2 viruses is accomplished by enumeration of plaque forming units (PFU) arising from assay of samples using an *E. coli* host in top agar on Petri plates

**Test Protocol:**
The test protocol was as follows:
1) Turn on the chamber AHU and circulating fan.
2) Allow the HEPA to clean the chamber air for at least 3 hours. Take background air sample.
3) Turn off AHU and turn on the Collison nebulizer and run at least 5 minutes.
4) Turn off the Collison nebulizer. Wait 1 minute and turn off the circulating fan
5) At the start of collection for the "0 min" sample the test unit is switched ON (device on test only)
6) Collect triplicate bioaerosol measurements at 0, 5, 10, and 15 minutes.

For the natural decay test, step 5 was omitted.

**Calculations:**
The performance of the air cleaner was evaluated by determining the Clean Air Rate (Microbial) or CARm, calculated as the CADR in the AHAM method. To calculate the CARm, the measured decay ($k_e$) and natural decay ($k_n$) rates are first calculated using the formula:

$$k = \frac{(\Sigma\, t * \ln C_t) - [(\Sigma\, t)(\Sigma \ln C_t)]/n}{(\Sigma\, t^2) - (\Sigma\, t)^2 / n} \qquad \text{Equation 1}$$

where:
$C_t$ = concentration at time, t
n = number of data points used in the regression
k = decay constant ($time^{-1}$)
t = time (minutes)
Then the CAR(m) was calculated for each measured decay rate, using the formula:

$CARm = V\,k_e - k_n$ Equation 2

where:

V = volume of the test chamber ($ft^3$)

$k_e$ = measured decay rate ($min^{-1}$)

$k_n$ = average natural decay rate ($min^{-1}$) for an organism.

Single Pass In-duct Test

The testing was conducted in the test duct shown schematically in Figure 2. The test section of the duct is 0.61 m by 0.61 m (24 in. by 24 in.). The locations of the major components, including the sampling probes, the device section (where the UV device is installed), and the aerosol generator (site of bioaerosol injection) are shown. The test duct is operated following procedures in the ANSI/ASHRAE (American National Standards Institute/American Society of Heating, Refrigerating and Air-Conditioning Engineers) Standard 52.2-1999, *Method of Testing General Ventilation Air-Cleaning Devices for Removal Efficiency by Particle Size.*

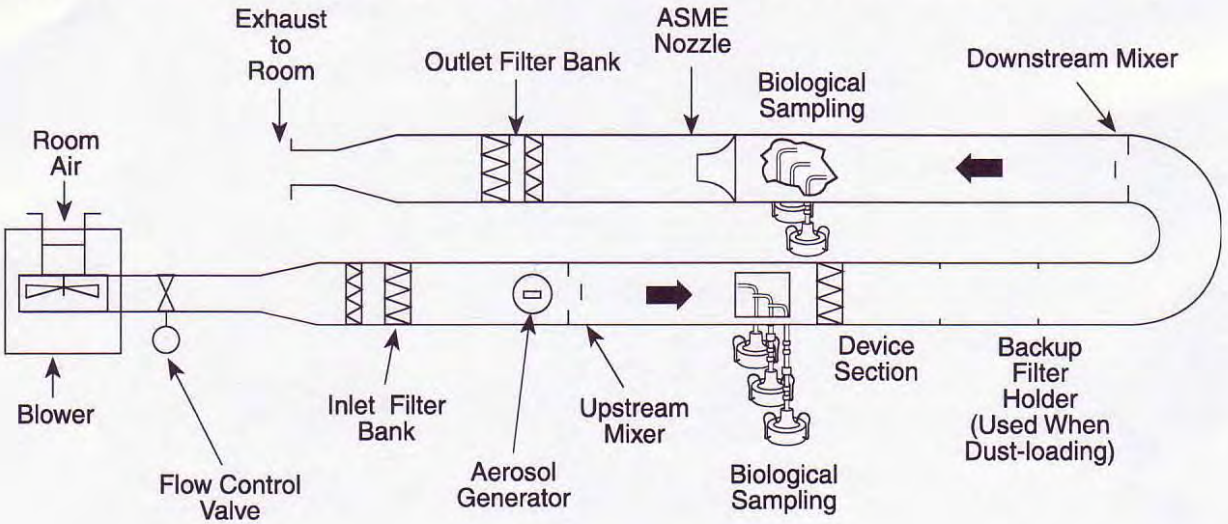



**Figure 2. Schematic of Test Duct. UV system is placed in device section.**

While Figure 2 shows the test duct without recirculation, during testing, the duct may be operated with or without recirculation. The decision for recirculation mode is based on building HVAC considerations. Because of the HEPA filters at the beginning and the end of the duct, the recirculation mode does not affect the test data as long as all other criteria are met.

The tested device consisted of three six-inch, photo-catalytic oxidation sections fabricated by Genesis Air and attached in tandem such that when installed, air flowing in the test duct would pass through each sequentially (see Appendix photograph 4). Each section had four integrated UV lamps positioned perpendicular to the direction of air flow. The lamps for each section were

connected to a ballast assembly (Philips) by cords that traversed the side of the test duct through sealed ports. The operation of each of the three assemblies was controlled by a toggle switch.

The challenge bioaerosol suspensions were aerosolized using a Collison modified MRE-type six-jet nebulizer (BGI, Waltham, MA). The output of the nebulizer was mixed with clean, dry air prior to its entry into the test duct to create the dry bioaerosol challenge.

Biological samples were collected from the air stream with sampling probes positioned within the test duct at both the upstream and downstream sampling sites. Samplers were employed and microorganism enumerated as described above for the chamber tests.

Collection of upstream and downstream air samples with the *E. coli* challenge was conducted with impingers to determine levels of endotoxin present. The impinger fluid was subjected to Limulus Amebocyte Lysate based assay that measures endotoxin activity relative to a series of standards. Likewise, impinger fluid from air samples collected using the *Cs* challenge was used to assess mycotoxin activity relative to a set of standards.

In a given run, one of the challenge bioaerosols was injected upstream of the device. A no-device transmission test was performed prior to installation of the test unit into the test duct, to determine the microorganism loss that would occur simply as the result of deposition in the test duct. For investigation of efficacy with respect to microbe culturability, the performance of the device is reported as the device's efficiency in inactivating the organism, corrected to account for the loss of organisms observed in the absence of the device. For investigation of endotoxin and mycotoxin levels in collected air samples, the performance of the device is reported as the net effect on toxin activity (, corrected to account for the loss of toxin activity observed in the absence of the device

**Test Protocol:**
The test protocol was as follows:
1) Turn on the test duct blower and adjust flow to 1800 CFM.
2) Supply power to the three ballast assemblies and switch ON.
3) Turn on the Collison nebulizer and drying air and run at least 5 minutes.
4) Collect upstream and downstream bioaerosol samples.
5) Turn OFF Collison and ballast assemblies

For the no-device test, step 2 was omitted.

**Calculations:**
The efficiency of the device for inactivating airborne bioaerosols was calculated as:

Airborne Inactivation Efficiency (%) = 100 (1- Corrected Survival Rate) Equation 3

The calculation of the test organism survival rate (culturable transmission) was based on the ratio of the downstream to upstream culturable organism counts. To remove system bias, the Survival Rate was corrected by the results of the no-device transmission test. The no-device transmission rate was calculated in the same manner as the survival rate test, but using the culturable organism counts from the no-device tests. The effect on toxin levels is calculated as percent increase in downstream versus upstream toxin activities after correction for the no-device transmission rate.

RESULTS

Chamber Air Cleaner Test

Figures 3-7 show the decay curves for the *Se* vegetative bacteria, the MS2 virus, the *Av* fungal spore, the *Bg* bacterial spore and the *Mp* vegetative bacteria, respectively. The numbers of CFUs (PFUs for MS2) per cubic foot in the chamber are plotted on the y-axis, versus the time in minutes on the x-axis. The data points for each time represent average results from the three sampling locations. The error bars indicate the standard deviations calculated for the multiple samples comprising each average. The natural decay curves are labeled "device OFF", while the air cleaner decay curves (with the air cleaner running) are labeled "device ON". In each case, the impact of the Genesis Air 2002B-MP test unit is readily visible in the graph. The decay rates with the device on are significantly and reproducibly higher than the decay rates with the device off over the time periods observed. The actual measured decay rates calculated according to the CARm method for each sampling location are shown in Table 1.

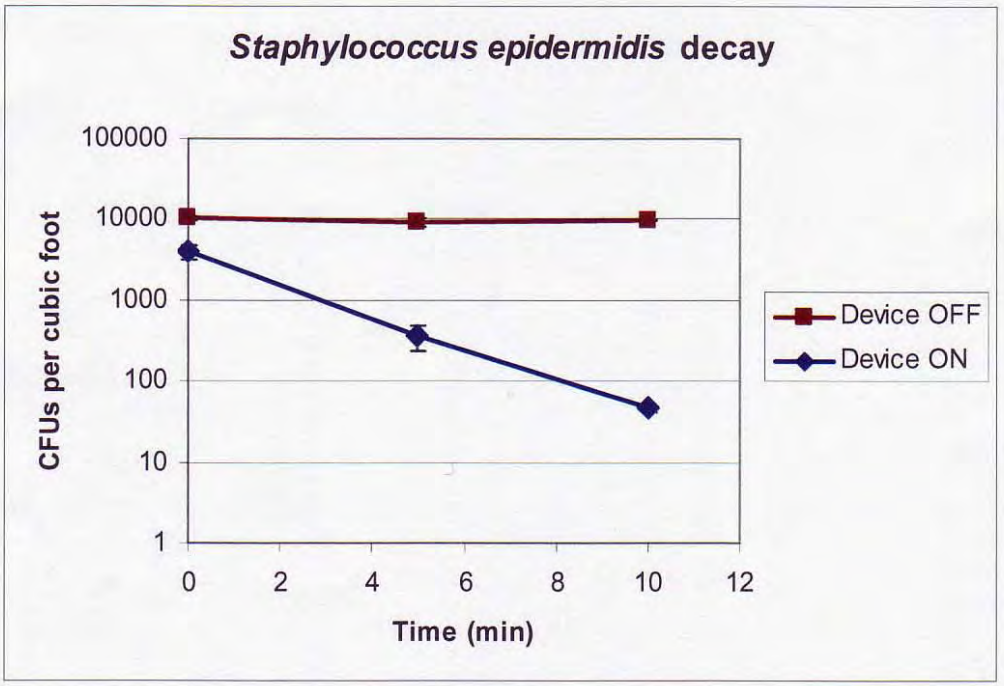



**Figure 3. Decay curves for *Staphylococcus epidermidis***

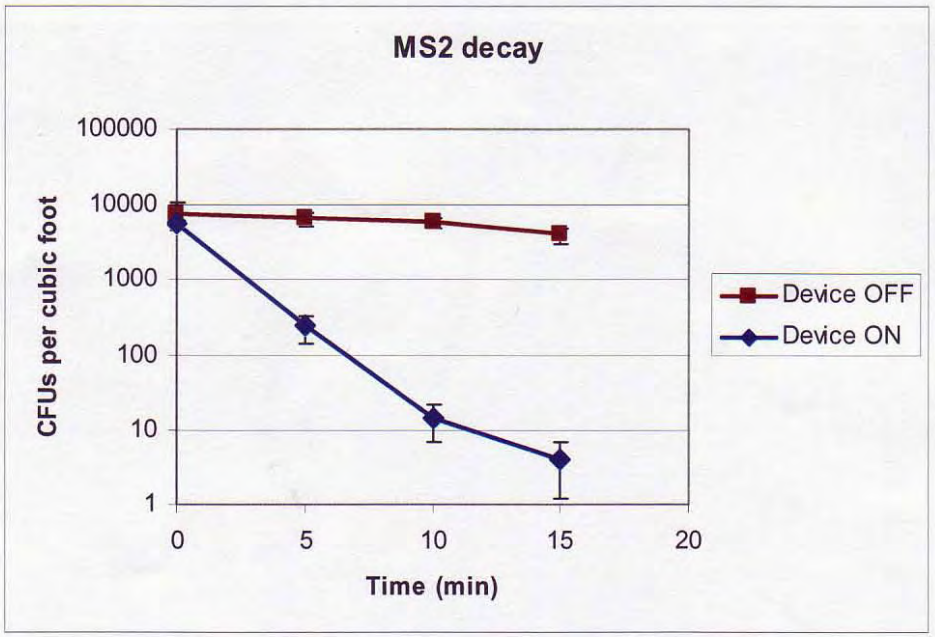



**Figure 4. Decay curves for the MS2 virus**

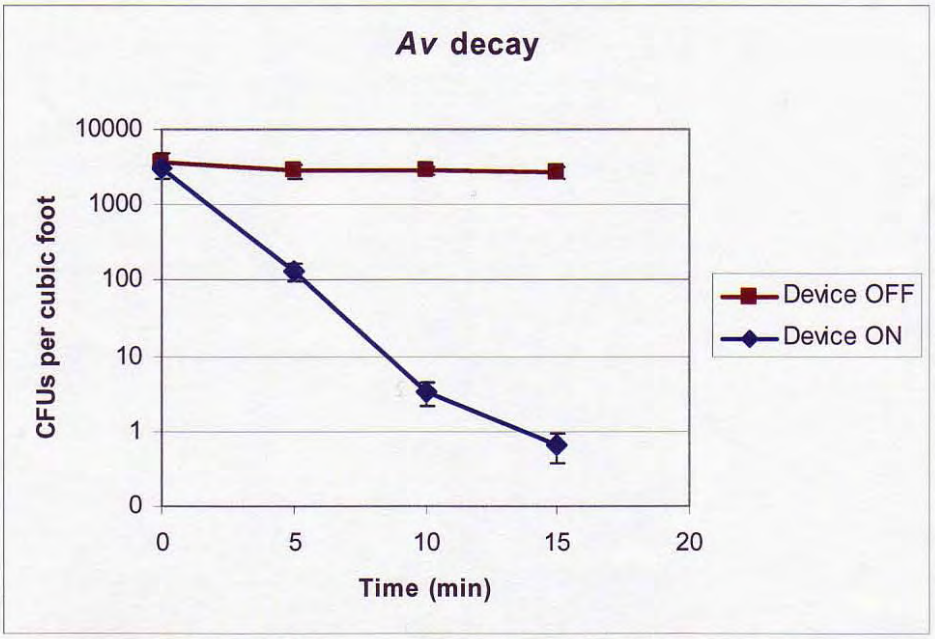



**Figure 5. Decay curves for the *Av* fungal spores**

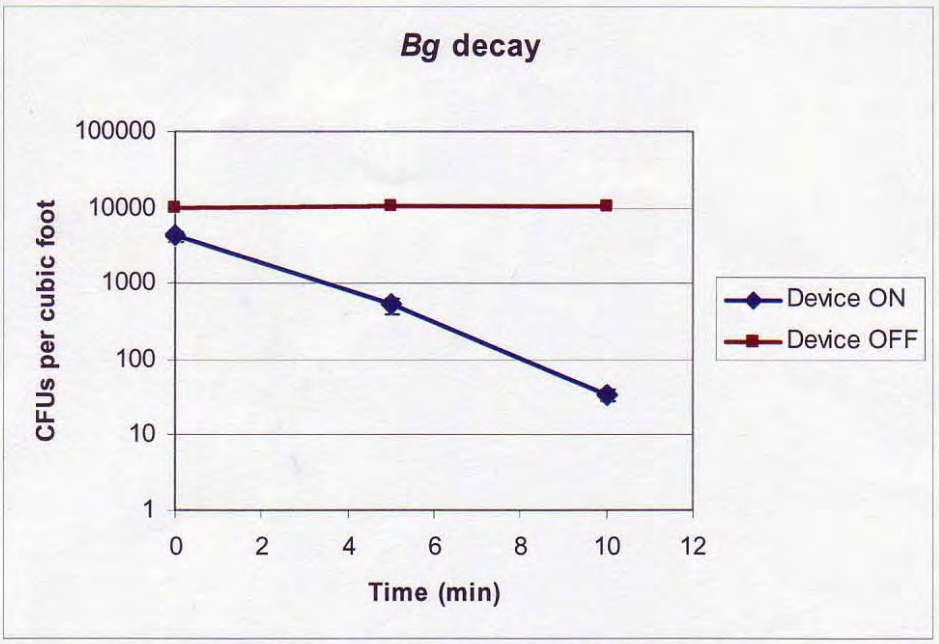



**Figure 6. Decay curves for the *Bg* bacterial spores**

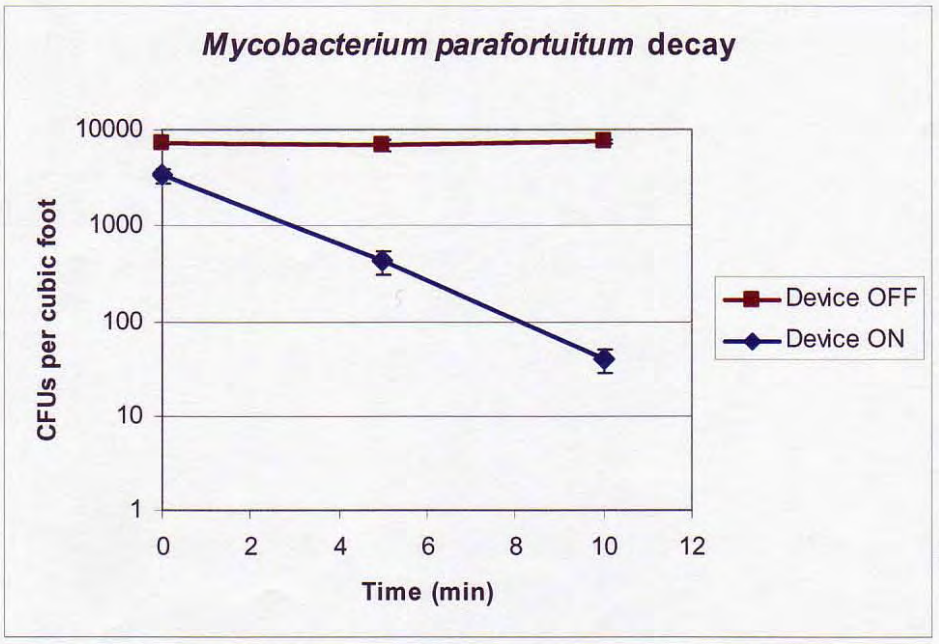



**Figure 7. Decay curves for the *Mycobacterium parafortuitum***

**Table 1. Decay rates measured for introduced microbial bioaerosols**

| | *Av* | MS2 | *Se* | *Mp* | *Bg* |
|---|---|---|---|---|---|
| **Natural Decay** | | | | | |
| knA | -0.041 | -0.069 | -0.008 | 0.008 | 0.008 |
| knB | -0.016 | -0.013 | -0.008 | 0.000 | 0.008 |
| knC | 0.009 | -0.032 | 0.000 | -0.026 | 0.000 |
| | | | | | |
| **Decay With Device** | | | | | |
| keA | -0.55 | -0.47 | -0.47 | -0.44 | -0.48 |
| keB | -0.62 | -0.57 | -0.43 | -0.47 | -0.47 |
| keC | -0.57 | -0.36 | -0.44 | -0.42 | -0.49 |

Table 2 presents the average CARm results and standard deviations. The CARm was calculated as shown in Eq. 2, and is a comparison of the two decay rates (natural and air cleaner) as a function of the volume of the test chamber (640 $ft^3$). These results are also displayed graphically in Figure 8.

**Table 2. CARm values calculated from mean decay rates**

| | *Av* | MS2 | *Se* | *Mp* | *Bg* |
|---|---|---|---|---|---|
| **AVE CARm** | 363 | 275 | 281 | 280 | 311 |
| **s.d.** | 29 | 76 | 13 | 26 | 6 |

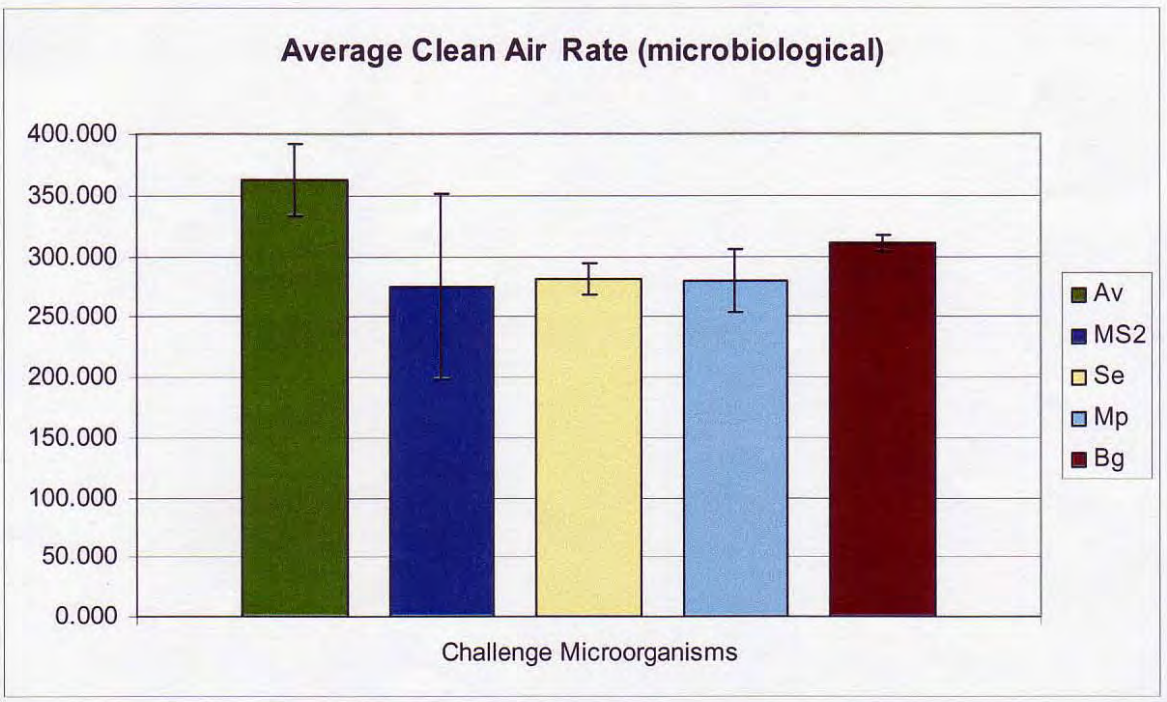



**Figure 8. CARm values for organisms tested**

In the ideal case where the air cleaner provides a well mixed chamber, the CARm is equivalent to the product of the air cleaner's flow rate and its inactivation/filtration efficiency for the challenge bioaerosol. In a chamber test, however, the test chamber is only "well-mixed" to the extent that the device itself provides this mixing by the air motions generated by its fan. Thus, the CARm combines the effects of efficiency of the air cleaner and the effectiveness of the air cleaner to draw the test chamber's air through it. Generally, the CARm should not exceed the air cleaner flow rate. The results show the CARm value for the *Av* challenge was indeed quite near the Genesis Air 2002B-MP air flow rate determined by Genesis Air as 330 CFM. Thus, for this microbial aerosol, the test unit achieved essentially the maximum performance that could be expected from a device operating at the designated air flow. The average CARm values for MS2, *Se*, *Mp* and *Bg* were slightly lower. *Av* spores are considerably larger than the other test microorganisms and thus are more effectively removed from the air by filtration. The higher efficiency observed with respect to *Av* is consistent with increased removal, relative to the smaller bioaerosols, by the unit's integrated filter.

Single Pass In-duct Test

Table 3 presents the efficacy results for the in-duct tripartite photo-catalytic oxidation (PCO) unit when operated at 1800 CFM. The inactivation efficiencies were calculated as shown in Eq. 3. The average values presented were calculated using the results from the five upstream and downstream measurements for each challenge. These results are also displayed graphically in Figure 9.

**Table 3. Inactivation efficiencies for introduced bioaerosols**

| Inactivation Efficiency | | |
|---|---|---|
| **Test Organism** | **mean** | **std. dev.** |
| *Staphylococcus epidermidis* | 99.9 | 0.02 |
| *Mycobacterium parafortuitum* | 88.7 | 2.7 |
| MS2 | 43.6 | 4.7 |
| *Bacillus globigii* | 34.6 | 8.9 |
| *Aspergillus versicolor* | 11.4 | 5.5 |

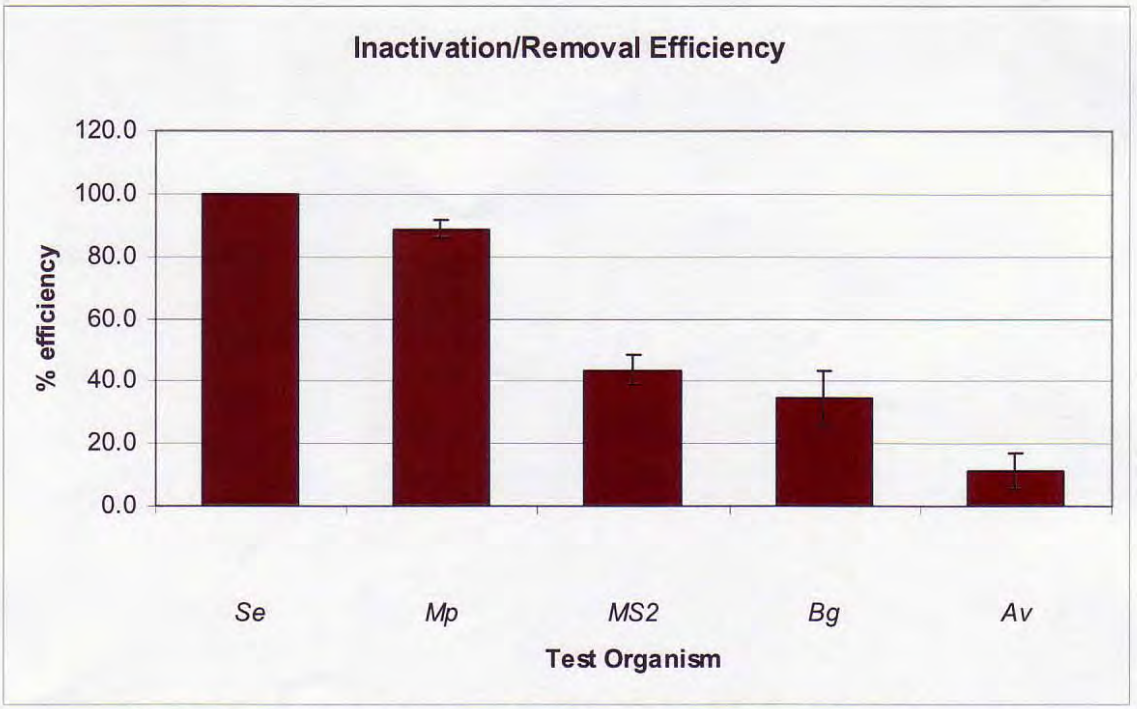



**Figure 9. Inactivation efficiency values for single pass efficacy tests**

As is apparent from the table and graph above, the efficacy was quite variable, depending on the nature of the challenge. While the inactivation efficiency was quite high with respect to the two vegetative bacteria challenges, it was clearly reduced with respect to the MS2 virus and *Bg* spores and lowest with respect to the *Av* fungal spore. This pattern of inactivation variability is not unexpected given the structural characteristics of this diverse range of microorganisms and is typical of devices that employ a mechanism aimed at inflicting damage to cell components rather than removal from the air stream by filtration.

**Table 4. Effect of device on microbial toxin levels**

| Percent change in toxin activity | | |
|---|---|---|
| **Analyte** | **mean** | **std. dev.** |
| Endotoxin | 5.5 | 22.0 |
| Mycotoxin | 19.1 | 18.9 |

Our results also show (table 4) that there was very little change in the levels of microbial toxins as a result of the toxin-producing organisms being exposed to the PCO unit at 1800 CFM. The upstream and downstream levels of endotoxin were essentially indistinguishable while a small increase in mycotoxin activity, which is nearly within the standard deviation for the measurement, was observed downstream. It may be that exposure to the PCO unit and consequent structural damage to the cells does not significantly increase the release of toxins from the microbes. Alternatively, an increase in microbial toxins may result from structural damage, but is offset by the destruction of toxins themselves by the PCO unit.

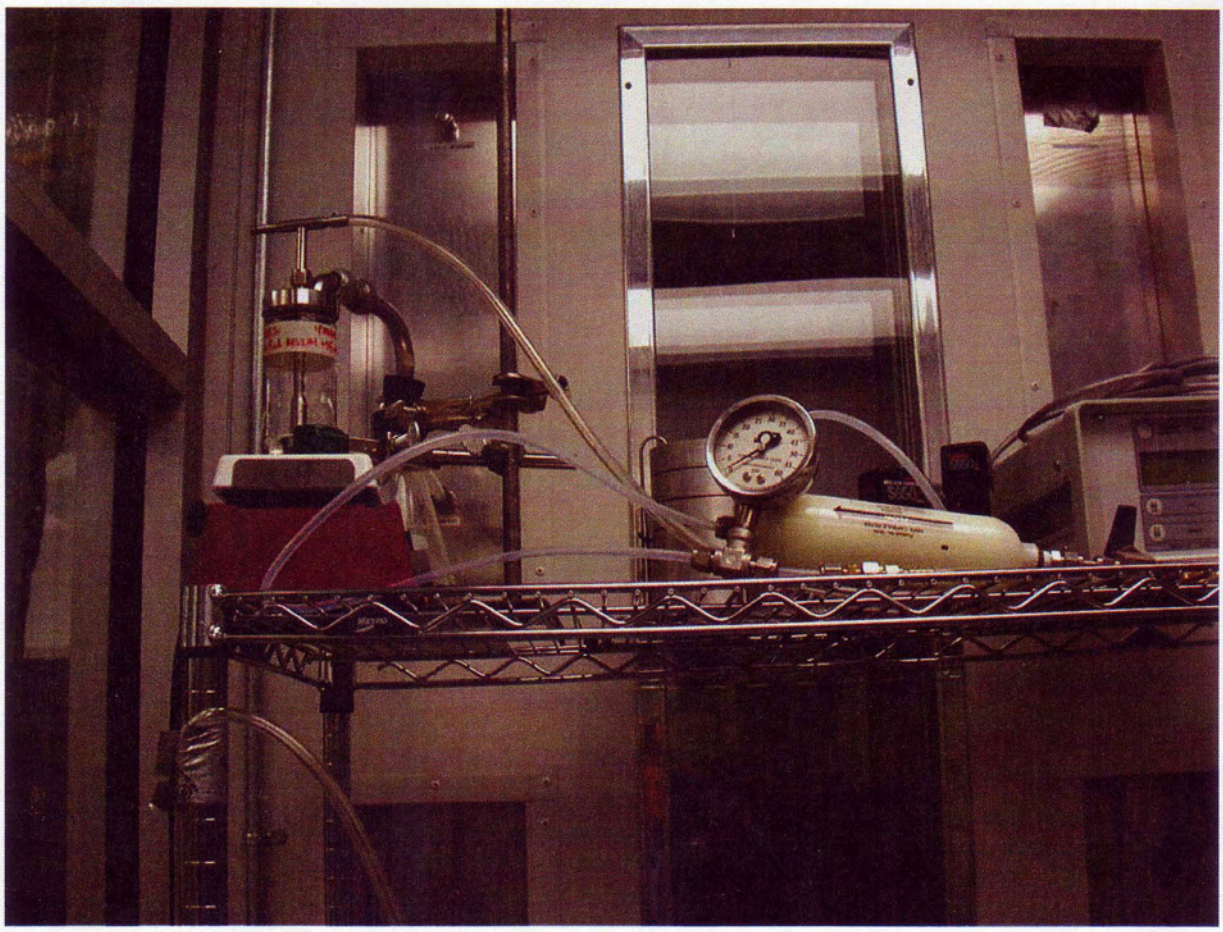
Please let me know if you have any additional questions, and feel free to call me at 919-541-6261 or email me at kesch@rti.org.

Sincerely,

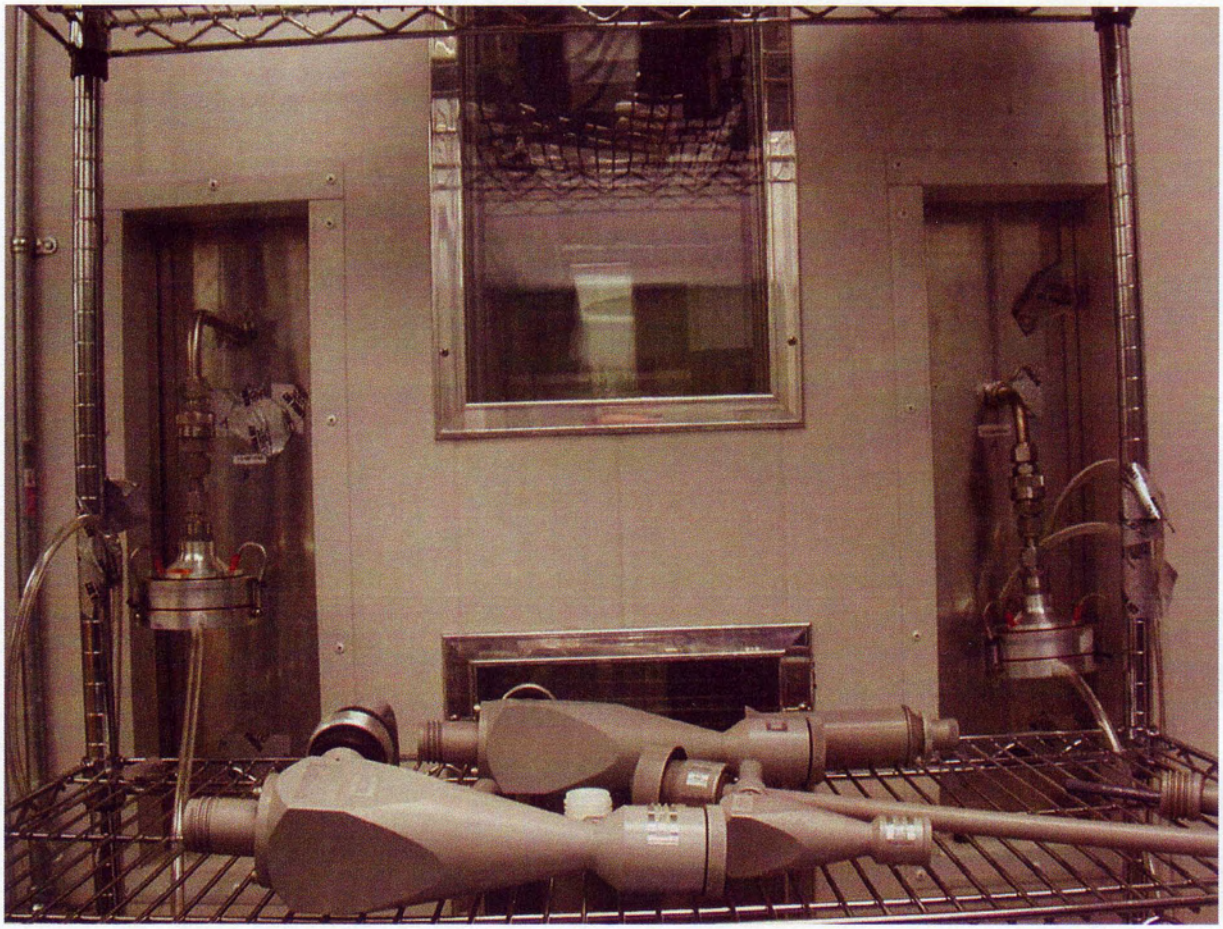
R. Keith Esch, Ph.D.
Research Microbiologist

cc: Karin Foarde
Lisa Bailey (0280800.182)
0210782.004 file

# APPENDIX to GSS Final Report
# 5/29/08

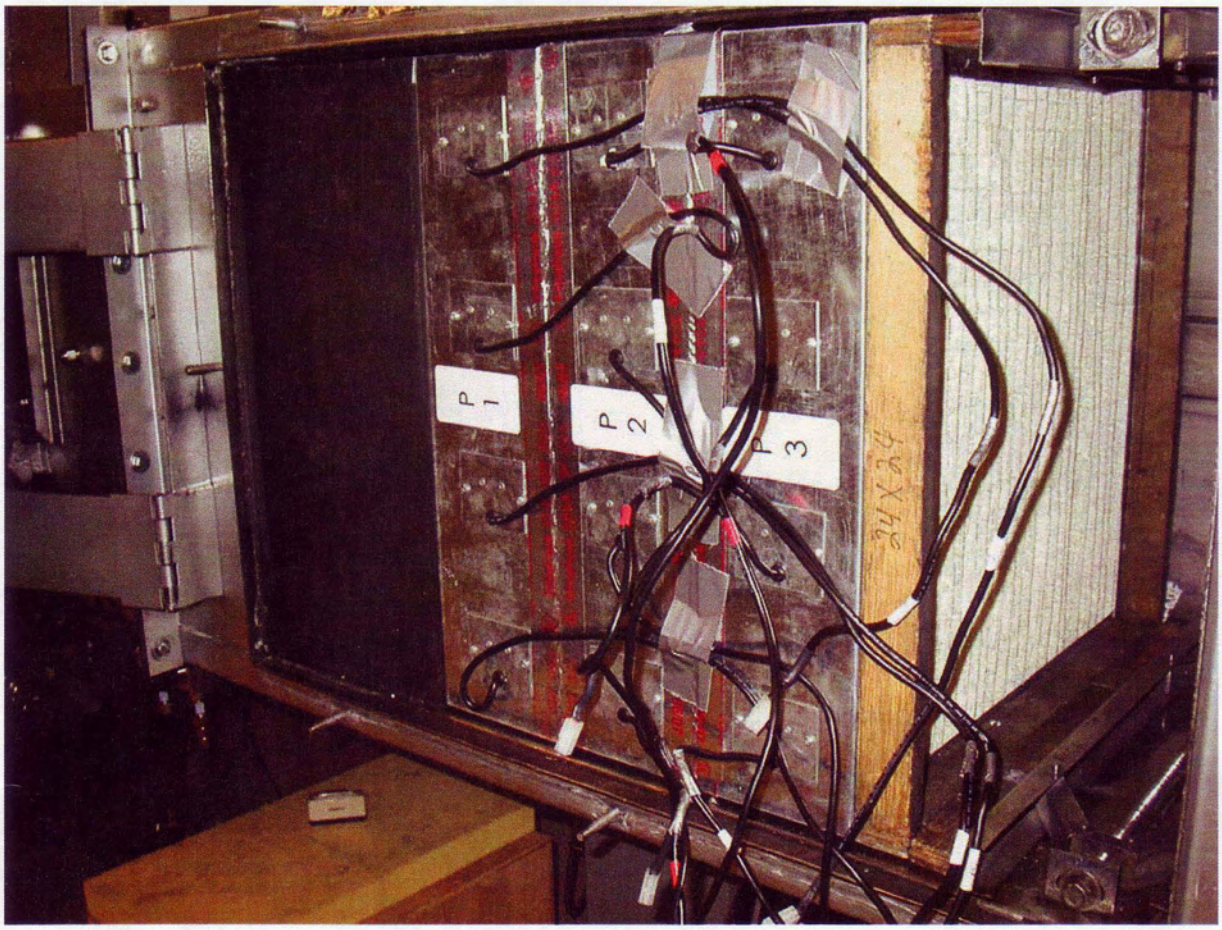


**Photograph 1. View of Bioaerosol Generation Components.** The glass collison jar, fitted with a nebulizer, is visible atop a magnetic stir plate. HEPA-filtered air (HEPA capsule visible near center) is supplied through tubing to the nebulizer at regulated pressure to aerosolize the microbes. The output of the collison nebulizer is directed through the glass drying flask and into the injection port in the chamber wall.



**Photograph 2. View of Samplers in Test Positions at Chamber Wall.** Each of two Anderson single-stage impactors used for *Se* and *Av* sampling is visible at the sides of the photo. Air samples are extracted from the chamber through tubes that lead to the samplers through ports in the chamber wall. The flexible tubing connected to the bottom of each sampler leads to vacuum pumps that regulate the flow of extracted air.

**Photograph 3. View of Samplers in Test Positions at Chamber Wall.** Each of two all glass impingers used for MS2 sampling is visible at the sides of the photo. Air samples are extracted from the chamber through tubes that lead to the samplers through ports in the chamber wall. The flexible tubing connected to the bottom of each sampler leads to vacuum pumps that regulate the flow of extracted air.




**Photograph 4. View of sequential photo-catalytic oxidation sections installed in test duct.** Air flow in the test duct is from left to right. The black section directly upstream of the device is the light baffle. Adjustable mechanical clamps and wooden force-transfer elements visible at the right of the photo secure the test assembly against a gasketed flange within the test duct. The entire side of the test duct section shown is covered by a sealed panel, through which the electrical cords pass.