Single Pass Efficacy of the Boskiair Device against Aerosolized *MS2 Bacteriophage (RNA virus)*

ARE Labs Project #: 10895.10
October 13, 2020

Jamie Balarashti [a], Jeffery Trolinger [a]

[a] Aerosol Research and Engineering Laboratories Inc. Olathe KS

Background: This in-vitro study characterized the decontamination efficacy of the Boskiair Device at multiple fan speeds against aerosolized *MS2 Bacteriophage (RNA virus)*. The Boskiair air sanitization device utilizes high intensity UV-C light to reduce bacteria, viruses and spores from the air. The device is designed to reduce airborne pathogens and thus mitigate disease transfer. MS2 Bacteriophage is a non-pathogenic RNA virus that is commonly used as a research surrogate for Influenza viruses and, more recently, as a potential surrogate for SARS-CoV2. The effectiveness of the Boskiair device was assessed in a 300ft^3 bioaerosol chamber. Testing was conducted in triplicate using three (3) different fan speed for a total of nine (9) trials.

Methods: *MS2 Bacteriophage* was continuously aerosolized into a sealed 300ft^3 environmental chamber containing the Boskiair Device. A sample probe was inserted into the Boskiair Device after the UV-C light chamber to sample the air downstream of the decontamination mechanism. The sample probe was then connected via tubing to a chamber sample port for extraction. The upstream sample consisted of an overall chamber concentration before entering the device. All impinger samples were serially diluted, plated, and enumerated in triplicate to yield the viable bioaerosol concentration at each sampling time point. Control trial data was subtracted from the Boskiair Device trial data to yield the net LOG reduction of viable bioaerosol concentration attributable to the device within the chamber.

Results: Three trials were conducted with the Boskiair device at three different fan speeds to determine its single pass efficacy at removing *MS2 Bacteriophage* from the air. The Boskiair device showed an average of 2.98 +/- 0.05 LOG reduction of MS2 bioaerosol with the device set at 100% fan speed. When the fan speed was turned down to 50% the single pass LOG reduction increased to 3.38 +/- 0.06 LOG. When the device was operated at 25% fan speed the single pass LOG reduction increased to 5.10 +/- 0.16 LOG.

Summary: Overall, the Boskiair Device performed extremely well at every fan speed. The decrease of fan speed on the device led to an increase in single pass LOG reduction. When the device was tested at 25% fan speed it is believed that the air in the device was moving so slow that the results of those trials could be a result of sampling air that wasn't being cycled through allowing for the longest exposure time. This is expected to be the cause of the large gap in single pass LOG reduction from the 50% to 25% fan speed trials.

This study was conducted in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

Overview

This study was conducted to evaluate the single pass efficacy of the Boskiair air sanitization device at removing MS2 bioaerosols. This device features high intensity UV-C light in order to sanitize the air. Testing was conducted in a 300ft^3 custom bioaerosol exposure chamber. The Boskiair Device's effectiveness was tested at 100%, 50%, and 25% fan speed against aerosolized MS2 Bacteriophage in order to evaluate the device's single pass LOG reduction of viable bioaerosol. Testing consisted of triplicate trials at the three fan speeds listed above for a total of nine (9) trials.

During the nine (9) test trials the Boskiair device was activated for the entirety of the trial. MS2 was continuously nebulized during the trial with the chamber mixing fan turned on to ensure the chamber remained a homogenous mixture. Midget impinger samples, up and downstream, were taken simultaneously for the same amount of time for every sample collected. Upstream and downstream concentrations were directly compared for each trial to determine the single pass efficacy of the device at each fan speed. A picture of the device can be found in **Figure 1**, a test matrix outlining all conducted single pass testing can be found in **Figure 2**.

Boskiair Device Picture	
	
Device:	Boskiair Air Sanitizer Device
Decontamination Method:	UV-C Light
Manufacturer:	Boskiair

Figure 1: *Front view (left) and back view (right) of the Boskiair Device.*

Test Location and Conditions

Testing was conducted at Aerosol Research and Engineering labs located at 15320 S. Cornice Street in Olathe, Kansas 66062. Laboratory conditions were approximately 75.0°F with 60% relative humidity during this study.

Testing Chamber

The primary aerosol exposure chamber containing the Boskiair device is a sealed 300ft^3 environmental chamber constructed of 3/8" Lexan and outfitted with all necessary pass-though and sub-systems sampling ports. The chamber is equipped with HEPA filtered house air in order to maintain a clean background environment prior to all testing and to allow rapid air flushing through the chamber after completion of each exposure to ensure a clean background at before conducting subsequent trials.

During the aerosolization of the bioaerosol, the chamber was operated in a balanced push/pull aerosol inlet and vacuum to eliminate over or under pressure in the chamber. The chamber was operated at a slightly negative pressure, -0.3 inH2O, for technician safety. Once aerosolization of the challenge organism at the beginning of each trial was complete, the inlet and vacuum balance were cut off and the chamber sat idly until air sample collections. The chamber is outfitted with two AGI-30 impinger sample ports located on the long ends and on top of the chamber.

The chamber was equipped with a mixing fan to ensure spatial homogeneity of bioaerosol during aerosolization and sampling. This fan was switched on during the aerosolization of the bioaerosol into the chamber and left on for the duration of all of the trials. A test chamber flow diagram can be found in **Figure 3**.

Boskiair Single Pass Testing Matrix

Trial	Run	Device Speed	Device	Organism	Target Monodispersed Particle Size	Sample Time (min)	Equipment
T1	Challenge	100%	Boskiair Air Sanitizer	*MS2 Bacteriophage - RNA Virus*	1.8-2.0um	5	*Chemglass Midget Impingers, 6-jet collison nebulizer*
T2	Challenge	100%					
T3	Challenge	100%					
T4	Challenge	50%	Boskiair Air Sanitizer	*MS2 Bacteriophage - RNA Virus*	1.8-2.0um	5	*Chemglass Midget Impingers, 6-jet collison nebulizer*
T5	Challenge	50%					
T6	Challenge	50%					
T7	Challenge	25%	Boskiair Air Sanitizer	*MS2 Bacteriophage - RNA Virus*	1.8-2.0um	5	*Chemglass Midget Impingers, 6-jet collison nebulizer*
T8	Challenge	25%					
T9	Challenge	25%					

Figure 2: *Testing Matrix*

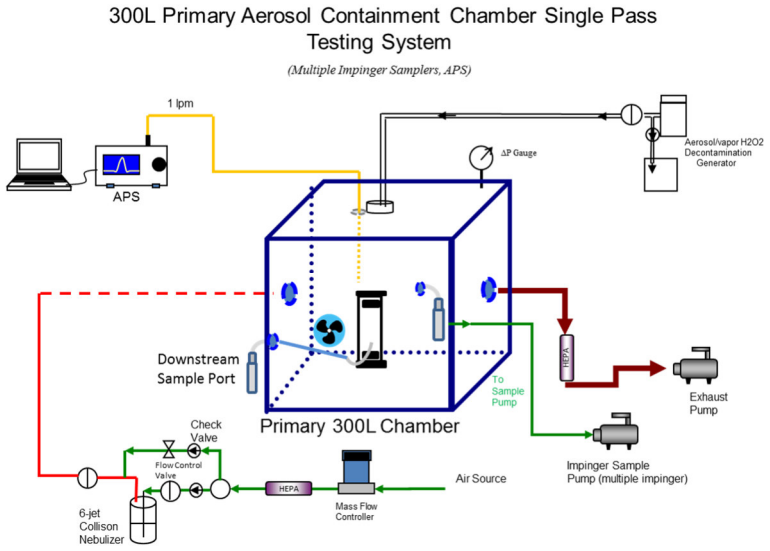

Figure 3: *Single Pass Testing System Exposure Chamber Flow Diagram*

Bioaerosol Generation System

MS2 was disseminated using a MiniHeart Hi-Flo medical nebulizer (Westmed Inc. Tuscon, AZ) driven by purified filtered house air supply. A pressure regulator allowed for control of disseminated particle size, use rate, and sheer force generated within the Collison nebulizer.

Bioaerosol Sampling and Monitoring System

Two ChemGlass midget impingers were used for bioaerosol collection to determine upstream and downstream concentrations. The upstream impinger sampled the ambient chamber air to determine initial concentrations. The downstream impinger was connected to a sample probe installed by the outlet of the Boskiair device. The midget impinger vacuum source was maintained at a negative pressure of 18 inches of Hg during all characterization and test sampling to assure critical flow conditions. The ChemGlass midget impingers were flow characterized using a calibrated TSI model 4040 mass flow meter.

The impingers were filled with 5 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection. The addition of Tween 80 was shown to increase the impinger collection efficiency and de-agglomeration of all microorganisms for proper plate counts.

Species Selection

Species selection is based on Biological Safety Level 1 (BSL1) surrogates for BSL3 pathogenic organisms. MS2 bacteriophage (ATCC 15597-B1) is positive-sense, single-stranded RNA virus that infects the bacterium Escherichia coli and other members of the Enterobacteriaceae family. MS2 is routinely used as a surrogate for pathogenic RNA viruses, such as influenza and is a tentative surrogate for coronavirus, SARS-CoV2. The species selection table for MS2 Bacteriophage is shown in **Figure 4**.

The US FDA guidance document; Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency; states that lipid enveloped viruses such as coronaviruses are the least resistant microorganisms to disinfectants. It is assumed that this trend is similar for other chemical and catalytic methods

Type	Surrogate Species	ATCC #	Host	Growth Media	Incubation	Incubation Temp	Notes
RNA Virus	MS2 Bacteriophage	15597-B1	E. coli 15597	Trypticase Soy	Aerobic	37°C	Infectious Agent

Figure 4: *Species selection table*

of kill. MS2 is an icosahedral, positive-sense, single-stranded viral RNA bacteriophage. It is non-lipid enveloped, which makes it more resistant to disinfection than lipid enveloped viruses. It therefore should be more resistant to kill. **Figure 4** is a graphic from the FDA document, COVID Sterilizers, Disinfectant Devices, and Air Purifiers Guidance, demonstrating microbial resistance to disinfection.

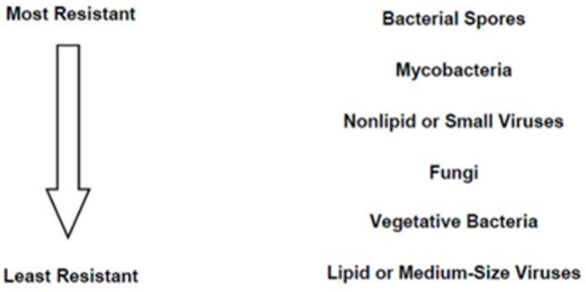

Figure 5: *Disinfection Resistance graphic from the FDA document*

Viral Culture & Preparation

Pure strain viral seed stock and host bacterium were obtained from ATCC. Host bacterium was grown in a similar fashion to the vegetative cells in an appropriate liquid media. The liquid media was infected during the logarithmic growth cycle with the specific bacteriophage. After an appropriate incubation time the cells were lysed and the cellular debris discharged by centrifugation. MS2 stock yields were greater than 1 x 10^{11} plaque forming units per milliliter (pfu/ml) with a single amplification procedure.

Bioaerosol Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate (multiple serial dilutions) using a standard spread plate assay technique onto tryptic soy agar plates. The plated cultures were incubated for 24 hours, enumerated and recorded.

Bioaerosol Particle Size Data

Aerosol particle size distributions were measured with the APS. The APS has a dynamic measurement range of 0.5 to 20µm and was programmed to take consecutive real time one minute aerosol samples throughout the duration of each aerosol trial. Data was logged in real time to an Acer laptop computer, regressed, and plotted. Aerosol particle size distribution for MS2 is shown in **Figure 5.**

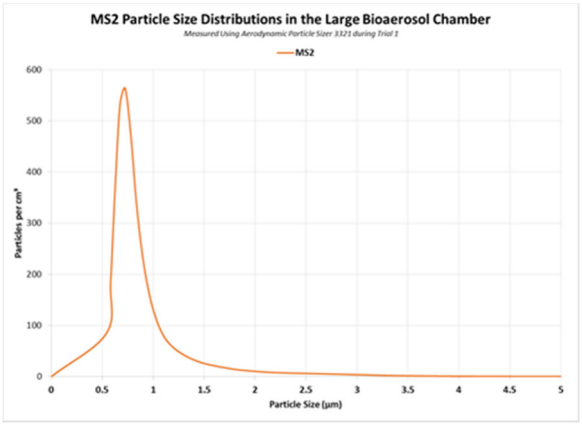

Figure 6*: Viral (MS2) Particle Size Distribution in Test Chamber*

Boskiair Device Testing Method

For each challenge test, the nebulizer was filled with approximately 30 mL of biological stock and operated at 35 psi for the duration of testing. During the Boskiair Device trials, the impingers were filled with 5 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection. The addition of Tween 80 has been shown to increase the impinger collection efficiency and de-agglomeration of micro-organisms.

The chamber mixing fans were turned on for the duration of every trial to assure a homogeneous bioaerosol concentration in the test chamber prior to the impinger samples. For the remainder of both control and test trials mixing fans remained off except during sampling periods in order to ensure bioaerosol homogeneity within the chamber for accurate sample collection.

Following bioaerosol generation, the upstream impinger sampling the ambient chamber and the downstream impinger sampling the Boskiair Device output air were sampled in unison for a duration of 3 minutes. After the three minutes the aliquots of impinger samples were collected and then used for plating. Impingers were rinsed 6x with sterile filtered water between each sampling interval, and re-filled with sterile PBS using sterile graduated pipettes for sample collection.

In order to test the various fan speeds for this experiment the fan speed of the device was characterized using an anemometer. The cord that controlled the fan for the device was then plugged into a variable auto transformer also known as a variac to determine at which voltage the device ran at 50% fan

speed and then at 25% fan speed. The UV light of the device was left unaltered for the duration of testing. It was plugged in to a separate power source and left at full power.

Plates were incubated and enumerated for viable plaque forming unit (pfu) counts to calculate bioaerosol challenge concentrations in the chamber and reduction of viable microorganisms. This testing method was designed to assess the viable bioaerosol reduction in the test chamber, it did not directly assess the kill of the microorganisms. The test matrix for this study is shown in **Figure 7.** A timeline diagram for all conducted trial testing is shown in **Figure 8.**

Post-Testing Decontamination and Prep

Following each test, the chamber was air flow evacuated/purged for a minimum of thirty minutes between tests and analyzed with a TSI Aerodynamic Particle Sizer (APS) for particle concentration decrease to baseline levels between each test. At the conclusion of testing the chamber was decontaminated using 35% vaporous food grade hydrogen peroxide. The nebulizer and impingers were cleaned at the conclusion of each day of testing by soaking in a 5% bleach bath for 20 minutes. The nebulizer and impingers were then submerged in a DI water bath, removed, and spray rinsed 6x with filtered DI water until use.

Data Analysis

The data analysis of this testing shows the results of the triplicate trials for each condition conducted for this study. All results indicate the calculated viable bioaerosol inside the exposure chamber and the concentration of viable aerosol post exposure through the device. All trials show individual and group average +/- standard deviations for LOG reduction on a per trial basis.

Boskiair Device Results

MS2 stock was prepared 48 hours in advance, and grew to a concentration greater than 10e9 cfu/ml the same *MS2* stock was used for all conducted testing. For the trials that were ran at 100 % fan speed the average single pass LOG reduction of the trials came out at 2.98 LOG with a standard deviation of 0.05 LOG.

When the Boskiair Device was tested at 50% fan speed the device single pass LOG reduction increased to 3.38 LOG with a standard deviation of 0.06.

The trials of the Boskiair Device where the fan speed was set to 25% fan speed the devices single pass LOG reduction showed an increase to 5.10 LOG reduction with a standard deviation of 0.16 LOG. The results of all of the trials as well as the group averages +/- standard deviation are pictured graphically in **Figure 7** and can be found in a summary table in **Figure 8**.

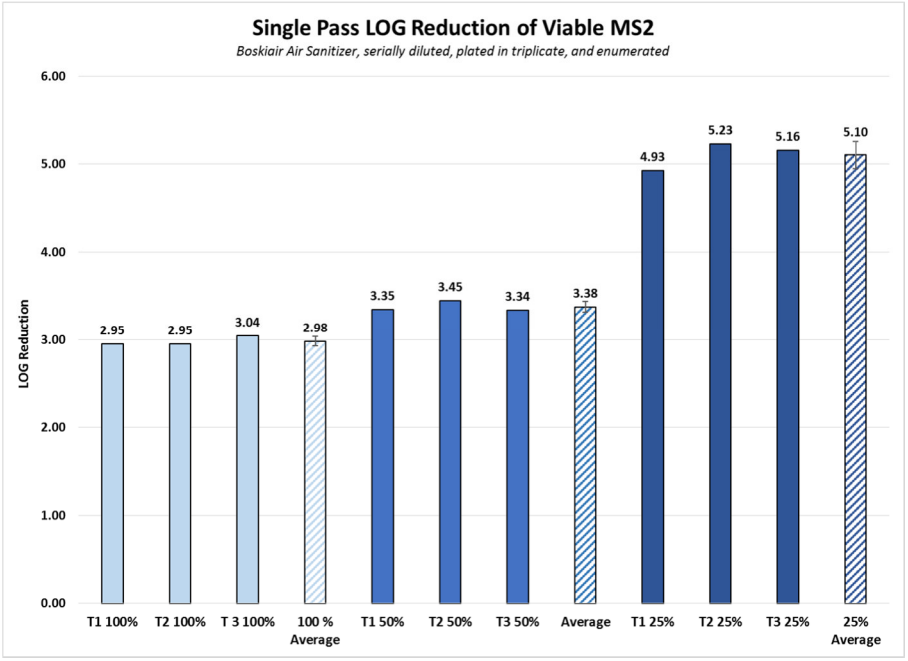

Figure 7: *LOG Reduction of MS2 Bacteriophage in control and Air Care 2 Device test trials.*

Boskiair Single Pass Testing Executive Summary Data

Trial ID	Upstream Conc.	Downstream Conc.	Percent remaining	Percent Reduction	LOG Reduction	Net LOG Reduction
T1 100%	2.05E+07	2.28E+04	0.111%	99.889%	-2.95	2.95
T2 100%	2.00E+07	2.25E+04	0.113%	99.888%	-2.95	2.95
T 3 100%	1.10E+07	1.00E+04	0.091%	99.909%	-3.04	3.04
100 % Average	2.03E+07	2.27E+04	0.105%	99.895%	-2.98	2.98
Standard Deviation	5.35E+06	7.32E+03	0.012%	0.012%	0.05	0.05
T1 50%	3.22E+07	1.45E+04	0.045%	99.955%	-3.35	3.35
T2 50%	2.40E+07	8.50E+03	0.035%	99.965%	-3.45	3.45
T3 50%	2.62E+07	1.20E+04	0.046%	99.954%	-3.34	3.34
Average	2.74E+07	1.17E+04	0.0421%	99.9579%	-3.38	3.38
Standard Deviation	4.23E+06	3.01E+03	0.006%	0.006%	0.06	0.06
T1 25%	4.15E+07	4.88E+02	0.0012%	99.9988%	-4.93	4.93
T2 25%	4.33E+07	2.57E+02	0.0006%	99.9994%	-5.23	5.23
T3 25%	4.18E+07	2.92E+02	0.0007%	99.9993%	-5.16	5.16
25% Average	4.22E+07	3.46E+02	0.0008%	99.9992%	-5.10	5.10
Std. Deviation	9.46E+05	1.25E+02	0.000%	0.000%	0.16	0.16

Figure 8: Net *LOG Reduction of MS2 in Boskiair Device device test trials with Average +/- Std. Deviation.*

Summary

Overall the Boskiair Device performed extremely well with a 2.98 LOG single pass reduction in viable airborne bioaerosol concentration when the devices fan speed was operating at 100%. The devices LOG reduction increased as the fan speed on the device decreased going down to a 3.38 LOG reduction when the fan was operated at 50%.

When the device was tested at 25% fan speed it appears that the speed was too low to move a steady stream of air through the device. Due to the low fan speed the resonance time of the MS2 was increased significantly which allows for longer and more intense exposures to the UV lights which increases single pass efficacy. This results in a very high LOG reduction of 5.10 LOG at a single pass.

This testing confirms that on each fan speed the Boskiair Device would be effective at reducing airborne influenza and norovirus pulmonary infections. Based on the FDA recommended surrogate organism chart this also implies that it would also have efficacy against airborne SARS-CoV2, although, specific claim would require testing against the SARS-CoV2 virus.

References

T. Reponen, K. Willeke, V. Ulevicius et al. *Techniques of Dispersion of Microorganisms in Air.* Aerosol Science and Technology. 27: 1997. pp. 405-421.

Ding, Pei & Wang, Chiu-sen. (2001). Effect of Sampling Time on the Total Recovery Rate of AGI-30 Impingers for E. coli Aerosols. Aerosol and Air Quality Research. 1. 10.4209/aaqr.2020.06.0010.

Analytical GLP Certificate

Aerosol Research and Engineering Labs, Inc.
15320 S. Cornice Street
Olathe, KS 66062

Project

10867.30

Study Director

Jamie Balarashti
Aerosol Research and Engineering Laboratories

GLP Statement

We, the undersigned, herby certify that the work described herein was conducted by Aerosol Research and Engineering Laboratories in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

Study Director:

Jamie D. Balarashti
Study Director
ARE Labs, Inc.

10/13/2020
Date

Principal Investigator:

Jeffery Trolinger
Principal Investigator
ARE Labs, Inc.

10/13/2020
Date

Appendix A: Calculations

To evaluate the viable aerosol delivery efficiency and define operation parameters of the system, calculations based on (theoretical) 100% efficacy of aerosol dissemination were derived using the following steps:

- Plating and enumeration of the biological to derive the concentration of the stock suspension (C_s) in pfu/mL or cfu/mL, or cfu/g for dry powder.
- Collison 24 jet nebulizer liquid use rate (R_{neb}) (volume of liquid generated by the nebulizer/time) at 30 psi air supply pressure = 1.0 ml/min.
- Collison 24 jet Generation time (t) = 3 or 4 minutes, test dependent.
- Chamber volume (V_c) = 1,000 Liters
- Nebulizer Generation efficiency (ε) (usually around 10%)

Assuming 100% efficiency, the quantity of aerosolized viable particles (V_P) per liter of air in the chamber for a given nebulizer stock concentration (C_s) is calculated as:

$$\text{Nebulizer: } V_P = \frac{C_s \cdot R_{neb}}{V_c} t \cdot \varepsilon$$

AGI – 30 impinger or 47mm filter collection calculation:

- Viable aerosol concentration collection (C_a) = cfu or pfu/L of chamber air.
- Viable Impinger concentration collection (C_{Imp}) = cfu or pfu/mL from enumeration of impinger sample or filter sample.
- Impinger sample collection volume (I_{vol}) = 20 mL collection fluid/impinger, or extraction fluid for filter.
- AGI–30 impinger or filter sample flow rate (Q_{imp}) = 12.5 L/min.
- AGI–30 impinger or filter sample time (t) = 2 to 10 minutes, test dependent.

For viable impinger or filter aerosol concentration collection (C_a) = cfu or pfu/L of chamber air:

$$C_a = \frac{\mathrm{C_{Imp} \cdot I_{vol}}}{\mathrm{Q_{imp}}} \mathrm{t}$$