October 20, 2021

Bioaerosol Inactivation Chamber Test of an Air Cleaner Challenged with MS2

Test Report

Prepared for

Carol A. Miller
Executive Director
Alliance for Manufacturing and Technology
5 South College Drive, Suite 104
Binghamton, NY 13905
607.774.0022 x316
cmiller@amt-mep.org

Prepared by

Jean H. Kim, Ph.D.
RTI International
P.O. Box 12194
Durham, NC 27709
(919) 541-8087
jeankim@rti.org

RTI Quote No.: 0282103.413
RTI Project No.: 0216376.053.001

Contents

Figures

Tables

1. Introduction

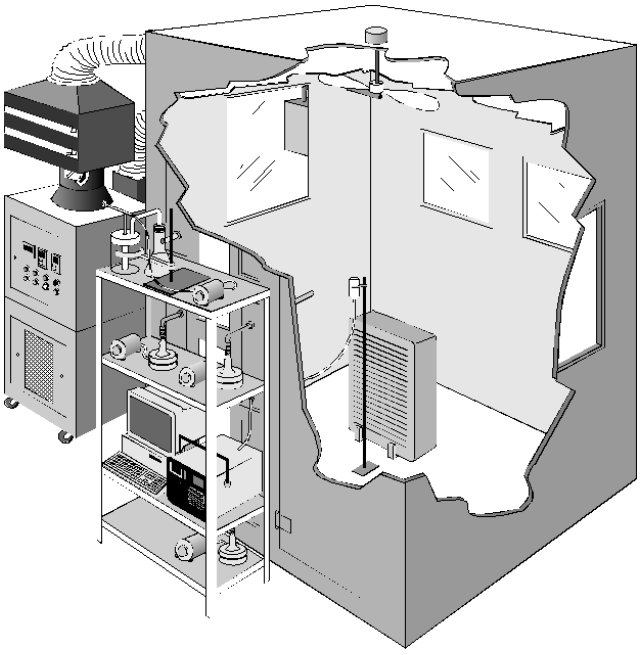
Under contract with Alliance for Manufacturing & Technology (AM&T), RTI performed a bioaerosol chamber test of a device provided by uvcPhyzx using MS2 bacteriophage[1]. MS2 was used to represent an infectious viral aerosol, and the device provided UV disinfection only. The objective of this study was to determine the Clean Air Rate (microbial) (CARm) of the device when challenged with the MS2 aerosol, which was accomplished by comparing the natural decay rate (device off) with the device decay rate (device on) for the microorganism.

2. Procedures

The device was placed into RTI's Dynamic Microbiological Test Chamber (DMTC) for testing (**Figures 1 & 2**). It was positioned in the corner of the room opposite from the entry point of the bioaerosol. The device was turned on by plugging into an outlet outside of the chamber.

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 (T) and relative humidity (RH) at the start of the test averaged 69.8 ± 0.3°F and 32.6 ± 0.0%, respectively, and at the end of the test, they averaged 69.3 ± 0.0°F and 32.9 ± 0.6%, respectively.

Figure 1. Schematic of DMTC with Sampling Instrumentation



The challenge microorganism selected for testing was as follows:

- MS2 bacteriophage, a common viral simulant for mammalian viruses such as influenza and norovirus.

The bioaerosol suspension was 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 (5.0 ft) above the floor of the chamber and 1.0 m (3.3 ft) from the

[1] See Appendix A-1 for justification for using MS2 as a viral aerosol simulant.

front wall. Port B was 1.52 m (5.0 ft) above the floor but was 0.25 m (0.8 ft) from the front wall of the chamber. The third port, C, was directly below Port A, but 0.65 m (2.1 ft) above the floor of the chamber. Sampling of the bioaerosol was accomplished using an all-glass impinger (AGI-4) 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 2. Location of uvcPhyzx Device Inside the DMTC.

2.1 Test Protocol:

The test protocol was as follows:

1. Start with the device in the DMTC but powered off.
2. Turn on the chamber ventilation system and ceiling fan.
3. Allow the HEPA to clean the chamber air for at least 30 minutes.
4. Turn off the ventilation system and ceiling fan.
5. Measure temperature (T) and relative humidity (RH) of the chamber.
6. Turn on the Collison nebulizer & ceiling fan. Run for 5 minutes with HEPA-filtered drying air flowing at 3.5 standard cubic feet per minute (SCFM).
7. Stop the Collison nebulizer and drying air. Mix the chamber using the ceiling fan in the chamber for 1 minute.
8. Turn the ceiling fan off and obtain a time 0 sample.
9. Collect triplicate bioaerosol measurements for 2 minutes at time intervals (5, 10 and 15 minutes).
10. Measure T & RH of the chamber.
11. Clean out the chamber for 20 minutes using the ventilation system with the ceiling fan on.
12. Repeat steps 4 – 11 with the device “on” after step 7.

2.2 Calculations:

The performance of the air cleaner was evaluated by determining the effective Clean Air Rate (microbial) or CARm, calculated as the CADR in the AHAM method[2]. To calculate the effective CARm, the measured decay (k_e) (device ON) and natural decay (k_n) (device OFF) rates are first calculated using the formula:

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

where:

C_t = concentration at time, t
n = number of data points used in the regression
k = decay constant (time^{-1})
t = time (min.)

[2] Association of Home Appliance Manufacturers (AHAM) Clean Air Delivery Rate: https://ahamverifide.org/

Then the effective CAR(m) was calculated for each measured decay rate, using the formula:

$$effective\ CARm = V(ke - kn) \qquad \text{Equation 2}$$

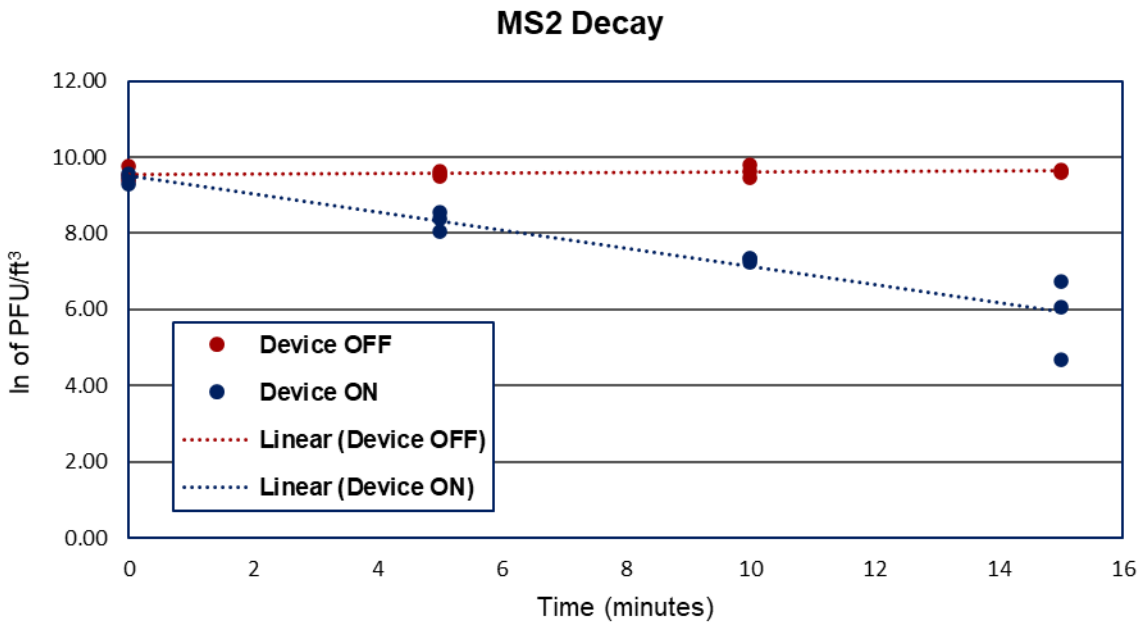
where:

V = volume of the test chamber (ft^3)
ke = measured decay rate (min^{-1})
kn = average natural decay rate (min^{-1}) for an organism.

3. Results

The decay curve for MS2 used to challenge the device are shown below (**Figure 3**). The natural log 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 curve is labeled "Device OFF," while the air cleaner decay curve (with the air cleaner running) is labeled "Device ON."

Figure 3. Decay Curve for MS2.



MS2 aerosol showed an increase in the decay rate with the device ON compared to the natural decay (device OFF) run. The measured decay rates calculated according to the CARm method for MS2 is shown in **Table 1**.

Table 1. Decay Rates Measured for Introduced MS2 Bioaerosol.

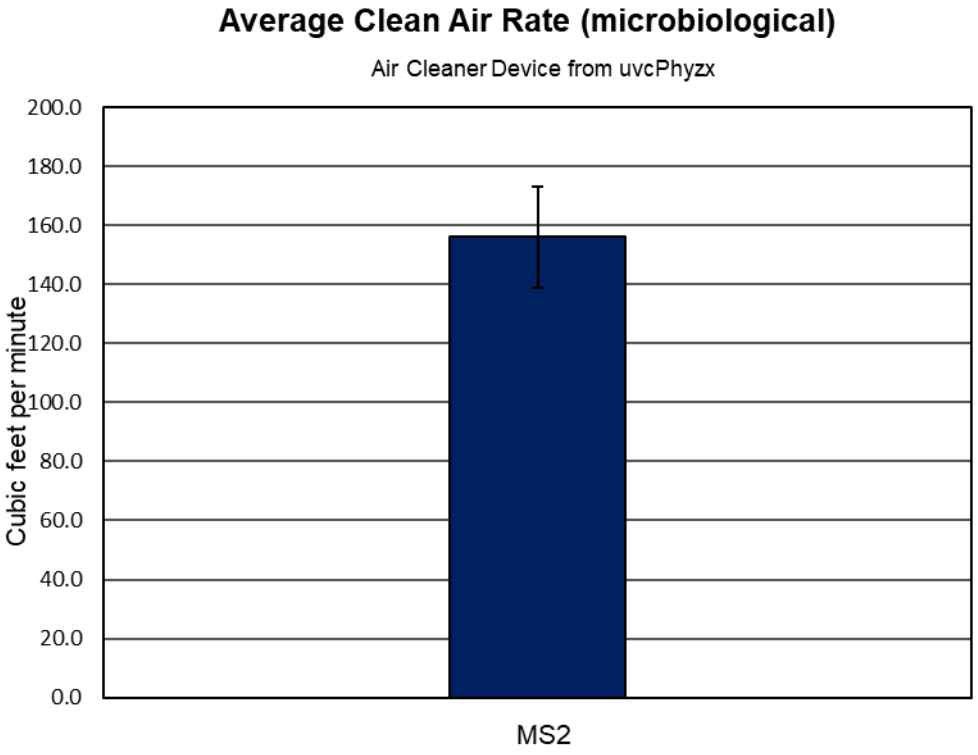
	Device OFF		Device ON	
	k_n	Std dev	k_e	Std dev
MS2	0.00664	0.0060	-0.2372	0.0260

Table 2 presents the average effective CARm result and standard deviation. The effective CARm was calculated using Eq. 2 and is a comparison of the two decay rates (natural and device) as a function of the volume of the test chamber (640 ft^3). A higher CARm rate indicates a large air cleaning capability. The result is also displayed graphically in **Figure 4**.

Table 2. Effective CARm Values Calculated from Mean Decay Rates.

	MS2
Average CARm	**156.0**
s.d.	**17.1**

Figure 4. Effective CARm Values for MS2 Tested.



4. Discussion and 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 disinfection 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 disinfection 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 which was provided by uvcPhyzx as 125

CFM. However, this device was shown to inactivate MS2 with a CARm of 156 CFM, which is actually higher than the flow rate of the air cleaner as provided by uvcPhyzx.

There are a couple factors that could result in a higher CARm compared to the device flow rate: 1) the flow rate of the device may be off or 2) since the device uses UV disinfection, there may be generation of reactive species, specifically ozone, resulting in the inactivation of virus outside of the exposure zone of the device. Both factors were assessed by uvcPhyzx. The air flow rate was assessed using a calibrated flow meter and measured to be between 125 – 135 CFM. This indicated that the air flow rate was not off from what was previously mentioned. Next, to address the potential for reactive species, uvcPhyzx performed measurements of ozone, a typical reactive species produced by UV light, using a NIST traceable, calibrated ozone meter, and found that there was no ozone detected by the meter (< 0.1 ppm). Based on these results, we concluded that there was another factor contributing to the higher CARm value compared to the device flow rate.

Later it was postulated that the cause for the higher CARm might be due to the function of the uvcPhyzx device and how it works to clean the air. The device is designed to draw heavier active viral concentrations from along the floor, sanitize it as it travels vertically up through the device and then discharge the sanitized air in a “fan” at the ceiling. The flow of air isn't enough to feel a draft but it does create a pressure differential for a generally downward flow from top to bottom. The concept is that shed virus is drawn downward away from others in the room and provide a source of clean air from the top. With the preferential downward movement of air, the homogenously mixed MS2 bioaerosol in the chamber, is moved downward when the device is on removing active virus from the air during sampling and expulsion of irradiated air out the top of the device preferentially distributing inactive virus in the air. Overall, this process provides a higher decay of active viral particles in the air during sampling and thus a higher CARm compared to the flow rate of the device.

Appendix A:
Supplemental Information for Interpreting Results

A-1. Suitability of MS2 bacteriophage as viral aerosol surrogate and use for COVID-19

MS2 bacteriophage has been used in many studies as a safe surrogate for human viral aerosols when Biosafety Level 2 or less is desired and to manage risk to researchers. Although MS2 is generally recognized as a good surrogate for a variety of human infectious bioaerosols, it is also regarded as typically hardier (i.e., resistant to inactivation) than human viral bioaerosols. Example studies and research on the topic:

- Turgeon, T., M-J. Toulouse, B. Martel, S. Moineau, and C. Duchaine. (2014) "Comparison of Five Bacteriophages as Models for Viral Aerosol Studies." *Applied and Environmental Microbiology*. 80 (14) 4242-4250; DOI: 10.1128/AEM.00767-14
- Rengasamy S., E. Fisher, R. E. Shaffer. (2010) "Evaluation of the survivability of MS2 viral aerosols deposited on filtering face piece respirator samples incorporating antimicrobial technologies," *American Journal of Infection Control.* 38 (1) 9-17 https://doi.org/10.1016/j.ajic.2009.08.006
- Verreault, D., M. Marcoux-Voiselle, N. Turgeon, S. Moineau, and C. Duchaine, Caroline. (2015). "Resistance of Aerosolized Bacterial Viruses to Relative Humidity and Temperature." *Applied and environmental microbiology*. DOI: 10.1128/AEM.02484-15

MS2 bacteriophage is being used as a surrogate for understanding inactivation of SARS-CoV-2, the causative virus for COVID-19. However, it is used as a surrogate with the understanding that MS2 is hardier that SARS-CoV-2, such that good inactivation of MS2 should translate to better inactivation of SARS-CoV-2. A couple of recent papers on the issue are:

- Hadi, J., M. Dunowska; S. Wu, and G. Brightwell. (2020) "Control Measures for SARS-CoV-2: A Review on Light-Based Inactivation of Single-Stranded RNA Viruses" *Pathogens* 9(9): 737. https://doi.org/10.3390/pathogens9090737
- Mohan, S.V, M. Hemalatha, H. Kopperi, I. Ranjith, and A. Kiran Kumar. (2021). "SARS-CoV-2 in environmental perspective: Occurrence, persistence, surveillance, inactivation and challenges." *Chemical Engineering Journal* 405: 126893, https://doi.org/10.1016/j.cej.2020.126893

A-2. Example use of CARm and CDC Guidelines

Example of using the CARm results for the device tested in this report to determine how long is needed for a room the same size as the test chamber to be clean and safe to use after it has been used, assuming release of a bacterial or viral aerosol of concern.

The needed information is:

- The size of the room
- CARm number for the bioaerosol of concern

- The heating, ventilation, and air conditioning (HVAC) information about the room and any clean/fresh air delivery rate to that room
- The desired safety level

Room size:

- The chamber is 8 × 8 × 10 ft
- Thus, the volume of room is 640 ft^3

Bioaerosol of concern and CARm Number:

- We will examine the viral aerosol represented by MS2 phage
- From the report results we have the effective air cleaning rate as:
  - CARm, MS2 = 156 ft^3/min (cfm)

Calculate the air exchange rate per hour (ACH) for the example room

- It is assumed that only the HVAC system provides air circulation in the room with a rate of air mixing used in the test chamber.
- For simplicity of calculation, it is assumed no additional air exchanges of fresh air are provided by the HVAC system
- ACH = (CARm × 60 min)/(Room Volume ft^3)
- ACH = (156 cfm × 60 min)/(640 ft^3)
  - ACH for virus = 14.6
- A typical HVAC system will provide between 1 and 2 ACH but with only about 30% fresh and filtered air dropping the effective ACH to approximately 0.3 to 0.6 ACH. Including these will only make minor improvements in the cleanliness of the air since the CARm for the tested device at the tested conditions provides more than 4 ACH on its own.

Time needed to remove percentage of contaminants from the room

- CCD guidelines: https://www.cdc.gov/infectioncontrol/guidelines/environmental/appendix/air.html#tableb1
- The data in the table were fitted to provide results for the ACH obtained in the scenarios provided in this example
- Table of results for time needed to achieve 99% and 99.9% removal of contaminants

	Time (min) to Achieve % Removal (Safety Level)	
Scenario & Bioaerosols	**99%**	**99.9%**
Case of only air cleaner running		
Virus	19	28.5