Efficacy of the Defend 1050 Recirculating Air Cleaner Against Three Respirable Microorganisms

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ABSTRACT

Purpose: This study was done to characterize the efficacy of the Defend 1050 air purification device, distributed by Protect ED™, against three aerosolized microorganisms.

Background: This study characterized the Defend 1050 air purifier's efficacy in removing three species of respirable bioaerosols using a sealed 16m^3 testing chamber. Two methods were used: 1) the single-pass efficiency was tested to calculate the CADR, and 2) chamber trials were performed to prove the device's efficacy in an indoor environment.

The species tested were *Enterobacter cloacae*, a gram-negative bacterium; *Salmonella enterica*, a gram-negative bacterium; and *Listeria innocua*, a gram-positive bacterium. These are typical food and airborne pathogens that can be found in public places and cause various illnesses. Consequently, the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) are concerned with controlling their spread.

Methods: Each microorganism was aerosolized into a sealed 16m3 environmental bioaerosol test chamber containing the test device using a Collison 24-jet nebulizer. For the single-pass testing, sampling was performed by simultaneously capturing bioaerosols directly at the inlet and outlet of the device and calculating the percent reduction. Three trials were performed for each organism and the results averaged. For the chamber trials, twelve (12) live bioaerosol trials were performed; three species each were tested in triplicate, including one control trial per organism. Bioaerosol samples were then taken from the chamber at multiple time points throughout each trial to quantify the reduction rate of the recirculating air cleaner. Chamber control trial data, or natural decay, was subtracted from the device trial data to yield the net log reduction for each bioaerosol challenge.

All samples were serially diluted, plated, incubated, and enumerated in triplicate to yield viable bioaerosol concentrations before and after passing through the device.

Results: The average single-pass reduction measured was 3.97 ± 0.03 log, or 99.989%, resulting in a CADR of 533 cfm.

In chamber trial tests, the device effectively reduced all three bioaerosol challenge organisms by a net log of 4.0 or greater (equivalent to 99.99% or greater) within 15 minutes, exceeding a 6 net log reduction in 20 minutes.

Conclusions: The device quickly reduced three species of airborne microorganisms in the chamber trials. This is due to the device's high efficiency, which resulted in a 533 cfm CADR.

Introduction

This study evaluated the efficacy of the Defend 1050 device, manufactured by Novaerus and distributed by Protect ED™, in reducing aerosolized microorganisms. The air filtration device utilizes a three-stage filter and Nanostrike™ technology for use in schools, medical buildings, and other large spaces. It's designed to purify air continuously while in operation. It filters out and inactivates airborne particles, including bacteria, viruses, and mold spores, with the filters and proprietary technology.

The Defend 1050 device has a 510(k) premarket notification to the U.S. Food and Drug Administration (FDA). The 510(k) submission provides comprehensive information about the device, its intended use, and data supporting its safety and efficacy. Clearance through the 510(k) process is essential for legally marketing the device in the United States.

The Defend 1050 device is designed to improve indoor air quality in commercial and medical applications. The test plan implemented in this study involved challenging the Defend 1050 device in a controlled environmental chamber. The objective was to determine the reduction rates and extent of three commonly found bacteria. Figure 1 shows a photo of the Defend 1050 device.

Figure 1: The Defend 1050 Recirculating Room Air Purifier.

Study Overview

The effectiveness of the Defend 1050 device (Figure 1) was evaluated against two Gram-negative and one Gram-positive bacteria. Testing was conducted to characterize the Defend 1050 device unit against three organism types to demonstrate the device's capability, when operating at the highest fan speed (533 CFM), to reduce viable bioaerosol concentrations, theoretically reducing the chances of airborne infection.

Test Device Description

The Defend 1050 is a free-standing recirculating air cleaner. It is designed to pull air through a pre-filter, and then the airflow is passed through the patented NanoStrike™ coils before being pushed out of a HEPA 13 filter and granulated carbon post-filter. The device has 5 fan speeds, and all testing was performed at the highest fan speed, which averaged 533 ft^3/minute (CFM).

Flow Rate Measurement

Before testing, the flow rate was measured on the test device when set to Speed 5 by measuring with a handheld digital anemometer (AOPUTTRIVER AP-856A). The average of 6 location linear flow rates and the total area the air passes through were measured. The volumetric flow rate was then calculated by multiplying the linear flow rate by the area of the device vent.

Chamber Use Justification

The 16m^3 stainless steel chamber was chosen for testing the device as it was tested for the 510k approval. The 16m^3 chamber has often been used to simulate an air cleaner running in a small room such as an office or residential space. The chamber has been used in previous FDA 510K submissions with devices from notable manufacturers such as Aeroclean (K223328) and The Pyure Company, formerly known as HGI Industries (K133800).

Trial	Run	Device	Device Fan Setting	Challenge Species (gram, description)	ATCC Ref #	Chamber Size (m^3)	Target Particle Size	Challenge Conc. (#/L)	Trial Time (min)	Bioaerosol Sampling Time Points (min)	Sampling Devices	Plating and Enumeration
1	Control	Defend 1050	Speed 5	*Enterococcus clocae* (gram -)	13047	16	1.0 - 3.0 µm	10^4-10^5	20	0, 5, 10, 15, 20	Impingers, Viable BioSamplers	All Samples in Triplicate
2	Challenge											
3	Challenge											
4	Challenge											
1	Control	Defend 1050	Speed 5	*Salmonella enterica* (gram -)	53648	16	1.0 - 3.0 µm	10^4-10^5	20	0, 5, 10, 15, 20	TSI 3321 APS, Impingers	All Samples in Triplicate
2	Challenge											
3	Challenge											
4	Challenge											
1	Control	Defend 1050	Speed 5	*Listeria innocua* (gram +)	33090	16	1.0 - 3.0 µm	10^4-10^5	20	0, 5, 10, 15, 20	TSI 3321 APS, Impingers	All Samples in Triplicate
2	Challenge											
3	Challenge											
4	Challenge											

Table 1: **Bioaerosol Challenge Test Matrix**. The Defend 1050 was operated at 533 CFM for all trials in this study.

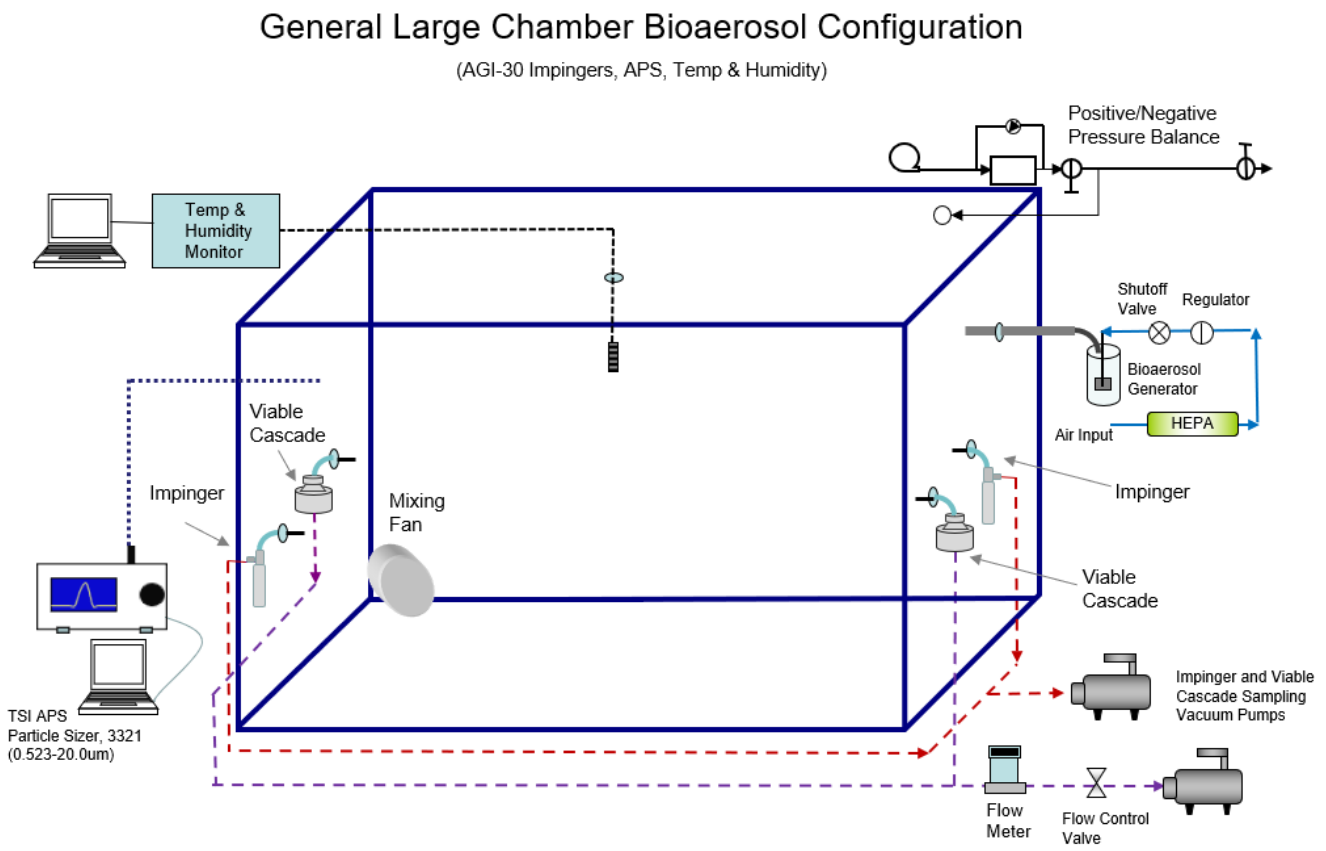

Figure 2 Bio-Aerosol Test Chamber Flow Diagram. The chamber includes bioaerosol induction, multiple bioaerosol sampling ports, Particle size monitoring, internal mixing fans, and temperature and humidity controls. The HEPA Evacuation System is not pictured.

Equipment

Bioaerosol Testing Chamber

A large, sealed aerosol test chamber was used to simulate a contaminated room environment and to contain any aerosols for lab and technician safety. 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's internal dimensions are 9.1 ft x 9.1 ft x 7 ft, with a displacement volume of 579 cubic feet, or 16,000 liters. **Figure 3** shows the bioaerosol chamber used for all testing in this study.

The chamber has filtered HEPA inlets, digital internal temperature and humidity monitors, a heater, a humidifier, a lighting system, multiple sampling ports, aerosol mixing fans, and a HEPA-filtered exhaust system operated with wireless remote control.

For testing, the chamber is equipped with four 3/8-inch diameter stainless steel probes for aerosol sampling and a 1-inch diameter port for bio-aerosol dissemination into the chamber using a Collison 24-jet nebulizer or dry powder eductor for the aerosolization of the microorganisms and spores, respectively. See **Figure 2** for a flow diagram of the testing chamber.

Figure 3: Stainless Steel Bioaerosol Test Chamber used for all Defend 1050 Device Testing. The Chamber has HEPA in/out, multiple bioaerosol sampling ports, decontamination, and pressure balance.

To avoid wall effects, all sample and dissemination ports were inserted approximately 18 inches from the chamber's

interior walls and at a height of approximately 40 inches from the floor. The aerosol sampling and dissemination probes are stainless steel and bulkheaded 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 -0.5 to 0.5 inches of H_2O (Dwyer instruments, Michigan City, IN) was used to monitor and balance the system pressure during aerosol generation, aerosol purge, and testing cycles.

Test Chamber Environmental Controls

For increased stability of bioaerosols, relative humidity inside the chamber is kept at 65% +/- 5% using a PID humidity controller in combination with an ultra-sonic humidifier to nebulize filtered DI water. Temperature controls maintain chamber trial conditions at typical ambient conditions of 72 ± 2 °F (22 ± 1 ^{o}C). These environmental controls ensure that the bioaerosols remain stable within the chamber, limiting the amount of natural decay seen during control tests. This subsequently allows for a better resolution of the reduction provided by the device, especially at lower chamber concentrations.

Bioaerosol Generation System

All test bioaerosols were disseminated using a Collison 24-jet nebulizer (BGI Inc. Waltham MA), similar to the one shown in **Figure 4**. A HEPA-purified, filtered house air supply drove the aerosolization of bioaerosols. A pressure regulator allowed for controlled dissemination, allowing for better control of particle size and standardizing the use rate and sheer force generated within the Collison nebulizer.

Figure 4. 6-Jet Collison nebulizer. Glass and 304 stainless steel construction, BGI Industries. The 24-jet variant was used for testing.

Before testing, the Collison nebulizer flow rate and usage rate were characterized using an air supply pressure of approximately 40-60 psi, which produced an output volumetric flow rate of 50-80 L/min with a fluid dissemination rate of approximately 1.25 mL/min. The flow of the Collison nebulizer was characterized by a calibrated TSI model 4040 mass flow meter (TSI Inc., St Paul, MN).

Bioaerosol Sampling and Monitoring System

Two AGI impingers (**Figure 5**, Ace Glass Inc. Vineland NJ) were used to collect all biological aerosols, allowing for precise back calculations to determine chamber concentrations. The two AGI Impingers were placed at opposite corners of the chamber, ensuring a better representative sample. The mixing fans inside the chamber provided a homogenous air mixture inside the chamber.

These procedural implementations are essential for consistency and accuracy during the bioaerosol sampling. The AGI-30 impingers were connected to a vacuum source maintained at a negative pressure of 18 inches of Hg during all characterization and test sampling to ensure critical flow conditions. The AGI-30 bio sampler impingers flow was characterized using a calibrated TSI model 4040 mass flow meter. This calibration, coupled with the impingers' critical orifice design, always allows for a consistent chamber airflow through the samplers.

Figure 5: Air samples were taken with an AGI-30 impinger.

TSI Aerodynamic Particle Sizer

A TSI Aerodynamic Particle Sizer (APS) model 3321 (TSI Inc., Shoreview, MN) was used to measure aerosol concentrations and particle size during trials. The APS provided real-time aerodynamic particle characterization with a size range from 0.54 to 20.0 µm and 52 size bins of resolution. Sampling is continuous with a data export interval of 1 second. The APS has a continuous flow rate of 5 liters per minute (LPM). A picture of the APS is shown in **Figure 6**.

Figure 6. TSI Aerodynamic Particle Sizer (APS) model 3321 was used to measure the challenge-bioaerosol's total particle concentration and particle size distribution. The range is 0.54-20.0 µm aerodynamic diameter, with 1 particle/L detection limits.

SKC Viable Bio-Sampler

Sample collections were also obtained using a pair of viable impactors during testing with less resilient organisms or those that fall out of the air more efficiently. A viable cascade impactor (SKC Inc., Valley View, PA) comprises an inlet cone, a precision-drilled 400-hole impactor stage, and a base with a standard-size agar plate (**Figure 7**). A high-flow pump pulls microorganisms in the air through the holes (jets) at 30 liters per minute, where they are collected directly onto the agar surface. This method is the most sensitive for detecting organisms at low concentrations.

Figure 7: SKC Single Stage BioStage Viable Cascade Impactor used for bacterial and spore sampling for select time points during bioaerosol trials. LOD is >0.01 cfu/L.

Single-Pass Sampling Configuration

To quantify the Defend 1050's single-pass reduction, stainless steel sample probes were mounted on the device for instantaneous sampling of its inflow and outflow. Figure 8 shows a picture of the probes mounted. A PVC pipe was mounted on the outflow to prevent particles from returning to the sample probe and measure the downstream bioaerosol concentrations. The upstream probe was mounted tangentially to the inflow of air on the front of the device.

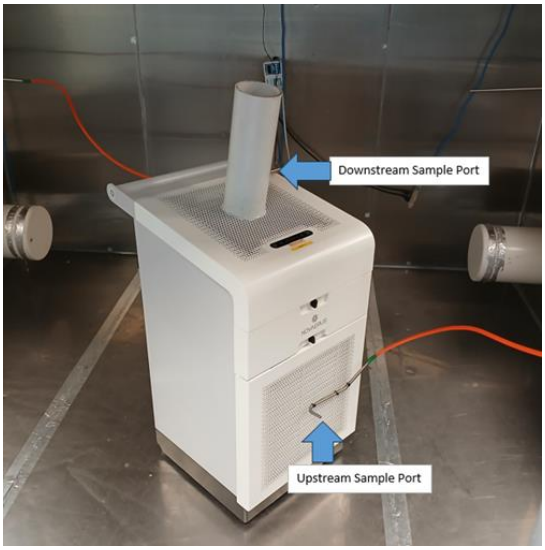

Figure 8: Aerodynamic Single pass sample port placement on the test device. Stainless steel probes were attached to PTE tubing for direct air sampling with AGI 30 impingers.

Species Selection and Justification

Reducing viable bioaerosols by 4 net log or 99.99% is the minimum requirement for FDA 510k-approved use. The organism species used were explicitly chosen for their natural abundance, particle size differences, and potential to cause infection. Due to safety concerns for bioaerosol testing, organism selection was based on Biological Safety Level 1 (BSL1) species.

Three different organisms with different particle sizes were selected to assess the filtration device fully. Determining the reduction rate by a filtration device with a range of particle sizes was one of the justifications for selecting these organisms and their predominance in commercial settings.

The first bacterium chosen was *Enterobacter cloacae* (ATCC # 13047), a gram-negative rod-shaped, facultatively anaerobic, and bears peritrichous flagella. *E. cloacae* is a bacteria commonly used in various forms of testing as it is a common pathogen found in a multiplicity of places, and it is also known as a simulant for medically significant gram-negative pathogens.

The second bacterium chosen was *Salmonella enterica* (ATCC # 53648), a rod-shaped, flagellate, facultatively anaerobic, Gram-negative bacterium. This is a common food-borne illness pathogen and is predominant in poultry processing factories.

The third bacterium was *Listeria innocua* (ATCC# 33090), a Gram-positive, rod-shaped bacterium. This species is motile, facultatively anaerobic, and non-spore-forming. Pathogenic forms of Listeria can form biofilms in food-processing plants, creating contamination problems.

Challenge Bioaerosol Aerodynamic Diameter

Bioaerosol particle size distributions were measured with a TSI Aerodynamic Particle Sizer model 3321 (APS) for all challenge species. The particle size distribution was taken shortly after aerosolization for each species via sampling through a sample probe into the test chamber. The APS has a dynamic measurement range of 0.54 to 20.0 µm and was programmed to take consecutive real-time one-minute aerosol samples.

Data was logged in real-time to a laptop computer, regressed, and plotted. All challenge bioaerosols' aerodynamic particle size distribution was within the respirable range for regional alveolar tract deposition. It showed a low geometric standard deviation (GSD), indicating that a monodispersed aerosol was generated in the chamber for each challenge species**.** The bioaerosol particle size distributions for the species tested are shown in **Figure 9**.

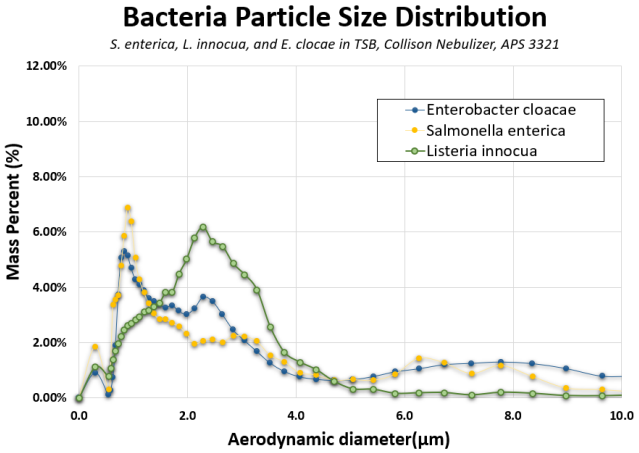

Figure 9: Aerodynamic Particle Size Distribution of the three bioaerosol species in the test chamber. MMAD for each species was approximately 1-3 µm.

Challenge Organism Culture & Preparation

Vegetative Cells Culture & Preparation

Pure strain seed stocks were purchased from ATCC (American Type Culture Collection, Manassas, VA). For ATCC reference numbers, see **Table 1** on page 3. Working stock cultures were prepared using aseptic techniques in a class 2 biological safety cabinet and followed standard seed preparation methodologies. Approximately 250mL of each biological stock was prepared in tryptic soy liquid broth media and incubated for 24-48 hours with oxygen infusion (1cc/min) at 37°C. Biological stock concentrations were around 1 x 10^{10} cfu/ml.

These stock cultures were then centrifuged for 10 minutes at 3000rpm in an LD-3 centrifuge in sterile 50mL conical tubes, growth media was removed, and the cells were resuspended in sterile Tryptic Soy Broth for aerosolization. For viable counts and stock concentration calculation, these suspensions were enumerated on tryptic soy agar plates (Hardy Diagnostics, Cincinnati, OH). For each organism, test working stocks were grown in sufficient volume to satisfy use quantities for all tests conducted using the same culture stock material.

Methods Bioaerosol Testing

Nebulizer Stock Preparation

The bacterial challenges were centrifuged to remove spent growth media, then resuspended in Tryptic Soy Broth (TSB) and Antifoam A (ThermoScientific). The Collison nebulizer was filled with approximately 50mL of biological stock suspension for all bioaerosol tests. The TSB acted as a soil and stabilizer for the challenge tests, adding sugars, proteins, and other particulate matter to simulate a more real-world scenario for the filter.

Bioaerosol Single Pass Methods

Using the custom sampling configuration described above, each organism was aerosolized individually for 10 minutes, and then simultaneous upstream and downstream impinger samples were taken 3 times with the Defend 1050 in operation. After the sampling, the nebulization was stopped, the device was powered off, and the chamber was evacuated.

Bioaerosol Trial Methods

To accurately assess the Defend 1050 device unit, test chamber pilot control trials were performed with all six organisms over 60 minutes to characterize the biological challenge of aerosol delivery/collection efficiency and viable concentration over time. Control testing was performed to provide baseline comparative data and verify that viable bioaerosol concentrations persisted above the required concentrations over the entire pilot control test period.

The control data, or natural decay, allowed quantifying the reduction from the Defend 1050 device challenge testing. During control runs, two low-velocity fans located in the corners of the bioaerosol test chamber were turned on for the trial to ensure a homogenous aerosol concentration within the chamber. The mixing fan was used for all control runs and was turned off during Defend 1050 device decontamination trials.

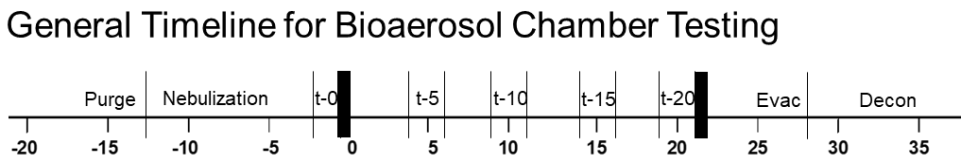

Figure 10 Bioaerosol Trial Timeline Example. Impinger samples were taken throughout each trial to determine the reduction rate of bioaerosols in the testing chamber, and trial times varied depending on the fan speed setting of the device. The figure shows the timeline of a 20-minute trial.

The two impingers for bioaerosol collection were pooled and mixed before plating and enumeration. A complete test matrix for the bioaerosol trials can be found at the beginning of the report in **Table 1**.

The Collison nebulizer was filled with the appropriate solution described above and then operated at 40 psi for 20 minutes. The TSB acted as a type of soil for the challenge tests. Then, the impingers were filled with 20 mL of sterilized PBS with an addition of 0.005% v/v Tween 80 for bioaerosol collection. The addition of Tween 80 was used to increase the impinger collection efficiency and deagglomeration of all microorganisms. The chamber mixing fans were turned on during bioaerosol dissemination to ensure a homogeneous bioaerosol concentration in the test chamber before taking the first impinger sample (T=0).

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and Defend 1050 device test by sampling simultaneously with two AGI-30 impingers at opposite corners of the chamber. AGI samples were collected for 2 to 10 minutes at different intervals (Liu, et al., 2013) throughout the 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 Defend 1050 device biological testing, the unit was turned on immediately following the T=0 timepoint sample and operated for the entirety of the test. Subsequent impinger samples were taken at various time points throughout the trial. These samples were enumerated for viable concentration to measure the effective viable bioaerosol reduction during the operation of the Defend 1050 device over time.

All samples were plated in triplicate on tryptic soy agar media over a minimum 3 log dilution range. Plates were incubated for 24-48 hours and enumerated for viable plaque-forming units (pfu) or colony-forming units (cfu) to calculate aerosol challenge concentrations in the chamber and reduction of viable microorganisms.

Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate. (Multiple serial dilutions) using a standard drop plaque assay technique onto tryptic soy agar plates. Depending on the species, the plated cultures were incubated for 24-48 hours and enumerated and recorded. Viable cascade sampling was used when working with microorganisms at extremely low concentrations. This method samples the chamber by pulling 30 liters per minute through the cascade device directly onto an agar plate.

Enumeration of colonies grown depends on the concentration of the sample. Colony counts totaling 400 can then be adjusted using the positive conversion table. This table is based on the principle that as the number of viable particles impinged on a given plate increases, the probability of the following particle entering an “empty hole” decreases. This can be corrected statistically using the conversion formula of Feller, W (1950). This viable cascade sampling method was not used in this study, given the level of resolution provided by the impingers at the end of these trials.

Post-Testing Decontamination and Prep

Following each test, the chamber was airflow evacuated/purged for at least twenty minutes between tests and analyzed with the APS for particle concentration decrease to baseline levels between each test. The chamber was decontaminated after the trials with aerosol/vaporous hydrogen peroxide (35%). The Collison nebulizer and impingers were cleaned after each day of testing by soaking in a 5% bleach bath for 20 minutes. The nebulizer and impingers were then submerged in a DI water bath, removed, and spray rinsed 6x with filtered DI water until use.

Data Analysis and Calculations

Single-pass upstream and downstream bioaerosol concentrations were used to calculate the single-pass reduction by the Defend 1050 device for each of the three organisms. This was done by dividing the downstream concentration by the upstream concentration and subtracting from one to determine the percent reduction after a single pass through the device. Each species was tested in triplicate for statistical significance. Also, once the single pass reduction was measured, the clean air delivery rate was calculated by multiplying the single pass reduction by the volumetric flow rate of the device, which was 533 CFM for all tests.

In addition, 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 Defend 1050 device to achieve at least 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 each organism's log reduction in viable bioaerosol. All data are normalized with time-zero enumerated concentrations. Subsequent samples are normalized and plotted to show the loss of viability over time. All raw data and supplemental graphs can be found in the report appendices. Example calculations used in this study can be found in **Appendix C.**

Single-Pass Reduction Results

The Defend 1050 device was challenged with three different bacteria species, and a minimum reduction of 99.988% was observed after a single pass through the device. Each species had similar reductions, proving that the device's robust efficacy captures nearly all of the bioaerosol generated. The percent reduction results can be found in Figure 11.

Single-Pass Conclusion and CADR

Given the high reduction observed from single-pass testing, the CADR can be calculated for each organism. This is the single pass reduction for each multiplied by the volumetric flow rate of the device, which was 533 CFM. Each of the CADRs averaged 532.9 CFM across the three species tested. The following equation was used to calculate the CADR:

$$CADR = \%\ Reduction \times V$$

CADR = Clean Air Delivery Rate
V = volumetric flow rate ft^3/min
% Reduction = Single-Pass Efficiency

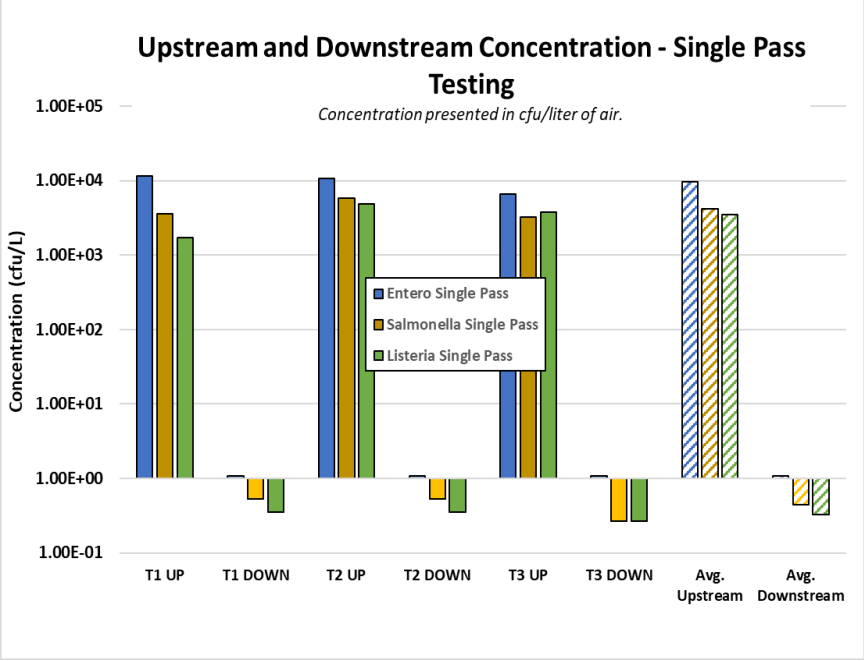

Figure 11: Up and downstream bioaerosol concentrations used in the calculations of the single-pass percent reduction results for all three species tested on the highest fan speed of the device (533 CFM).

Test Species	Number of Trials	Volumetric Flow Rate (ft^3/min)	Data Type	Trial 1	Trial 2	Trial 3	Average
Enterobacter clocae	3	533	CADR	532.95	532.95	532.91	532.94 ± 0.02
			Single Pass Efficiency	99.991%	99.990%	99.984%	99.988% ± 0.004%
Salmonella enterica	3	533	CADR	532.92	532.95	532.96	532.94 ± 0.02
			Single Pass Efficiency	99.985%	99.991%	99.992%	99.989% ± 0.004%
Listeria innocua	3	533	CADR	532.89	532.96	532.96	532.94 ± 0.04
			Single Pass Efficiency	99.979%	99.993%	99.993%	99.988% ± 0.008%

Figure 12: This table summarizes each trial's single pass reduction results and the group average and standard deviation. The CADR was calculated for each trial by multiplying the single pass efficiency by the volumetric flow rate of 533 CFM.

Chamber Trial Reduction Results

The Defend 1050 device performed quickly and efficiently, reducing all three organisms by over 4 log or 99.99% in under 15 minutes in the testing chamber. Samples were taken every 5 minutes after the device was activated, and no bacteria was detected after 20 minutes of operation. The average total net reduction of *E. cloacae* was 6.95 ± 0.14 log or 99.999988% in 20 minutes. The average total reduction of *S. enterica* was 6.25 ± 0.22 log or 99.999939% in 20 minutes. The average reduction of *L. innocua* was 6.81 ± 0.23 log or 99.999983% in 20 minutes.

The reproducibility of the results and consistent performance demonstrate that Defend 1050 is very effective at reducing airborne bacteria. The net log reduction results can be found in Figure 13, and an executive summary detailing the test results can be found in Figure 14. Supplementary graphs and raw data are in Appendixes **A** and **B.**

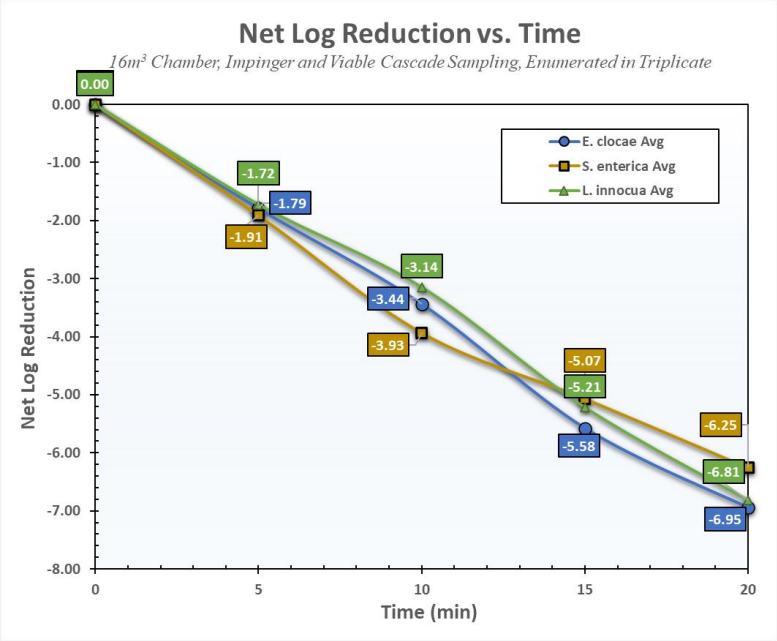

Figure 13: Net Log Reduction for the bacterial species challenged against the Defend 1050 device. The device was set to 533 CFM for all trials.

Bioaerosol Type	Species (description)	Trial Name	Reduction Type	Trial Time (minutes)			
				5	10	15	20
Bacteria	*E. clocae* (gram -)	*Entero-T1*	*Net Log Reduction*	-1.50	-3.21	-5.51	-7.05
			Net % Reduction	96.8301%	99.9390%	99.9997%	100.0000%
Bacteria	*E. clocae* (gram -)	*Entero-T2*	*Net Log Reduction*	-1.82	-3.72	-5.64	-7.01
			Net % Reduction	98.4983%	99.9809%	99.9998%	100.0000%
Bacteria	*E. clocae* (gram -)	*Entero-T3*	*Net Log Reduction*	-2.05	-3.38	-5.59	-6.78
			Net % Reduction	99.1019%	99.9580%	99.9997%	100.0000%
All Trial Averages +/- St. Dev.			***Net Log Reduction***	***-1.79 ± 0.28***	***-3.44 ± 0.26***	***-5.58 ± 0.07***	***-6.95 ± 0.14***
			Net % Reduction	*98.143% ± 1.177%*	*99.959% ± 0.021%*	*99.9997% ± 0.00004%*	*99.999988% ± 0.000004%*
Bacteria	*S. enterica* (gram -)	*Salmonella-T1*	Net Log Reduction	-1.73	-3.85	-5.14	-6.30
			Net % Reduction	98.1478%	99.9858%	99.9993%	100.0000%
Bacteria	*S. enterica* (gram -)	*Salmonella-T2*	Net Log Reduction	-1.99	-3.96	-4.81	-6.02
			Net % Reduction	98.9669%	99.9890%	99.9985%	99.9999%
Bacteria	*S. enterica* (gram -)	*Salmonella-T3*	Net Log Reduction	-2.02	-3.99	-5.24	-6.45
			Net % Reduction	99.0345%	99.9898%	99.9994%	100.0000%
All Trial Averages ± St. Dev.			Net Log Reduction	***-1.91 ± 0.16***	***-3.93 ± 0.08***	***-5.07 ± 0.23***	***-6.25 ± 0.22***
			Net % Reduction	*98.716% ± 0.494%*	*99.988% ± 0.002%*	*99.9991% ± 0.00052%*	*99.999939% ± 0.000032%*
Bacteria	*L. innocua* (gram +)	*Listeria-T1*	Net Log Reduction	-1.51	-3.32	-5.29	-6.62
			Net % Reduction	96.9078%	99.9525%	99.9995%	100.0000%
Bacteria	*L. innocua* (gram +)	*Listeria-T2*	Net Log Reduction	-1.87	-3.07	-5.16	-6.74
			Net % Reduction	98.6433%	99.9155%	99.9993%	100.0000%
Bacteria	*L. innocua* (gram +)	*Listeria-T3*	Net Log Reduction	-1.78	-3.03	-5.19	-7.07
			Net % Reduction	98.3561%	99.9074%	99.9993%	100.0000%
All Trial Averages ± St. Dev.			Net Log Reduction	***-1.72 ± 0.19***	***-3.14 ± 0.16***	***-5.21 ± 0.07***	***-6.81 ± 0.23***
			Net % Reduction	*97.969% ± 0.93%*	*99.925% ± 0.024%*	*99.9994% ± 0.00009%*	*99.999983% ± 0.000008%*

Figure 14: Net Log Reduction and net percent reduction for the bacterial species challenged against the Defend 1050 device.

Overall Study Summary:

In conclusion, the Defend 1050 device, with its high efficiency and CADR, achieved greater than a 4 net log reduction of all three bioaerosols within 20 minutes of operation in the 16m3 environmental chamber. The device proved highly effective in reducing the aerosol bioburden of three distinct microbial species. It is anticipated that such a reduction should reduce the likelihood of individuals contracting airborne infectious diseases in an enclosed environment.

Deviations and Data Analysis:

No deviations from the protocol were noted throughout the trials. All results were ≤0.30 standard deviations from the mean. Following ARE Lab's standard practice and in compliance with GLPs, all data were verified for accuracy. All raw data and supplemental graphs can be found in the appendices following the report.

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Analytical Testing Facility

Aerosol Research and Engineering Labs, Inc.
12880 Metcalf Avenue
Overland Park, KS 66213

Project #

11007.10

Study Director

Richard Ludwick
Aerosol Research and Engineering Laboratories

GLP Statement

We, the undersigned, certify that Aerosol Research and Engineering Laboratories conducted the work described herein in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

Conflict of Interest Statement

Aerosol Research and Engineering Laboratories, Inc. has no affiliations with, or involvement in any capacity, with Protect ED & Novaerus' financial interests, such as membership, employment, stock ownership, or other equity interests.

Study Director:

Richard Ludwick
Study Director
ARE Labs, Inc.

7/11/2024
Date

Principal Investigator:

Sean McLeod
Principal Investigator
ARE Labs, Inc.

7/11/2024
Date

Appendix A - Additional Figures:
Reduction Graphs by Organism

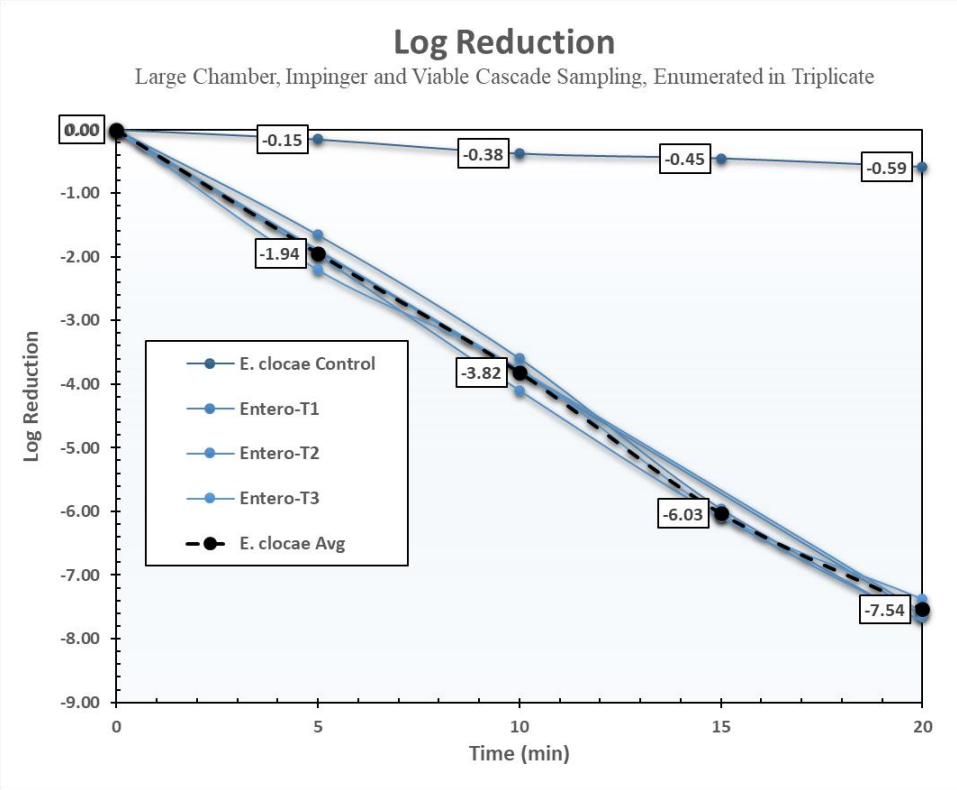


Figure 1A: *Enterobacter cloacae* Defend 1050 device Log Reduction

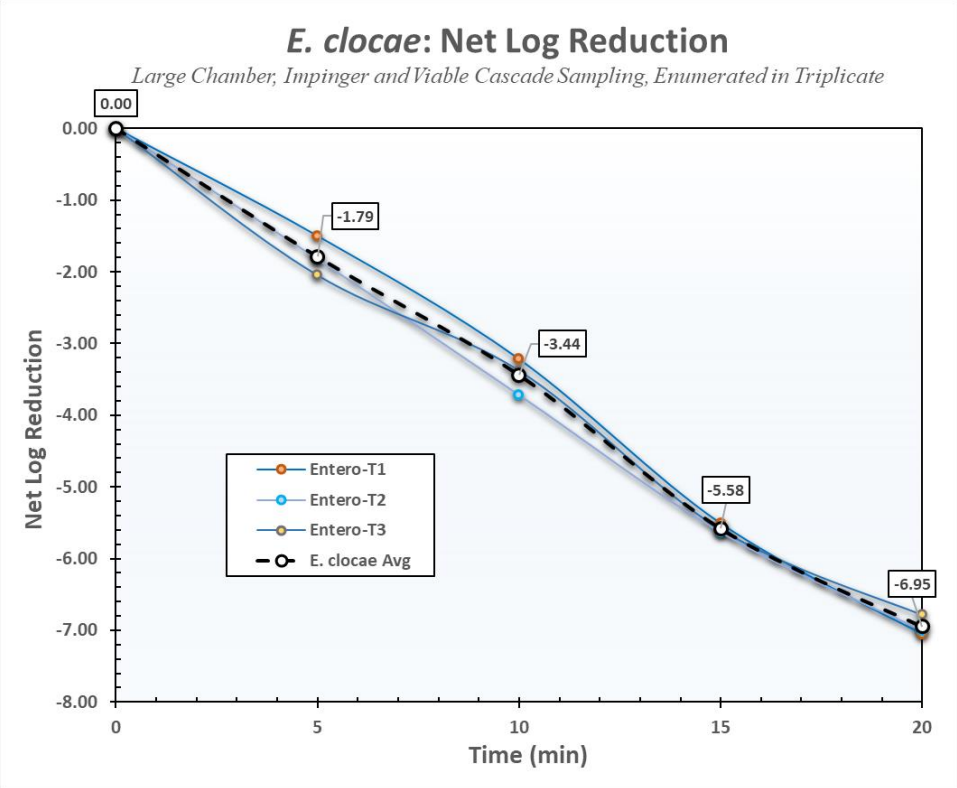


Figure 2A: *Enterobacter cloacae* Defend 1050 device Net Log Reduction

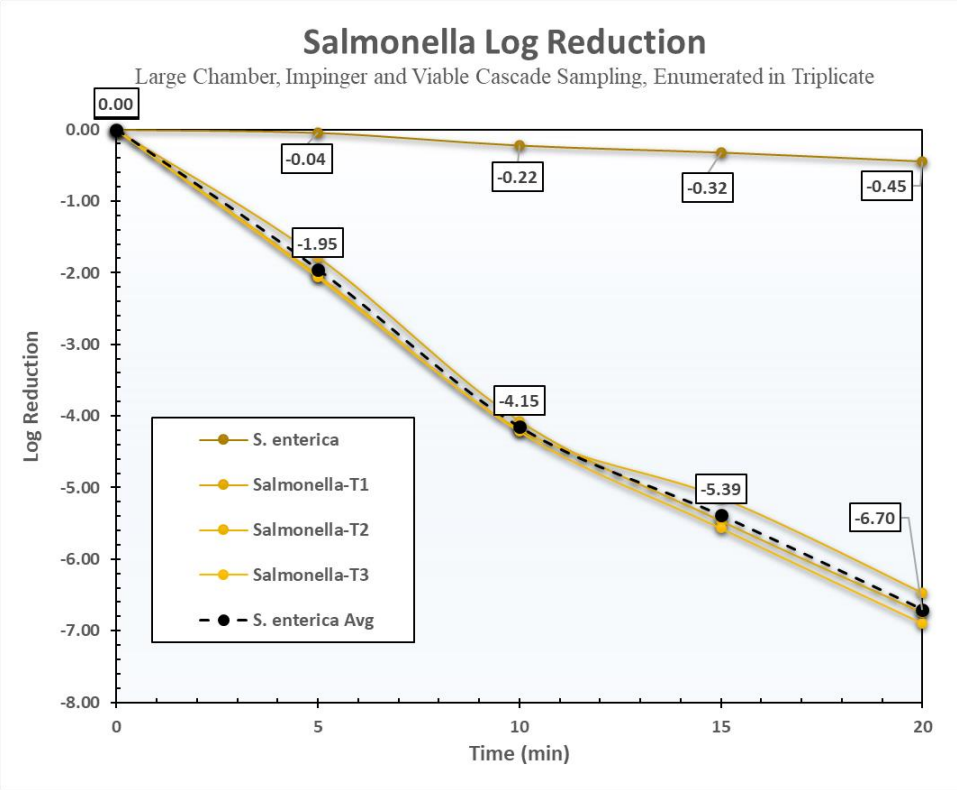


Figure 3A: *Salmonella enterica* Defend 1050 device Log Reduction

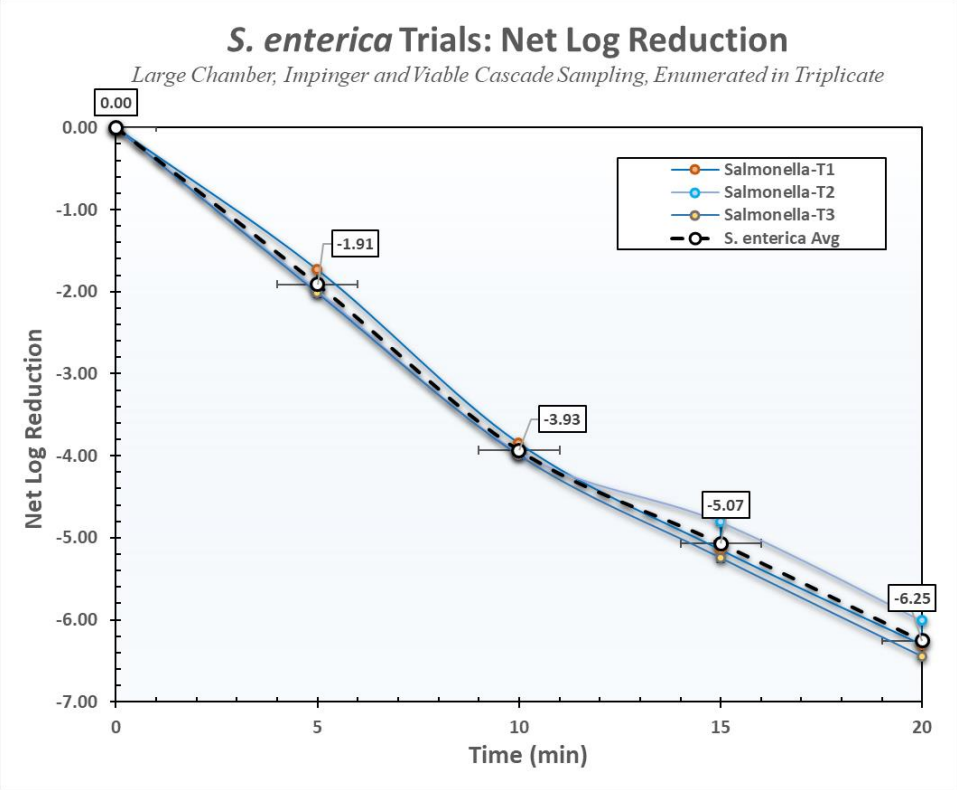


Figure 4A: *Salmonella enterica* Defend 1050 device Net Log Reduction

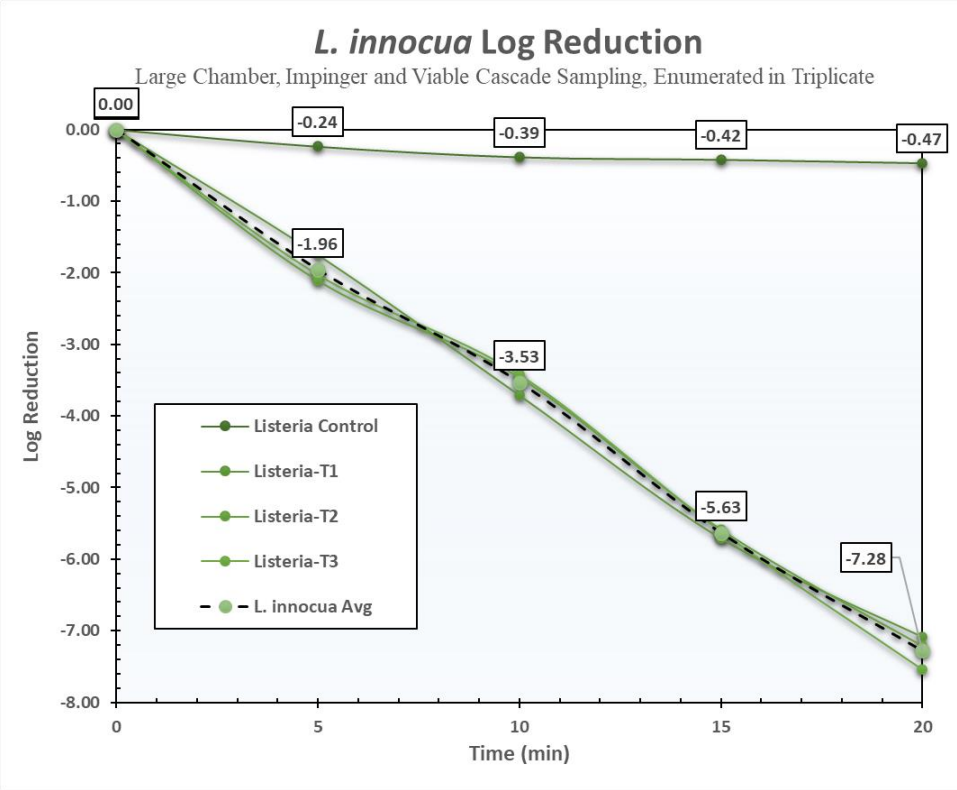


Figure 5A: *Listeria innocua* Defend 1050 device Log Reduction

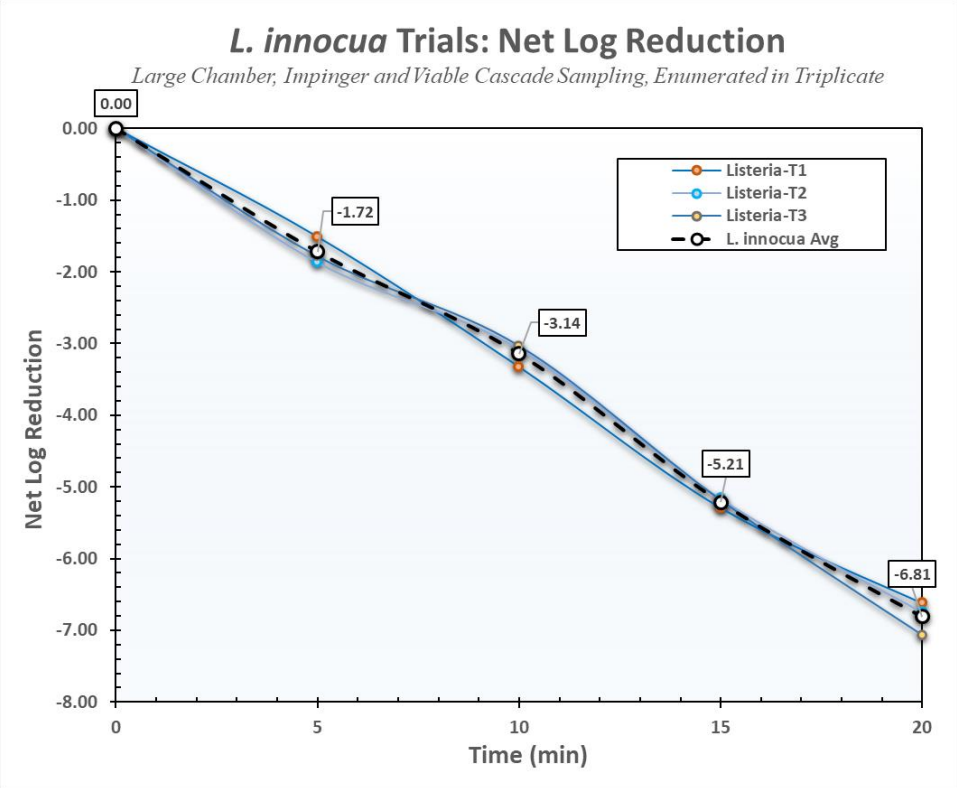


Figure 6A: *Listeria innocua* Defend 1050 device Net Log Reduction

Appendix B: Raw Data

Trial Information

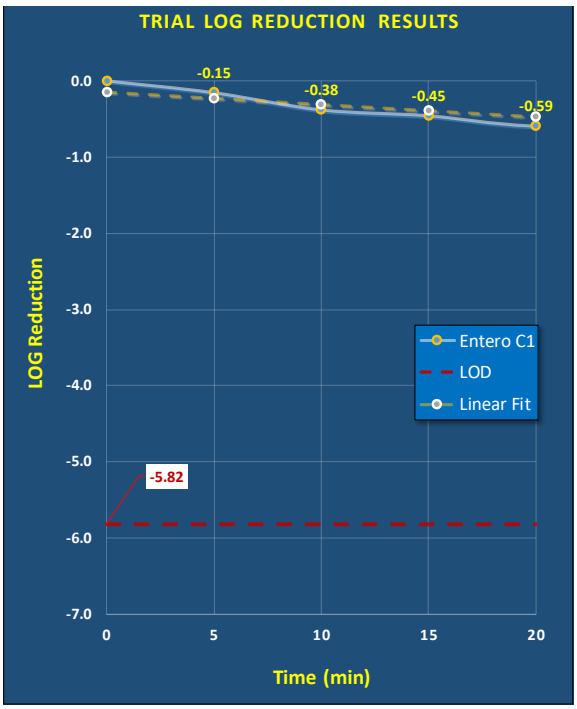
TEST DATE:	Tuesday, July 2, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	C1
TEST ORGANISM:	E. clocae
TRIAL NAME ID (GRAPHS/TABLES):	Entero C1

Device Information

MANUFACTURER:	NA
UNIT MODEL:	NA
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	9.422E+04	6.613E+04	3.947E+04	3.307E+04	2.416E+04
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	23.33%	45.00%	31.82%	45.00%	38.21%
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**9.42E+04**	**66133.33**	**39466.67**	**33066.67**	**2.416E+04**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**70.1887%**	**41.8868%**	**35.0943%**	**25.6415%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**29.8113%**	**58.1132%**	**64.9057%**	**74.3585%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.15**	**-0.38**	**-0.45**	**-0.59**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	15	20
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-4**	**-4**	**-4**	**-3**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	1 2 3	4 3 1	1 1 1	2 1 1	2 11 15
	PLATE AVERAGE COUNT (# / drop)	2.00	2.67	1.00	1.33	9.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	200,000	266,667	100,000	133,333	93,333
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.07E+05	8.53E+04	3.20E+04	4.27E+04	2.99E+04
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-3**	**-3**	**-3**	**-2**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	12 18 16	14 19 11	14 16 14	6 11 5	51 60 62
	PLATE AVERAGE COUNT (# / drop)	15.33	14.67	14.67	7.33	57.67
	IMPINGER CONCENTRATION (cfu or pfu/ml)	153,333	146,667	146,667	73,333	57,667
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	8.18E+04	4.69E+04	4.69E+04	2.35E+04	1.85E+04

Figure 1B: *Enterobacter cloacae* Control

Trial Information

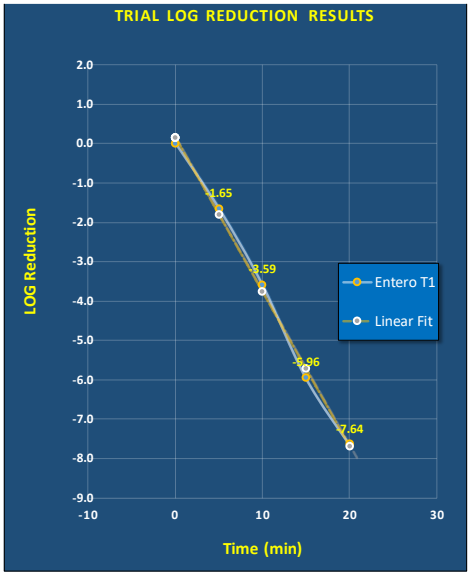
TEST DATE:	Tuesday, July 2, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T1
TEST ORGANISM:	E. clocae
TRIAL NAME ID (GRAPHS/TABLES):	Entero T1

Device Information

MANUFACTURER:	NV
UNIT MODEL:	Defend 1050
FAN SPEED (CFM):	533
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	n
VIABLE CASCADE USED (y / n)	n	n	n	n	y
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	2.924E+05	6.507E+03	7.467E+01	3.200E-01	
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					0.007
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	26.84%	25.71%	60.00%		
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**2.92E+05**	**6506.67**	**74.67**	**0.32**	**6.667E-03**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**2.2249%**	**0.0255%**	**0.0001%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**97.7751%**	**99.9745%**	**99.9999%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.65**	**-3.59**	**-5.96**	**-7.64**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	15	20
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-3**	**-2**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**500**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	5 8 6	1 4 2	1 0 0	1 0	
	PLATE AVERAGE COUNT (# / drop)	6.33	2.33	0.33	0.50	
	IMPINGER CONCENTRATION (cfu or pfu/ml)	633,333	23,333	333	1	
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	3.38E+05	7.47E+03	1.07E+02	3.20E-01	
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-2**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**500**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	46 38 55	15 20 17	1 1 2		
	PLATE AVERAGE COUNT (# / drop)	46.33	17.33	1.33		
	IMPINGER CONCENTRATION (cfu or pfu/ml)	463,333	17,333	133		
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	2.47E+05	5.55E+03	4.27E+01		

Viable Cascade Sampling Conditions ****Statistical Correction Applied Automatically for counts>60**

	SAMPLE TIME (min)	0	5	10	15	20
Viable Cascade #1	**VIABLE CASCADE SAMPLING TIME (min)**	**0.5**	**0.5**	**1.0**	**2.0**	**5.0**
	VIABLE CASCADE FLOW RATE (lpm)	**30**	**30**	**30**	**30**	**30**
	ENUMERATED PLATE COUNTS (# / plate)					1
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)					1
	PLATE AVERAGE COUNT (# / plate)					1.00
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)					0.007

Figure 2B: *Enterobacter cloacae* Trial 1

Trial Information

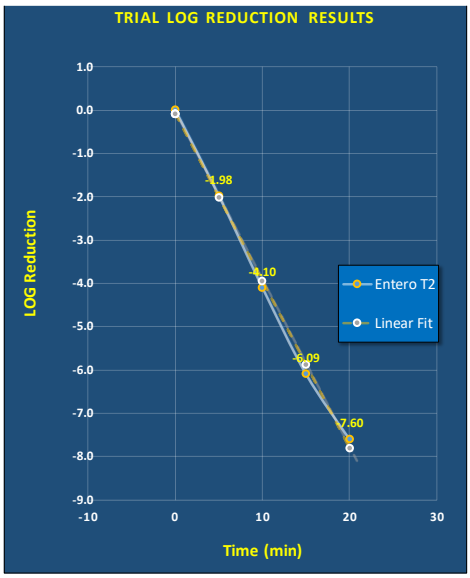
TEST DATE:	Wednesday, July 3, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T2
TEST ORGANISM:	E. clocae
TRIAL NAME ID (GRAPHS/TABLES):	Entero T2

Device Information

MANUFACTURER:	NV
UNIT MODEL:	Defend 1050
FAN SPEED (CFM):	533
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	n
VIABLE CASCADE USED (y / n)	n	n	n	n	y
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	2.631E+05	2.773E+03	2.101E+01	2.133E-01	
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					0.007
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	25.88%	7.41%	38.52%		
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**263111.11**	**2773.33**	**21.01**	**0.21**	**6.667E-03**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**1.0541%**	**0.0080%**	**0.0001%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**98.9459%**	**99.9920%**	**99.9999%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.98**	**-4.10**	**-6.09**	**-7.60**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	5	10	15	20
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5

Dilution Range #1						
	DILUTION RATIO (10^x)	**-4**	**-2**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**750**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	4 7 6	7 9 11	1 0	1 0	
	PLATE AVERAGE COUNT (# / drop)	5.67	9.00	0.50	0.50	
	IMPINGER CONCENTRATION (cfu or pfu/ml)	566,667	9,000	50	1	
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	3.02E+05	2.88E+03	1.60E+01	2.13E-01	

Dilution Range #1						
	DILUTION RATIO (10^x)	**-3**	**-1**	**0**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**750**	**500**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	27 43 56	88 75 87	61		
	PLATE AVERAGE COUNT (# / drop)	42.00	83.33	61.00		
	IMPINGER CONCENTRATION (cfu or pfu/ml)	420,000	8,333	81		
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	2.24E+05	2.67E+03	2.60E+01		

Viable Cascade Sampling Conditions ****Statistical Correction Applied Automatically for counts>60**

Viable Cascade #1						
	SAMPLE TIME (min)	0	5	10	15	20
	VIABLE CASCADE SAMPLING TIME (min)	**0.5**	**0.5**	**1.0**	**2.0**	**5.0**
	VIABLE CASCADE FLOW RATE (lpm)	**30**	**30**	**30**	**30**	**30**
	ENUMERATED PLATE COUNTS (# / plate)					1
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)					1
	PLATE AVERAGE COUNT (# / plate)					1.00
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)					0.007

Figure 3B: *Enterobacter cloacae* Trial 2

Trial Information

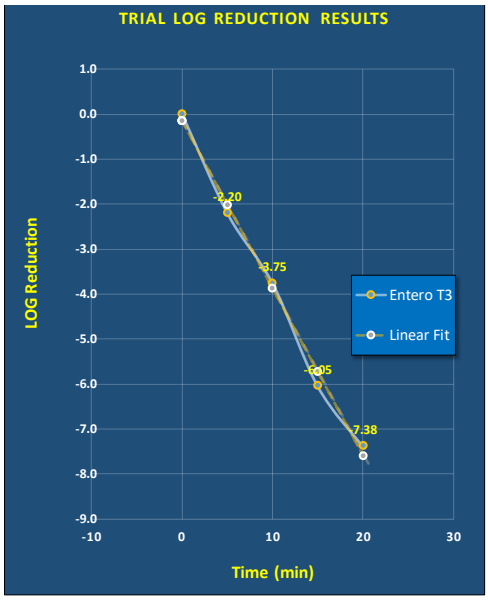
TEST DATE:	Wednesday, July 3, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T3
TEST ORGANISM:	E. clocae
TRIAL NAME ID (GRAPHS/TABLES):	Entero T3

Device Information

MANUFACTURER:	NV
UNIT MODEL:	Defend 1050
FAN SPEED (CFM):	533
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	n
VIABLE CASCADE USED (y / n)	n	n	n	n	y
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.582E+05	9.973E+02	2.784E+01	1.422E-01	
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					0.007
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	18.37%	30.00%	26.00%		
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	158222.22	997.33	27.84	0.14	6.667E-03
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	0.6303%	0.0176%	0.0001%	0.0000%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	99.3697%	99.9824%	99.9999%	100.0000%
LOG REDUCTION FROM T=0 (log_{10})	0.00	-2.20	-3.75	-6.05	-7.38

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	15	20
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	-4	-2	-1	0	0
	DROPLET SIZE (µl)	100	100	100	750	500
	ENUMERATED PLATE COUNTS (# / drop)	1 3 4	2 5 4	1 1 1	1 0 0	
	PLATE AVERAGE COUNT (# / drop)	2.67	3.67	1.00	0.33	
	IMPINGER CONCENTRATION (cfu or pfu/ml)	266,667	3,667	100	0	
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.42E+05	1.17E+03	3.20E+01	1.42E-01	

Dilution Range #1	**DILUTION RATIO (10^x)**	-3	-1	0	0	0
	DROPLET SIZE (µl)	100	100	500	500	500
	ENUMERATED PLATE COUNTS (# / drop)	26 36 36	24 32 21	37		
	PLATE AVERAGE COUNT (# / drop)	32.67	25.67	37.00		
	IMPINGER CONCENTRATION (cfu or pfu/ml)	326,667	2,567	74		
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.74E+05	8.21E+02	2.37E+01		

Viable Cascade Sampling Conditions ****Statistical Correction Applied Automatically for counts>60**

	SAMPLE TIME (min)	0	5	10	15	20
Viable Cascade #1	**VIABLE CASCADE SAMPLING TIME (min)**	0.5	0.5	1.0	2.0	5.0
	VIABLE CASCADE FLOW RATE (lpm)	30	30	30	30	30
	ENUMERATED PLATE COUNTS (# / plate)					1
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)					1
	PLATE AVERAGE COUNT (# / plate)					1.00
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)					0.007

Figure 4B: *Enterobacter cloacae* Trial 3

Trial Information

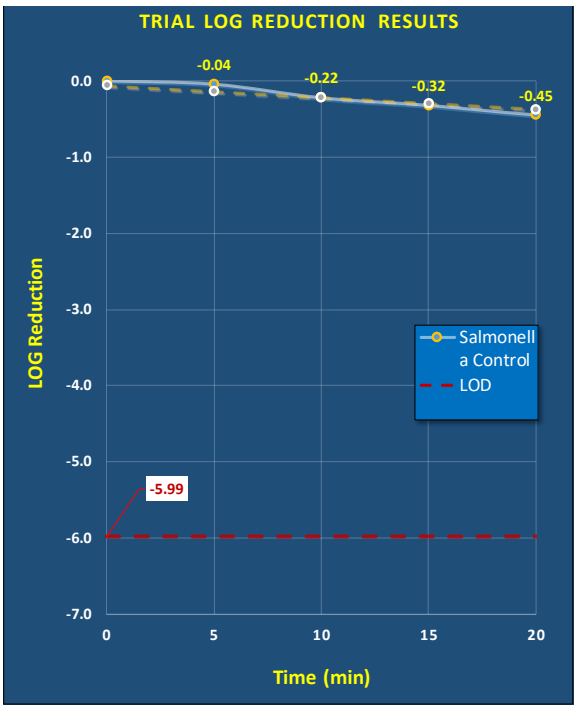
TEST DATE:	Monday, July 8, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	C1
TEST ORGANISM:	S. enterica
TRIAL NAME ID (GRAPHS/TABLES):	Salmonella Control

Device Information

MANUFACTURER:	NA
UNIT MODEL:	NA
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.378E+05	1.248E+05	8.267E+04	6.560E+04	4.907E+04
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	17.65%		6.25%	4.76%	23.08%
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**137777.78**	**124800.00**	**82666.67**	**65600.00**	**4.907E+04**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**90.5806%**	**60.0000%**	**47.6129%**	**35.6129%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**9.4194%**	**40.0000%**	**52.3871%**	**64.3871%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.04**	**-0.22**	**-0.32**	**-0.45**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	15	20
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-4**	**-4**	**-4**	**-4**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	3		3	2	1
		2		4	1	2
		2		1	3	1
	PLATE AVERAGE COUNT (# / drop)	2.33		2.67	2.00	1.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	233,333		266,667	200,000	133,333
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.24E+05		8.53E+04	6.40E+04	4.27E+04
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-3**	**-3**	**-3**	**-3**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	30	44	26	24	16
		26	43	25	19	15
		29	30	24	20	21
	PLATE AVERAGE COUNT (# / drop)	28.33	39.00	25.00	21.00	17.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	283,333	390,000	250,000	210,000	173,333
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.51E+05	1.25E+05	8.00E+04	6.72E+04	5.55E+04

Figure 5B: *Salmonella enterica* Control

Trial Information

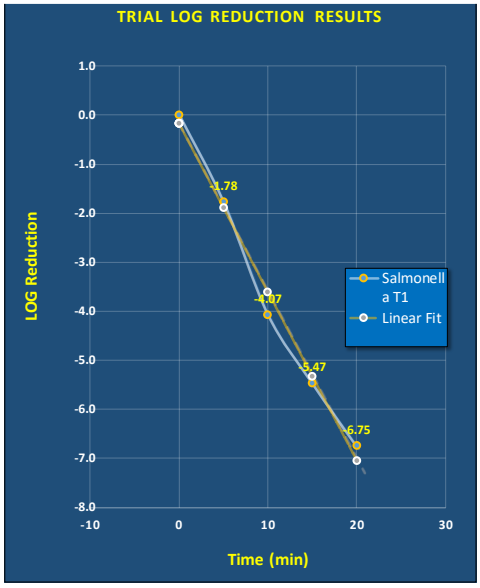
TEST DATE:	Tuesday, July 2, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T1
TEST ORGANISM:	S. enterica
TRIAL NAME ID (GRAPHS/TABLES):	Salmonella T1

Device Information

MANUFACTURER:	NV
UNIT MODEL:	Defend 1050
FAN SPEED (CFM):	533
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	n
VIABLE CASCADE USED (y / n)	n	n	n	n	y
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.876E+05	3.147E+03	1.600E+01	6.400E-01	
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					0.033
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	24.17%	52.50%			
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**187555.56**	**3146.67**	**16.00**	**0.64**	**3.333E-02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**1.6777%**	**0.0085%**	**0.0003%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**98.3223%**	**99.9915%**	**99.9997%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.78**	**-4.07**	**-5.47**	**-6.75**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	5	10	15	20
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1					
DILUTION RATIO (10^x)	**-4**	**-3**	**-1**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**500**	**500**
ENUMERATED PLATE COUNTS (# / drop)	5 4 3	0 1 3	1 0	1	
PLATE AVERAGE COUNT (# / drop)	4.00	1.33	0.50	1.00	
IMPINGER CONCENTRATION (cfu or pfu/ml)	400,000	13,333	50	2	
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	2.13E+05	4.27E+03	1.60E+01	6.40E-01	
Dilution Range #1					
DILUTION RATIO (10^x)	**-3**	**-2**	**-1**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**500**	**500**
ENUMERATED PLATE COUNTS (# / drop)	32 27 32	6 7 6			
PLATE AVERAGE COUNT (# / drop)	30.33	6.33			
IMPINGER CONCENTRATION (cfu or pfu/ml)	303,333	6,333			
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.62E+05	2.03E+03			

Viable Cascade Sampling Conditions ****Statistical Correction Applied Automatically for counts>60**

SAMPLE TIME (min)	0	5	10	15	20
Viable Cascade #1					
VIABLE CASCADE SAMPLING TIME (min)	**0.5**	**0.5**	**1.0**	**2.0**	**5.0**
VIABLE CASCADE FLOW RATE (lpm)	**30**	**30**	**30**	**30**	**30**
ENUMERATED PLATE COUNTS (# / plate)					5
STATISTICALLY CORRECTED PLATE COUNTS (# / plate)					5
PLATE AVERAGE COUNT (# / plate)					5.00
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)					0.033

Figure 6B: *Salmonella enterica* Trial 1

Trial Information

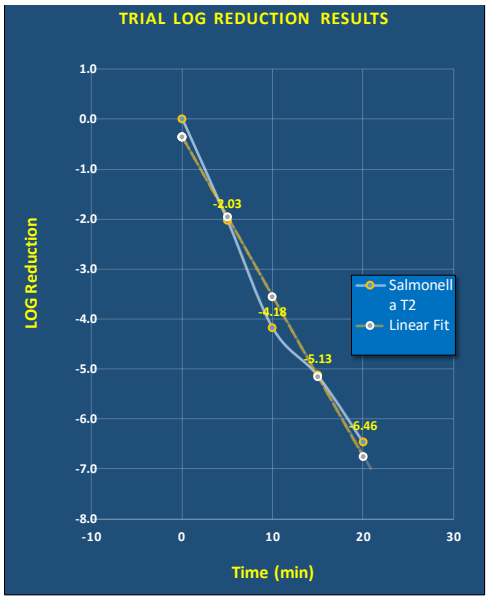
TEST DATE:	Wednesday, July 3, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T2
TEST ORGANISM:	S. enterica
TRIAL NAME ID (GRAPHS/TABLES):	Salmonella T2

Device Information

MANUFACTURER:	NV
UNIT MODEL:	Defend 1050
FAN SPEED (CFM):	533
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	n
VIABLE CASCADE USED (y / n)	n	n	n	n	y
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.938E+04	1.813E+02	1.280E+00	1.422E-01	
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					0.007
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	42.03%	30.00%			
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**19377.78**	**181.33**	**1.28**	**0.14**	**6.667E-03**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**0.9358%**	**0.0066%**	**0.0007%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**99.0642%**	**99.9934%**	**99.9993%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-2.03**	**-4.18**	**-5.13**	**-6.46**

Impinger Sampling Conditions

	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1 **DILUTION RATIO (10^x)**	**-3**	**-2**	**0**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**500**	**750**	**500**
ENUMERATED PLATE COUNTS (# / drop)	3 3 2	1 1 0	2	1 0 0	
PLATE AVERAGE COUNT (# / drop)	2.67	0.67	2.00	0.33	
IMPINGER CONCENTRATION (cfu or pfu/ml)	26,667	667	4	0	
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.42E+04	2.13E+02	1.28E+00	1.42E-01	
Dilution Range #1 **DILUTION RATIO (10^x)**	**-2**	**-1**	**-1**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**500**	**500**
ENUMERATED PLATE COUNTS (# / drop)	47 38 53	2 6 6			
PLATE AVERAGE COUNT (# / drop)	46.00	4.67			
IMPINGER CONCENTRATION (cfu or pfu/ml)	46,000	467			
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	2.45E+04	1.49E+02			

Viable Cascade Sampling Conditions ****Statistical Correction Applied Automatically for counts>60**

	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
Viable Cascade #1 **VIABLE CASCADE SAMPLING TIME (min)**	**0.5**	**0.5**	**1.0**	**2.0**	**5.0**
VIABLE CASCADE FLOW RATE (lpm)	**30**	**30**	**30**	**30**	**30**
ENUMERATED PLATE COUNTS (# / plate)					1
STATISTICALLY CORRECTED PLATE COUNTS (# / plate)					1
PLATE AVERAGE COUNT (# / plate)					1.00
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)					0.007

Figure 7B: *Salmonella enterica* Trial 2

Trial Information

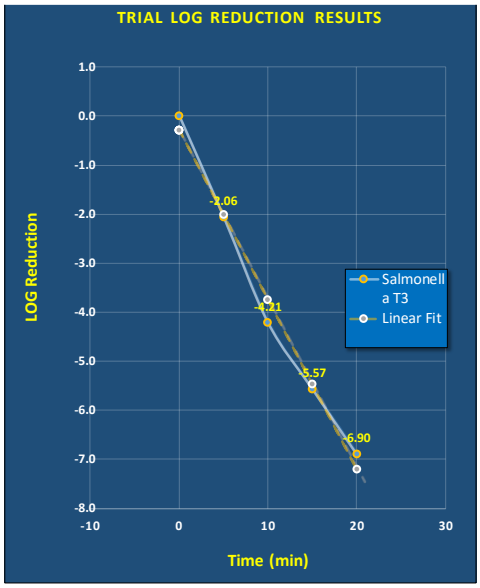
TEST DATE:	Wednesday, July 3, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T3
TEST ORGANISM:	S. enterica
TRIAL NAME ID (GRAPHS/TABLES):	Salmonella T3

Device Information

MANUFACTURER:	NV
UNIT MODEL:	Defend 1050
FAN SPEED (CFM):	533
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	n
VIABLE CASCADE USED (y / n)	n	n	n	n	y
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	5.244E+04	4.587E+02	3.200E+00	1.422E-01	
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					0.007
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	3.33%	28.00%			
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**52444.44**	**458.67**	**3.20**	**0.14**	**6.667E-03**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**0.8746%**	**0.0061%**	**0.0003%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**99.1254%**	**99.9939%**	**99.9997%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-2.06**	**-4.21**	**-5.57**	**-6.90**

Impinger Sampling Conditions

	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1 **DILUTION RATIO (10^x)**	**-4**	**-2**	**0**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**500**	**750**	**500**
ENUMERATED PLATE COUNTS (# / drop)	1	2	5	1	
	1	1		0	
	1	2		0	
PLATE AVERAGE COUNT (# / drop)	1.00	1.67	5.00	0.33	
IMPINGER CONCENTRATION (cfu or pfu/ml)	100,000	1,667	10	0	
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	5.33E+04	5.33E+02	3.20E+00	1.42E-01	
Dilution Range #1 **DILUTION RATIO (10^x)**	**-3**	**-1**	**-1**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**500**	**500**
ENUMERATED PLATE COUNTS (# / drop)	7	12			
	8	12			
	14	12			
PLATE AVERAGE COUNT (# / drop)	9.67	12.00			
IMPINGER CONCENTRATION (cfu or pfu/ml)	96,667	1,200			
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	5.16E+04	3.84E+02			

Viable Cascade Sampling Conditions ****Statistical Correction Applied Automatically for counts>60**

	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
Viable Cascade #1 **VIABLE CASCADE SAMPLING TIME (min)**	**0.5**	**0.5**	**1.0**	**2.0**	**5.0**
VIABLE CASCADE FLOW RATE (lpm)	**30**	**30**	**30**	**30**	**30**
ENUMERATED PLATE COUNTS (# / plate)					1
STATISTICALLY CORRECTED PLATE COUNTS (# / plate)					1
PLATE AVERAGE COUNT (# / plate)					1.00
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)					0.007

Figure 8B: *Salmonella enterica* Trial 3

Trial Information

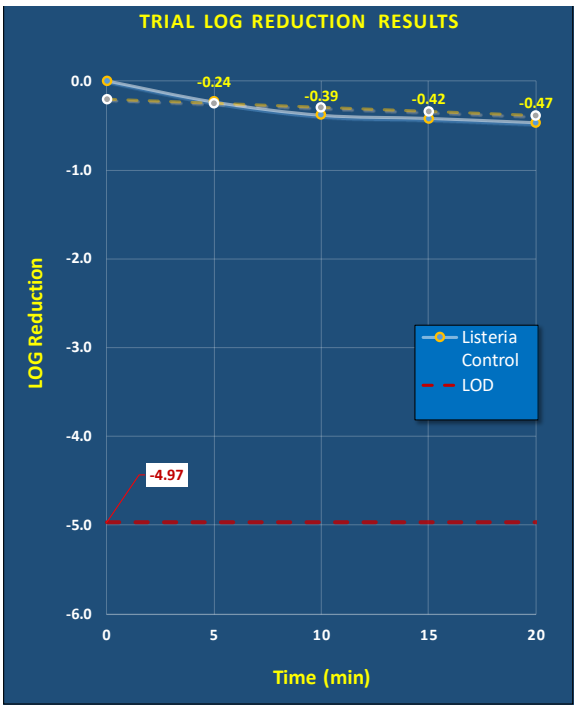
TEST DATE:	Thursday, July 4, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	C1
TEST ORGANISM:	L. innocua
TRIAL NAME ID (GRAPHS/TABLES):	Listeria Control

Device Information

MANUFACTURER:	NA
UNIT MODEL:	NA
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.333E+04	7.733E+03	5.493E+03	5.067E+03	4.533E+03
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	50.00%	18.75%	28.33%	27.27%	45.45%
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**13333.33**	**7733.33**	**5493.33**	**5066.67**	**4.533E+03**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**58.0000%**	**41.2000%**	**38.0000%**	**34.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**42.0000%**	**58.8000%**	**62.0000%**	**66.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.24**	**-0.39**	**-0.42**	**-0.47**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	15	20
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-3**	**-3**	**-3**	**-3**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	5	3	2	1	1
		4	2	2	1	1
		1	3	2	2	1
	PLATE AVERAGE COUNT (# / drop)	3.33	2.67	2.00	1.33	1.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	33,333	26,667	20,000	13,333	10,000
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.78E+04	8.53E+03	6.40E+03	4.27E+03	3.20E+03
Dilution Range #1	**DILUTION RATIO (10^x)**	**-2**	**-2**	**-2**	**-2**	**-2**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	19	28	18	17	20
		15	21	15	22	20
		16	16	10	16	15
	PLATE AVERAGE COUNT (# / drop)	16.67	21.67	14.33	18.33	18.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	16,667	21,667	14,333	18,333	18,333
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	8.89E+03	6.93E+03	4.59E+03	5.87E+03	5.87E+03

Figure 9B: *Listeria innocua* Control

Trial Information

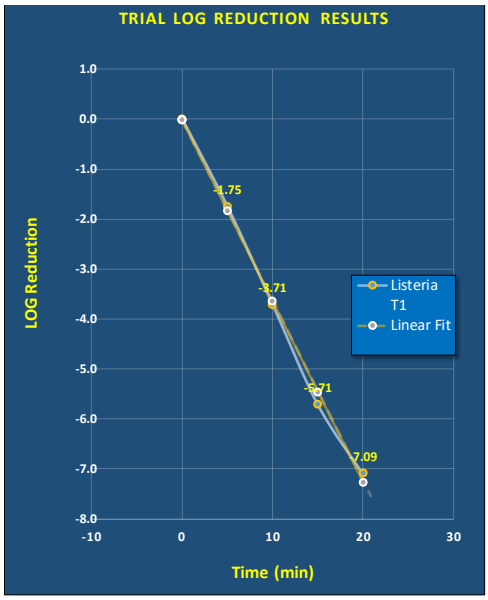
TEST DATE:	Tuesday, July 2, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T1
TEST ORGANISM:	L. innocua
TRIAL NAME ID (GRAPHS/TABLES):	Listeria T1

Device Information

MANUFACTURER:	NV
UNIT MODEL:	Defend 1050
FAN SPEED (CFM):	533
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	n
VIABLE CASCADE USED (y / n)	n	n	n	n	y
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.636E+05	2.933E+03	3.200E+01	3.200E-01	
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					0.013
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	4.26%	16.67%			
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**163555.56**	**2933.33**	**32.00**	**0.32**	**1.333E-02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**1.7935%**	**0.0196%**	**0.0002%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**98.2065%**	**99.9804%**	**99.9998%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.75**	**-3.71**	**-5.71**	**-7.09**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	15	20
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-3**	**-2**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**500**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	2 3 4	1 1 1		1 0	
	PLATE AVERAGE COUNT (# / drop)	3.00	1.00		0.50	
	IMPINGER CONCENTRATION (cfu or pfu/ml)	300,000	10,000		1	
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.60E+05	3.20E+03		3.20E-01	
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-2**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**500**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	35 27 32	6 8 11	1 1 1		
	PLATE AVERAGE COUNT (# / drop)	31.33	8.33	1.00		
	IMPINGER CONCENTRATION (cfu or pfu/ml)	313,333	8,333	100		
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.67E+05	2.67E+03	3.20E+01		

Viable Cascade Sampling Conditions **Statistical Correction Applied Automatically for counts>60

	SAMPLE TIME (min)	0	5	10	15	20
Viable Cascade #1	**VIABLE CASCADE SAMPLING TIME (min)**	**0.5**	**0.5**	**1.0**	**2.0**	**5.0**
	VIABLE CASCADE FLOW RATE (lpm)	**30**	**30**	**30**	**30**	**30**
	ENUMERATED PLATE COUNTS (# / plate)					2
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)					2
	PLATE AVERAGE COUNT (# / plate)					2.00
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)					0.013

Figure 10B: *Listeria innocua* Trial 1

Trial Information

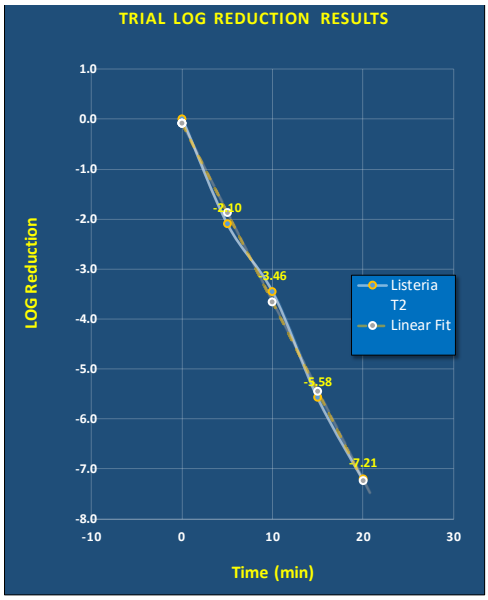
TEST DATE:	Wednesday, July 3, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T2
TEST ORGANISM:	L. innocua
TRIAL NAME ID (GRAPHS/TABLES):	Listeria T2

Device Information

MANUFACTURER:	NV
UNIT MODEL:	Defend 1050
FAN SPEED (CFM):	533
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	n
VIABLE CASCADE USED (y / n)	n	n	n	n	y
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.084E+05	8.533E+02	3.776E+01	2.844E-01	
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					0.007
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	30.56%	22.22%			
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**108444.44**	**853.33**	**37.76**	**0.28**	**6.667E-03**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**0.7869%**	**0.0348%**	**0.0003%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**99.2131%**	**99.9652%**	**99.9997%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-2.10**	**-3.46**	**-5.58**	**-7.21**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	15	20
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-2**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**750**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	2 2 1	2 2 5		1 1 0	
	PLATE AVERAGE COUNT (# / drop)	1.67	3.00		0.67	
	IMPINGER CONCENTRATION (cfu or pfu/ml)	166,667	3,000		1	
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	8.89E+04	9.60E+02		2.84E-01	
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-1**	**0**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**500**	**500**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	20 26 26	28 19 23	59		
	PLATE AVERAGE COUNT (# / drop)	24.00	23.33	59.00		
	IMPINGER CONCENTRATION (cfu or pfu/ml)	240,000	2,333	118		
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.28E+05	7.47E+02	3.78E+01		

Viable Cascade Sampling Conditions ****Statistical Correction Applied Automatically for counts>60**

	SAMPLE TIME (min)	0	5	10	15	20
Viable Cascade #1	**VIABLE CASCADE SAMPLING TIME (min)**	**0.5**	**0.5**	**1.0**	**2.0**	**5.0**
	VIABLE CASCADE FLOW RATE (lpm)	**30**	**30**	**30**	**30**	**30**
	ENUMERATED PLATE COUNTS (# / plate)					1
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)					1
	PLATE AVERAGE COUNT (# / plate)					1.00
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)					0.007

Figure 11B: *Listeria innocua* Trial 2

Trial Information

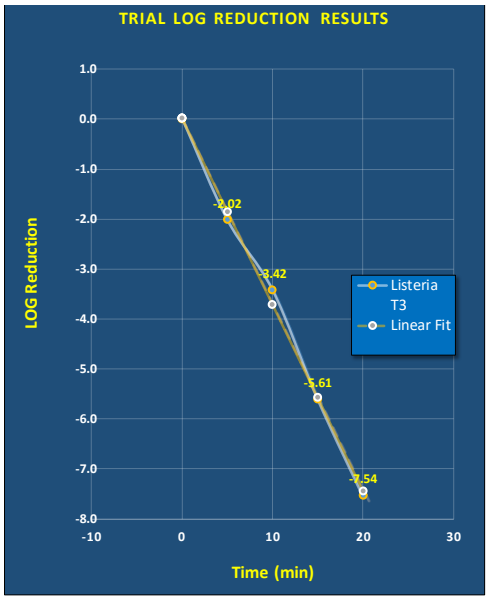
TEST DATE:	Wednesday, July 3, 2024
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T3
TEST ORGANISM:	L. innocua
TRIAL NAME ID (GRAPHS/TABLES):	Listeria T3

Device Information

MANUFACTURER:	NV
UNIT MODEL:	Defend 1050
FAN SPEED (CFM):	533
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	57
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	100 mL overnight stock centrifuged with soil and antifoam



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	5	10	15	20
IMPINGER USED (y / n)	y	y	y	y	n
VIABLE CASCADE USED (y / n)	n	n	n	n	y
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	4.587E+05	4.373E+03	1.749E+02	1.138E+00	
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					0.013
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		42.31%	50.91%		
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**458666.67**	**4373.33**	**174.93**	**1.14**	**1.333E-02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**0.9535%**	**0.0381%**	**0.0002%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**99.0465%**	**99.9619%**	**99.9998%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-2.02**	**-3.42**	**-5.61**	**-7.54**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	15	20
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-3**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**750**	**500**
	ENUMERATED PLATE COUNTS (# / drop)		1	8	3	
			2	8	3	
			0	6	2	
	PLATE AVERAGE COUNT (# / drop)		1.00	7.33	2.67	
	IMPINGER CONCENTRATION (cfu or pfu/ml)		10,000	733	4	
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		3.20E+03	2.35E+02	1.14E+00	
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-2**	**0**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**500**	**500**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	69	20	180		
		80	17			
		109	15			
	PLATE AVERAGE COUNT (# / drop)	86.00	17.33	180.00		
	IMPINGER CONCENTRATION (cfu or pfu/ml)	860,000	17,333	360		
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	4.59E+05	5.55E+03	1.15E+02		

Viable Cascade Sampling Conditions **Statistical Correction Applied Automatically for counts>60

	SAMPLE TIME (min)	0	5	10	15	20
Viable Cascade #1	**VIABLE CASCADE SAMPLING TIME (min)**	**0.5**	**0.5**	**1.0**	**2.0**	**5.0**
	VIABLE CASCADE FLOW RATE (lpm)	**30**	**30**	**30**	**30**	**30**
	ENUMERATED PLATE COUNTS (# / plate)					2
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)					2
	PLATE AVERAGE COUNT (# / plate)					2.00
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)					0.013

Figure 12B: *Listeria innocua* Trial 3

Appendix C: Calculations

To evaluate the viable aerosol delivery efficiency and define the operation parameters of the system, calculations based on (theoretical) 100% efficacy of aerosol dissemination were derived using the following steps:

- Plating and enumerating the biological to derive the stock suspension concentration (Cs) in pfu/mL, 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:

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

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 the impinger or filter sample enumeration.
- Impinger sample collection volume (I_{vol}) = 20 mL collection fluid/impinger or extraction fluid for the filter.
- AGI–30 impingers 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:

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