Efficacy of the Bluezone Model 450 against Aerosolized MS2 Virus in a Large Chamber

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Background: This in vitro study characterized the efficacy of the Bluezone Model 450 at removing aerosolized MS2 Bacteriophage. The Bluezone device is designed to reduce airborne bacteria, viruses, and fungal spores in order to decrease infection rates from airborne pathogens. For this study the Bluezone 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 SARS-CoV-2 due to its size similarity to influenza and its RNA genome. This study evaluated the efficacy of the device against aerosolized MS2 bacteriophage in a stainless steel bioaerosol chamber. The study consisted of a total of three (3) live bioaerosol trials, and a single (1) bioaerosol control run.

Methods: MS2 bacteriophage was aerosolized into a sealed environmental bioaerosol chamber containing the Bluezone Model 450. AGI Impinger samples were taken from the chamber in order to quantify the reduction speed and capabilities of the Bluezone device. 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. The chamber control trial data was subtracted from the Bluezone trial data to yield net LOG reduction in the chamber for the bioaerosol challenges.

Results: When tested against the MS2 bacteriophage, the Bluezone Model 450 device showed a consistent net LOG reduction throughout the testing. The average net LOG reduction went from 3.39 at the 30-minute time point down to 5.32 at the 60-minute time point. These results indicate the Bluezone 450 device produced a rapid reduction in viable MS2 within the 60 minute test period. The 450 is estimated to reach a 4.0 net LOG reduction of viable MS2 bioaerosol in a 16m^3 chamber at 35 minutes.

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 Bluezone Model 450 at reducing aerosolized MS2 bacteriophage. The Bluezone device is an air purification system intended for use in medium to large sized rooms. The unit was used at full capacity for the duration of the trials.

The Bluezone device is equipped with UV lights to reduce the MS2 bacteriophage. The Bluezone device is an air purifier designed for use in occupied spaces such as restaurants, classrooms, offices and conference rooms. The test plan incorporated challenging the Bluezone device in a closed environmental chamber to determine the destruction rate of MS2 bacteriophage by the Bluezone device. A picture of the Bluezone Model 450 is shown in **Figure 1,** on the following page.

Study Overview

The effectiveness of the Bluezone device was evaluated against a single RNA virus which was MS2 bacteriophage. For more information on the MS2 bacteriophage please see species selection section in the body of this report.

Testing was conducted to characterize a single Bluezone unit against MS2 with triplicate (3) independent trials as well as a single (1) control trial to demonstrate the capability of the Bluezone 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*: Bluezone Model 450 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.

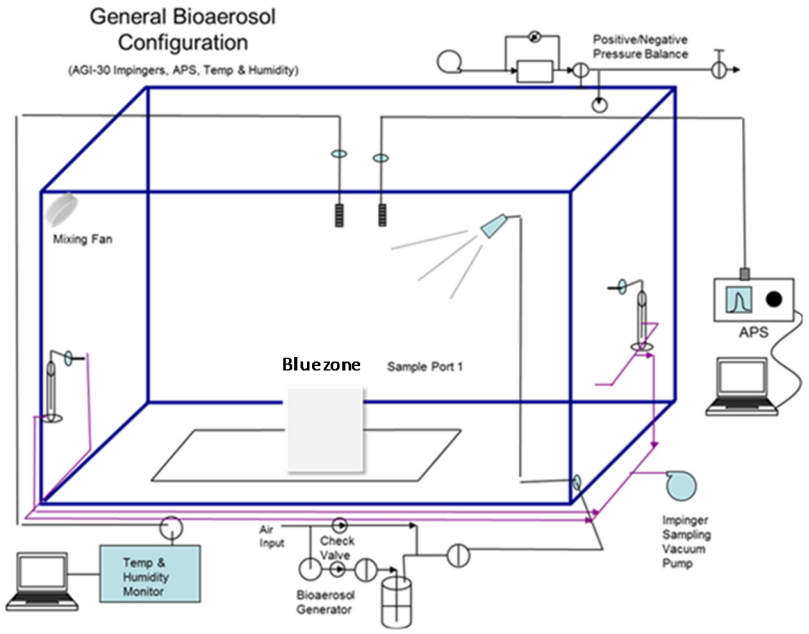

Figure 3: *Bio-Aerosol Test Chamber Flow Diagram.*

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 shear 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 Bluezone 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 with 1 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 bacteriophage (ATCC 15597-B1) is positive-sense, single-stranded RNA virus that infects the bacterium Escherichia coli and other members of the Enterobacteriaceae family. MS2 is routinely used as a surrogate for pathogenic RNA viruses, such as influenza and is a tentative surrogate for coronavirus, SARS-CoV2.

The 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 5** is a graphic

Test Matrix for the Bluezone Model 450 Device

Trial	Run	Device	Organism	Target Monodispersed Particle Size	Trial Time (min)	Sampling Period (min)	Sampling
C1 T1 T2 T3	Control Challenge Challenge Challenge	Bluezone Model 450	*MS2 Bacteriophage - RNA Virus*	0.5-1.0 um	60	0, 15, 30, 45, 60	AGI Impingers, APS

Figure 4: *Bioaerosol Test Matrices for all trials*

from the FDA document, COVID Sterilizers, Disinfectant Devices, and Air Purifiers Guidance, demonstrating resistance to disinfection.

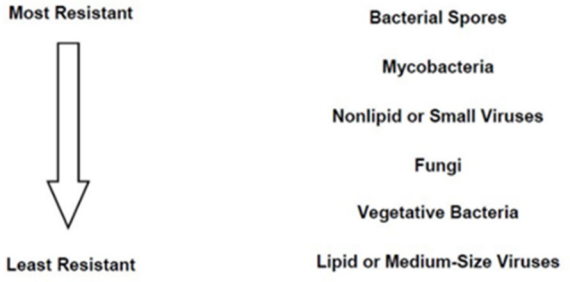

Figure 5: *FDA Graphic showing resistance to disinfection for various organisms.*

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 MS2 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 MS2 bacteriophage cultures were serially diluted and plated in quadruplicate (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.

Bioaerosol Control Testing

To accurately assess the Bluezone unit, test chamber pilot control trials were performed with MS2 bacteriophage 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 Bluezone 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 remained on during Bluezone 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**.

Bluezone 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 Bluezone 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 MS2 bacteriophage dissemination and remained on for the duration of the test to assure a homogeneous

bioaerosol concentration in the test chamber prior to taking the first impinger sample.

Following bioaerosol generation, baseline MS2 concentrations were established for each pilot control and Bluezone test by sampling simultaneously with two AGI-30 impingers located at opposite corners of the chamber. AGI samples were collected for 2 to 5 minutes at intervals of 15 minutes throughout the entire test period.

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 Bluezone biological testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the test (60 minutes). Subsequent impinger samples were taken at 0, 15, 30, 45 & 60 minutes and samples enumerated for viable concentration to measure the effective viable MS2 bacteriophage reduction during operation of the Bluezone device over time. All samples were plated in quadruplicate 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 after the device was removed 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 throughout the trials. 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 a Microsoft Surface, regressed, and plotted. The aerosol particle size distribution for MS2 in the large chamber during the trials is shown in **Figure 6**.

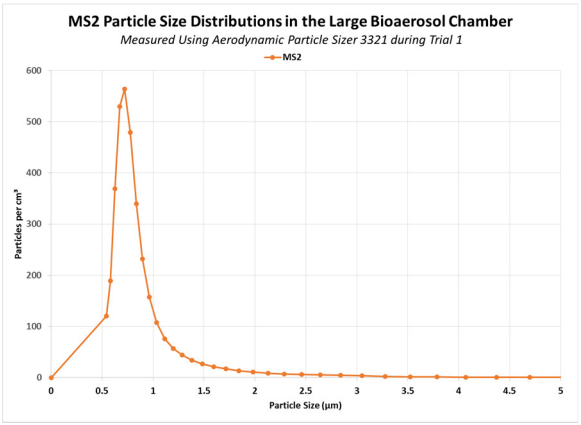

Figure 6: *Viral (MS2) Number Particle Size Distribution in Test Chamber.*

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. The key particle size distribution values for MS2 bacteriophage in the chamber can be found in **Figure 7.**

	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.*

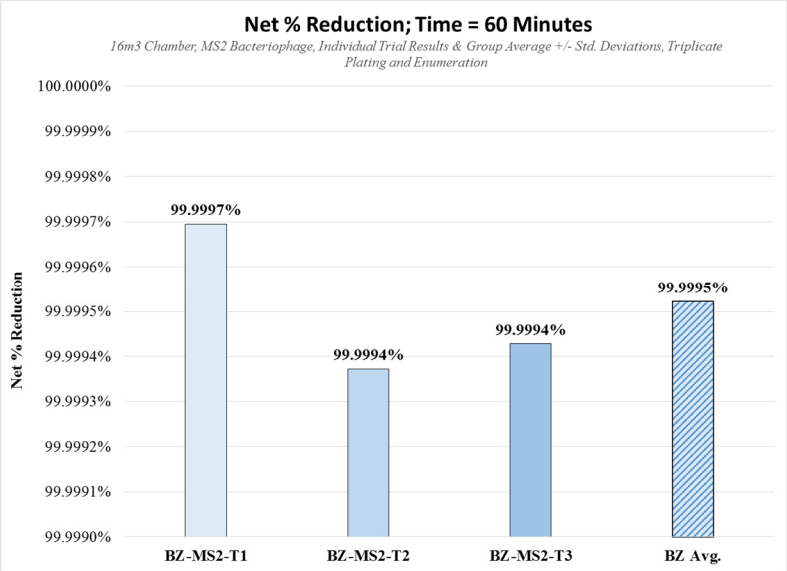

Figure 8: *MS2 Bluezone Net % Reduction at T-60*

Data Analysis

Results from the control trial were graphed and plotted to show natural viability loss over time in the chamber. This control run served as the basis to determine the time required for the Bluezone Model 450 to reduce 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 (**Figure 10**). 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. Due to the speed of the Bluezone reduction efficacy, control data at T=15 and 45 were interpolated from the average of two different control trials.

Results

When tested against the *MS2 bacteriophage* the device showed a consistent net log reduction throughout the duration of the trial. The net log reduction for the three trials averaged 5.32 log which is equivalent to a 99.9995% reduction. A graphic displaying the net percent reduction for each trial as well as an average for all of the trials can be found in **Figure 8.** The net log reduction at the same time point ranged from 5.20 to 5.52 log. This is represented graphically in **Figure 9**.

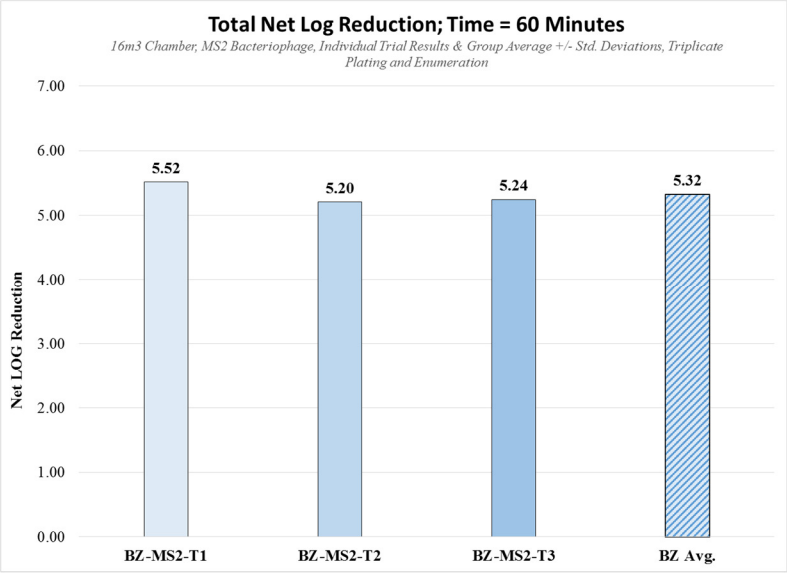

Figure 9: *MS2 Bluezone Net Log Reduction at T-60*

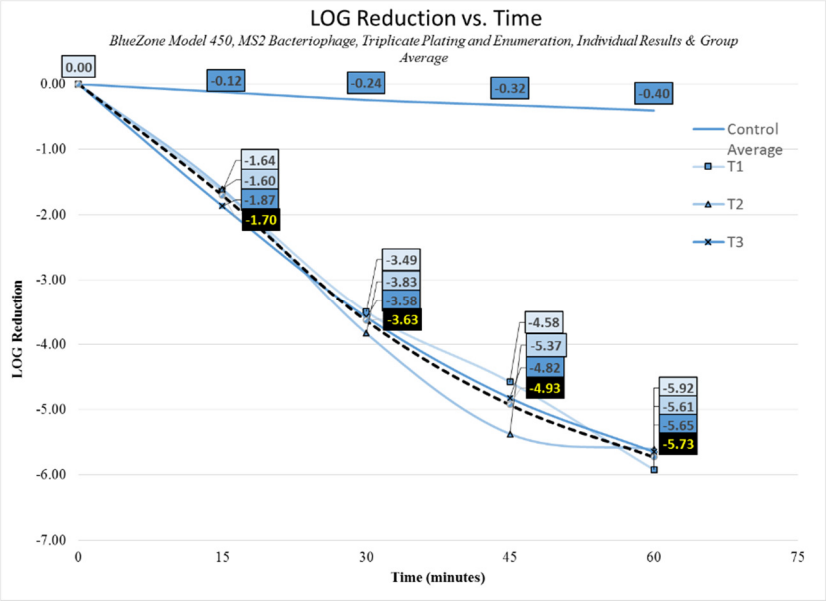

Figure 10: *MS2 Bluezone LOG Reduction all trials*

Summary of Results

When tested against the MS2 bacteriophage, a surrogate traditionally used for influenza and now considered as a possible surrogate for SARS-COV-2, the device showed consistent net log reduction throughout each trial. By the 30-minute time point results showed an average 3.63 LOG reduction which equates to an average 3.39 net LOG reduction. LOG reduction results can be found in **Figure 10**, Net LOG reduction results can be found in **Figure 11**.

After 60 minutes the device had an average LOG reduction of 5.73 LOG which is equivalent to a 5.32 net LOG reduction. For all trials conducted the device achieved greater than a 4 net LOG reduction by the 45 minute time point which is equivalent to a 99.99% reduction. By the 60 minute sample point the Bluezone 450 had an average net percent reduction of 99.9995%. These results indicate that in theory the Bluezone device would help prevent the spread of airborne infection. A table showing the results in net log reduction can be found in **Figure 12.**

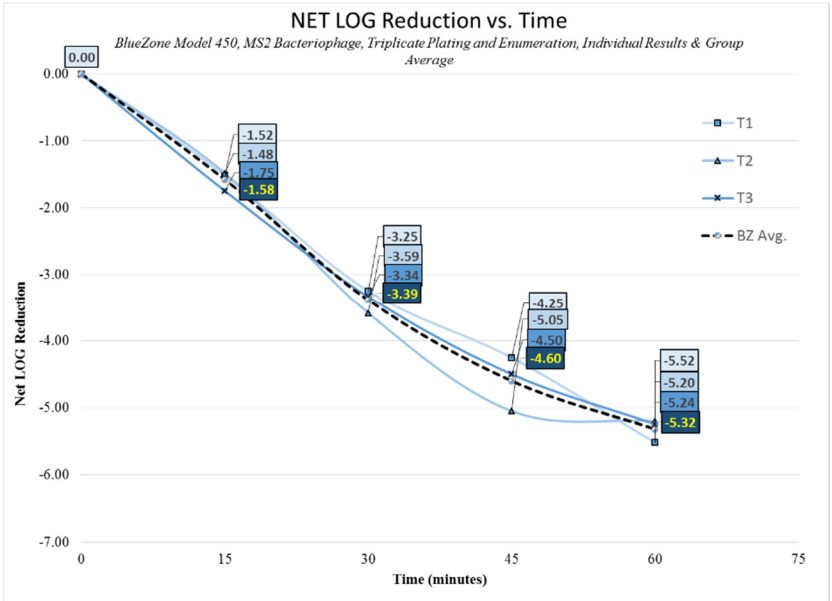

Figure 11: *MS2 Bluezone Net LOG Reduction all trials*

Summary Data for Reduction of MS2 By Bluezone Model 450

Bioaerosol Type	Species	Surrogate	Trial ID		15min	30min	45min	60min
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*Trial 1*	Net Log Reduction	**-1.52**	**-3.25**	**-4.25**	**-5.52**
				Net Percent Reduction	***96.9552%***	***99.9439%***	***99.9944%***	***99.9997%***
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*Trial 2*	Net Log Reduction	**-1.48**	**-3.59**	**-5.05**	**-5.20**
				Net Percent Reduction	***96.7060%***	***99.9740%***	***99.9991%***	***99.9994%***
Virus	*MS2 bacteriophage (RNA E. coli phage)*	*Influenza*	*Trial 3*	Net Log Reduction	**-1.75**	**-3.34**	**-4.50**	**-5.24**
				Net Percent Reduction	***98.2128%***	***99.9540%***	***99.9968%***	***99.9994%***
Average NET LOG Reduction					**-1.58 +/- 0.14**	**-3.39 +/- 0.17**	**-4.6 +/- 0.41**	**-5.32 +/- 0.17**
Average NET Percent Reduction					**97.291% +/- 0.808%**	**99.957% +/- 0.015%**	**99.9968% +/- 0.002%**	**99.9995% +/- 0.0002%**

Figure 12: *Bluezone Model 450 Executive Summary table*

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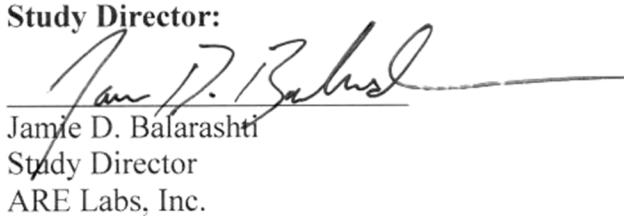
GLP Statement

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:



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