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Bioaerosol Inactivation Chamber Test of an Air Cleaner for Three Microorganisms

Test Report

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Bioaerosol Inactivation Chamber Test of Three Microorganisms for the Clensair Air Sanitation System

1. Introduction

Under contract with Prodew Inc./Aguair, RTI performed bioaerosol chamber tests of three microorganisms (*Staphylococcus epidermidis*, *Bacillus atrophaeus* (Bg) spores, and MS2 bacteriophage) using an air cleaner device provided by Aguair. The device was an Aguair Clensair Air Sanitation System (Part No. CLNS10K). The objective for this study was to determine the Clean Air Rate (microbial) (CARm) of the device when challenged with microbiological aerosols. This was accomplished by comparing the natural decay rates (device off) with the device decay rates (device on) for each organism.

2. Procedures

A single device was provided to RTI. The device was placed into RTI's Dynamic Microbiological Test Chamber (DMTC) on a cart raising the device ~3 ft from the floor (Figure 1). The DMTC is a room-sized environmental chamber contained within the microbiological aerosol test facility, a nominally Class 1,000 cleanroom. The chamber is 2.44 x 2.44 x 3.05 m (18.16 m^3 or 640 ft^3), and contains a ceiling-mounted mixing fan with two aluminum blades 61 cm in diameter attached to a shaft extending 61 cm from the ceiling into the center of the chamber. The temperature and relative humidity for all of the test runs averaged 72 ± 1°F and 35 ± 2%, respectively.

The challenge microorganisms selected for testing were as follows:

1) *Staphylococcus epidermidis* – gram-positive bacteria; simulant for *Staphylococcus aureus* which is commonly associated with nosocomial infections and MRSA (Methicilliin-Resistant *Staphylococcus aureus*).
2) *Bacillus atrophaeus*, a common simulant for *Bacillus anthracis*. The spore form is most resistant to inactivation.
3) MS2 bacteriophage, a common viral simulant for mammalian viruses such as influenza, and norovirus.

The bioaerosol suspensions were aerosolized using a Collison nebulizer (BGI, Waltham, MA) attached to a drying tower. The aerosol was generated at 15 psi air pressure and the drying tower supplied 3.5 SCFM of HEPA-filtered drying air. Three sampling ports designated A, B, and C were used to collect triplicate simultaneous samples. Port A was positioned near the center of the chamber wall, 1.52 m above the floor of the chamber and 1.0 m from the front wall. Port B was 1.52 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. Sampling of the bioaerosols was accomplished using either a single-stage Andersen viable bioaerosol sampler or an all-glass impinger (AGI-4). The first two organisms listed above utilized the single-stage sampler which allowed for direct collection of the bioaerosol challenge onto Petri dishes containing solid media appropriate for the specific microorganism. Colony forming units (CFU) were counted and recorded. MS2 utilized the AGI-4s which contained 20 mL of impinger fluid. The collected viral particles were counted for plaque forming units (PFU) on a lawn of *E. coli*.

Figure 1. Location of the air cleaner device from Aguair inside the DMTC.

Test Protocol:

The test protocol was as follows:

1) Turn on the chamber ventilation system and ceiling fan.
2) Allow the HEPA to clean the chamber air for at least 1 hour.
3) Turn off the ventilation system and turn on the Collison nebulizer. Run for 5 minutes with HEPA-filtered drying air flowing at 3 SCFM.
4) Stop the Collison nebulizer and drying air. Mix the chamber using the ceiling fan in the chamber for 1 minute.
5) Turn the ceiling fan off and obtain a time 0 sample.
6) Collect triplicate bioaerosol measurements at appropriate time intervals (0, 5, 10 and 15 minutes).
7) Clean out the chamber for 20 minutes using the ventilation system with the ceiling fan on.
8) Repeat steps 3 – 7 with the air cleaning device "on" after step 5.

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) (device on) and natural decay (k_n) (device off) rates are first calculated using the formula:

$$k = \frac{(\Sigma\, t * \ln C_t) - [(\Sigma\, t)(\Sigma \ln C_t)]/n}{(\Sigma\, t^2) - (\Sigma\, t)^2 / n}$$ 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 (min.)
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.

3. Results

The decay curves for each of the microorganisms used to challenge the air cleaning device from Aguair are shown below (Figures 2-4). The numbers of CFUs or 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 natural decay curves are labeled "Device OFF," while the air cleaner decay curves (with the air cleaner running) are labeled "Device ON."

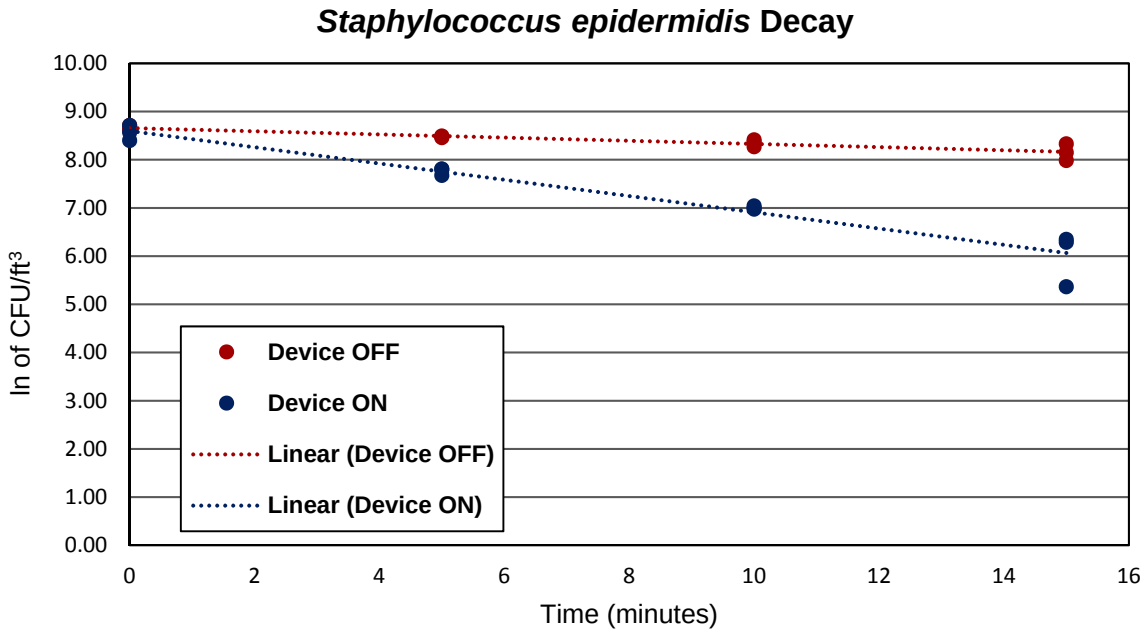

Figure 2. Decay curves for *Staphylococcus epidermidis*

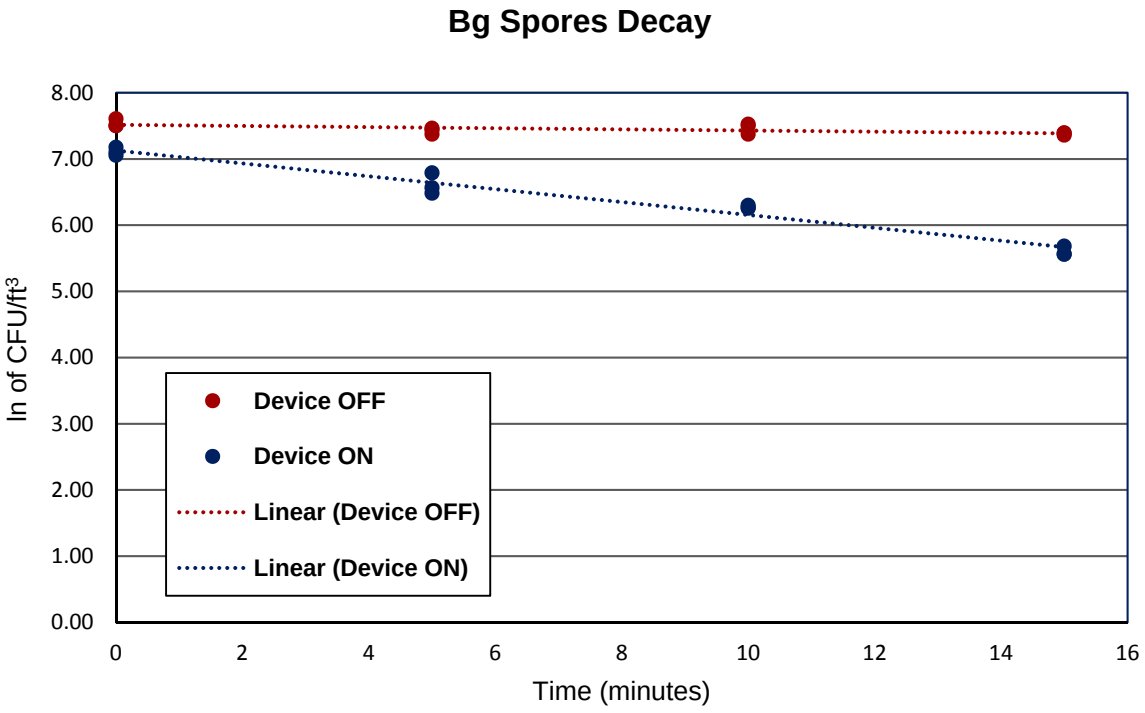

Figure 3. Decay curves for the Bg spores

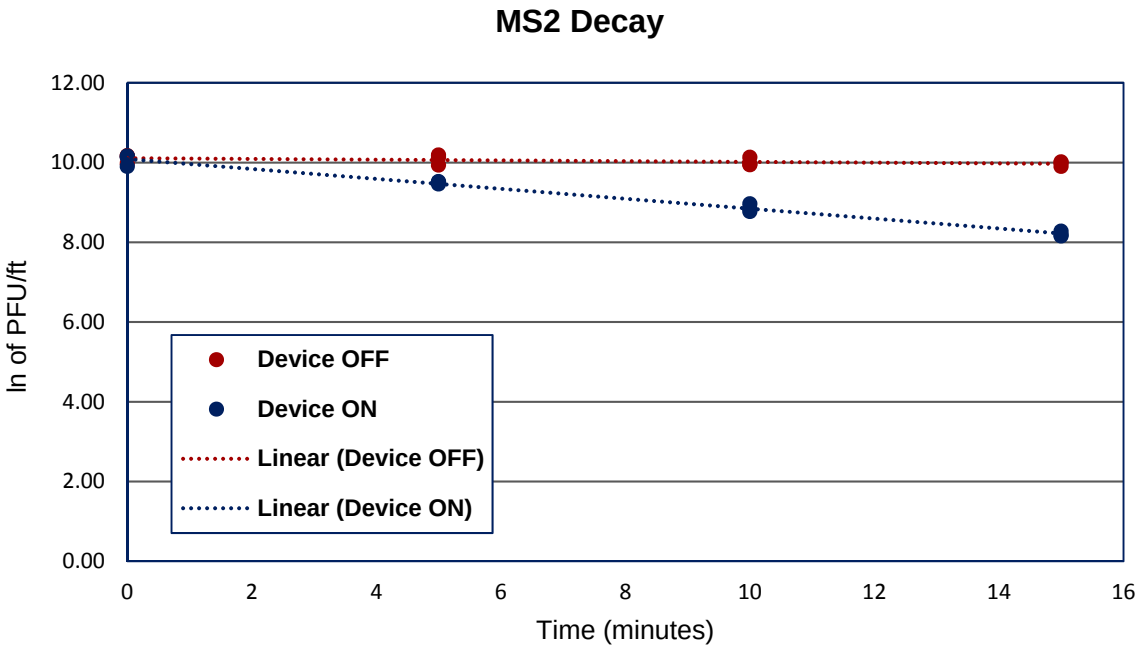

Figure 4. Decay curves for the MS2

All of the challenge microorganisms showed an increase in decay rates with the device ON compared to the natural decay (device OFF) run. This was evident based on the slopes of the linear regressions. The measured decay rates calculated according to the CARm method for each organism are shown in Table 1.

Table 1. Decay rates measured for introduced microbial bioaerosols

	Device OFF		**Device ON**	
	k_n	Std dev	k_e	Std dev
Staphylococcus epidermidis	**-0.0328**	**0.0046**	**0.1687**	**0.0138**
Bg spores	**-0.0086**	**0.0032**	**0.0972**	**0.0138**
MS2	**-0.0093**	**0.0048**	**0.1244**	**0.0045**

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 6.

Table 2. CARm values calculated from mean decay rates

	Staphylococcus epidermidis	Bg spores	MS2
CARm (cfm)	**87.0**	**56.7**	**73.7**
s.d.	**13.6**	**4.0**	**7.7**

One way to interpret this data set is to look at what the percentage of organisms that would be removed if this device operated for one hour. Assuming the same room conditions, including lack of leaks, no sources of organisms, and size, and looking at only the influence of the air cleaner (actual removal level would be slightly higher due to, for example, gravity), the predicted removal/inactivations of organisms over one hour are shown in Table 3. To put this in perspective, remember that the CARm is in units of clean air supplied by the unit in one minute. An hour's use would mean that 60 times as much cleaned air would be supplied to the room.

Table 3. Predicted removal level in one hour of continuous use (applies to the chamber in the test)

	Staphylococcus epidermidis	Bg spores	MS2
Removal/Inactivation level (%)	**99.97**	**99.51**	**99.90**

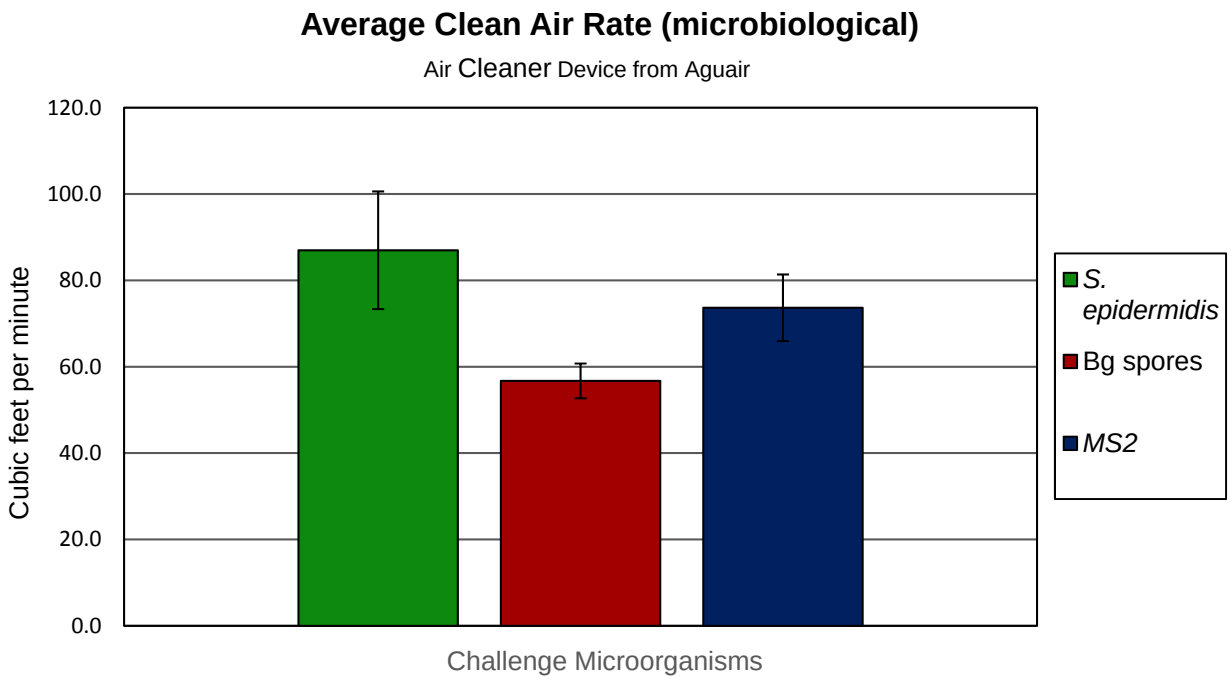

Figure 6. CARm values for organisms tested

3. Conclusion

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 will not exceed the air cleaner flow rate.

This device is shown to inactivate all three organisms with different efficacy depending on the organism. In one hour in a similar room, this device could be expected to remove 99.97% of *Staphylococcus epidermidis*; 99.51% of Bg spores; or 99.90% of MS2.