

May 2, 2008

SANYO Commercial Solutions
ATTN: DEEPAK MISTRY
1062 THORNDALE AVE
BENSENVILLE, IL 60106

Dear Mr. Deepak Mistry:

RTI is pleased to submit this letter report to SANYO Commercial Solutions on our project to test the efficacy of microbe inactivation by the Virus Washer model VW-VF10BG. The test device unit was supplied by SANYO and tested as supplied with the integrated fan operated at the highest (10 m³/min) setting and the louver in “swing” mode. The testing program included three organisms: one fungus, one virus, and one vegetative bacterium. *Aspergillus versicolor* (*Av*), the representative fungus, is frequently reported as a causative agent of hypersensitivity pneumonitis and as been isolated from a number of problem buildings. The MS2 *E. coli* bacteriophage is commonly used by the scientific and testing community as a representative virus and roughly approximates the aerosol-related physical characteristics of human viruses. *Staphylococcus epidermidis* (*Se*) is a common gram-positive human shedding organism and was the representative vegetative bacterium. Separate interim reports were issued for the first and second microbes tested and this cumulative report covers the total testing program.

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 m³ or 640 ft³) 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 Virus Washer unit was positioned near the center of the chamber. As per instructions from SANYO, the unit was operated at the maximum fan speed. According to SANYO, this results in a flow rate of approximately 10 m³/min.

The challenge bioaerosol suspensions were aerosolized using a Collison modified MRE-type six-jet nebulizer (BGI, Waltham, MA) at 15psi. The Collison generates particles or droplets with an approximate mean diameter of 2µm. 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.

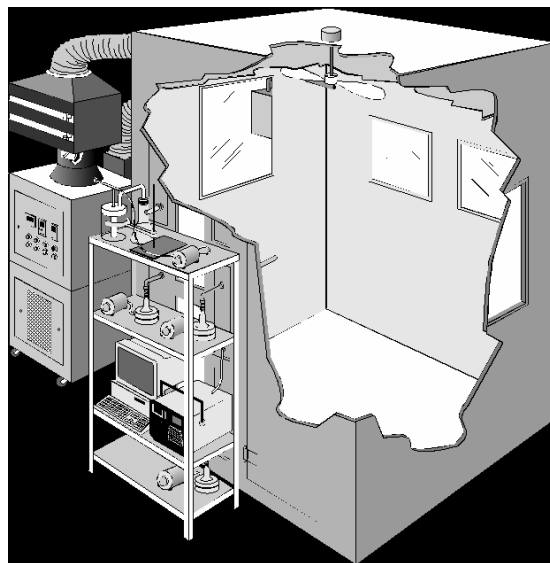


Figure 1 Artist's Rendition of the DMTC with sampling instrumentation.

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 and Av was accomplished using one-stage Andersen viable bioaerosol samplers (see Appendix photograph 2) loaded with Petri dishes containing growth media. The one-stage Andersen sampler is a 400-hole multiple-jet impactor operating at 28 L/min. The mean aerodynamic diameter of captured particles is 0.65 μm . After sampling, the Petri dishes were removed from the sampler and incubated overnight at 37 deg. C for Se , 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) containing 20 ml of impinger fluid (AGI-4). The AGI-4 sampler has a flowrate of 12.3-12.6 LPM and the mean aerodynamic diameter of captured particles is $\sim 0.3\mu\text{m}$. 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) Three minutes prior to the start of collection for the “0 min” sample the Virus washer is switched ON (device on test only)
- 5) Turn off the Collison nebulizer. Wait 1 minute and turn off the circulating fan
- 6) Collect triplicate bioaerosol measurements at 0, 5, 10, and 15 minutes.

Two modifications were used in the natural decay test:

- 1) Step 4 was omitted.
- 2) A humidifier positioned in the chamber was started at “0 min” simultaneously with collection of the first samples.

Calculations:

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

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

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:

$$CARm = V k_e - k_n \quad \text{Equation 2}$$

where:

V = volume of the test chamber (ft³)

k_e = measured decay rate (min⁻¹)

k_n = average natural decay rate (min⁻¹) for an organism.

RESULTS

Figure 2 shows the decay curves for the *Se* vegetative bacteria. The numbers of CFUs 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”.

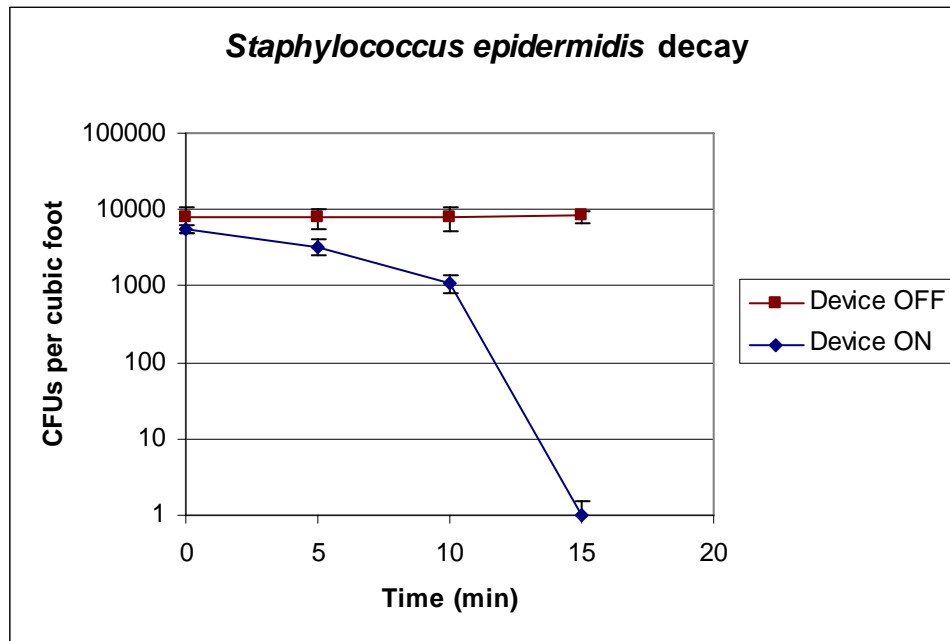


Figure 2. Decay curves for *Staphylococcus epidermidis*

The impact of the VW-VF10BG test unit is readily visible in the graph. The concentration of viable *Se* in the chamber is reduced by over 5000-fold within 15 minutes of operation. This corresponds to an inactivation efficiency of 99.98%. The decay rate with the device on is significantly and reproducibly higher than the decay rate with the device off over the time period

observed. The actual measured decay rates calculated according to the CARm method for each sampling location are shown in Table 1. Relative Humidity (RH) within the chamber at the beginning of each test was approximately 45%. In the device ON testing RH reached approximately 75% by the end of the test period. In the device OFF testing the RH reached approximately 90%.

Figure 3 shows the decay curves for the MS2 virus. The numbers of PFUs 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”.

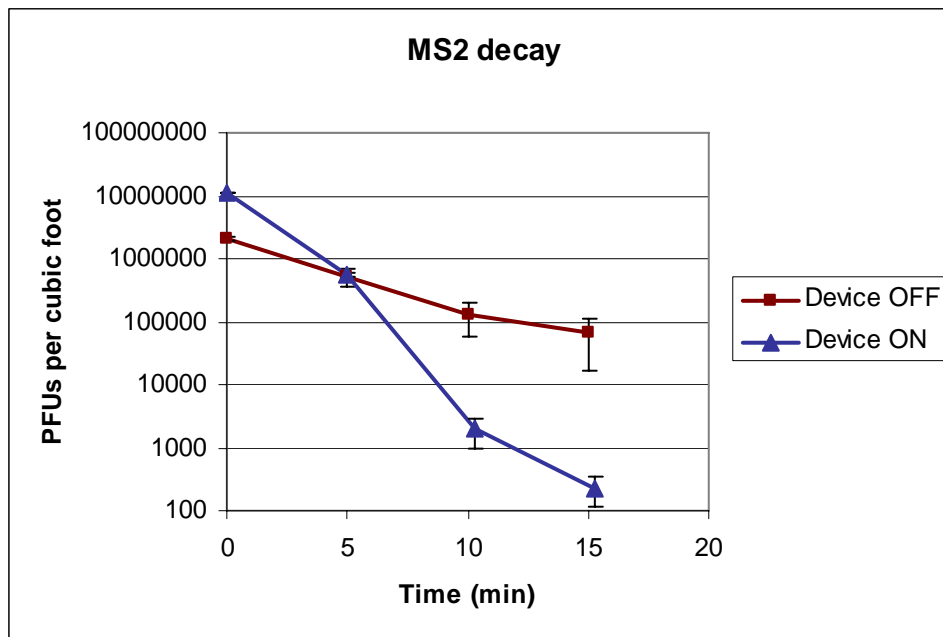


Figure 3. Decay curves for the MS2 virus

The high efficacy of the VW-VF10BG test unit with respect to the virus challenge is readily visible in the graph. The decay rate of viable MS2 with the device on is significantly and reproducibly higher than the decay rate with the device off over the time period observed. The actual measured decay rates calculated according to the CARm method for each sampling location are shown in Table 1. Relative Humidity (RH) within the chamber at the beginning of each test was approximately 45%. In the device ON testing, RH reached approximately 75% by the end of the test period. In the device OFF testing the RH reached approximately 85%. The natural decay losses of MS2 in the chamber determined in the device OFF condition was 99.96%, corresponding to a reduction of 32-fold. This is much higher than what is typically observed over this time period when chamber RH remains stable during the course of the experiment. Nonetheless, a greatly increased decay rate was produced when the VW-VF10BG test unit was operating. By comparing the 49,235-fold reduction observed in the Device ON

condition with the 32-fold observed for the device OFF, the inactivation attributable to the device correcting for the impact of the increased RH is 99.998%.

Figure 4 shows the decay curves for the *Av* fungal spores. The numbers of CFUs 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”.

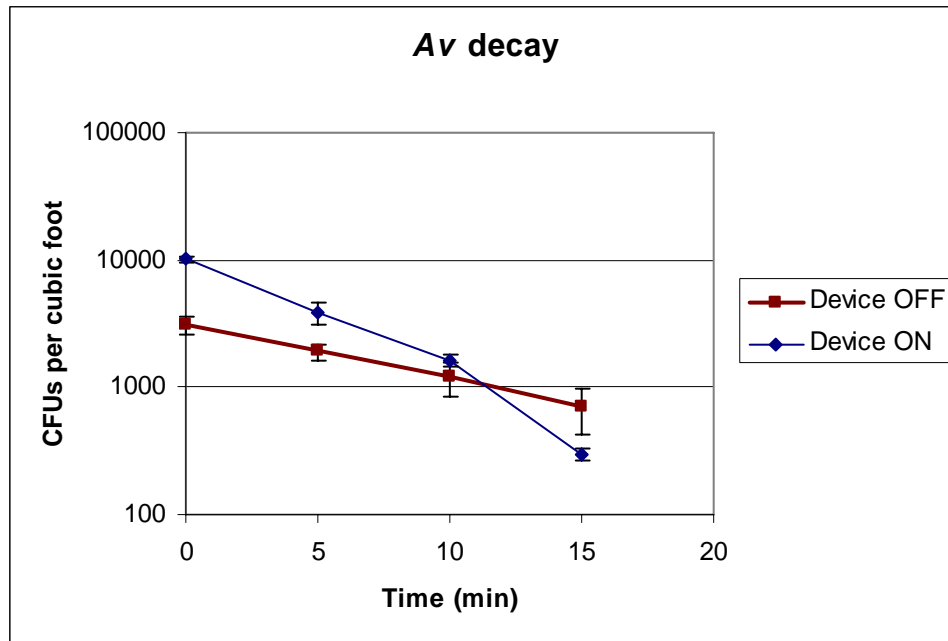


Figure 4. Decay curves for the *Av* fungal spores

The increased inactivation of *Av* by the VW-VF10BG test unit is shown in the graph. The decay rate with the device on is significantly and reproducibly higher than the decay rate with the device off over the time period observed. The actual measured decay rates calculated according to the CARm method for each sampling location are shown in Table 1. Relative Humidity (RH) within the chamber at the beginning of each test was approximately 45%. In the device ON testing, RH reached approximately 75% by the end of the test period. In the device OFF testing the RH reached approximately 95%. The natural decay losses of *Av* in the chamber determined in the device OFF condition was 77.1%, corresponding to a reduction of 4.4-fold. This is somewhat higher than what is typically observed over this time period when chamber RH remains stable during the course of the experiment. Nonetheless, a significantly increased decay rate was produced when the VW-VF10BG test unit was operating. By comparing the 34.2-fold reduction observed in the Device ON condition with the 4.4-fold observed for the device OFF, the inactivation attributable to the device correcting for the impact of the increased RH is 96.6%.

Table 1. Decay rates measured for introduced microbial bioaerosols

	<i>A. versicolor</i>	MS2	<i>Staph epidermidis</i>
Natural Decay			
knA	-0.09	-0.22	0.000
knB	-0.09	-0.21	-0.006
knC	-0.13	-0.36	0.022
Decay With Device			
keA	-0.24	-0.73	-0.54
keB	-0.23	-0.77	-0.52
keC	-0.24	-0.76	-0.57

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 ft³). These results are also displayed graphically in Figure 5.

Table 2. CARm values calculated from mean decay rates

	<i>A. versicolor</i>	MS2	<i>Staph epidermidis</i>
AVE			
CARm	84	313	353
s.d.	12	51	27

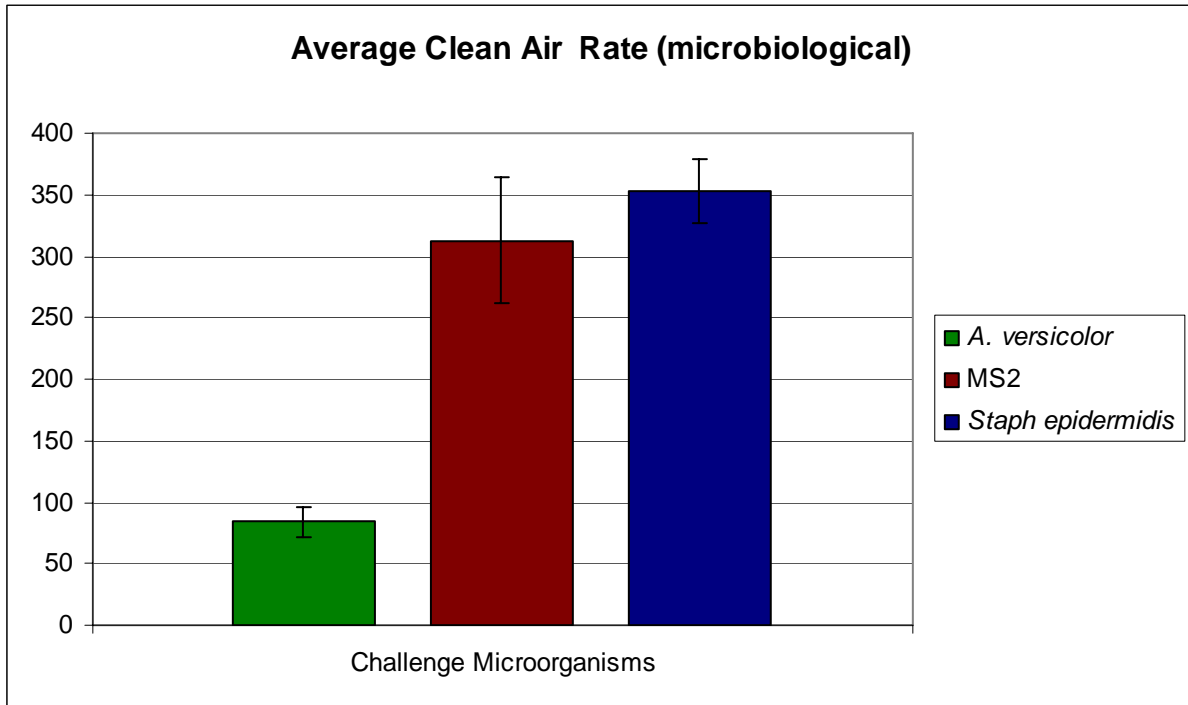


Figure 5. CARm values for organisms tested

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 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 filtration 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 *Se* and MS2 challenges were indeed quite near the VW-VF10BG air flow rate determined by SANYO as 353 CFM. Thus, for the bacteria and virus tested, the VW-VF10BG achieved near the maximum performance that could be expected from a device operating at the designated air flow. The CARm for the *Av* fungal spores was significantly lower. This is consistent with the higher resistance to inactivation technologies generally observed for fungal spores and may be a result of the highly protective coat that surrounds these spores.

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)
0210782.005 file

APPENDIX



Photograph 1. View of Bioaerosol Generation Components. The glass collision 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 collision 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 S_e and A_v 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.