Efficacy of the YFLife AIR6+ device against Aerosolized *MS2* bacteriophage in a 1m^3 chamber

ARE Labs Project #10918.20 - March 25th, 2021

Jamie Balarashti [a], Jeffery Trolinger [a]

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

Background: This in vitro study characterized the efficacy of the YFLife AIR6+ at removing aerosolized MS2 bacteriophage. The AIR6+ device is designed as a home air purifier, and features filtration as well as photocatalytic oxidation as well as an ionizer to reduce airborne bacteria, viruses, and fungal spores in order to decrease infections rates from airborne pathogens. For this study, the device was challenged at the highest setting using aerosolized MS2 bacteriophage which has been historically used as a surrogate for numerous respiratory viruses, such as influenza, rhinovirus, and is a tentative surrogate for SARS-CoV-2. This study evaluated the efficacy of the device against aerosolized MS2 bacteriophage, in a 1m^3 bioaerosol chamber. The study consisted of three (3) trials on the highest speed setting which is speed 3.

Methods: *MS2* bacteriophage was aerosolized into a sealed 1m^3 environmental chamber containing the YFLife AIR6+ system. AGI impingers were used to determine chamber bioaerosol concentrations at pre-determined sampling times. All impinger samples were serially diluted, plated and enumerated in triplicate to yield viable bioaerosol concentration at each sampling point and time. Chamber control trial data was subtracted from YFLife AIR6+ trial data to yield net LOG reduction in the chamber for viable bioaerosol concentration.

Results: Three trials were conducted to evaluate the YFLife AIR6+ system efficacy at removing viable *MS2* bacteriophage from the air. The YFLife AIR6+ device yielded a 2.14 +/- 0.33 net LOG reduction in a 60-minute time period and on average showed a 3.20 +/- 0.17 net LOG reduction in a 120 minute period in a 1m^3 test chamber. This corresponds to a 99.9333% +/- 0.025% net reduction in viable bioaerosol on average compared to the control in 120 minutes.

Summary: Overall, the YFLife AIR6+ system performed very well with an average 99.9333% net reduction in viable bioaerosol concentration within a 120 minute period. Testing was conducted using aerosolized *MS2* bacteriophage. This testing confirms that, in theory, the YFLife AIR6+ system should show efficacy at reducing the risk of viral pathogen infection.

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 efficacy of the YFLife AIR6+ at removing viable airborne MS2 bacteriophage. A picture of the device can be found in **Figure 1**. Testing was conducted in a 1m^3 custom bioaerosol exposure chamber. The efficacy of the device was determined by sampling of the chamber and evaluating the reduction capabilities of the device.

The YFLife AIR6+ contains a nano sterilization washable filter to block large particles, 3 dimensional Photocatalytic Oxidation (PCO) structure to purify incoming air, and an ionizer to reduce airborne particles. The bioaerosol chosen to determine the effectiveness of the YFLife air purification system was a single RNA non-enveloped virus which was the *MS2* bacteriophage. Testing was conducted in triplicate trials plus a control trial to demonstrate the capability of reducing viable bioaerosol concentrations. The control run served as a representative of the natural occurring reduction over the time allotted in the trials. There were a total of four (4) independent trials in this study.

During the control trial, the AIR6+ system remained inside the test chamber but was never turned on. During test trials, the system was turned on after initial chamber concentration sampling and remained running until the completion of the trial. *MS2* bacteriophage was aerosolized into the test chamber to begin the trial and then impinger samples were collected at set time points throughout each trial to characterize the efficacy of the device.

Manufacturer:	YFLife
Model:	AIR6+
Notes:	Purify by PCO and ionization

Figure 1*: YFLife Air Pro 6 Device*

Trials with the YFLife device turned on were compared to control trials in order to determine net LOG reduction of viable bioaerosols within the chamber. ARELabs does not assess the kill mechanism or kill rate of devices, only the reduction capabilities.

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 76°F with 41% relative humidity.

Testing Chamber

The primary aerosol exposure chamber containing the YFLife AIR6+ system is a sealed 1m^3 environmental chamber constructed of 3/8" Lexan and outfitted with all necessary pass-through 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 bioaerosols, 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 an AGI-30 impinger sample port located near the center of the box. A second sample port located at the front left corner of the box was used for viable cascade impactor sampling.

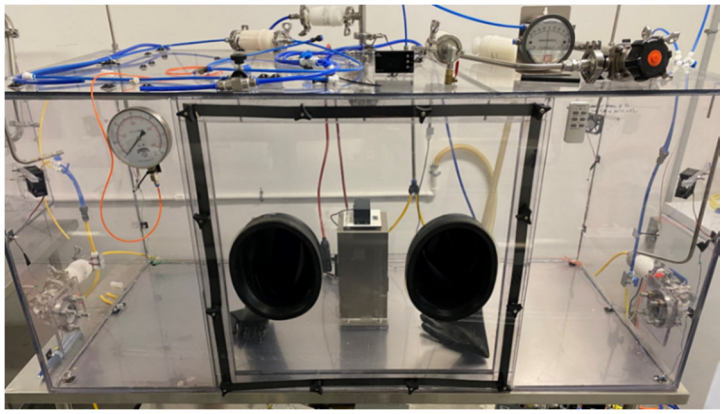


Figure 2: *Exposure Test Chamber*

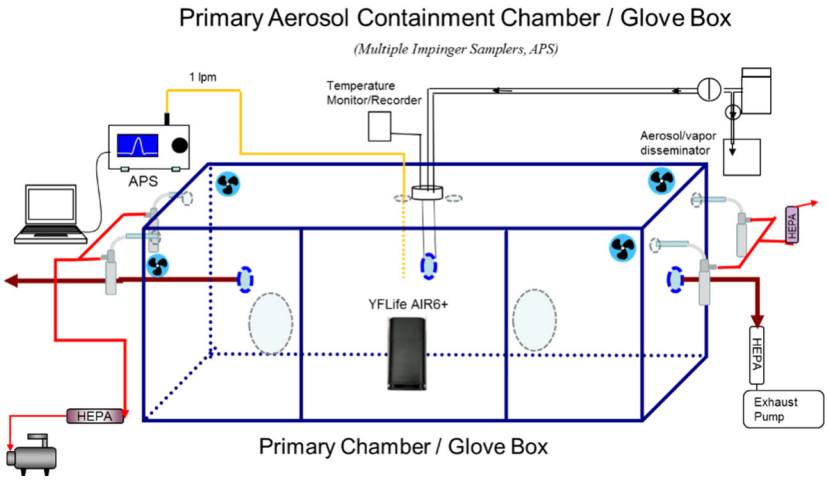


Figure 3: *Test Chamber Flow diagram for testing*

The chamber was equipped with four (4) mixing fans to ensure spatial homogeneity of bioaerosols during their aerosolization and sampling. These fans were switched on during the aerosolization of the bioaerosol into the chamber and remained on for the duration of the trials to ensure spatial homogeneity. **Figure 2** shows a picture of the full chamber setup containing the device.

Bioaerosol Generation System

Test bioaerosols were disseminated using a Collison 24-jet nebulizer (BGI Inc., Waltham MA) 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.

Prior to testing, the Collison nebulizer flow rate and use rate were characterized using an air supply pressure of approximately 35 psi, which obtained an output volumetric flow rate of approximately 50 lpm with a fluid dissemination rate of approximately 1 ml/min. The Collison nebulizer was flow characterized using a calibrated TSI model 4040 mass flow meter (TSI Inc., St Paul MN).

Bioaerosol Sampling and Monitoring System

An AGI-30 impinger (Ace Glass Inc., Vineland NJ) was used for bioaerosol collection of biological aerosols to determine the chamber concentration. This impinger was connected to the bioaerosol chamber via a sample port located near the center of the exposure box.

The AGI-30 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 AGI-30 sample impingers were flow characterized using a calibrated TSI model 4040 mass flow meter.

The impingers were filled with 20 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. Impingers were taken in quadruplicate and pooled for an overall average of chamber concentration.

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 a tentative surrogate for coronavirus, SARS-CoV2.

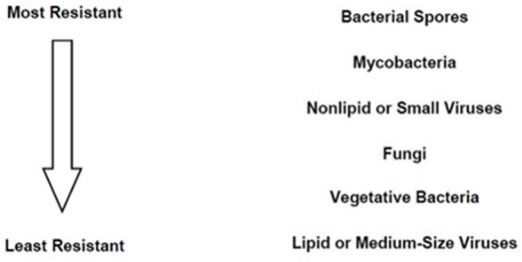


Figure 4: *FDA Graphic showing resistance to disinfection for various organisms.*

Biological Test Matrix

Trial	Run	Pathogenic Organism	Surrogate Species (gram, description)	ATCC Ref	Target Monodispersed Particle Size	Challenge Conc. (#/L)	Trial Time (min)	Sample Time (min)	Sampling	Plating and Enumeration
1	Control	Influenza, (tentative surrogate for Sars-cov2)	*MS2 bacteriophage (E. coli phage)*	15597-B1	<1.0um	10^4-10^6	120	0, 30, 60, 90, 120	APS, Impingers	all samples in triplicate
2	High Speed Challenge									
3	High Speed Challenge									
4	High Speed Challenge									

Figure 5: *Biological Test Matrix*

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 of kill. MS2 is a non-lipid enveloped virus. This appears to make it more resistant to disinfection than lipid viruses and therefore should be more resistant to kill when compared to SARS-CoV-2. **Figure 4** is a graphic from the FDA document, *COVID Sterilizers, Disinfectant Devices, and Air Purifiers Guidance, demonstrating resistance to disinfection*.

Test Matrix

To accurately test the YFLife AIR6+ device, triplicate challenge trials were performed in the test chamber. In order to characterize the device's performance while taking into account the natural reduction of the bioaerosol in the chamber, a control trial was ran. A testing matrix for the device can be found in **Figure 5.**

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 separated by centrifugation. MS2 stock yields were greater than 1 x 10^{11} plaque forming units per milliliter (pfu/mL) with a single amplification procedure. This stock MS2 viral solution was then diluted with PBS to approximately 1 x 10^{10} plaque forming units per milliliter (pfu/mL) for use in the Collision nebulizer.

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 in a class 2 biosafety cabinet. The plated cultures were incubated for 24 hours, enumerated and recorded for data analysis.

Bioaerosol Particle Size Data

Aerosol particle size distributions were measured with the APS. The APS has a dynamic measurement range of 0.5 to 20 µm 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 6**

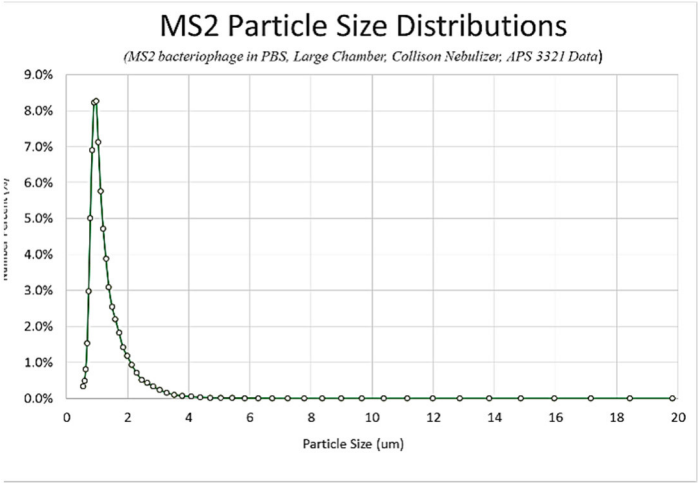


Figure 6: MS2 *Particle Size Distribution*

Control Testing Method

To accurately assess the YFLife AIR6+ unit, test chamber pilot control trials were performed with *MS2* bacteriophage for 120 minute periods without the system in operation to characterize the biological challenge aerosol for aerosol delivery/collection efficiency, decay rate and viable concentration over time.

General Timeline for Bioaerosol Chamber Testing

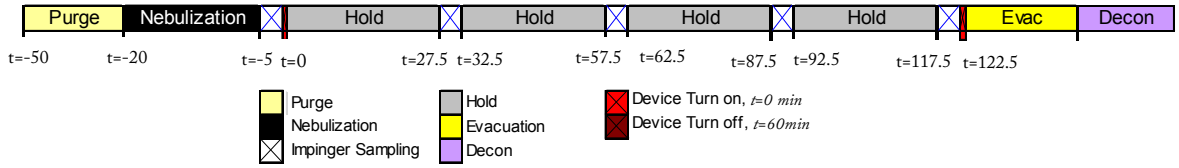


Figure 7: *General trial timeline for bioaerosol testing.*

Control testing was performed to provide baseline comparative data in order to assess the actual viable bioaerosol reduction from the AIR6+ challenge testing and verify that contaminant concentrations persisted above the required concentrations over the entire pilot control test period.

YFLife AIR6+ Testing Method

For each control and challenge test, the Collison nebulizer was filled with approximately 50 mL of biological stock and operated at 35 psi for a period of 5 minutes. For control and system trials, the impinger was filled with 20 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 during bioaerosol generation to assure a homogeneous bioaerosol concentration in the test chamber prior to the first impinger sample. For the remainder of both control and test trials, mixing fans remained on to ensure bioaerosol homogeneity.

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and challenge test by sampling with an AGI-30 impinger located near the center of the chamber. AGI samples were collected for 2 or 5 minutes depending on which time point the sample was taken. Longer samples were taken towards the end of each test in order to collect enough viable bioaerosol for plating and enumeration.

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.

For device testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the trial length of 120 minutes. Subsequent impinger samples were taken at intervals of 30 minutes and samples enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the system over time.

Figure 7 outlines the general timeline for the testing procedure with the YFLife AIR6+ system. All samples were plated in triplicate on tryptic soy agar media over a minimum of a 2 log dilution range.

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 killing of the microorganism.

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 Collison 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 shows the results of the triplicate trials conducted for this study, as well as an average at each time point for the group. All trials show individual and group average +/- standard deviations for Net LOG reduction on a per trial basis. The values depicted on each graph represents the group average at that time point.

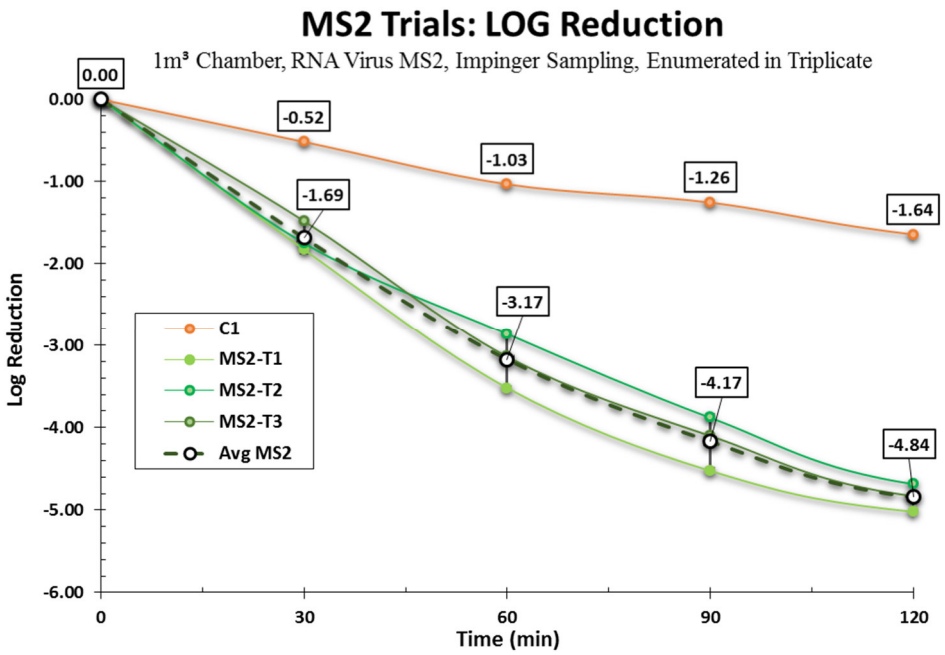


Figure 8: *LOG reduction of* MS2 *in control and device challenge trials.*

YFLife AIR6+ Results

MS2 bacteriophage cultures were initiated the day prior to testing and grew to a concentration greater than $1e^{10}$ pfu/ml. The control trial experienced a 1.64 LOG reduction of *MS2* after 120 minutes of sample collections using the AGI-30 impingers. The device showed a fast reduction throughout all of the trials. At the 60 minute time point, the device showed an average 3.17 LOG reduction. After accounting for the natural losses seen in the control that comes out to an average 2.14 net LOG reduction. At the 90 minute time point, the device had an average 2.91 net LOG reduction. This amount of reduction is equivalent to a 99.8547% reduction. The reduction rate of the device as well as an average for all the trials can be found in LOG reduction in **Figure 8** and net LOG reduction in **Figure 9**.

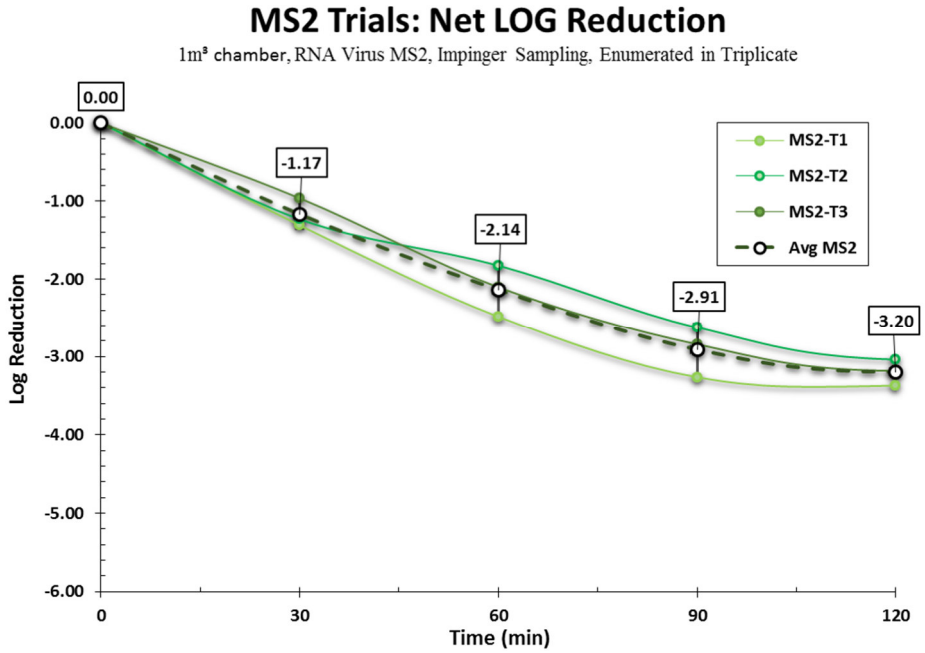


Figure 9: *Net LOG reduction of* MS2 *in challenge trials*

Bioaerosol Reduction Summary Table

Bioaerosol Type	Species (description)	Trial Name	Reduction Type	Trial Time (minutes)			
				30	60	90	120
Virus	*MS2* (RNA E. coli phage)	*MS2-T1*	*Net Log Reduction*	-1.31	-2.49	-3.27	-3.38
			Net % Reduction	95.1437%	99.6750%	99.9463%	99.9579%
Virus	*MS2* (RNA E. coli phage)	*MS2-T2*	*Net Log Reduction*	-1.23	-1.83	-2.62	-3.04
			Net % Reduction	94.1440%	98.5065%	99.7618%	99.9080%
Virus	*MS2* (RNA E. coli phage)	*MS2-T3*	*Net Log Reduction*	-0.96	-2.10	-2.84	-3.18
			Net % Reduction	89.1349%	99.2128%	99.8560%	99.9341%
All Trial Averages			***Net Log Reduction***	**-1.17 +/- 0.18**	**-2.14 +/- 0.33**	**-2.91 +/- 0.33**	**-3.20 +/- 0.17**
			Net % Reduction	***92.8075% +/- 3.2196%***	***99.1314% +/- 0.5885%***	***99.8547% +/- 0.0923%***	***99.9333% +/- 0.025%***

Figure 10: *Summary Table of the MS2 trials with the AIR6+ device*

Summary

Overall the YFLife AIR6+ device performed well in the 1m³ chamber. At the final time point of 120-minutes the device yielded an average 4.84 +/- 0.17 LOG reduction. When you take into account the natural losses seen in the control that equates to a 3.20 +/- 0.17 average net LOG reduction. When that is converted to percent reduction the device yielded an average reduction of 99.933% +/- 0.025% greater than seen in the control trial.

With a reduction of that magnitude on the MS2 bacteriophage, assuming MS2 is a suitable surrogate, the AIR6+ device should help reduce the risk of infection caused by airborne viral pathogens by reducing concentrations in the air.

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 and Wing. *Effects of Sampling Time on the Total Recovery rate of AGI-30 Impingers for E. coli*. Aerosol and Air Quality Research, Vol. 1, No. 1, 2001, pp. 31-36.

Analytical GLP Certificate

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

Project

10918.20

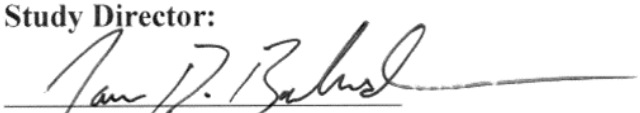
Study Director

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.

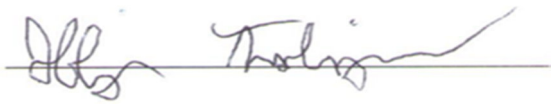
Study Director:



3/25/2021
Date

Jamie D. Balarashti
Study Director
ARE Labs, Inc.

Principal Investigator:



3/25/2021
Date

Jeffery Trolinger
Associate Research Scientist
ARE Labs, In