Efficacy of The Novaerus Defend 400 device against Aerosolized MS2 Virus and Bacillus Globigii

Jamie Balarashti [a], Zach Conley [a]
[a] Aerosol Research and Engineering Laboratories Inc. Olathe KS

Background: This in vitro study characterized the efficacy of the Novaerus Defend 400 device at removing aerosolized MS2 Bacteriophage well as *Bacillus Globigii* bacterial endospores. The Novaerus Defend 400 device is designed to reduce airborne bacteria, viruses, and fungal spores in order to decrease infections rates from airborne pathogens. For this study the Novaerus Defend 400 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. In addition the device was tested against aerosolized *Bacillus Globigii* bacterial endospores a commonly used surrogate for *Bacillus Anthraxis*. This study also evaluated the efficacy of the device against aerosolized MS2 bacteriophage, *Bacillus Globigii* in a stainless steel bioaerosol chamber. The Defend 400 was also tested against multiple sizes of polystyrene latex microspheres in order to determine the reduction speed and capabilities of the device prior to live bioaerosol testing. The study consisted of a total of six (6) live bioaerosol trials as well as two (2) bioaerosol control runs, one (1) for each organism tested.

Methods: MS2 bacteriophage was aerosolized into a sealed environmental bioaerosol chamber containing the Novaerus Defend 400 device. AGI Impinger samples were taken every at 0, 15, 30, 45 and 60 minutes from the chamber in order to quantify the reduction speed and capabilities of the Novaerus Defend 400. 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. Chamber control trial data was subtracted from the Defend 400 trial data to yield net LOG reduction in the chamber for the bioaerosol challenges.

Results: The Defend 400 device showed a high reduction in viable bioaerosols within the 45 to 60 minute test period for both organisms. The device showed increased performance with the MS2 bacteriophage when compared to the Bg spores. The device had a final net LOG reduction of MS2 after 45 minutes of 5.31 which is equivalent to a net percent reduction of 99.9995%. When tested against Bacillus globigii endospores the device showed an average net LOG reduction of 4.55 LOG which is equivalent to a net percent reduction of 99.9969%. An additional test was conducted for 24-hours in order to determine whether the Defend 400 was causing re-aerosolization of impacted bioaerosols from the walls of the chamber, after sampling 300 liters of air from the chamber a single colony forming unit was collected indicating that the device was not causing re-aerosolization.

Introduction

This study was conducted to evaluate the efficacy of the Novaerus Defend 400 air cleaner device at reducing aerosolized MS2 bacteriophage. The Novaerus Defend 400 device is an air cleaner device intended for use in small to medium sized rooms. The unit has several different settings ranging from low to high. All testing conducted during this study utilized at the highest device setting.

The Novaerus Defend 400 is intended as a room recirculating air cleaner. The system is used for filtering out and inactivating airborne particles from the air. The Defend 400 uses a dielectric plasma discharge and filtration media including HEPA grade and carbon filters. The test plan incorporated challenging the Novaerus Defend 400 device in a closed environmental chamber to determine the reduction rate of MS2 bacteriophage and Bacillus *globigii* by the Novaerus Defend 400 device. A picture of the Defend 400 device is shown in **Figure 1,** on the following page.

Study Overview

The effectiveness of the Novaerus Defend 400 device was evaluated against a single RNA virus which was MS2 Bacteriophage as well as a single bacterial endospore which was Bacillus *globigii*. For more organism information please see species selection section in the body of this report.

Figure 1*: Novaerus Defend 400 device*

Testing was conducted to characterize a single Novaerus Defend 400 unit against a single (1) non-enveloped RNA bacteriophage as well as a single (1) capability of the Novaerus Defend 400 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.

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 microorganisms tested for this study.

bacterial endospore with triplicate (3) independent trials as well as a single (1) control trial to demonstrate the

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.

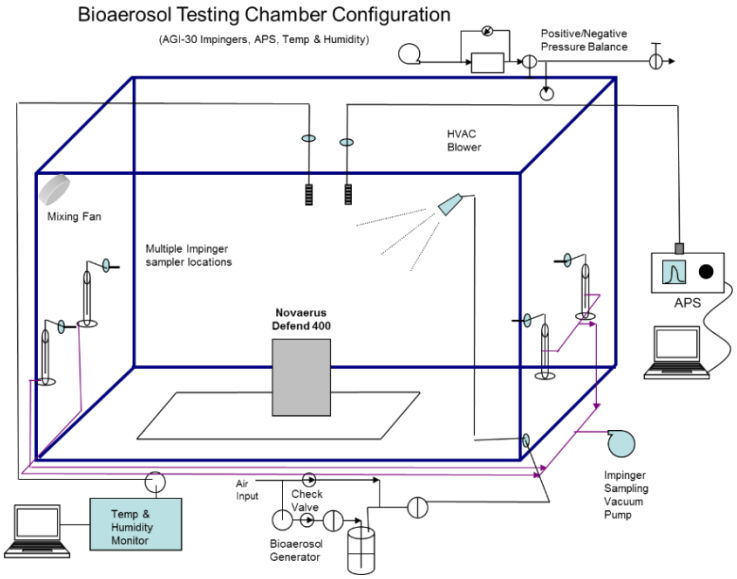

Figure 3: *Bio-Aerosol Test Chamber Flow Diagram.*

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.

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 at the beginning of all control and Novaerus Defend 400 trial runs using a model 3321 Aerodynamic Particle Sizer (APS) (TSI Inc., St Paul, MN). 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. Bacillus *globigii* is a bacterial endospore that is commonly used as a surrogate for Anthrax a biological warfare agent.

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.

Endospore Culture & Preparation

Bacillus globigii spore preparations were grown sporelated, purified and spray dried to yield a pure dry powder with greater than $1e^{11}$ cfu/gram. The dry endospore preparation were suspended in PBS + 0.005% Tween 80 at a mass: volume ratio to obtain a concentration of approximately 1 x 10^9 cfu/ml.

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 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, 2.0µm and 4.0µm.

Figure 4, below, shows the results for the control and NV 1050 trials 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 Defend 400 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 Defend 400 in operation (please note the LOG scale of the y-axis). This figure also shows that after 15 minutes of operation by the Defend 400 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.

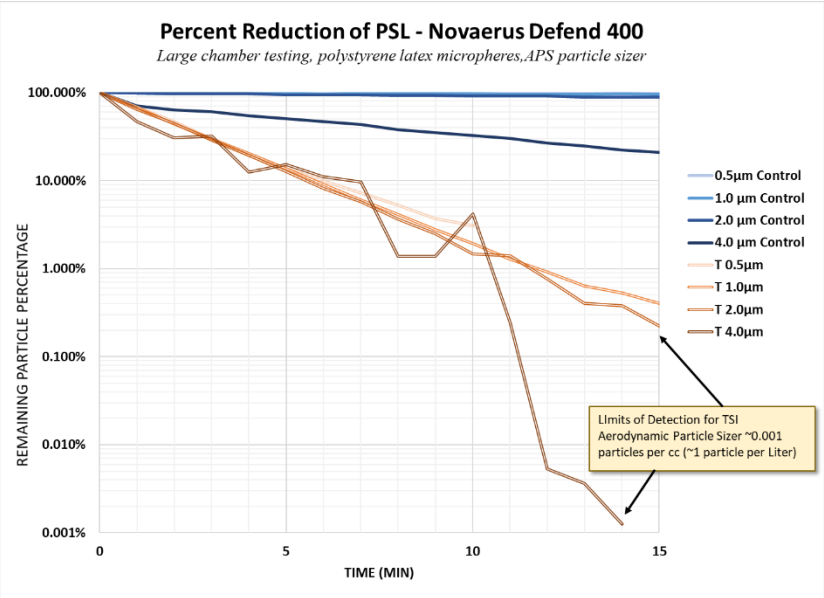

Figure 4: PSL Microspheres Chamber Trials for the Control and Novaerus Defend 400 Device. Chart shows percent reduction versus time. Note that the y-axis is a LOG scale.

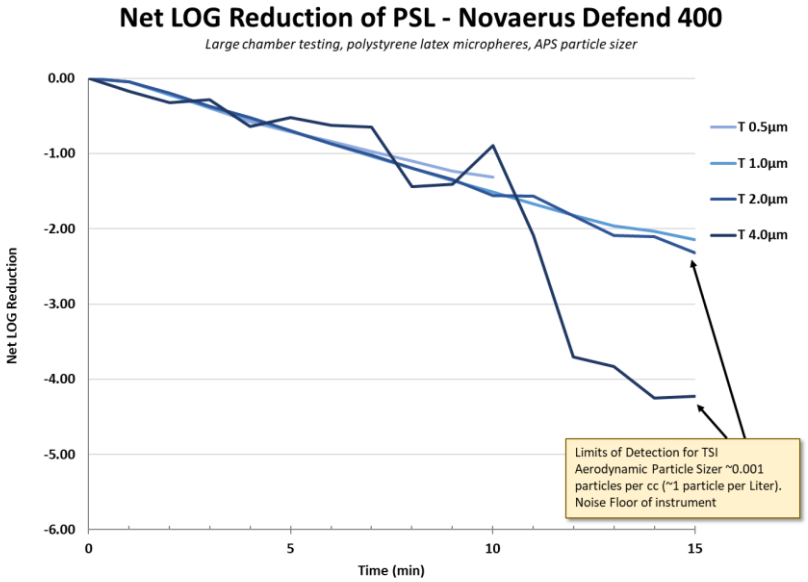

Figure 5: *Net LOG Reduction of PSL Microspheres by Novaerus Defend 400 Device. Control minus Defend 400 Trial data for 0.5, 1.0 and 2.0 µm particle sizes in bioaerosol test chamber.*

Bioaerosol Control Testing

To accurately assess the Novaerus Defend 400 unit, test chamber pilot control trials were performed with all Bioaerosols over a 60-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 Novaerus Defend 400 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 also turned on during Novaerus Defend 400 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 6**.

Novaerus Defend 400 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 (organism dependent). For control and Novaerus Defend 400 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.

Biological Test Matrix

Trial	Run	Species (description)	ATCC Ref #	Target Monodispersed Size	Challenge Conc.	Trial Time (min)	Sample Times	Sampling Device	Plating and Enumeration
1	Control	*Bacillus Globigii (Bacterial Endospore)*	49760	1.0 - 2.4 µm	10^4-10^6	60	0, 15, 30, 45, 60	APS 3321, AGI-30 impingers	All samples plated in triplicate multiple dilutions
2	Challenge								
3	Challenge								
4	Challenge								
5	Control	*MS2* bacteriophage (E.coli phage)	13706-B1	<1.0 µm	10^4-10^6	45	0, 15, 30, 45	APS 3321, AGI-30 impingers	All samples plated in triplicate multiple dilutions
6	Challenge								
7	Challenge								
8	Challenge								

Figure 6: *Bioaerosol Test Matrices for all trials*

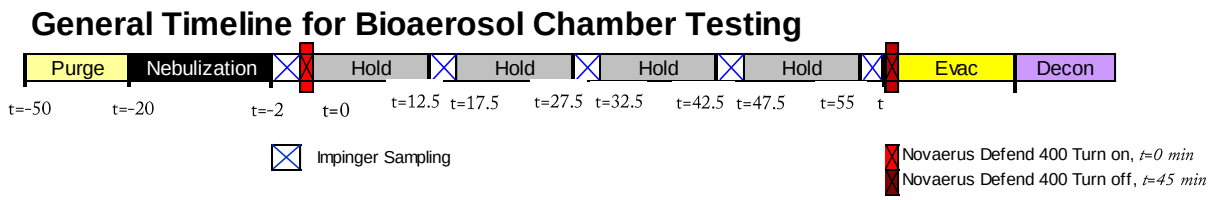

Figure 7: General *Trial Timeline for Novaerus Defend 400 Decontamination Trials.*

Following bioaerosol generation, baseline bioaerosol concentrations were established for each 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. The chamber mixing fan was in operation for the duration of the trial in order to ensure a homogenous aerosol concentration throughout the trial.

pilot control and Novaerus Defend 400 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 minutes throughout the entire test period. **Figure 7** the general timeline for each Novaerus Defend 400 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 soaked in a 3% bleach bath and then rinsed 6x with sterile filtered water between each sampling interval, and re-filled with sterile PBS using sterile graduated pipettes for sample collection.

For Novaerus Defend 400 biological testing, the unit was turned on to the highest setting immediately following a time 0 baseline sample and operated for the entirety of the test (60min). Subsequent impinger samples were taken at 0, 15, 30, 45 and 60 minutes and samples enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the Novaerus Defend 400 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 or colony forming units (pfu/cfu) 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 each organism using 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 at the onset of each test for initial particle concentration and particle size.

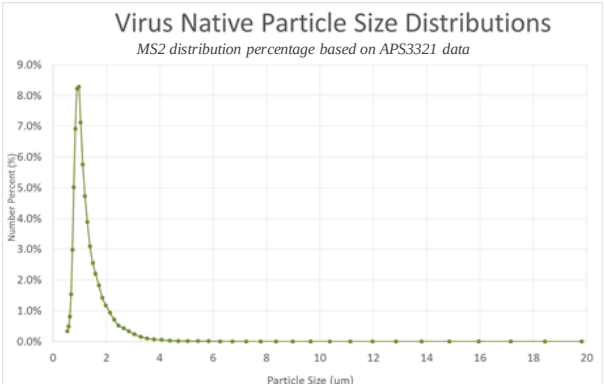

Figure 8: *Viral (MS2) Particle Size Distribution in Test Chamber.*

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 8**.

	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 9: *Key Particle Size Distribution Values for MS2 Bioaerosol in 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. The aerosol particle size distribution for Bacillus Globigii can be found in **Figure 10**.

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 Novaerus Defend 400 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.

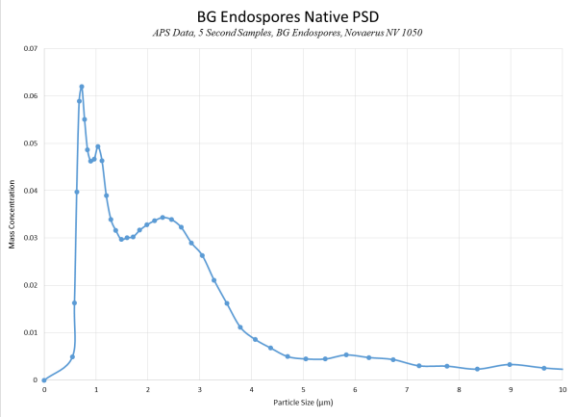

Figure 10: *Bacterial Endospore (BG) Particle Size Distribution in Test Chamber.*

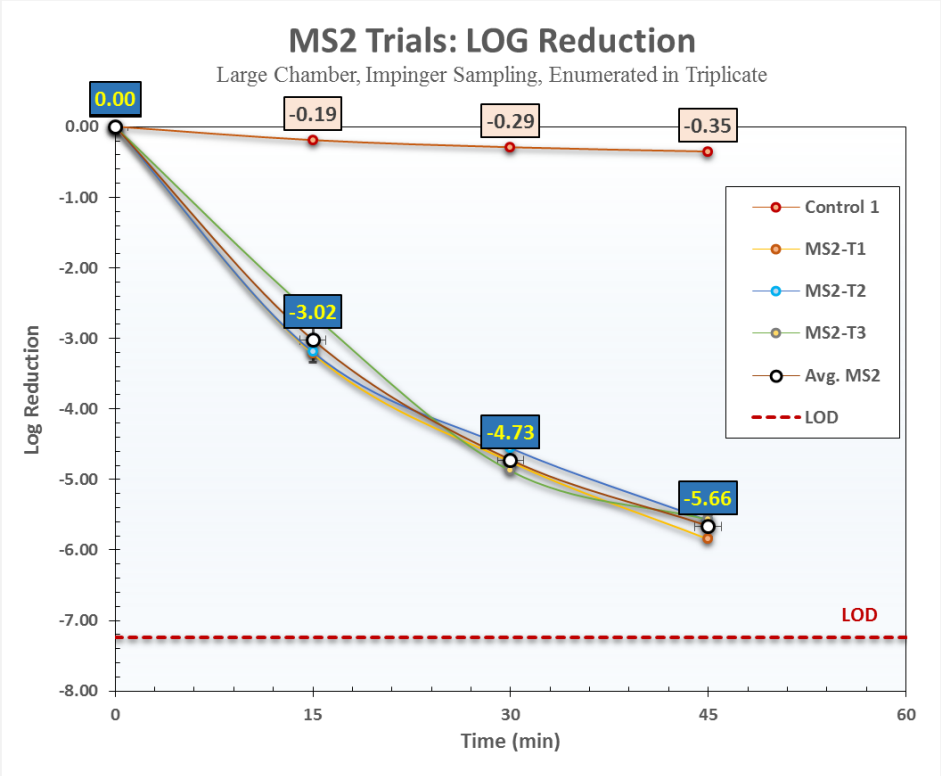

Figure 11: *MS2 Novaerus Defend 400 Log Reduction.*

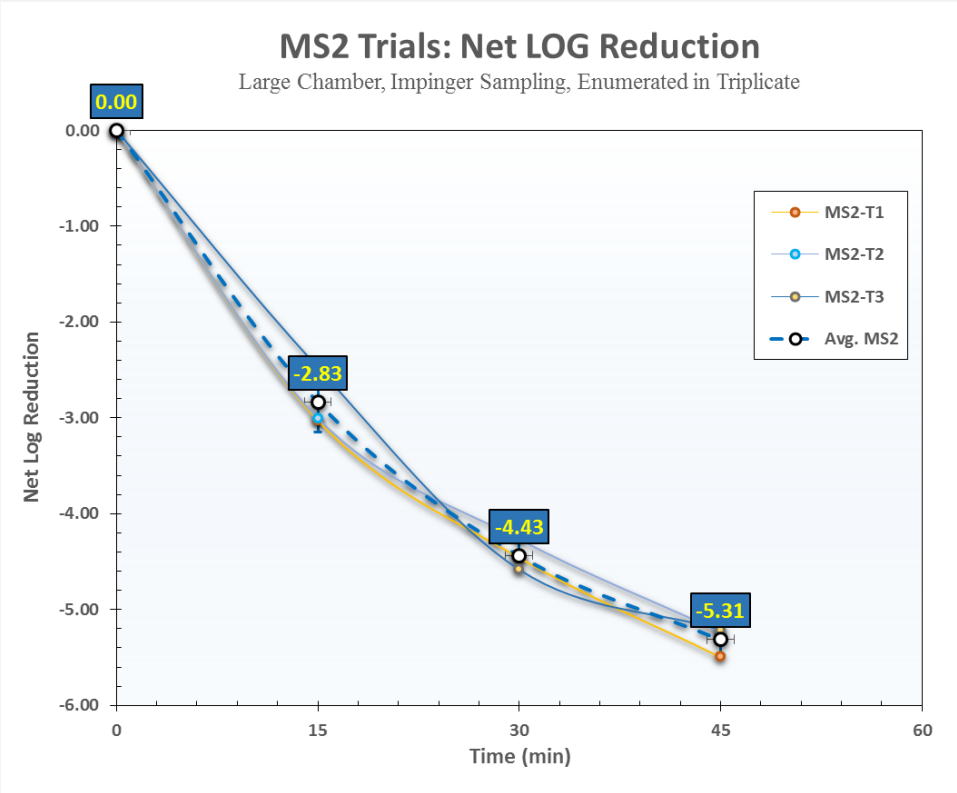

Figure 12: *MS2 Novaerus Defend 400 Net LOG Reduction.*

MS2 Results

When tested against the *MS2 bacteriophage* the device showed high LOG reduction values in very short periods of time, by the 15-minute time point the device achieved a 3.02 LOG reduction of MS2 bacteriophage. At the final 45-minute time point the Defend 400 device achieved an average LOG reduction of 5.66. This is represented graphically in **Figure 11** which shows the log reduction for each trial as well as an overall average.

The Novaerus Defend 400 achieved net LOG reductions of over 99.99% in 30 minutes with all trials showing net LOG reductions over 4.0 LOG and an overall average net LOG reduction of 4.43 which is equivalent to a net percent reduction of 99.9961%. By the 45-minute time point the device achieved an average net LOG reduction of 5.31 which is equivalent to a net percent reduction of 99.9995%. Net LOG reduction is displayed graphically in **Figure 12**, a summary table containing all net LOG and net percent reduction values can be found in **Figure 13**.

Bacillus Globigii Results

When tested against Bacillus globigii the device showed a rapid reduction within the test chamber achieving over a 99.99% reduction in 45 minutes with an average LOG reduction of 4.51. **Figure 14** shows the LOG reduction of Bacillus *globigii* by the Defend 400.

Novaerus Defend 400 MS2 Summary Data

Bioaerosol Type	Species (description)	Trial Name	Reduction Type	Trial Time (minutes)		
				15	30	45
Virus	*MS2* (RNA E. coli phage)	*MS2-T1*	*Net Log Reduction*	-3.03	-4.46	-5.49
			Net % Reduction	99.9057%	99.9965%	99.9997%
Virus	*MS2* (RNA E. coli phage)	*MS2-T2*	*Net Log Reduction*	-3.00	-4.26	-5.23
			Net % Reduction	99.9005%	99.9945%	99.9994%
Virus	*MS2* (RNA E. coli phage)	*MS2-T3*	*Net Log Reduction*	-2.47	-4.58	-5.21
			Net % Reduction	99.6641%	99.9974%	99.9994%
All Trial Averages			***Net Log Reduction***	**-2.83 +/- 0.31**	**-4.43 +/- 0.16**	**-5.31 +/- 0.16**
			Net % Reduction	***99.8234% +/- 0.138%***	***99.9961% +/- 0.0015%***	***99.9995% +/- 0.0002%***

Figure 13: *Net Log Reduction summary table*

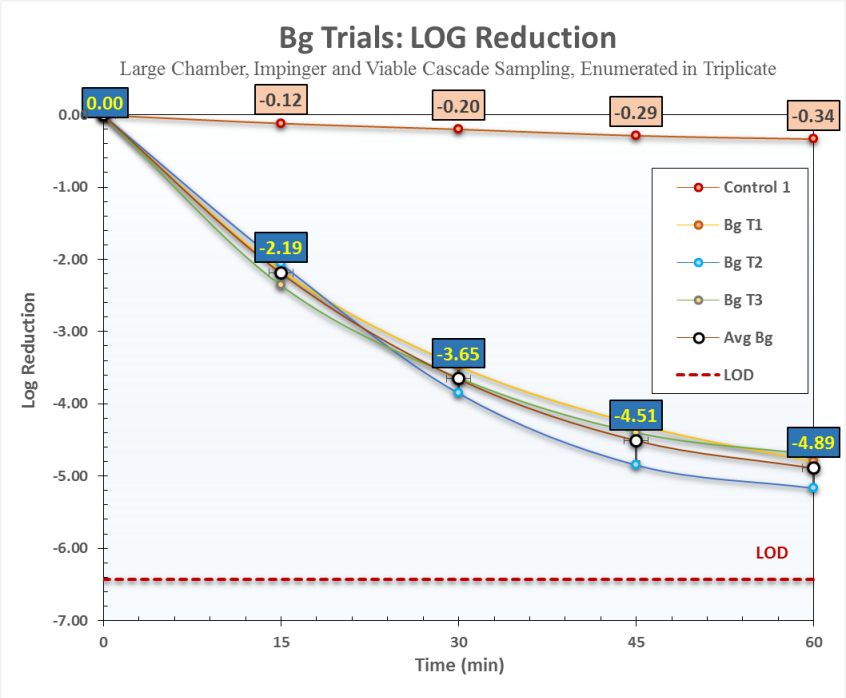

Figure 14: *Bacillus Globigii Novaerus Defend 400 Log Reduction*

The Defend 400 showed high net LOG reduction values with an average net LOG reduction of 4.22 within 45 minutes which equates to an over 99.99% net reduction in viable bioaerosols. At the final 60-minute time point the device showed an average net LOG reduction of 4.55 which equates to a net percent reduction of 99.9969%. **Figure 15** shows the net LOG reduction of the Defend 400 device, **Figure 16** on the following page shows a summary table with all net LOG and net percent reduction values.

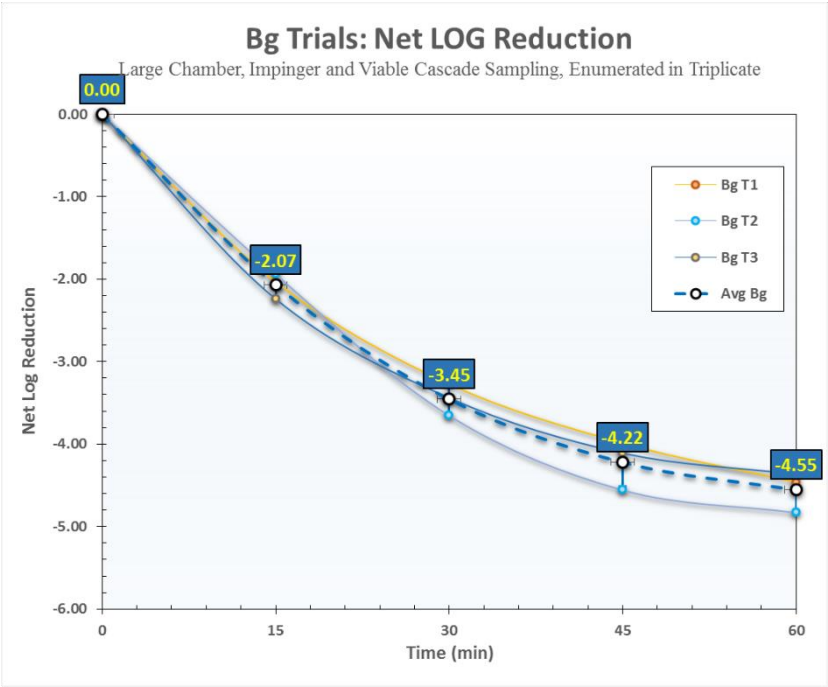

Figure 15: *Bacillus Globigii Novaerus Defend 400 Net LOG Reduction.*

Novaerus Defend 400 BG Trial Summary Data

Bioaerosol Type	Species (description)	Trial Name	Reduction Type	Trial Time (minutes)			
				15	30	45	60
Virus	*BG* (RNA E. coli phage)	*Bg T1*	*Net Log Reduction*	-2.03	-3.27	-3.99	-4.47
			Net % Reduction	99.0739%	99.9469%	99.9898%	99.9966%
Virus	*BG* (RNA E. coli phage)	*Bg T2*	*Net Log Reduction*	-1.94	-3.65	-4.56	-4.83
			Net % Reduction	98.8519%	99.9777%	99.9972%	99.9985%
Virus	*BG* (RNA E. coli phage)	*Bg T3*	*Net Log Reduction*	-2.24	-3.44	-4.11	-4.36
			Net % Reduction	99.4239%	99.9635%	99.9922%	99.9957%
All Trial Averages			***Net Log Reduction***	**-2.07 +/- 0.15**	**-3.45 +/- 0.19**	**-4.22 +/- 0.3**	**-4.55 +/- 0.24**
			Net % Reduction	***99.1166% +/- 0.2884%***	***99.9627% +/- 0.0154%***	***99.9931% +/- 0.0038%***	***99.9969% +/- 0.0014%***

Figure 16: *Bacillus Globigii Net Log Reduction summary table*

Bacillus Globigii Re-Aerosolization Test

In addition to the Bacillus Globigii testing conducted in the body of this report an additional test was run for a 24-hour period to determine if the velocity of the fan within the Defend 400 device was causing re-aerosolization of biological contaminants that had impacted on the walls of the testing chamber.

Test methods remained the same for this testing as all other testing conducted for this study. After nebulization a T-0 sample was collected via AGI-30 impinger and the device was operated at high speed for a period of 24-hours. After 24-hours an SKC viable cascade impactor sampled at a flow rate of 30 LPM for a period of ten (10) minutes resulting in a 300-liter sample from the chamber. The plate was then incubated for 24-hours and returned a single (1) colony forming unit. These results indicate that the Defend 400 is not causing re-aerosolization of impacted bioaerosols. These results equate to an 8.22 LOG reduction in 24-hours. These results are represented graphically in **Figure 17**.

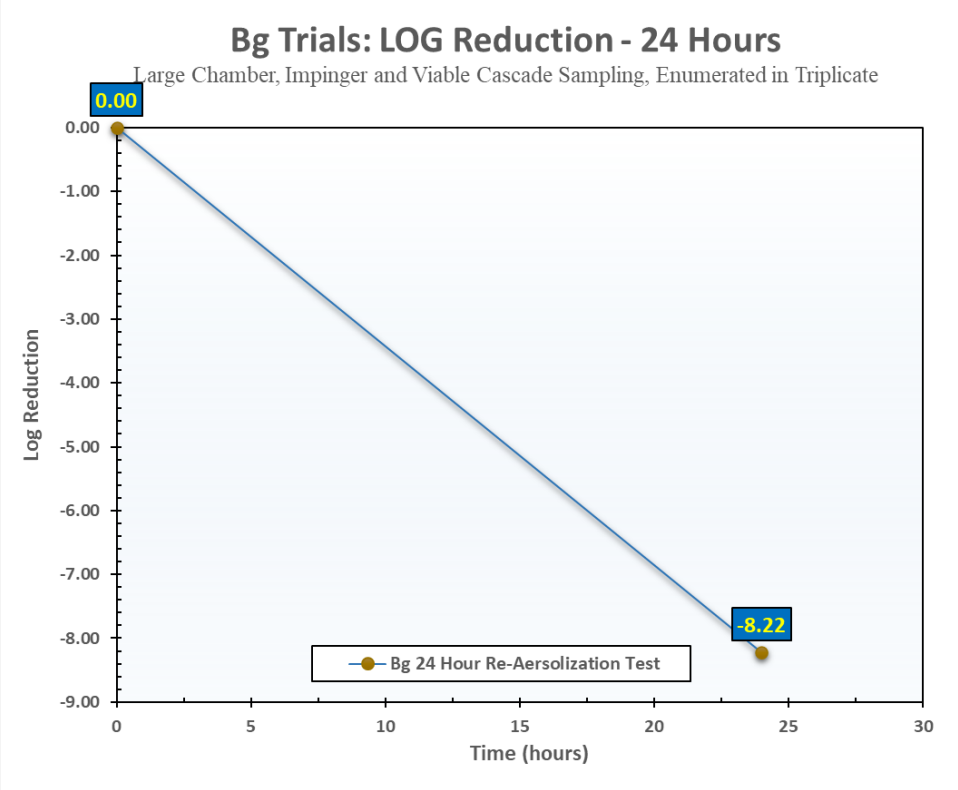

Figure 17: *Bacillus Globigii LOG Reduction after 24-hours*

Summary of Results

The Defend 400 device showed a high reduction in viable bioaerosols within the 45 to 60 minute test period for both organisms. The device showed increased performance with the MS2 bacteriophage when compared to the Bg spores. The device had a final net LOG reduction of MS2 after 45 minutes of 5.31 which is equivalent to a net percent reduction of 99.9995%. When tested against Bacillus *globigii* endospores the device showed an average net LOG reduction of 4.55 LOG which is equivalent to a net percent reduction of 99.9969%.

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Analytical Testing Facility

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

Project

10814.40

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.

04/08/2020
Date

Principal Investigator:

Jeffery Trolinger
Principal Investigator
ARE Labs, Inc.

04/08/2020
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 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:

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