Efficacy of the Erlab Halo P Device against Aerosolized MS2 Virus

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Background: This in vitro study characterized the efficacy of the Erlab device, Halo P, at removing aerosolized MS2 bacteriophage. The Halo P device is designed to reduce airborne bacteria, viruses, and fungal spores from the air. For this study the Halo P device was challenged using aerosolized MS2 bacteriophage, which has been historically used as a surrogate for influenza and now a tentative surrogate for *SARS CoV-2*. The worldwide pandemic has infected millions and methods of deterring airborne pathogens has become at the forefront of public health. As such, this study evaluated the efficacy of the device against aerosolized MS2 bacteriophage, as well as various sizes of polystyrene latex microspheres (PSL) in a stainless steel bioaerosol chamber. The study consisted of a total of three (3) live bioaerosol trials, a single (1) bioaerosol control run plus a PSL (1) challenge trial as well as a PSL (1) control trial.

Methods: MS2 bacteriophage was aerosolized into a sealed environmental bioaerosol chamber containing the Halo P device. AGI Impinger samples were taken at 0, 15, 30, 45, 60, and 90 minute time points from the chamber in order to quantify the reduction speed and capabilities of the Halo P. AGI impingers were used to sample chamber bioaerosol concentrations. All impinger samples were serially diluted, plated and enumerated in triplicate to yield viable bioaerosol concentration at each sampling point and time. Testing was conducted with the device on the high setting in triplicate. Chamber control trial data was subtracted from the Halo P trial data to yield net LOG reduction in the chamber for the bioaerosol challenges.

Results: When tested against the MS2 bacteriophage, the device showed a steady net log reduction in a relatively short amount of time with average log reduction values ranging from 0.82 average net Log in 15 minutes to 4.13 average net log reduction in 90 minutes. A net log reduction over 4.0 in 90 minutes indicates the speed and efficiency of this device against MS2 bacteriophage.

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

Introduction

This study was conducted to evaluate the efficacy of the Halo P air purification device at reducing aerosolized MS2 bacteriophage. The Halo P device is an air purification system intended for use in medium to large sized offices, schools and laboratories. The unit has several smart settings to allow for ease of use and monitoring of air quality. Once connected to a network via ethernet, the device can be controlled using the online application. In addition, Halo P device contains a pre-filter and a HEPA filter for removal of bioaerosols.

The test plan incorporated challenging the Halo P device in a closed environmental chamber to determine the reduction rate of MS2 bacteriophage by the Halo P device. Demonstrating the reduction in potentially hazardous organisms is key in determining efficacy of the device. A diagram entailing the technical specifications of the Halo P device is shown below in **Figure 1**.

Study Overview

The effectiveness of the Halo P device was evaluated against MS2 bacteriophage, a single-stranded, nonenveloped RNA virus. For more organism information please see species selection section in the body of this report. Particulate testing was also performed with polydispersed latex microspheres ranging from 0.5 to 5um.

Testing was conducted to characterize a single Halo P unit challenged with MS2 with triplicate (3) independent trials as well as a single (1) control trial to demonstrate the capability of the Halo P device to reduce viable bioaerosol concentrations therefore theoretically reducing chances of airborne infection. This study does not make any claims regarding the efficacy of this device at reducing airborne infections.

Figure 1*: Halo P device*

Bioaerosol Testing Chamber

A large sealed aerosol test chamber was used to replicate a potentially contaminated room environment and to contain any potential release of aerosols into the surrounding environment.

The aerosol test chamber is constructed of 304 stainless steel and is equipped with three viewing windows and an air-tight lockable chamber door for system setup and general ingress and egress. The test chamber internal dimensions are 9.1ft x 9.1ft x 7ft, with a displacement volume of 579 cubic feet, or 16,000 liters. **Figure 2** shows the bioaerosol chamber used for all testing in this study.

The chamber is equipped with filtered HEPA inlets, digital internal temperature and humidity monitor, external humidifiers (for humidity control), lighting system, multiple sampling ports, aerosol mixing fans, and a HEPA filtered exhaust system that are operated with wireless remote control. For testing, the chamber was equipped with four 3/8-inch diameter stainless steel probes for aerosol sampling, a 1-inch diameter port for bio-aerosol dissemination into the chamber using a Collison 24-jet nebulizer for the aerosolization of the bacteriophage.

A ¼ inch diameter probe was used for continuous aerosol particle size monitoring via a TSI Aerodynamic Particle Sizer (APS) model 3321. All sample and dissemination ports were inserted approximately 18 inches from the interior walls of the chamber to avoid wall effects and at a height of approximately 40 inches from the floor.

Figure 2*: Bioaerosol Test Chamber Exterior.*

The aerosol sampling and aerosol dissemination probes are stainless steel and bulk headed through the chamber walls to provide external remote access to the aerosol generator and samplers during testing.

The test chamber is equipped with two high-flow HEPA filters for the introduction of filtered purified air

into the test chamber during aerosol evacuation/purging of the system between test trials and a HEPA filtered exhaust blower with a 500 ft^3/min rated flow capability for rapid evacuation of remaining bioaerosols.

A Magnehelic gauge with a range of 0.0 +/- 0.5 inch H_2O (Dwyer instruments, Michigan City IN) was used to monitor and balance the system pressure during aerosol generation, aerosol purge and testing cycles.

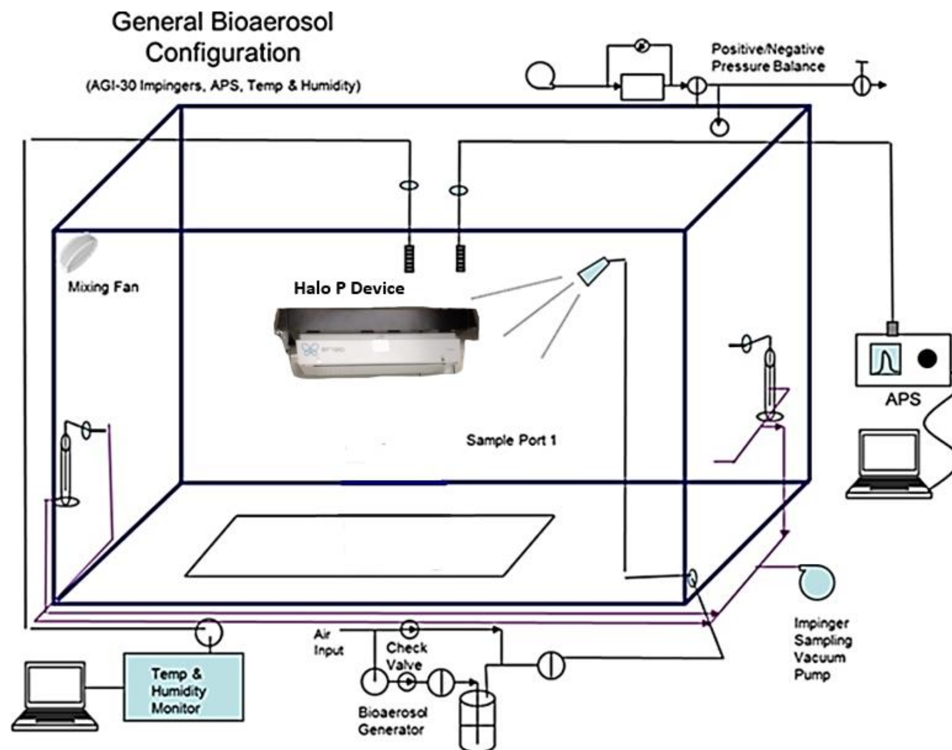

Figure 3: *Bio-Aerosol Test Chamber Flow Diagram.*

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 60 psi, which obtained an output volumetric flow rate of 50-80 lpm with a fluid dissemination rate of approximately 1.25 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

Two AGI impingers (Ace Glass Inc., Vineland NJ) were used for bio-aerosol collection of all biological aerosols to determine chamber concentration. 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.

Aerosol particle size distributions and count concentrations were measured in real-time through the duration of all control and Halo P trial runs using a model 3321 Aerodynamic Particle Sizer (APS) (TSI Inc., St Paul, MN). The APS sampled for the entire duration of all trials (90 minutes) with 2-10 minute sampling intervals. A general flow diagram of the aerosol test system is shown above in **Figure 3** above.

Species Selection

Species selection is based on Biological Safety Level 1 (BSL1) surrogates for BSL2/BSL3 pathogenic organisms. MS2 is a viral RNA bacteriophage that is commonly used as a surrogate for the influenza virus and a tentative surrogate for *SARS-CoV-2*. As mentioned the ability of a device at reducing a surrogate of a pandemic causing organism is crucial in the day to day setting for everyone. Minimizing risk of infection will be the key point in all filtration devices moving forward.

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.

Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate (multiple serial dilutions) using a small drop plaque assay technique onto tryptic soy agar plates. The plated cultures were incubated for 24-48 hours and enumerated and recorded.

Inert Particle Characterization

In order to calculate the dissemination efficiency and stability of the bioaerosol, polystyrene latex microspheres (PSL microspheres) were used to characterize the various aspects of the chamber system. Polydispersed PSL microspheres (Phosphorex Inc., Hopkinton, MA) with aerodynamic diameters of 0.5 - 5.0µm were nebulized, in DI water, and chamber concentrations were recorded using the APS. The APS recorded individual particle count from 0.5 to 20.0 µm in size with 52 separate size bins of resolution. In additional to these trial separate monodispersed PSL microspheres of the following sizes were also used for characterization: 500 nm, 1.0 µm, 2.0 µm and 4.0 µm.

Figure 4, below, shows the results for the control and Halo P for 0.5, 1.0, 2.0 µm and 4.0 µm PSL microsphere testing in the chamber. This data has been normalized to show percent reduction as a function of time in the chamber. Control trials were performed with chamber mixing fans "on" during the entirety of the trial. Additionally, the Halo P trials also had the mixing fans "on" during the entire trial also for consistency of test methods. When the data is plotted, there is a drop in particle number concentration with the Halo P in operation (please note the LOG scale of the y-axis). This figure also shows that after 22 minutes of operation by the Halo P device limits-of-detection for the APS are reached (0.001 particle per cc or 1 particle per liter)

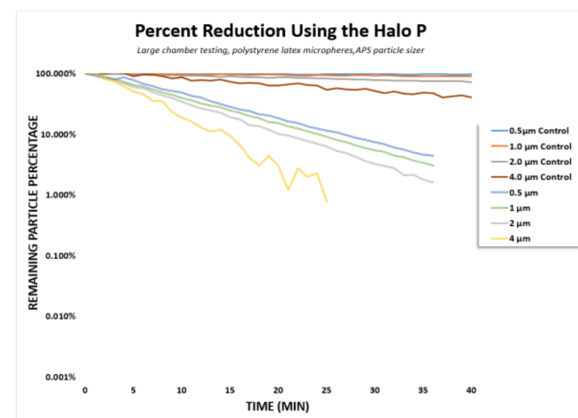

Figure 4: *PSL Microspheres Chamber Trials for the Control and Halo P Device. Chart shows percent reduction versus time. Note that the y-axis is a LOG scale*

Figure 5, on the following page**,** shows the NET LOG reduction for 0.5, 1.0, 2.0 µm and 4.0 µm PSL microspheres. The net log reduction of all tested sizes of PSL microspheres follows a precise logarithmic function for both cases. The figure shows the comparison between the reduction with and without the device in operation. It is notable that this size (0.5 um) is smaller than all vegetative bacteria, bacterial endospores, mold spores and pollens; it is also smaller than most soot particles.

The PSL microsphere trial data were used to estimate nebulization efficiencies, particle stability and AGI-30 collection times and aerosol persistence prior to bioaerosol testing.

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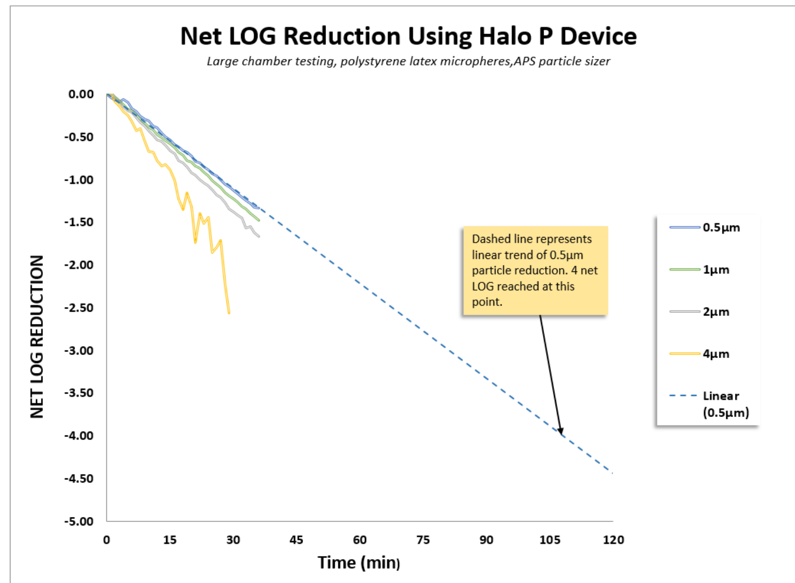

Figure 5: *Net LOG Reduction of PSL Microspheres by Halo P Device. Control minus Halo P Trial data for 0.5, 1.0, 2.0 µm and 4.0 particle sizes in bioaerosol test chamber.*

Bioaerosol Control Testing

To accurately assess the Halo P unit, test chamber pilot control trials were performed with both bioaerosols over a 90-minute period without the device in operation to characterize the biological challenge aerosol for particle size distribution, aerosol delivery/collection efficiency, and viable concentration over time. Control testing was performed to provide baseline comparative data in order to assess the actual reduction from the Halo P challenge testing and verify that viable bioaerosol concentrations persisted above the required concentrations over the entire pilot control test period.

During control runs, a single low velocity fan located in the corner of the bioaerosol test chamber was turned on for the duration of trial to ensure a homogenous aerosol concentration within the aerosol chamber. The mixing fan was used for all control runs and was turned off during Halo P decontamination trials. The two impingers used for bacteriophage collection were pooled and mixed prior to plating and enumeration. A complete test matrix for all bioaerosol trials can be found below in **Figure 4**.

Halo P Testing

For each control and challenge test, the Collison nebulizer was filled with approximately 40 mL of biological stock and operated at 50 psi for a period of 20 minutes. For control and Halo P trials, 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.

The chamber mixing fan was turned on during bioaerosol dissemination to assure a homogeneous bioaerosol concentration in the test chamber prior to taking the first impinger sample. Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and Halo P test by sampling simultaneously with two AGI-30 impingers located at opposite corners of the chamber. AGI samples were collected for 2 to 10 minutes at intervals of 15 or 30 minutes throughout the entire test period. **Figure 5** the general timeline for each Halo P live bioaerosol challenge trial can be seen below.

Trial	Run	Device	Organism	Target Monodispersed Particle Size	Trial Time (min)	Sampling Period (min)	Sampling
C1 T1 T2 T3	Control Challenge Challenge Challenge	Halo P	*MS2 Bacteriophage - RNA bacteriophage*	1.8 to 2.0µm	90	0,15,30,45,60,90	AGI Impingers, APS

Figure 4: *Bioaerosol Test Matrices for all trials*

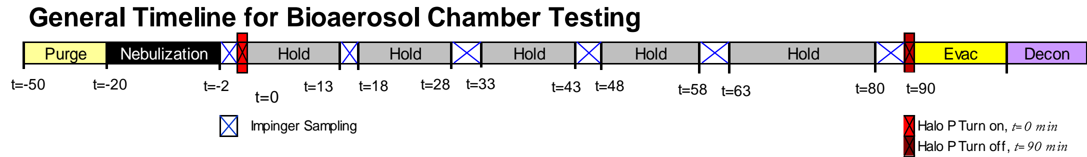

Figure 5: General *Trial Timeline for Halo P Decontamination Trials*

Collected impinger chamber samples were pooled and mixed at each sample interval for each test. 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 Halo P biological testing, the unit was turned on to the highest setting, which correspond to a flow rate of ~174ft^3/min, immediately following a time 0 baseline sample and operated for the entirety of the test (90 min). Subsequent impinger samples were taken at 15, 30, 45, 60, and 90 minutes and samples enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the Halo P device over time. All samples were plated in triplicate on tryptic soy agar media over a minimum of a 3 log dilution range.

Plates were incubated for 24 hours and enumerated for viable plaque forming units (pfu) to calculate aerosol challenge concentrations in the chamber and reduction of viable microorganisms.

Post-Testing Decontamination and Prep

Following each test, the chamber was air flow evacuated/purged for a minimum of twenty minutes between tests and analyzed with the APS for particle concentration decrease to baseline levels between each test. The chamber was decontaminated at the conclusion of the trials with aerosol/vaporous hydrogen peroxide (35%). 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.

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. The aerosol particle size distribution for MS2 is shown in **Figure 6**.

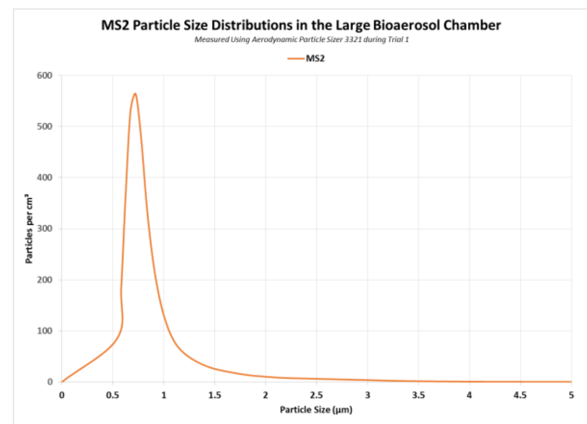

Figure 6: *Viral (MS2) Particle Size Distribution in Test Chamber.*

The particle size distribution for MS2 bioaerosols are shown to be within the respirable range for alveolar region tract lung deposition and show a low geometric standard deviation (GSD) indicating a monodispersed aerosol was generated into the test chamber.

Data Analysis

Results from the control trials were graphed and plotted to show natural viability loss over time in the chamber. These control runs served as the basis to determine the time required for the Halo P to achieve a 4 log (99.99%) reduction in viable bioaerosol above the natural losses from the control runs. The control and trial runs are plotted showing log reduction in viable bioaerosol for each organism. All data is normalized with time zero (*t=0* minutes) enumerated concentrations. Subsequent samples are normalized and plotted to show the loss of viability over time.

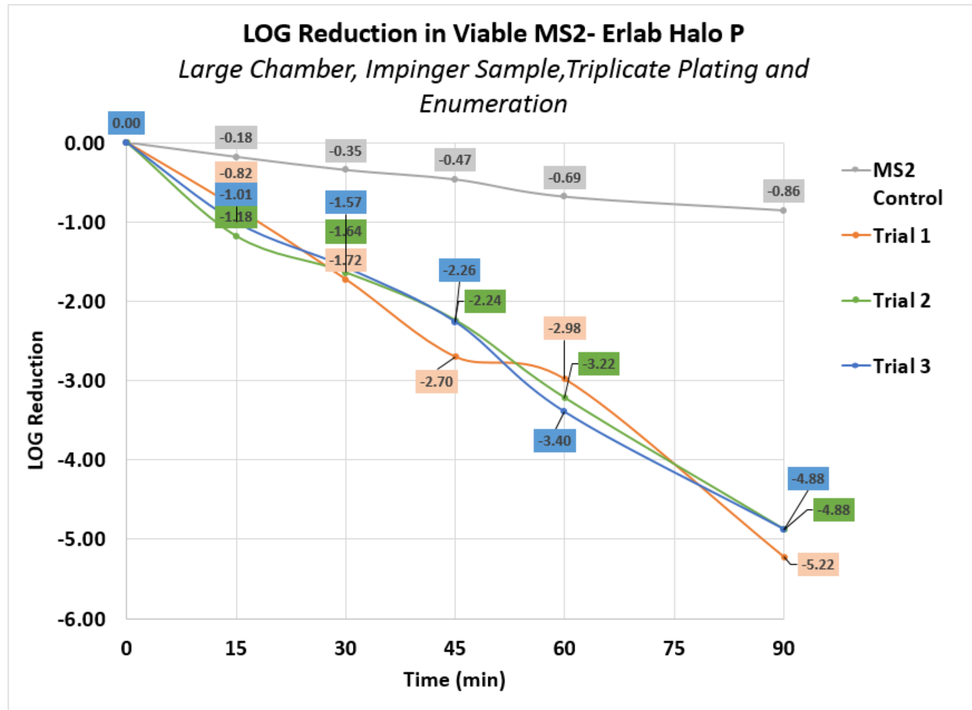

Figure 7: *MS2 Halo P Log Reduction.*

Results

When tested against the MS2 bacteriophage the device showed a steady net log reduction throughout the trials. At the 15-minute time point, there was an average 0.82 net LOG reduction. At the 90-minute time point, the Halo P device had average net log reduction 4.13 log. This is represented graphically in **Figure 7** which shows the log reduction for each trial.

Summary of Results

When tested against the MS2 bacteriophage, a surrogate traditionally used for influenza, the device showed a steady net log reduction in a relatively short amount of time. By the 90-minute time point results showed an average 4.99 LOG reduction and an average 4.13 net LOG reduction. This equates to over a 99.99% reduction in viable MS2. Net LOG reduction results can be found in **Figure 8.**

Overall the trials showed how efficient the Halo P device is against aerosolized MS2 in a relatively short time frame.

Average NET LOG Reduction of MS2 By Halo P Device

Bioaerosol Type	Species	Surrogate	Trial ID	15min	30min	45min	60min	90min
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*1*	0.64	1.38	2.23	2.30	4.36
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*2*	1.00	1.29	1.77	2.53	4.02
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*3*	0.82	1.23	1.79	2.71	4.02
Average				**0.82 +/- 0.18**	**1.3 +/- 0.07**	**1.93 +/- 0.26**	**2.51 +/- 0.21**	**4.13 +/- 0.2**

Figure 8: *Net LOG Reduction summary table for all trials*

References

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Analytical Testing Facility

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Project #

10903.10

Study Director

Dr. Michael Hornback
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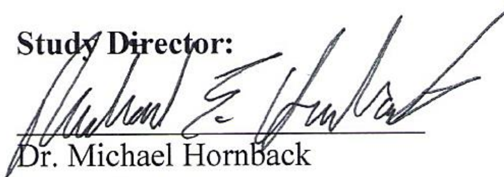
GLP Statement

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.

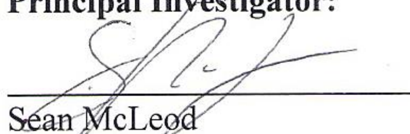
Study Director:



Dr. Michael Hornback
Study Director
ARE Labs, Inc.

10/29/2020
Date

Principal Investigator:



Sean McLeod
Principal Investigator
ARE Labs, Inc.

10/29/2020
Date

Appendix A: Raw Plate Enumerations

Test Date	28-Oct-20
Trial Number	Control
Test Organism	MS2
Nebulizer	Collison 24-Jet; approx. 20 min nebulization
Sampling Systems	Impinger for T=0,15,30, 45,60, 90
Comments/Notes	NA

Sample Time	Plate Dilution Factor	Plate volume ul	Plate counts 100ul 1	2	3	Average counts	Average cfu/ml	Enumerated Concentration cfu/mL	Impinger Sample Rate (lpm)	Impinger Sample Time (min)	Impinger vol PBS (ml)	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
0	2x	100	TNTC	TNTC	TNTC				12.5	5	20.0			
	3x	100	8	6	3	6	57	5.67E+04				1.13E+06	1.8E+04	
	4x	100	1	1	1	1	10	1.00E+05				2.00E+06	3.2E+04	
						Average		7.83E+04				1.57E+06	2.51E+04	100.0%
15	2x	100	68	59	52	60	597	5.97E+04	12.5	5	20.0	1.19E+06	1.9E+04	76.2%
	3x	100	4	4	5	4	43	4.33E+04				8.67E+05	1.4E+04	55.3%
	4x	100												
						Average		5.15E+04				1.03E+06	1.65E+04	65.7447%
30	2x	100	32	41	48	40	403	4.03E+04	12.5	5	20.0	8.07E+05	1.3E+04	51.5%
	3x	100	4	2	3	3	30	3.00E+04				6.00E+05	9.6E+03	38.3%
	4x													
						Average		3.52E+04				7.03E+05	1.13E+04	44.8936%
45	2x	100	32	27	21	27	267	2.67E+04	12.5	5	20.0	5.33E+05	8.5E+03	34.0%
	3x	100	2	3	3	3	27	2.67E+04				5.33E+05	8.5E+03	34.0%
	4x													
						Average		2.67E+04				5.33E+05	8.53E+03	34.0426%
60	2x	100	17	11	9	12	123	1.23E+04	12.5	5	20.0	2.47E+05	3.9E+03	15.7%
	3x	100	2	2	2	2	20	2.00E+04				4.00E+05	6.4E+03	25.5%
	4x													
						Average		1.62E+04				3.23E+05	5.17E+03	20.6383%
90	2x	100	15	16	4	12	117	1.17E+04	12.5	5	20.0	2.33E+05	3.7E+03	14.9%
	3x	100	2	1	0	1	10	1.00E+04				2.00E+05	3.2E+03	12.8%
	4x													
						Average		1.08E+04				2.17E+05	3.47E+03	13.8298%

Test Date	27-Oct-20
Trial Number	Trial 1
Test Organism	MS2
Nebulizer	Collison 24-Jet; approx. 20 min nebulization
Sampling Systems	Impinger for T=0,15,30, 45,60, 90
Comments/Notes	NA

Sample Time	Plate Dilution Factor	Plate Vol ul	Plate counts 1	2	3	Average count	Average cfu/ml	Enumerated Concentration cfu/mL	Impinger Sample Rate (lpm)	Impinger Sample Time (min)	Impinger vol PBS (ml)	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
0	4x	100							12.5	2	20.0			
	4x	100	1	1	0	1	7	6.67E+04				1.33E+06	5.3E+04	
	5x	750												
						Average		6.67E+04				1.33E+06	5.33E+04	100.0%
15	2x	100	20	22	28	23	233	2.33E+04	12.5	5	20.0	4.67E+05	7.5E+03	14.0%
	3x	100	4	2	2	3	27	2.67E+04				5.33E+05	8.5E+03	16.0%
	4x	750												
						Average		2.50E+04				5.00E+05	8.00E+03	15.0000%
30	3x	100												
	2x	100	3	3	1	2	23	2.33E+03				4.67E+04	7.5E+02	1.4%
	1x	100	39	41	39	40	397	3.97E+03				7.93E+04	1.3E+03	2.4%
						Average		3.15E+03				6.30E+04	1.01E+03	1.8900%
45									12.5	10	20.0			
	1x	100	4	5	1	3	33	3.33E+02				6.67E+03	1.1E+02	0.2%
	0x	750												
						Average		3.33E+02				6.67E+03	1.07E+02	0.2000%
60	0x	750	185	129	77	130	174	1.74E+02	12.5	10	20.0	3.48E+03	5.6E+01	0.1%
	1x													
	2x													
						Average		1.74E+02				3.48E+03	5.56E+01	0.1043%
90	0x	750	3	0		2	2	2.00E+00	12.5	10	20.0	4.00E+01	3.2E-01	0.0%
	1x													
	2x													
						Average		2.00E+00				4.00E+01	3.20E-01	0.0006%

Test Date	27-Oct-20
Trial Number	Trial 2
Test Organism	MS2
Nebulizer	Collison 24-Jet; approx. 20 min nebulization
Sampling Systems	Impinger for T=0,15,30, 45,60, 90
Comments/Notes	NA

Sample Time	Plate Dilution Factor	Plate Vol ul	Plate counts 1	2	3	Average count	Average cfu/ml	Enumerated Concentration cfu/mL	Impinger Sample Rate (lpm)	Impinger Sample Time (min)	Impinger vol PBS (ml)	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
0	3x	100							12.5	2	20.0			
	4x	100	2	1	1	1	13	1.33E+05				2.67E+06	1.1E+05	
						Average		***1.33E+05***				***2.67E+06***	***1.07E+05***	***100.0%***
15	2x	100	31	16	15	21	207	2.07E+04	12.5	5	20.0	4.13E+05	6.6E+03	6.2%
	3x	100	6	1	0	2	23	2.33E+04				4.67E+05	7.5E+03	7.0%
	4x	750												
						Average		***2.20E+04***				***4.40E+05***	***7.04E+03***	**6.6000%**
30	3x	100	1	1	0	1	7	6.67E+03	12.5	5	20.0	1.33E+05	2.1E+03	2.0%
	2x	100	5	11	10	9	87	8.67E+03				1.73E+05	2.8E+03	2.6%
	1x	100												
						Average		***7.67E+03***				***1.53E+05***	***2.45E+03***	**2.3000%**
45									12.5	10	20.0			
	1x	100												
	1x	100	35	40	40	38	383	3.83E+03				7.67E+04	6.1E+02	0.6%
						Average		***3.83E+03***				***7.67E+04***	***6.13E+02***	**0.5750%**
60	0x	750	388	229	288	302	402	4.02E+02	12.5	10	20.0	8.04E+03	6.4E+01	0.1%
	1x													
	2x													
						Average		***4.02E+02***				***8.04E+03***	***6.44E+01***	**0.0603%**
90	0x	750	9	7	4	7	9	8.89E+00	12.5	10	20.0	1.78E+02	1.4E+00	0.0%
	1x													
	2x													
						Average		***8.89E+00***				***1.78E+02***	***1.42E+00***	**0.0013%**

Test Date	27-Oct-20
Trial Number	Trial 3
Test Organism	MS2
Nebulizer	Collison 24-Jet; approx. 20 min nebulization
Sampling Systems	Impinger for T=0,15,30, 45,60, 90
Comments/Notes	NA

Sample Time	Plate Dilution Factor	Plate Vol ul	Plate counts 1	2	3	Average count	Average cfu/ml	Enumerated Concentration cfu/mL	Impinger Sample Rate (lpm)	Impinger Sample Time (min)	Impinger vol PBS (ml)	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
0	3x	100							12.5	2	20.0			
	4x	100	1	2	0	1	10	1.00E+05				2.00E+06	8.0E+04	
						Average		***1.00E+05***				***2.00E+06***	***8.00E+04***	***100.0%***
15	2x	100	22	22	30	25	247	2.47E+04	12.5	5	20.0	4.93E+05	7.9E+03	9.9%
	3x	100												
	4x	750												
						Average		***2.47E+04***				***4.93E+05***	***7.89E+03***	**9.8667%**
30									12.5	5	20.0			
	2x	100	9	9	2	7	67	6.67E+03				1.33E+05	2.1E+03	2.7%
	1x	750												
						Average		***6.67E+03***				***1.33E+05***	***2.13E+03***	**2.6667%**
45									12.5	10	20.0			
	1x	100	28	30	24	27	273	2.73E+03				5.47E+04	4.4E+02	0.5%
	0x	750												
						Average		***2.73E+03***				***5.47E+04***	***4.37E+02***	**0.5467%**
60	0x	750	153	152	145	150	200	2.00E+02	12.5	10	20.0	4.00E+03	3.2E+01	0.0%
	1x													
	2x													
						Average		***2.00E+02***				***4.00E+03***	***3.20E+01***	**0.0400%**
90	0x	750	8	4	3	5	7	6.67E+00	12.5	10	20.0	1.33E+02	1.1E+00	0.0%
	1x													
	2x													
						Average		***6.67E+00***				***1.33E+02***	***1.07E+00***	**0.0013%**

Appendix B: 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 use rate (R_{neb}) (volume of liquid generated by the nebulizer/time) at 28 psi air supply pressure = 1.0 ml/min.
- Collison 24 jet Generation time (t) = 20 or 30 minutes, test dependent.
- Chamber volume (V_c) = 15,993 Liters

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:

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

Plating and enumeration of the biological to derive the concentration of the dry powder (C_p) in cfu/g.

- Eductor use rate (M_p) (Mass of powder generated by the eductor in grams)
- Chamber volume (V_c) = 15,993 Liters

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

$$\text{Eductor: } V_P = \frac{C_p \cdot M_p}{V_c}$$

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) = 5 or 10 minutes, test dependent.

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

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

The aerosol system viable delivery efficiency (expressed as %) is:

$$Efficiency = \frac{C_a}{V_p} \cdot 100$$