Efficacy of The Purafil Purashield 500 device against Aerosolized MS2 Virus

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Background: This in vitro study characterized the efficacy of the Purafil device, Purashield 500, at removing aerosolized MS2 Bacteriophage. The Purashield 500 device is designed to reduce airborne bacteria, viruses, and fungal spores in order to decrease infections rates from airborne pathogens. For this study the Purashield 500 device was challenged using aerosolized MS2 bacteriophage which has been historically used as a surrogate for influenza, and is now being considered as a surrogate for coronaviruses such as COVID-19 due to the size similarity to influenza and RNA genome. The CDC estimates that the influenza virus is responsible for 140,000 to 810,000 hospitalizations and 12,000 to 61,000 deaths annually. 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 control trial.

Methods: MS2 bacteriophage was aerosolized into a sealed environmental bioaerosol chamber containing the Purashield 500 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 Purashield 500. 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 Purashield 500 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 high net log reduction in a relatively short amount of time with average log reduction values ranging from 3.45 net Log in 15 minutes to 5.30 net log reduction in 30 minutes. A net log reduction over 4.0 in under 30 minutes indicates the speed and efficiency of this device against the 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 Purashield 500 air purification device at reducing aerosolized MS2 bacteriophage. The Purashield 500 device is an air purification system intended for use in medium to large sized rooms. The unit has several different settings ranging from low to high. All testing conducted during this study utilized the device at the highest setting.

The Purashield filters contain (1) a pre-filter, (2) a chemical and microbial gas filter using Purafil SP patented filtration media, (3) a filter comprised of patented Puraward fiber with antimicrobial properties, and (4) a HEPA final filter. The test plan incorporated challenging the Purashield 500 device in a closed environmental chamber to determine the destruction rate of MS2 bacteriophage by the Purashield 500 device. A picture of the Purashield 500 device is shown in **Figure 1,** on the following page.

Study Overview

The effectiveness of the Purashield 500 device was evaluated against a single RNA virus which was the MS2 Bacteriophage. 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 Purashield 500 unit against a single non-enveloped RNA bacteriophage with triplicate (3) independent trials for each fan speed as well as a single (1) control trial to demonstrate the capability of the Purashield 500 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*: PuraShield 500 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.

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.

Figure 2*: Bioaerosol Test Chamber Exterior.*

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.

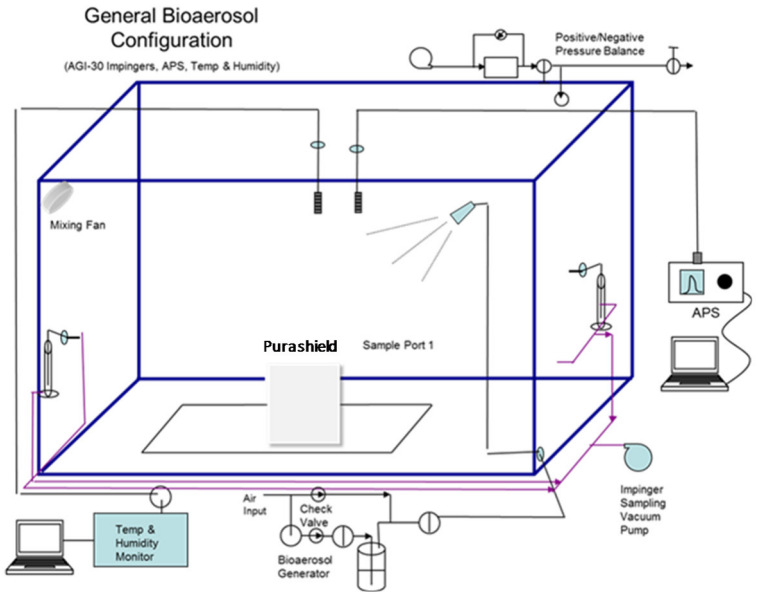

Figure 3: *Bio-Aerosol Test Chamber Flow Diagram.*

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 Purashield 500 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 BSL3 pathogenic organisms. *MS2* is a viral RNA bacteriophage that is commonly used as a surrogate for the influenza virus, and is now being considered as a possible surrogate for other RNA viruses such as COVID-19. This is due to its similar size to influenza and RNA genome. The major difference is the enveloping of COVID-19.

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 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 20.0µm 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: 500nm, 1.0µm and 2.0µm.

Figure 4, below, shows the results for the control and Purashield 500 for 0.5, 1.0 and 2.0 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 Purashield 500 trials also had the mixing fans "on" during the entire trial also for consistency of test methods. Looking at the data we can see the sharp drop in particle number concentration with the Purashield 500 in operation (please note the LOG scale of the y-axis). This figure also shows that after 22 minutes of operation by the Purashield 500 device limits-of-detection for the APS are reached (0.001 particle per cc or 1 particle per liter)

Figure 5, on the following page**,** shows the NET LOG reduction for 0.5, 1.0 and 2.0 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 (0.5um) 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.

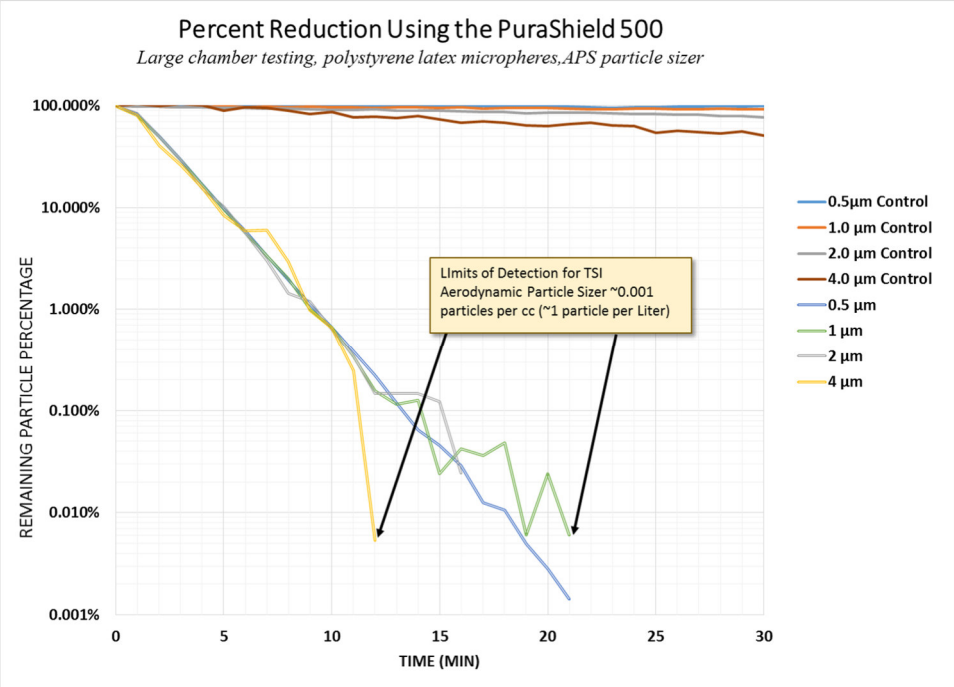

Figure 4: PSL Microspheres Chamber Trials for the Control and Purashield 500 Device. Chart shows percent reduction versus time. Note that the y-axis is a LOG scale.

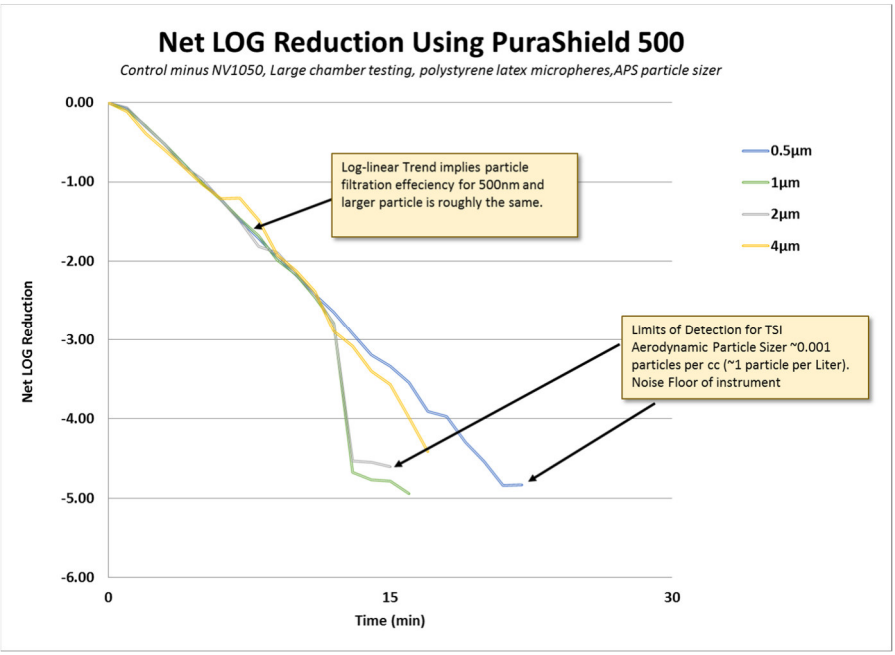

Figure 5: *Net LOG Reduction of PSL Microspheres by Purashield 500 Device. Control minus Purashield 500 Trial data for 0.5, 1.0 and 2.0 µm particle sizes in bioaerosol test chamber.*

Bioaerosol Control Testing

To accurately assess the Purashield 500 unit, test chamber pilot control trials were performed with all 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 Purashield 500 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 Purashield 500 decontamination trials. The two impingers used for bacteriophage were pooled and mixed prior to plating and enumeration. A complete test matrix for all bioaerosol trials can be found above in **Figure 4**.

Purashield 500 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 15 minutes (organism dependent). For control and Purashield 500 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.

Trial	Run	Device	Organism	Target Monodispersed Particle Size	Trial Time (min)	Sampling Period (min)	Sampling
C1 T1 T2 T3	Control Challenge Challenge Challenge	Purashield 500	*MS2 Bacteriophage - RNA bacteriophage*	1.8 to 2.0um	90	0,15,30,45,60,90	AGI Impingers, APS

Figure 4: *Bioaerosol Test Matrices for all trials*

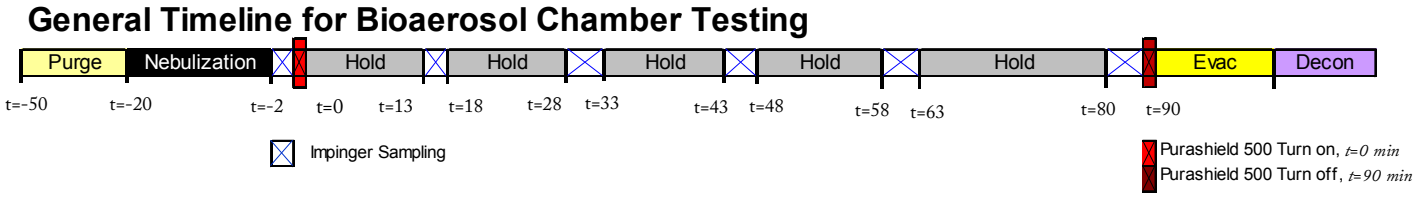

Figure 5: General *Trial Timeline for Purashield 500 Decontamination Trials.*

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and Purashield 500 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 (organism dependent) at intervals of 15 or 30 minutes throughout the entire test period. **Figure 5** the general timeline for each Purashield 500 live bioaerosol challenge trial.

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 Purashield 500 biological testing, the unit was turned on to the highest setting, which correspond to a flow rate of 600 m3/h or ~353ft3/min, immediately following a time 0 baseline sample and operated for the entirety of the test (90min). Subsequent impinger samples were taken at 0, 15, 30, 45, 60, and 90 minutes and samples enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the Purashield 500 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 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. The aerosol particle size distribution for MS2 is shown in **Figure 6**.

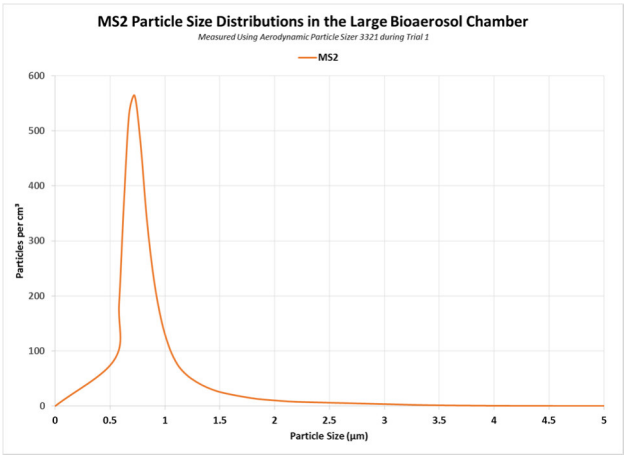

Figure 6: *Viral (MS2) Particle Size Distribution in Test Chamber.*

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.

	Number Particle Size
Median (µm)	0.857
Mean (µm)	0.938
Geo. Mean (µm)	0.893
Mode (µm)	0.777
Geo. St. Dev.	1.35
Total Conc.	4.99e+03(#/cm³)

Figure 7: *Key Particle Size Distribution Values for MS2 Bioaerosol in 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 Purashield 500 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.

Results

When tested against the *MS2 bacteriophage* the device showed a high net log reduction in a relatively short amount of time at the 15-minute time point there was an average 3.45 Net LOG reduction. At the 30-minute time point the Purashield 500 device had log reduction values ranging from 5.09 to 5.45 log. This is represented graphically in **Figure 7** which shows the log reduction for each trial as well as an overall average.

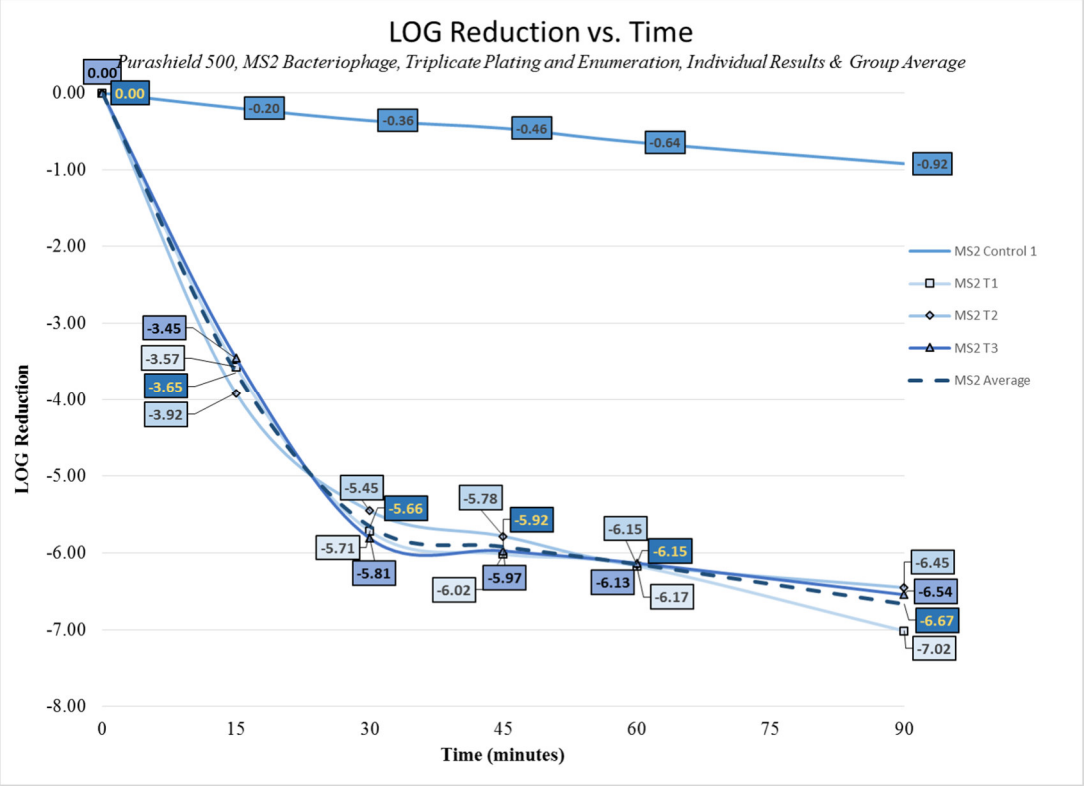

Figure 7: *MS2 Purashield 500 Log Reduction.*

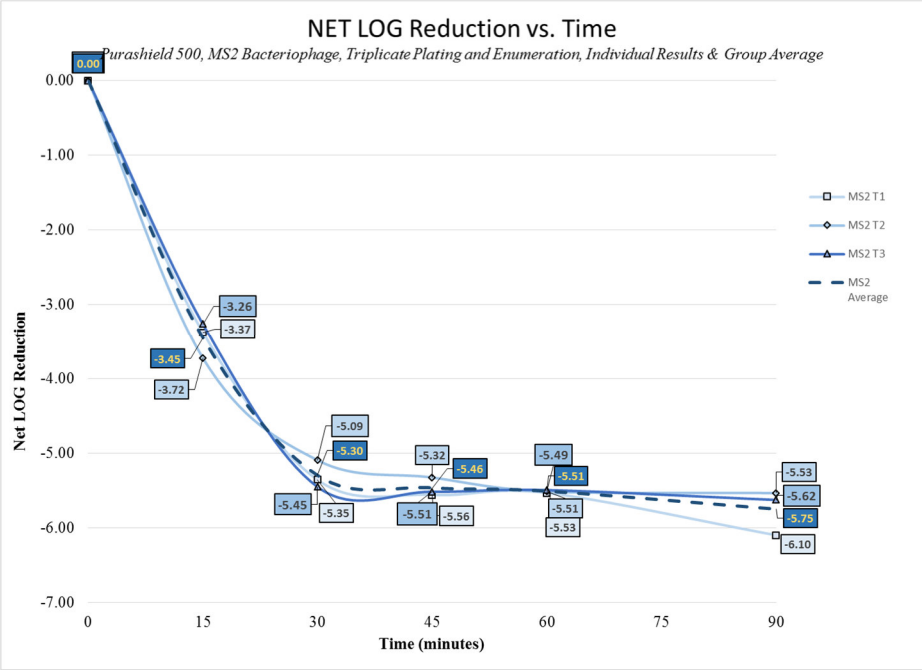

Figure 8: *MS2 Purashield 500 Net LOG Reduction.*

Summary of Results

When tested against the *MS2 bacteriophage*, a surrogate traditionally used for influenza and now considered as a possible surrogate for COVID-19, the device showed a high net log reduction in a relatively short amount of time. By the 30-minute time point results showed an average 5.66 LOG reduction which equates to an average 5.30 net LOG reduction. This equates to over a 99.999% reduction in viable MS2. LOG reduction results can be found in **Figure 9.** Net percent reduction can be found in **Figure 10**

From the 45 minute time point to the 90 minute time point on the device continued to show more reduction. The average net log reduction at 45 minutes was at 5.46 and dropped to 5.75 at the 90 minute sample.

Overall the trials showed how efficient the Purashield 500 device is against aerosolized MS2 in a relatively short time frame.

Average NET LOG Reduction of MS2 By Purashield 500

Bioaerosol Type	Species	Surrogate	Trial ID	15min	30min	45min	60min	90min
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*1*	-3.37	-5.35	-5.56	-5.53	-6.10
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*2*	-3.72	-5.09	-5.32	-5.51	-5.53
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*3*	-3.26	-5.45	-5.51	-5.49	-5.62
Average				**-3.45 +/- 0.24**	**-5.3 +/- 0.19**	**-5.46 +/- 0.12**	**-5.51 +/- 0.02**	**-5.75 +/- 0.3**

Figure 9: *Net LOG Reduction summary table for all trials*

Average reduction percentage of MS2 By Purashield 500

Bioaerosol Type	Species	Surrogate	Trial ID	15min	30min	45min	60min	90min
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*1*	99.9577%	99.9996%	99.9997%	99.9997%	99.9999%
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*2*	99.9449%	99.9996%	99.9997%	99.9997%	99.9998%
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*3*	99.9646%	99.9995%	99.9997%	99.9997%	99.9998%
Average +/- Standard Deviation				99.9646% +/- 0.0183%	99.9995% +/- 0.0002%	99.9997% +/- 0.0001%	99.9997% +/- 0.000%	99.9998% +/- 0.0001%

Figure 10: Net Percentage Reduction summary table for all trials

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Analytical Testing Facility

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Project #

10883.10

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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.

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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 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:

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