Efficacy of the CerroZone Mobile Against Aerosolized *Aspergillus brasiliensis* Spores in a Controlled Test Chamber

Sean McLeod [a], Jeffery Trolinger[a], Richard Ludwick [a], Jamie Balarashti [a]

[a] **Aerosol Research and Engineering Laboratories Inc. Overland Park, KS**

www.arelabs.com
(p) 913-850-6630 (f) 913-850-6635

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Sean McLeod [a], Richard Ludwick [a], Jamie Balarashti [a], Jeffery Trolinger [a]
[a] Aerosol Research and Engineering Laboratories Inc. Olathe KS

Report Info

Submitter:

CerroZone LLC
13975 Riverport Place Dr
Suite 102
Maryland Heights, MO 63043

Testing Lab:

Aerosol Research and Engineering Laboratories, Inc.
12880 Metcalf Ave
Overland Park, KS 66213

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

Conflict of Interest:

Aerosol Research and Engineering Laboratories, Inc. have no affiliations with, or involvement in any capacity, with CerroZone's financial interests such as membership, employment, stock ownership, or other equity interest.

ABSTRACT

Purpose:

The purpose of this in-vitro study was to measure the efficacy of the CerroZone Mobile air purification device at reducing aerosolized *Aspergillus brasiliensis* mold spores in a sealed, environmental test chamber.

Background:

The CerroZone Mobile is an air purification system using a proprietary ozone technology. Air is passed through a pre-filter then exposed to a chamber of ozone producing ultraviolet bulbs for inactivation. The air is then blown through a catalyst filter which converts the ozone back to oxygen before exiting the device through a post-filter.

All testing was conducted in a 30m³ bioaerosol test chamber. The species selected for this study was *Aspergillus brasiliensis* (formerly known as *A. niger*), a black mold spore, that is a recognized surrogate for more dangerous mold species. Triplicate bioaerosol test trials were performed at two different fan speeds (Speed 2 and 3) for the CerroZone Mobile device, as well as three control bioaerosol trials.

Methods:

The CerroZone Mobile was sealed in a custom 30m³ environmental test chamber for all testing. *A. brasiliensis* was aerosolized into the sealed test chamber, using a dry powder eductor pressurized with house filtered air. *A. brasiliensis* was the microorganism used for all aerosol trials. Bioaerosol samples were taken, with AGI 30 glass impingers at multiple time points throughout each trial, to quantify the reduction rate capability of the air purification device. The impinger samples were serially diluted, plated, incubated, and enumerated in triplicate to yield the viable bioaerosol concentration of the chamber at each sampling time point. Chamber *A. brasiliensis* control trial data, or the natural decay rate of the species, was subtracted from the device trial data to yield the net log reduction which was attributable to the device for each of the bioaerosol challenges.

Results:

The CerroZone Mobile was able to reduce the *A. brasiliensis* bioaerosol by 4.07 +/- 0.12 on speed 2 and 4.58 +/- 0.18 on speed 3 in an hour of testing in the chamber. The clean air delivery rate was calculated for this unit based off of triplicate single pass trials conducted with the device. It achieved an average CADR of 215.69 +/- 3.68 and 290.60 +/- 6.17 CFM on speeds 2 and 3 respectively.

Conclusion:

The test device was capable of reducing the *Aspergillus brasiliensis* bioaerosol consistently and showed a log-linear reduction trend in the 30m³ test chamber.

Introduction

The purpose of this study was to evaluate and quantify the bioaerosol elimination rate and capability of the CerroZone Mobile air purification device. The CerroZone is a Mobile air purifier designed to reduce the viability of pathogens in medical facilities, classrooms, and other indoor spaces. The device has three speed settings. For this study, the device was tested on speeds two and three.

Testing was conducted in a controlled bioaerosol chamber to simulate an average size room. The effectiveness of the device was measured and then adjusted using control trial data. The control trials were done to determine the natural loss of the biological species over the same time as the test trials. The results of this study may be utilized to support the development of future qualification testing protocols for CerroZone air purification devices.

Overview

Testing, see test matrix **Figure 1**, was conducted in an environmentally controlled bioaerosol test chamber. The chamber is constructed from a nonporous material and is designed to simulate an average room size environment (1060ft³). The testing environment was maintained at a temperature of 25°C ± 3°C with a relative humidity of approximately 60% ± 5%.

Trial	Run	Device	Device Fan Speed (ft^3/min)	Surrogate Species (gram, description)	Pathogenic Species Represented	ATCC Ref #	Chamber Size (m3)	Target Particle Size (µm)	Challenge Conc. (#/L)	Trial Time (min)	Bioaerosol Sampling Time Points (min)	Sampling Devices	Plating and Enumeration
1	Control	N/A	N/A	*Aspergillus brasiliensis* (Mold Spore)	Toxis Black Mold Species	16404	30.0	>3.0um	10^4-10^5	60	0, 5, 10, 20, 30, 45, 60	TSI 3321 APS, AGI-30 Impingers	all samples in triplicate
2	Challenge	CerroZone Mobile	311 +/- 40	*Aspergillus brasiliensis* (Mold Spore)	Toxis Black Mold Species	16404	30.0	>3.0um	10^4-10^5	60	0, 5, 10, 20, 30, 45, 60	TSI 3321 APS, AGI-30 Impingers	all samples in triplicate
3	Challenge												
4	Challenge												
5	Challenge	CerroZone Mobile	226 +/- 40	*Aspergillus brasiliensis* (Mold Spore)	Toxis Black Mold Species	16404	30.0	>3.0um	10^4-10^5	60	0, 5, 10, 20, 30, 45, 60	TSI 3321 APS, AGI-30 Impingers	all samples in triplicate
6	Challenge												
7	Challenge												

Figure 1: Test Matrix for Bioaerosol Testing.

Testing was performed in three separate test trials on both two speeds. Control tests, that measure the natural decay rate of these species in the chamber, were also performed. These control tests are used to calculate the net reduction in bioaerosol attributed to the device. The mold spores were dispersed into the sealed environmental bioaerosol chamber containing the CerroZone Mobile device. The chamber starting concentration was approximately 1 x 10^5 cfu per liter.

AGI impingers were used to capture viable chamber bioaerosols at set sampling times. All impinger samples were serially diluted, plated, and enumerated in triplicate to yield viable bioaerosol concentration at each sampling point. Chamber control log reduction test data was subtracted from the operational device log reduction test data to yield net reduction in the chamber for each species bioaerosol challenge and each sampling time point.

Figure 2: CerroZone Mobile air purifier.

TEST DEVICE DESCRIPTION

The CerroZone Mobile air purifier utilizes proprietary oxidation technology. It consists of ozone generating bulbs that emit light at 254 nm and 185 nm wavelength and a catalyst within the device that converts the ozone fully back into oxygen before the airstream exits the unit. The CerroZone device had a measured air flow rate of 226 +/- 40 CFM on Speed 2 and 311 +/- 40 CFM on Speed 3 and exposes airborne pathogens to Ozone and UVC light within the device. A picture of the Mobile is shown in **Figure 2.**

TESTING EQUIPMENT

Bioaerosol Testing Chamber

The test chamber is the main component in bioaerosol testing used for controlled manipulation and testing of microorganisms. It allows for the introduction, sampling, and secure confinement of microorganisms, thus contributing to the precision and reproducibility of testing outcomes. ARE Lab's 30m^3 test chamber adheres to the stringent guidelines in AHAM AC-5 and aligns with both AHAM and ASHRAE 241 criteria.

Structurally, the chamber has dimensions of 30 $\pm$ 1.5 cubic meters, or approximately 1060 ft^3, with the width deliberately maintained within 85 to 100% of its length. This dimensional consistency ensures a uniform testing space, which allows for reliable experimentation. Constructed from a non-porous material, the chamber's walls exhibit notable qualities. Beyond its physical attributes, this material emits minimal volatile organic compounds (VOCs), is non-reactive, non-reflective, and has a non-ionizing quenching nature. This creates an environment conducive to reliable and repeatable testing conditions.

Airtight integrity is monitored and controlled, within the chamber achieving a controlled air change rate (ACH) below 0.05, as per the benchmark set by ASTME 741. This characteristic provides the operator with the ability to isolate the testing environment, thus enhancing result reliability. The chamber is designed to prevent external microbial contamination while maintaining internal atmospheric conditions. These features include an aseptic maintenance system, HEPA filtration, cross-contamination-free item transfer mechanisms, external power control, real-time observation facilitated by multiple viewing windows, and the capability to produce and evenly disperse aerosolized microbes.

Sampling ports, positioned approximately 48 inches from the floor and 18 inches from the walls, ensure optimal sample collection while maintaining prescribed device separation. The chamber's temperature and humidity are maintained, within ASHRAE 241 limits, with a programmable controller.

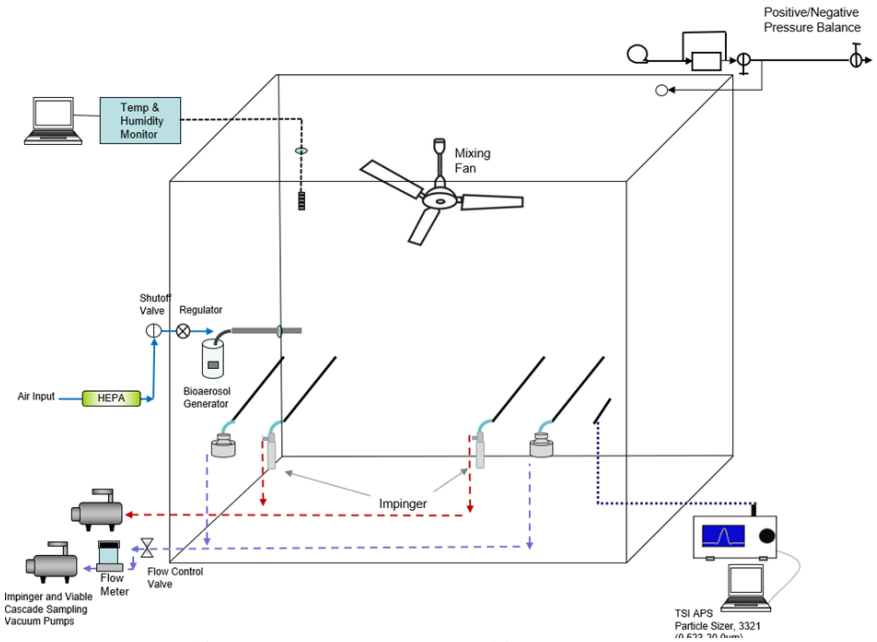


Figure 3: 30m³ Environmental Test Chamber Flow Diagram. The chamber includes bioaerosol induction, multiple bioaerosol sampling ports, particle size monitoring, internal mixing fans, temperature and humidity controls, and a main system HEPA evacuation system (not pictured).

The incorporation of negative pressure airflow allows for controlled purging, and a HEPA filter adds an additional layer of protection, inhibiting potential contamination. The 30m³ testing chamber at ARE Labs fulfills both AHSRAE 241 and AHAM AC-5 requirements.

Figure 4: The CerroZone Mobile device in the 30 m³ bioaerosol testing chamber at ARE Labs adheres to AHAM AC-5 standards and ASHRAE 241 criteria. The chamber is equipped with HEPA filtered air in/out, multiple bio aerosol sampling ports, decontamination, and pressure balance.

A general flow diagram of the aerosol test system is shown in **Figure 4**. A Magnehelic gauge (Dwyer instruments, Michigan City IN), with a range of -0.5 to 0.5 inches of H_2O, is used to monitor and balance the system pressure during aerosol generation, aerosol purge, and testing cycles. **Figure 3** shows the bioaerosol chamber used for all testing in this study.

Bioaerosol Generation System

Test bioaerosols were disseminated using a dry-powder eductor driven by purified filtered house air supply. A pressure regulator allowed for control of disseminated particle size, use rate and sheer force generated within the eductor. The spore powder was weighed prior to dispersal to allow for consistent chamber concentrations at the beginning of each trial. The eductor was adapted to the nebulization port of the chamber for dispersal into the chamber.

Bioaerosol Sampling System

Two AGI-30 impingers (Ace Glass Inc. Vineland NJ) were used for bioaerosol collection to determine chamber concentrations. These two AGI-30 Impingers were placed on opposite sides of the chamber in order to better represent the entire room. A picture of the AGI-30 is shown in **Figure 5** below. The mixing fans inside the chamber worked to ensure a homogenous air mixture inside the chamber. 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.

Figure 5: AGI-30 Impinger, Ace Glass Inc. Vineland NJ.

The AGI-30 impingers sample at a rate of 12.5 LPM impinger flows were characterized using a calibrated TSI model 4040 mass flow meter.

Temperature and Humidity Monitor/Controller

The temperature and humidity within the chamber are monitored and controlled with an AC Infinity Controller 69. This controller allows for real-time monitoring and control of the temperature in the 30m^3 bioaerosol chamber used for testing. Temperature and humidity control is essential for the stability of aerosolized micro-organisms during testing. During testing the range for humidity was kept at 50% $\pm$ 10% while the temperature range was maintained at 73°F + 5° (23°C + 3°C). A picture of the controller is shown in **Figure 6.**

Figure 6: AC Infinity Controller 69 Temperature and Humidity Controller.

TSI Aerodynamic Particle Sizer (APS)

A TSI model 3321 Aerodynamic Particle Sizer (APS) (TSI Inc., Shoreview, MN) was used to measure aerosol concentrations and the particle size distribution within the chamber during the test trials. The APS provided real-time aerodynamic particle characterization with a size range from 0.54-20.0 µm with 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 7.**

Figure 7. TSI Aerodynamic Particle Sizer (APS) model 3321 used to measure total particle concentration and particle size distribution of the challenge bioaerosol. It has a range of 0.54-20.0 µm aerodynamic diameter, with 1 particle/L detection limits.

Species Selection

Due to safety concerns for bioaerosol testing, organism selection was based on Biological Safety Level 1 (BSL1) species which serve as surrogates for more dangerous pathogens. *A. brasiliensis* is a spore-forming mold species that has an average particle size less than 5 µm in diameter. It is commonly used as a surrogate for pathogenic black mold species and other spore-forming fungal species such as *Golovinomyces spadiceu* which is found throughout the country and causes powdery mildew. *A. brasiliensis* spores are much smaller than many mold species making it a worst-case scenario for removal from the air.

Fungal Spore Culture & Preparation

A. brasiliensis fungal spores were obtained in purified bulk powder form at a concentration of 1 x 10^9 cfu/g. To verify the bulk powder spore concentration, an aliquot of weighed dry powder was prepared in suspension in PBS + 0.005% Tween 80 at a mass: volume ratio to obtain a concentration of 1 x 10^9 cfu/ml. This aliquoted spore suspension was plated prior to testing to verify concentration.

Challenge Bioaerosol Aerodynamic Diameter

Bioaerosol particle size distributions were measured with a TSI Aerodynamic Particle Sizer model 3321 (APS) for the challenge species. The particle size distribution was taken shortly after aerosolization 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 an Acer laptop computer, regressed, and plotted. **Figure 8** shows the particle size distribution of *Aspergillus brasiliensis* used during testing. The graph shows that the *A. brasiliensis* aerosol is in a respirable range with a peak concentration occurring at 2.5µm to 4.0µm particle size range.

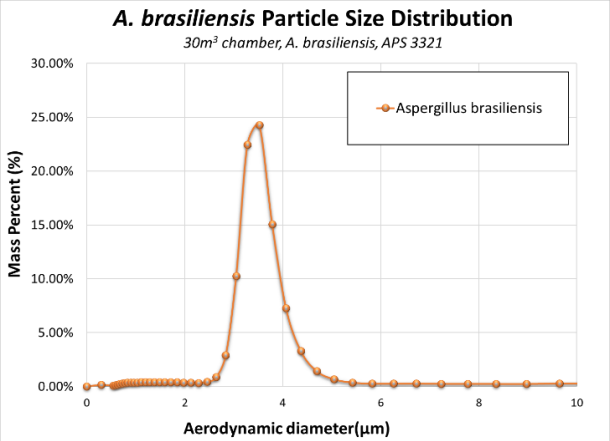


Figure 8: Aerodynamic Particle Size Distribution of the mold spore *Aspergillus brasiliensis* in the test chamber. The MMAD for this viral species averaged approximately 3.0 µm.

BIOAEROSOL TESTING

Chamber Bioaerosol Control Trials

Chamber bioaerosol control trials involved assessing the natural decay rate of the test bioaerosol within the chamber over time without the air purifier in operation. This time aligns with the intended operational testing time of the air purifier, with multiple sampling point intervals to establish a robust natural decay curve. Bioaerosols were collected using a AGI 30 impingers filled with phosphate-buffered saline (PBS) solution with 0.005% of the surfactant Tween 80, ensuring a representative and homogeneous sample. The sampling rate and volume were precisely defined. If necessary, multiple impingers were employed in series to enhance collection efficiency.

The samples collected in the impingers were then carefully processed through serial dilution, plating, and enumeration in triplicate (see plating and enumeration section for more information). This meticulous analysis provided viable bioaerosol concentrations at each sampling point and contributed to accurate data interpretation during analysis. For increased stability of bioaerosols, the relative humidity inside the chamber was kept at 50% +/- 10% 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 73°F +/- 5°F.

Air Purifier Efficacy Evaluation Procedure

The process of evaluating the efficacy of air cleaners in reducing airborne microbial concentrations was run similar to the control test trial, but the test chamber contained the air purifier in operation. The pre-weighed powder of test microbes was disseminated into the chamber air, and an initial measurement of the microbial concentration is taken before activating the air purifier.

Once the baseline was set, the air purifier was activated, with the operation time varying according to the specific characteristics of the unit. See **Figure 9**, at the bottom of the page, for an example sampling timeline. For testing with this air cleaner the trial was set to a total length of 60 minutes with samples occurring at the 0, 5, 10, 20, 30, 45, and 60 minute time points. During the air cleaner's operation, air samples were systematically collected at these time points for lengths of 3, 5, or 10 minutes intervals over the 60-minute duration of the trial. The collected samples were pooled and mixed to provide an encompassing sample of the chamber's concentration at each time point.

Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate. (Multiple drop samples for each dilution) using a standard drop plate technique onto tryptic soy agar plates. The drop plate technique is a widely utilized method in microbiology for determining bacterial or viral concentrations in liquid samples.

In this technique, known volumes of the liquid sample are serially diluted, and each dilution is carefully dispensed onto solid agar plates. These plates provide a nutrient-rich environment that supports bacterial growth. Once the drops are evenly spread across the surface, the plates are incubated for 24-48 hours, depending on the species, then enumerated and recorded. The number of colonies that form on the plates is counted and used to calculate the original bacterial concentration in the liquid sample.

Post-Testing Decontamination and Prep

Following each test, the chamber was air flow evacuated/purged for a minimum of 20 minutes and analyzed with the APS for particle concentration decrease to baseline levels. The chamber was decontaminated with vaporous hydrogen peroxide (35%).

The eductor and impingers were cleaned at the conclusion of each day of testing by soaking them in a 10% bleach bath for 20 minutes. The nebulizer and impingers were then submerged in a DI water bath, spray rinsed 6 times with filtered DI water, and finally air dried.

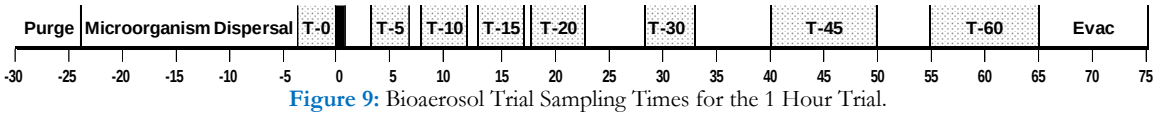


Figure 9: Bioaerosol Trial Sampling Times for the 1 Hour Trial.

DATA ANALYSIS

Results from the control trials were plotted to show natural viability loss over time in the chamber. These control trials served as the basis for determining the reduction of the CerroZone device at two different fan speeds over an hour trial, above the natural losses from the control trials. The control and test trials averages are plotted showing log reduction in viable *A. brasiliensis* bioaerosol. Graphs showing individual trial results can be found in **Appendix A** following the report.

All data was normalized with time zero enumerated concentrations. Subsequent samples were normalized and plotted to show the reduction of viable bioaerosol over time. All raw data was recorded in a dedicated lab notebook, and analysis performed using Microsoft Excel. See **Appendix B** for raw data for the control and challenge tests.

RESULTS

When tested against the mold spore Aspergillus brasiliensis the device yielded a steady reduction on both speeds it was tested. When tested on speed 2 the device yielded a 4.62 log reduction in 60 minutes. When the control was taken into account the net log reduction of the device was calculated at 4.07 net log reduction. The testing on speed 3 yielded a greater reduction in the same amount of time, as expected. At the 60-minute time point the device yielded a net log reduction of 4.58 on speed 3. See **Figures 10, 11, and 12** for a total graphical overview of both log and net log reduction. All trials were performed in the 30m³ chamber under the same conditions per testing standard.

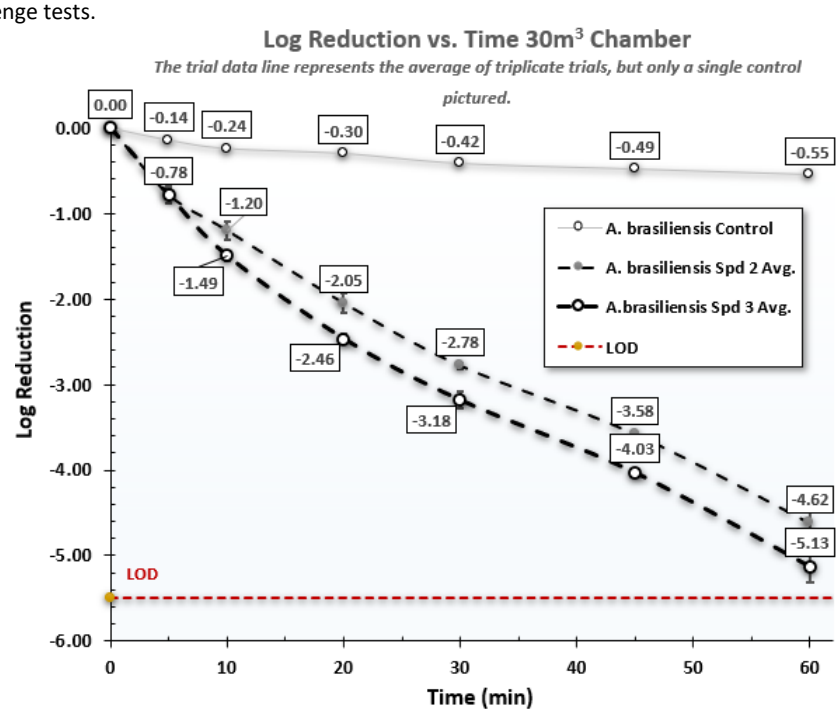


Figure 10: Log Reduction of Aerosolized Mold Spores by the CerroZone Mobile. Each line represents the average of three trials performed under the same conditions in the 30m³ chamber for statistical significance.

CerroZone Mobile A. brasiliensis Trial Summary Data

Trial Name	Species (description)	Reduction Type	Trial Time (minutes) 5	10	20	30	45	60
A.brasiliensis Spd 2 T1	*A. brasiliensis* (Mold Spore)	*Net Log Reduction*	-0.72	-0.84	-1.81	-2.37	-3.08	-4.03
		Net % Reduction	81.0678%	85.6375%	98.4613%	99.5729%	99.9172%	99.9907%
A.brasiliensis Spd 2 T2	*A. brasiliensis* (Mold Spore)	*Net Log Reduction*	-0.66	-1.01	-1.61	-2.33	-3.09	-3.97
		Net % Reduction	78.3017%	90.1931%	97.5500%	99.5286%	99.9190%	99.9894%
A.brasiliensis Spd 2 T3	*A. brasiliensis* (Mold Spore)	*Net Log Reduction*	-0.53	-1.02	-1.82	-2.39	-3.11	-4.20
		Net % Reduction	70.5957%	90.5509%	98.4706%	99.5902%	99.9225%	99.9937%
Speed 2 Trial Averages +/- St. Dev.		***Net Log Reduction***	***-0.64 +/- 0.10***	***-0.96 +/- 0.10***	***-1.75 +/- 0.12***	***-2.36 +/- 0.03***	***-3.09 +/- 0.01***	***-4.07 +/- 0.12***
		Net % Reduction	*76.655% +/- 5.427%*	*88.794% +/- 2.739%*	*98.161% +/- 0.529%*	*99.564% +/- 0.032%*	*99.92% +/- 0.003%*	*99.9913% +/- 0.0022%*
A.brasiliensis Spd 3 T1	*A. brasiliensis* (Mold Spore)	*Net Log Reduction*	-0.74	-1.29	-2.20	-2.76	-3.57	-4.38
		Net % Reduction	81.8531%	94.8735%	99.3705%	99.8274%	99.9730%	99.9958%
A.brasiliensis Spd 3 T2	*A. brasiliensis* (Mold Spore)	*Net Log Reduction*	-0.58	-1.27	-2.10	-2.67	-3.53	-4.66
		Net % Reduction	73.5207%	94.6731%	99.2068%	99.7843%	99.9705%	99.9978%
A.brasiliensis Spd 3 T3	*A. brasiliensis* (Mold Spore)	*Net Log Reduction*	-0.61	-1.19	-2.19	-2.87	-3.55	-4.71
		Net % Reduction	75.5794%	93.5531%	99.3538%	99.8646%	99.9718%	99.9980%
Speed 3 Trial Averages +/- St. Dev.		***Net Log Reduction***	***-0.64 +/- 0.09***	***-1.25 +/- 0.05***	***-2.16 +/- 0.05***	***-2.77 +/- 0.10***	***-3.55 +/- 0.02***	***-4.58 +/- 0.18***
		Net % Reduction	*76.984% +/- 4.34%*	*94.367% +/- 0.712%*	*99.31% +/- 0.09%*	*99.825% +/- 0.04%*	*99.972% +/- 0.001%*	*99.9972% +/- 0.0012%*

Figure 11: Executive Summary. Net log, and associated percentage reduction, values at each timepoint and fan speed.

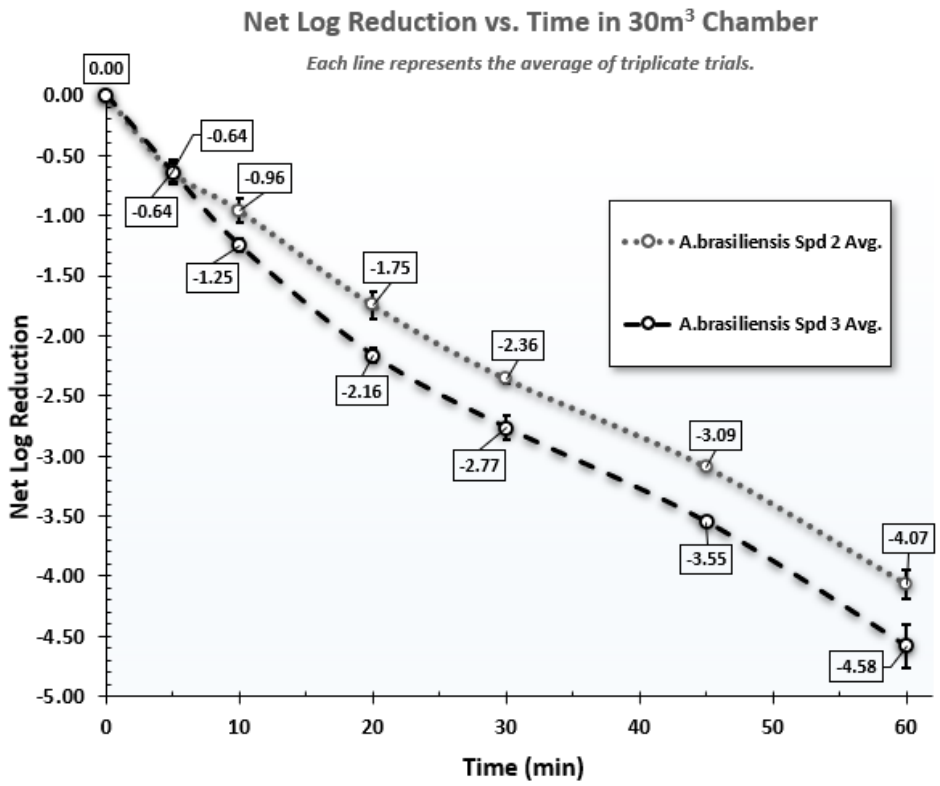


Figure 12: Net Log Reduction of Aerosolized Mold Spores by the CerroZone Mobile. Each line represents the average of three trials performed under the same conditions in the 30m³ chamber for statistical significance.

Single Pass Testing Method

To test the single pass efficiency of the device, one sample probe was installed upstream of the device near the inlet. This probe allowed for characterization of the air going into the device during testing. Another probe was installed in the outlet of the device to allow for the characterization of the air coming out of the device. The probes were connected to the stainless-steel probes of the chamber by 3/8-inch tubing to be sampled from outside of the chamber.

Each test incorporated nebulizing the same *A. brasiliensis* spores, used in the chamber tests, during each test. After a ten-minute wait period to build up bioaerosol concentration in the chamber, the device was turned on and sampling upstream and downstream of the device commenced simultaneously with AGI-30 impingers. The sampling was done for 5 minutes during each trial. The upstream and downstream samples were turned on and off at the same time for each trial. The two samples were then collected in clearly labeled conical tubes to be diluted, plated, and incubated in the same method performed in the chamber bioaerosol testing.

Single Pass Testing Data Analysis

Impinger data was plotted to show the upstream and downstream concentrations from each trial which was used to calculate the log reduction for the CerroZone Mobile device tests and control. A graph depicting the average upstream and downstream concentrations from each speed can be found below in **Figure 13.** The net log reduction attributable to the device was calculated by subtracting the average control reduction from each of the test device trials. The net log reduction for the device was plotted for each trial. All data shows individual and group average +/- standard deviation.

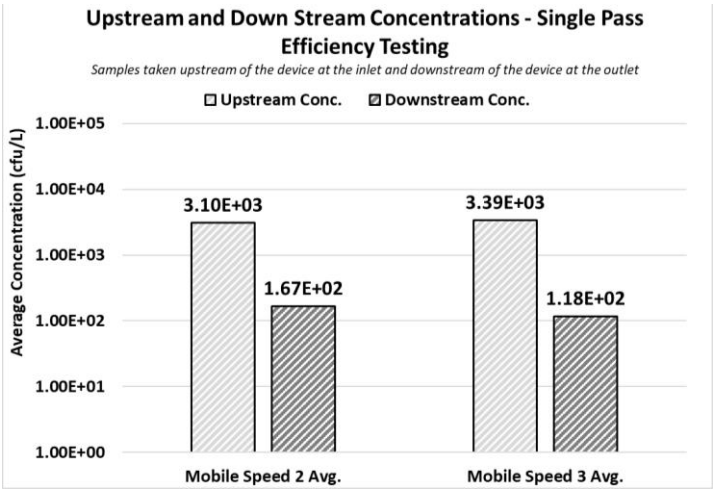


Figure 13: *Upstream vs. downstream average concentrations from the single pass test trials.*

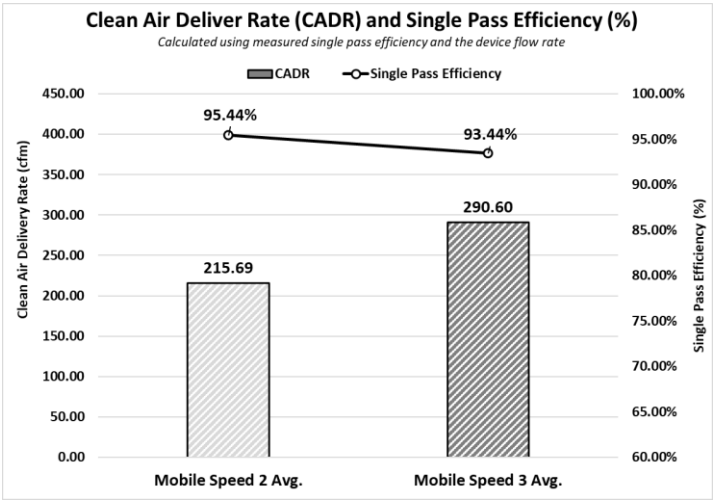


Figure 14: **CADR calculation from Single Pass Efficiency**. The CADR was calculated using the single pass efficiency determined through testing multiplied by the volumetric flow rate of the device.

Single Pass Testing Results

During the single pass testing, the CerroZone Mobile yielded consistent reduction throughout each trial at each speed. On the speed two setting the device ranged from a 93.68% to a 96.90% single pass efficiency with an overall average of 95.44%. When analyzing the speed three setting the device showed a slightly lower single pass efficiency. When tested on speed 3 the single pass efficiency ranged from a low of 91.03% to a high of 95.13%. Overall, the device averaged a single pass efficiency of 93.44% on speed 3. The average single pass efficiency for each group can be found in the graph in **Figure 14** above. Results for each individual trial can be found in **Appendix A** after the report.

Clean Air Delivery Rate

Clean air delivery rate (CADR) is a measure of how much clean air is being delivered by an air purification device as it is in operation. CADR is calculated by considering the flow rate of the air purification device and the single pass efficiency of that device.

To calculate the CADR for each fan speed, the measured flow rate of the device, which was 226 cubic feet per minute (cfm) on speed 2 and 311 cfm on speed 3, was multiplied by the single pass efficiency percentage of each trial. Using this method, the average CADR of the speed 2 trials is 215.69 cfm and the CADR of the speed 3 trials is 290.60 cfm. A graph depicting the average single pass efficiency and CADR of each speed setting can be found above in **Figure 14**.

Deviations and Acceptance Criteria

No deviations from the protocol were noted throughout the test trials. All final endpoints were ≤0.30 standard deviations from the mean. In accordance with ARE Lab's standard practices, and in compliance with GLP, all data was verified for accuracy. Neither ASHRAE 241 nor AHAM AC-5 have specific guidelines regarding standard deviation across triplicate trials.

References

AHAM. (2023). AHAM AC-5: Performance Evaluation of Room Air Cleaners. Association of Home Appliance Manufacturers.

ASHRAE 241. (July 2023). Control of Infection Aerosols. American Society of Heating, Refrigerating, and Air Conditioning Engineers.

Ding and Wing. (2001). Effects of Sampling Time on the Total Recovery Rate of AGI-30 Impingers for E. coli. Aerosol and Air Quality Research, Vol. 1, No. 1, pp. 31-36.

Feller, W. (1950). An Introduction to Probability Theory and Its Applications. Wiley.

T. Reponen, K. Willeke, V. Ulevicius et al. (1997). Techniques of Dispersion of Microorganisms in Air. Aerosol Science and Technology, 27, pp. 405-421.

U.S. Department of Health and Human Services Food and Drug Administration. (March 2009). Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency Guidance for Industry and Food and Drug Administration Staff.

Dietrich, Watts L., et al. (2020). Laboratory Modeling of SARS-CoV-2 Exposure Reduction Through Physically Distanced Seating in Aircraft Cabins Using Bacteriophage Aerosol — November 2020. Morbidity and Mortality Weekly Report, 69(46), pp. 1744-1750.

Analytical Testing Facility

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

Project

10880.160

Study Director

Richard Ludwick
Aerosol Research and Engineering Laboratories

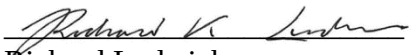
GLP Statement

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

Conflict of Interest Statement

Aerosol Research and Engineering Laboratories, Inc. have no affiliations with, or involvement in any capacity, with CerroZone's financial interests such as membership, employment, stock ownership, or other equity interest.

Study Director:



10/5/2023
Richard Ludwick — Date
Study Director
ARE Labs, Inc.

Principal Investigator:

10/5/2023
Jeffery Trolinger — Date
Staff Research Scientist
ARE Labs, Inc.

APPENDIX A: Supplemental Graphs

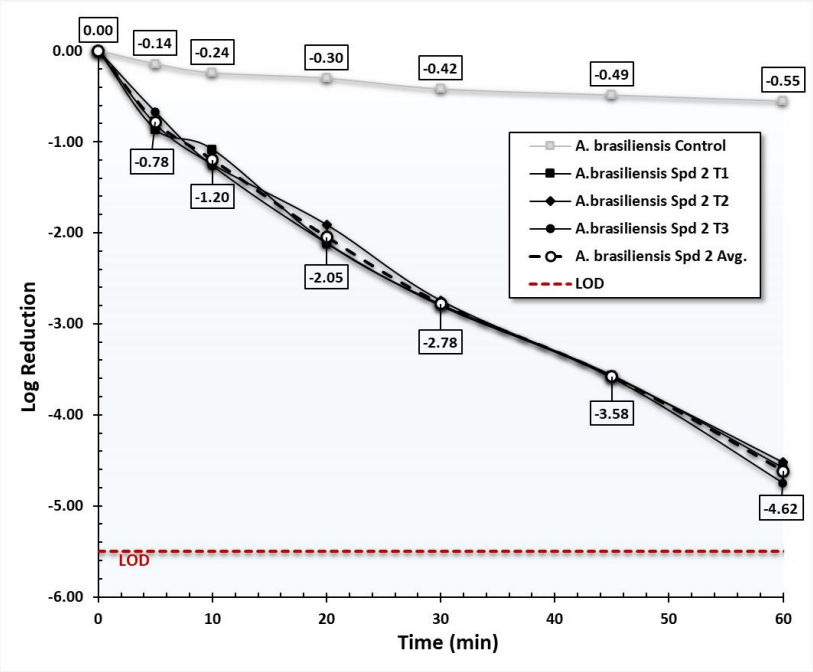


Figure 1A: *Log reduction of the triplicate Mobile speed 2 trials as well as the average of the group and the control*

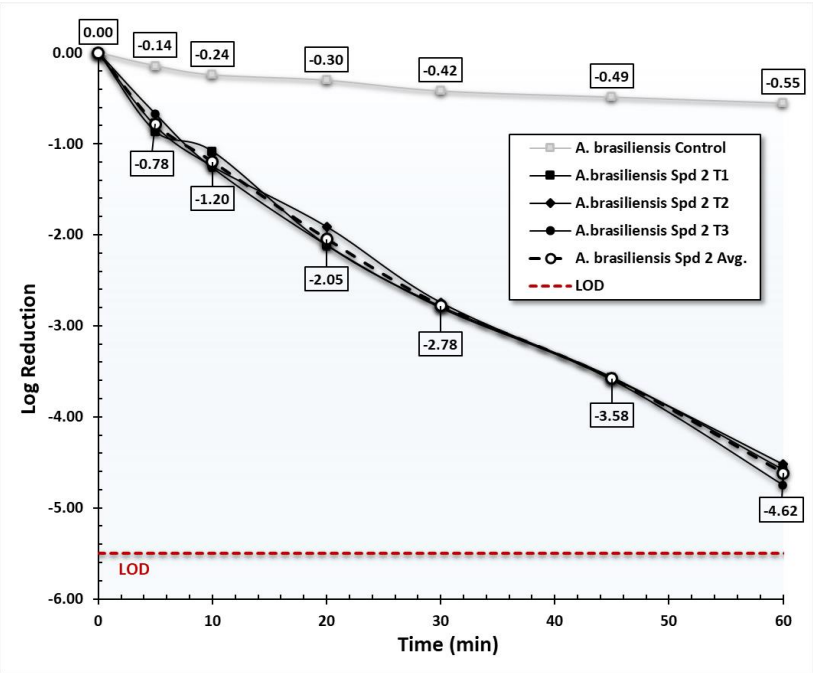


Figure 2A: *Net log reduction of the triplicate Mobile speed 3 trials as well as the average of the group*

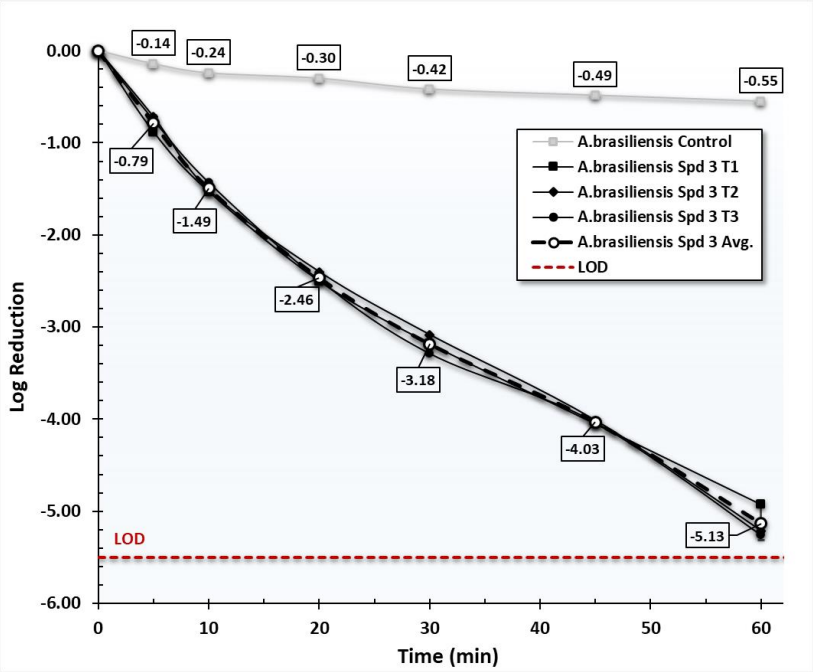


Figure 3A: *Log reduction of the triplicate Mobile speed 3 trials as well as the average of the group and the control*

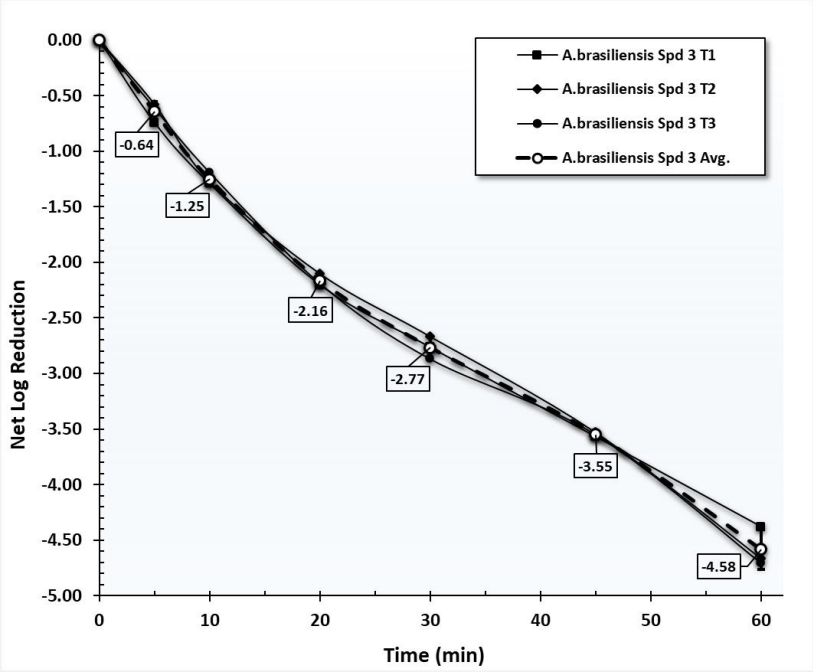


Figure 4A: *Net log reduction of the triplicate Mobile speed 3 trials as well as the average of the group*

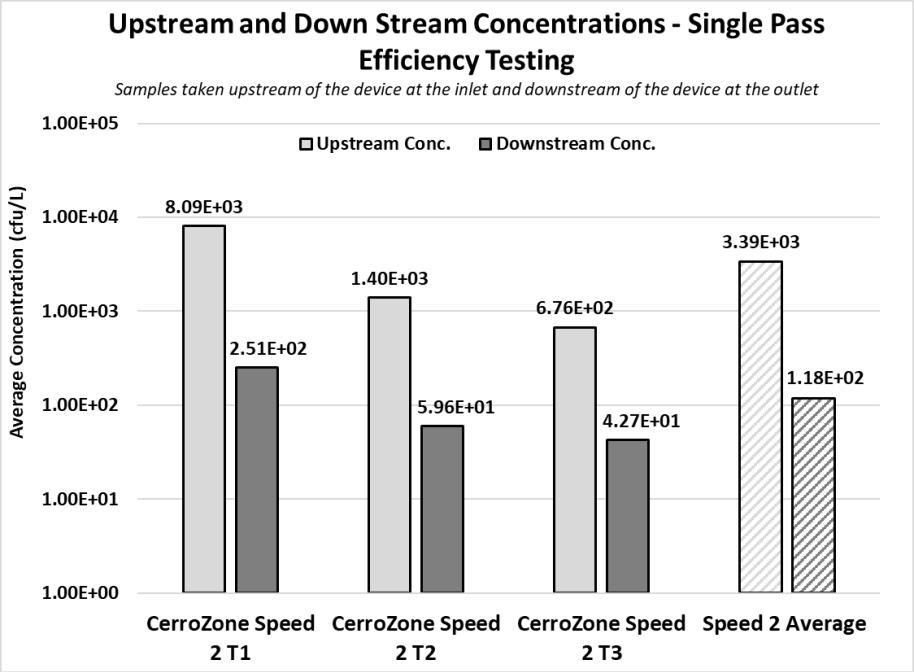


Figure 5A: *Up and downstream concentrations of each trial and the group average for Mobile speed 2 testing.*

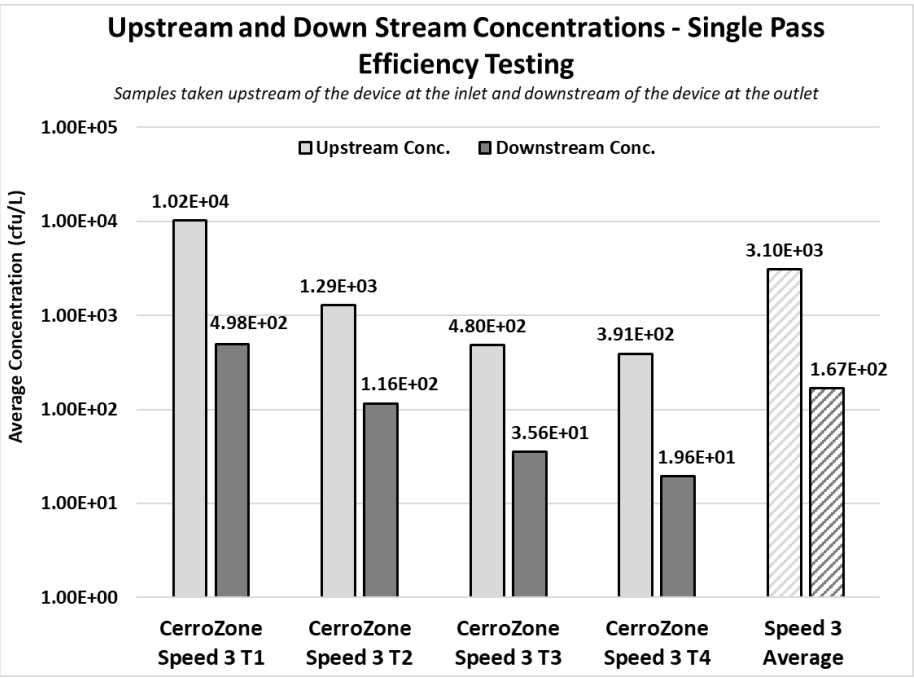


Figure 6A: *Up and downstream concentrations of each trial and the group average for Mobile speed 3 testing.*

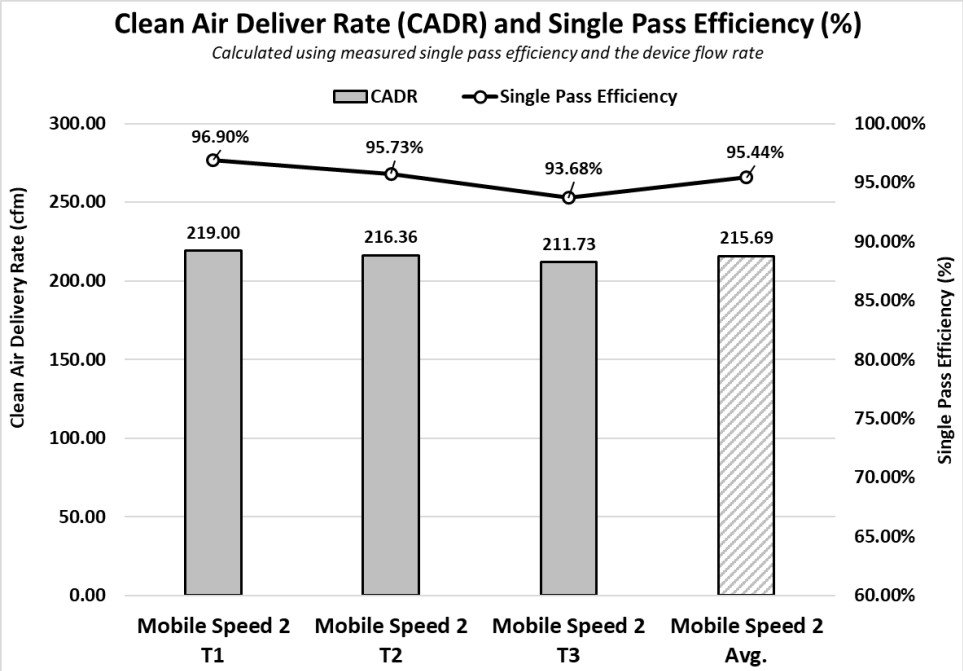


Figure 7A: *Single pass efficiency and clean air delivery rate for each trial as well as the group average of the Mobile speed 2 testing.*

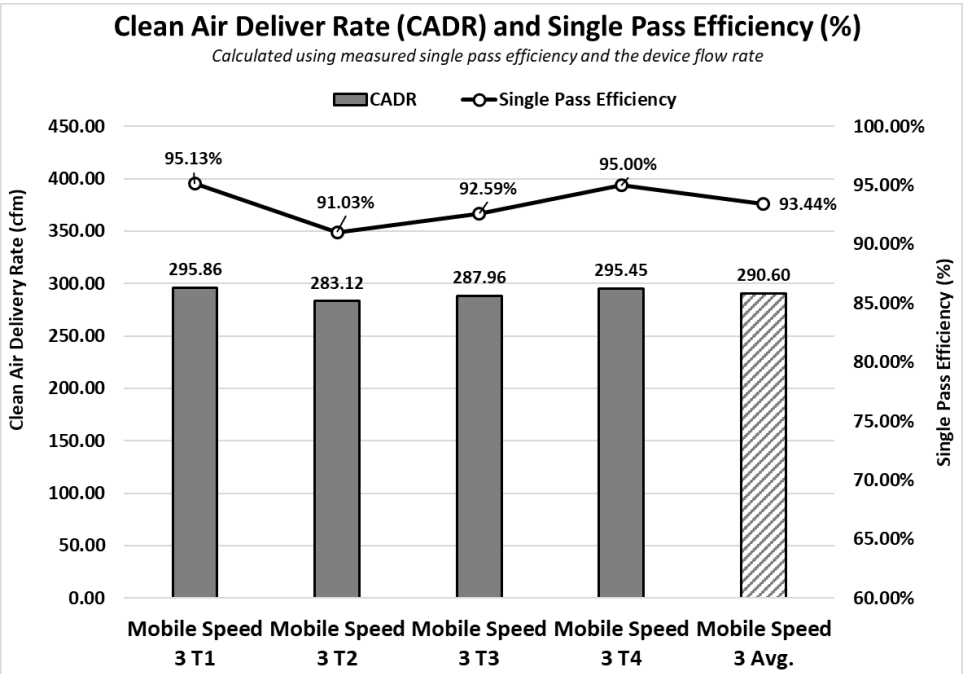


Figure 8A: *Single pass efficiency and clean air delivery rate for each trial as well as the group average of the Mobile speed 3 testing.*

APPENDIX B: Bioaerosol Raw Data

Trial Information

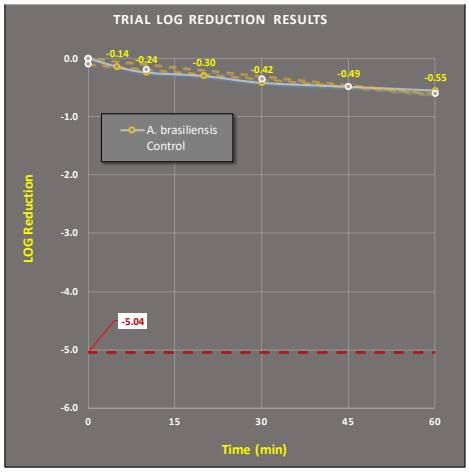
TEST DATE: Wednesday, September 27, 2023
TRIAL PERFORMED BY: JCT
TRIAL NUMBER: A. brasiliensis Control
TEST ORGANSIM: Aspergillus brasiliensis
TRIAL NAME ID (GRAPHS/TABLES): A. brasiliensis Control

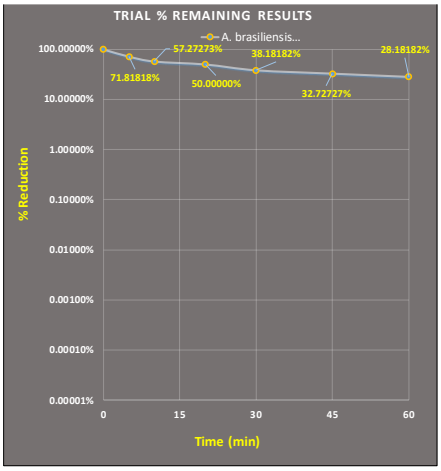
Device Information

MANUFACTURER: N/A
UNIT MODEL: N/A
FAN SPEED (CFM): N/A
UNIT SERIAL #: N/A
FITER ID #: N/A
FILTER LOT #: N/A

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3): 30
NEBULIZER CONDITIONS: Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD: Impinger & Cascade
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: Picarro, Interscan, Tiger
TRIAL COMMENTS/NOTES





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	LOD
SAMPLE TIME (min)	0	5	10	20	30	45	60				LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y				y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n				n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.173E+05	8.427E+04	6.720E+04	5.867E+04	4.480E+04	3.840E+04	3.307E+04				1.067E+00
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)											
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)											100.00%
VIABLE CONSISTENCY CHECKS (% agreement)											
IMP & VIABLE CROSS CHECK (% agreement)											
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.173E+05**	**8.427E+04**	**6.720E+04**	**5.867E+04**	**4.480E+04**	**3.840E+04**	**3.307E+04**				**1.0667**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**71.8182%**	**57.2727%**	**50.0000%**	**38.1818%**	**32.7273%**	**28.1818%**				**0.0009%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**28.1818%**	**42.7273%**	**50.0000%**	**61.8182%**	**67.2727%**	**71.8182%**				**99.9991%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.14**	**-0.24**	**-0.30**	**-0.42**	**-0.49**	**-0.55**				**-5.04**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	20	30	45	60			LOD
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0			20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0	5.0	5.0			5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5			12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-3**	**-3**	**-3**	**-3**	**-3**	**-3**			**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**			**100**
	ENUMERATED PLATE COUNTS (# / drop)	23	23	20	18	12	11	11			1
		26	26	20	17	16	15	11			0
		17	30	23	20	14	10	9			0
	PLATE AVERAGE COUNT (# / drop)	22.00	26.33	21.00	18.33	14.00	12.00	10.33			0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	220,000	263,333	210,000	183,333	140,000	120,000	103,333			3
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.17E+05**	**8.43E+04**	**6.72E+04**	**5.87E+04**	**4.48E+04**	**3.84E+04**	**3.31E+04**			**1.07E+00**
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-3**	**-2**	**-2**	**-2**	**-2**	**-2**			
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**			
	ENUMERATED PLATE COUNTS (# / drop)										
	PLATE AVERAGE COUNT (# / drop)										
	IMPINGER CONCENTRATION (cfu or pfu/ml)										
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)										

Figure 1B: *Control Trial 1 Bioaerosol Raw Data.*

Trial Information

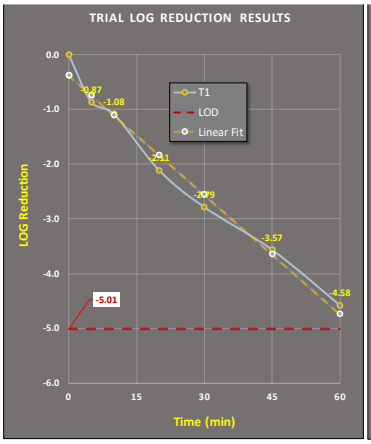
TEST DATE: Thursday, September 28, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T1
TEST ORGANSIM: A. brasiliensis
TRIAL NAME ID (GRAPHS/TABLES): T1

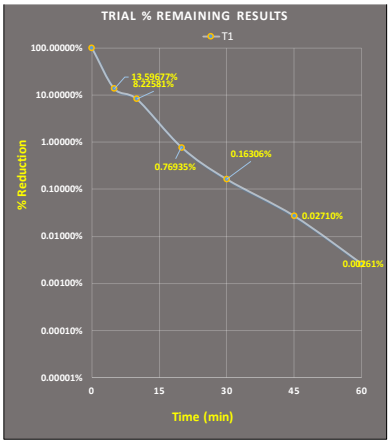
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mobile
FAN SPEED (CFM): 226
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3): 30
NEBULIZER CONDITIONS: Dry Powder Eductor
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: 5g of spores used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S6	S7	S8	LOD
SAMPLE TIME (min)	0	5	10	20	30	45	60					LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y					y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n					n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.102E+05	1.499E+04	9.067E+03	8.480E+02	1.797E+02	2.987E+01	2.880E+00					1.067E+00
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)												
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		12.67%		1.25%	39.52%							100.00%
VIABLE CONSISTENCY CHECKS (% agreement)												
IMP & VIABLE CROSS CHECK (% agreement)												
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.102E+05**	**1.499E+04**	**9.067E+03**	**8.480E+02**	**1.797E+02**	**2.987E+01**	**2.880E+00**					**1.0667**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**13.5968%**	**8.2258%**	**0.7694%**	**0.1631%**	**0.0271%**	**0.0026%**					**0.0010%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**86.4032%**	**91.7742%**	**99.2306%**	**99.8369%**	**99.9729%**	**99.9974%**					**99.9990%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.87**	**-1.08**	**-2.11**	**-2.79**	**-3.57**	**-4.58**					**-5.01**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	5	10	20	30	45	60		LOD
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0		20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0	10.0	10.0		5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5		12.5
DILUTION RATIO (10^x)	**-3**	**-3**	**-2**	**-2**	**0**	**0**	**0**		**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**500**		**100**
Dilution Range #1 ENUMERATED PLATE COUNTS (# / drop)	20	4	36	2	25	11	9		1
	20	3	22	3	61	9			0
	22	8	27	3	41	36			0
PLATE AVERAGE COUNT (# / drop)	20.67	5.00	28.33	2.67	42.33	18.67	9.00		0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	206,667	50,000	28,333	2,667	423	187	18		3
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.10E+05**	**1.60E+04**	**9.07E+03**	**8.53E+02**	**1.35E+02**	**2.99E+01**	**2.88E+00**		**1.07E+00**
DILUTION RATIO (10^x)	**-2**	**-2**	**0**	**-1**	**-1**	**0**	**-2**		
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**500**	**100**		
Dilution Range #1 ENUMERATED PLATE COUNTS (# / drop)		50		26	6				
		40		27	7				
		41		26	8				
PLATE AVERAGE COUNT (# / drop)		43.67		26.33	7.00				
IMPINGER CONCENTRATION (cfu or pfu/ml)		43,667		2,633	700				
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		**1.40E+04**		**8.43E+02**	**2.24E+02**				

Figure 2B: *CerroZone Mobile Speed 2 T1 Bioaerosol Raw Data.*

Trial Information

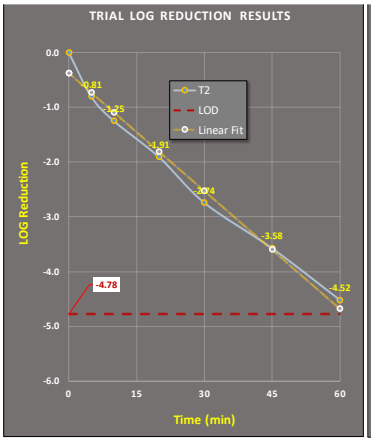
TEST DATE: Thursday, September 28, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T2
TEST ORGANSIM: A. brasiliensis
TRIAL NAME ID (GRAPHS/TABLES): T2

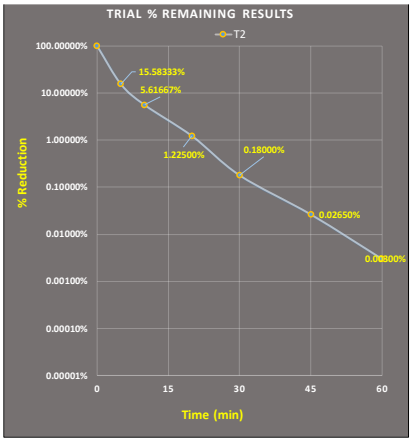
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mobile
FAN SPEED (CFM): 226
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3): 30
NEBULIZER CONDITIONS: Dry Powder Eductor
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: 5g of spores used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S6	S7	S8	LOD
SAMPLE TIME (min)	0	5	10	20	30	45	60					LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y					y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n					n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	6.400E+04	9.973E+03	3.595E+03	7.840E+02	1.152E+02	1.696E+01	1.920E+00					1.067E+00
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)												
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		30.00%	43.26%	36.67%	20.00%	7.27%						100.00%
VIABLE CONSISTENCY CHECKS (% agreement)												
IMP & VIABLE CROSS CHECK (% agreement)												
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**6.400E+04**	**9.973E+03**	**3.595E+03**	**7.840E+02**	**1.152E+02**	**1.696E+01**	**1.920E+00**					**1.0667**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**15.5833%**	**5.6167%**	**1.2250%**	**0.1800%**	**0.0265%**	**0.0030%**					**0.0017%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**84.4167%**	**94.3833%**	**98.7750%**	**99.8200%**	**99.9735%**	**99.9970%**					**99.9983%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.81**	**-1.25**	**-1.91**	**-2.74**	**-3.58**	**-4.52**					**-4.78**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	5	10	20	30	45	60		LOD
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0		20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0	10.0	10.0		5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5		12.5
DILUTION RATIO (10^x)	**-3**	**-3**	**-2**	**-2**	**0**	**0**	**0**		**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**500**		**100**
Dilution Range #1 ENUMERATED PLATE COUNTS (# / drop)	13	5	20	2	30	10	6		1
	15	3	11	3	30	8			0
	8	3	12	4	36	15			0
PLATE AVERAGE COUNT (# / drop)	12.00	3.67	14.33	3.00	32.00	11.00	6.00		0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	120,000	36,667	14,333	3,000	320	110	12		3
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**6.40E+04**	**1.17E+04**	**4.59E+03**	**9.60E+02**	**1.02E+02**	**1.76E+01**	**1.92E+00**		**1.07E+00**
DILUTION RATIO (10^x)	**-2**	**-2**	**-1**	**-1**	**-1**	**0**	**-2**		
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**500**	**100**		
Dilution Range #1 ENUMERATED PLATE COUNTS (# / drop)		16	88	20	3	51			
		24	90	21	4				
		37	66	16	5				
PLATE AVERAGE COUNT (# / drop)		25.67	81.33	19.00	4.00	51.00			
IMPINGER CONCENTRATION (cfu or pfu/ml)		25,667	8,133	1,900	400	102			
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		**8.21E+03**	**2.60E+03**	**6.08E+02**	**1.28E+02**	**1.63E+01**			

Figure 3B: *CerroZone Mobile Speed 2 T2 Bioaerosol Raw Data.*

Trial Information

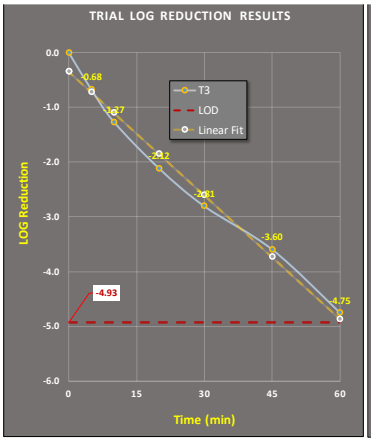
TEST DATE: Thursday, September 28, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T3
TEST ORGANSIM: A. brasiliensis
TRIAL NAME ID (GRAPHS/TABLES): T3

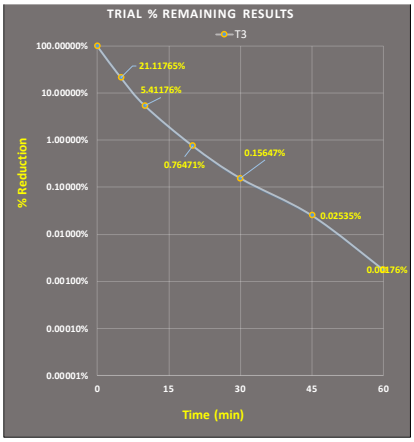
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mobile
FAN SPEED (CFM): 226
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3): 30
NEBULIZER CONDITIONS: Dry Powder Eductor
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: 5g of spores used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S6	S7	S8	LOD
SAMPLE TIME (min)	0	5	10	20	30	45	60					LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y					y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n					n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	9.067E+04	1.915E+04	4.907E+03	6.933E+02	1.419E+02	2.299E+01	1.600E+00					1.067E+00
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)												
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		36.82%		14.29%		34.23%						100.00%
VIABLE CONSISTENCY CHECKS (% agreement)												
IMP & VIABLE CROSS CHECK (% agreement)												
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**9.067E+04**	**1.915E+04**	**4.907E+03**	**6.933E+02**	**1.419E+02**	**2.299E+01**	**1.600E+00**					**1.0667**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**21.1176%**	**5.4118%**	**0.7647%**	**0.1565%**	**0.0254%**	**0.0018%**					**0.0012%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**78.8824%**	**94.5882%**	**99.2353%**	**99.8435%**	**99.9746%**	**99.9982%**					**99.9988%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.68**	**-1.27**	**-2.12**	**-2.81**	**-3.60**	**-4.75**					**-4.93**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	20	30	45	60	LOD
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0	10.0	10.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-3**	**-2**	**-2**	**0**	**0**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**500**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	17	9	16	2	38	12	5	1
		19	9	12	3	47	20		0
		15	4	18	2	48	20		0
	PLATE AVERAGE COUNT (# / drop)	17.00	7.33	15.33	2.33	44.33	17.33	5.00	0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	170,000	73,333	15,333	2,333	443	173	10	3
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**9.07E+04**	**2.35E+04**	**4.91E+03**	**7.47E+02**	**1.42E+02**	**2.77E+01**	**1.60E+00**	**1.07E+00**
Dilution Range #1	**DILUTION RATIO (10^x)**	**-2**	**-2**	**-1**	**-1**	**-1**	**0**	**-2**	
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**500**	**100**	
	ENUMERATED PLATE COUNTS (# / drop)		52		23		57		
			45		18				
			42		19				
	PLATE AVERAGE COUNT (# / drop)		46.33		20.00		57.00		
	IMPINGER CONCENTRATION (cfu or pfu/ml)		46,333		2,000		114		
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		**1.48E+04**		**6.40E+02**		**1.82E+01**		

Figure 4B: *CerroZone Mobile Speed 2 T3 Bioaerosol Raw Data.*

Trial Information

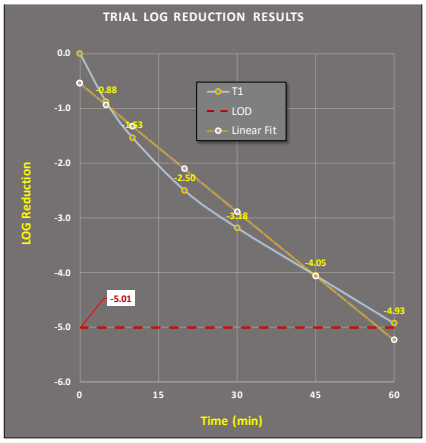
TEST DATE: Thursday, September 28, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T1
TEST ORGANSIM: A. brasiliensis
TRIAL NAME ID (GRAPHS/TABLES): T1

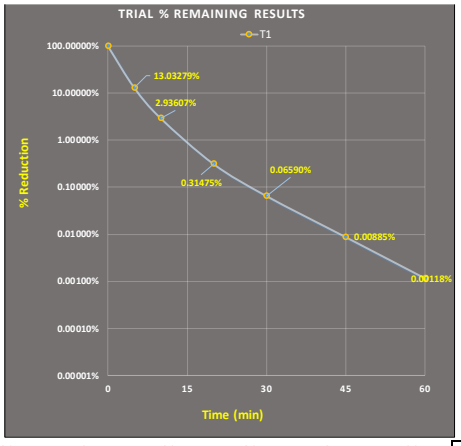
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mobile
FAN SPEED (CFM): 311
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3): 30
NEBULIZER CONDITIONS: Dry Powder Eductor
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: 5g of spores used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S6	S7	S8	LOD
SAMPLE TIME (min)	0	5	10	20	30	45	60					LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y					y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n					n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.084E+05	1.413E+04	3.184E+03	3.413E+02	7.147E+01	9.600E+00	1.280E+00					1.067E+00
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)												
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		34.38%	46.92%	11.76%								100.00%
VIABLE CONSISTENCY CHECKS (% agreement)												
IMP & VIABLE CROSS CHECK (% agreement)												
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.084E+05**	**1.413E+04**	**3.184E+03**	**3.413E+02**	**7.147E+01**	**9.600E+00**	**1.280E+00**					**1.0667**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**13.0328%**	**2.9361%**	**0.3148%**	**0.0659%**	**0.0089%**	**0.0012%**					**0.0010%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**86.9672%**	**97.0639%**	**99.6852%**	**99.9341%**	**99.9911%**	**99.9988%**					**99.9990%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.88**	**-1.53**	**-2.50**	**-3.18**	**-4.05**	**-4.93**					**-5.01**

Impinger Sampling Conditions

	S1	S2	S3	S4	S5	S6	S7	LOD
SAMPLE TIME (min)	0	5	10	20	30	45	60	LOD
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0	10.0	10.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
DILUTION RATIO (10^x)	**-3**	**-3**	**-2**	**-2**	**0**	**0**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**500**	**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	14 16 31	3 6 7	10 16 13	1 1 1	18 20 29		4	1 0 0
PLATE AVERAGE COUNT (# / drop)	20.33	5.33	13.00	1.00	22.33		4.00	0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	203,333	53,333	13,000	1,000	223		8	3
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.08E+05**	**1.71E+04**	**4.16E+03**	**3.20E+02**	**7.15E+01**		**1.28E+00**	**1.07E+00**
DILUTION RATIO (10^x)	**-2**	**-2**	**-1**	**-1**	**-1**	**0**	**-2**	
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**500**	**100**	
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)		25 36 44	64 76 67	12 13 9		30		
PLATE AVERAGE COUNT (# / drop)		35.00	69.00	11.33		30.00		
IMPINGER CONCENTRATION (cfu or pfu/ml)		35,000	6,900	1,133		60		
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		**1.12E+04**	**2.21E+03**	**3.63E+02**		**9.60E+00**		

Figure 5B: *CerroZone Mobile Speed 3 T1 Bioaerosol Raw Data.*

Trial Information

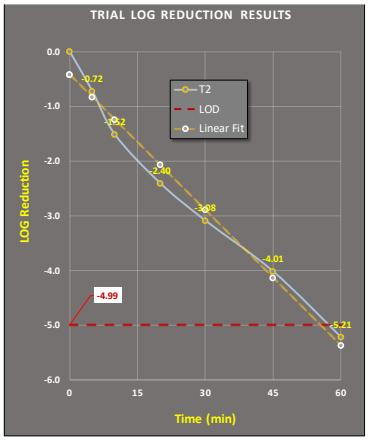
TEST DATE: Friday, September 29, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T2
TEST ORGANSIM: A. brasiliensis
TRIAL NAME ID (GRAPHS/TABLES): T2

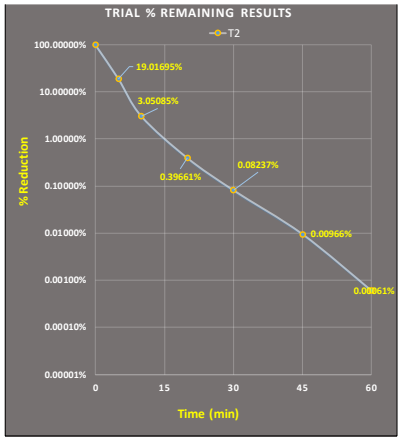
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mobile
FAN SPEED (CFM): 311
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3): 30
NEBULIZER CONDITIONS: Dry Powder Eductor
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: 5g of spores used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S6	S7	S8	LOD
SAMPLE TIME (min)	0	5	10	20	30	45	60					LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y					y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n					n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.049E+05	1.995E+04	3.200E+03	4.160E+02	8.640E+01	1.013E+01	6.400E-01					1.067E+00
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)												
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		66.43%										100.00%
VIABLE CONSISTENCY CHECKS (% agreement)												
IMP & VIABLE CROSS CHECK (% agreement)												
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.049E+05**	**1.995E+04**	**3.200E+03**	**4.160E+02**	**8.640E+01**	**1.013E+01**	**6.400E-01**					**1.0667**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**19.0169%**	**3.0508%**	**0.3966%**	**0.0824%**	**0.0097%**	**0.0006%**					**0.0010%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**80.9831%**	**96.9492%**	**99.6034%**	**99.9176%**	**99.9903%**	**99.9994%**					**99.9990%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.72**	**-1.52**	**-2.40**	**-3.08**	**-4.01**	**-5.21**					**-4.99**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	5	10	20	30	45	60		LOD
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0		20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0	10.0	10.0		5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5		12.5
DILUTION RATIO (10^x)	**-3**	**-3**	**-2**	**-1**	**0**	**0**	**0**		**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**500**		**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	20	8	12	13	27	10	2		1
	20	10	8	8	25	4			0
	19	10	10	18	29	5			0
PLATE AVERAGE COUNT (# / drop)	19.67	9.33	10.00	13.00	27.00	6.33	2.00		0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	196,667	93,333	10,000	1,300	270	63	4		3
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.05E+05**	**2.99E+04**	**3.20E+03**	**4.16E+02**	**8.64E+01**	**1.01E+01**	**6.40E-01**		**1.07E+00**
DILUTION RATIO (10^x)	**-2**	**-2**	**-1**	**0**	**-1**	**0**	**-2**		
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**500**	**100**		
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)		29							
		29							
		36							
PLATE AVERAGE COUNT (# / drop)		31.33							
IMPINGER CONCENTRATION (cfu or pfu/ml)		31,333							
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		**1.00E+04**							

Figure 6B:. *CerroZone Mobile Speed 3 T2 Bioaerosol Raw Data.*

Trial Information

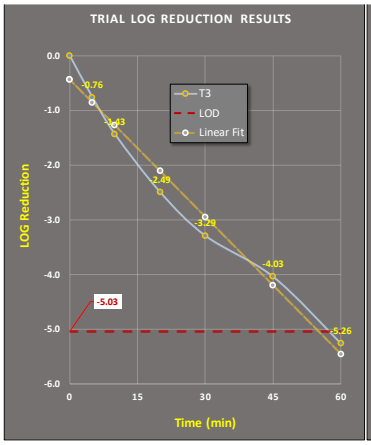
TEST DATE: Friday, September 29, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T3
TEST ORGANSIM: A. brasiliensis
TRIAL NAME ID (GRAPHS/TABLES): T3

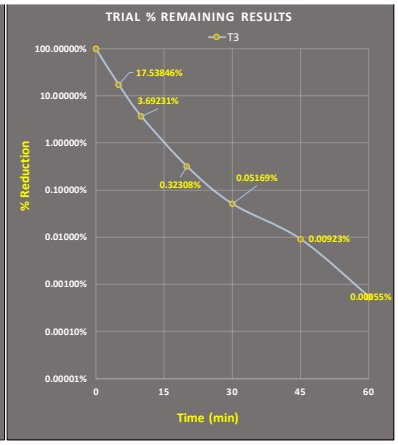
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mobile
FAN SPEED (CFM): 311
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: NA

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3): 30
NEBULIZER CONDITIONS: Dry Powder Eductor
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: 5g of spores used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S6	S7	S8	LOD
SAMPLE TIME (min)	0	5	10	20	30	45	60					LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y					y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n					n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.156E+05	2.027E+04	4.267E+03	3.733E+02	5.973E+01	1.067E+01	6.400E-01					1.067E+00
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)												
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)												100.00%
VIABLE CONSISTENCY CHECKS (% agreement)												
IMP & VIABLE CROSS CHECK (% agreement)												
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.156E+05**	**2.027E+04**	**4.267E+03**	**3.733E+02**	**5.973E+01**	**1.067E+01**	**6.400E-01**					**1.0667**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**17.5385%**	**3.6923%**	**0.3231%**	**0.0517%**	**0.0092%**	**0.0006%**					**0.0009%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**82.4615%**	**96.3077%**	**99.6769%**	**99.9483%**	**99.9908%**	**99.9994%**					**99.9991%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.76**	**-1.43**	**-2.49**	**-3.29**	**-4.03**	**-5.26**					**-5.03**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	5	10	20	30	45	60				LOD
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0				20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0	5.0	10.0	10.0				5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5				12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-3**	**-2**	**-1**	**0**	**0**	**0**				**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**500**				**100**
	ENUMERATED PLATE COUNTS (# / drop)	20	5	16	10	18	7	2				1
		23	10	12	13	20	5					0
		22	4	12	12	18	8					0
	PLATE AVERAGE COUNT (# / drop)	21.67	6.33	13.33	11.67	18.67	6.67	2.00				0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	216,667	63,333	13,333	1,167	187	67	4				3
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.16E+05**	**2.03E+04**	**4.27E+03**	**3.73E+02**	**5.97E+01**	**1.07E+01**	**6.40E-01**				**1.07E+00**
Dilution Range #1	**DILUTION RATIO (10^x)**	**-2**	**-2**	**-1**	**0**	**-1**	**0**	**-2**				
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**500**	**100**				
	ENUMERATED PLATE COUNTS (# / drop)											
	PLATE AVERAGE COUNT (# / drop)											
	IMPINGER CONCENTRATION (cfu or pfu/ml)											
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)											

Figure 7B: *CerroZone Mobile Speed 3 T3 Bioaerosol Raw Data.*

Appendix C: Calculations

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

- Plating and enumeration of the biological to derive the concentration of the stock suspension (C_s) in pfu/mL or cfu/mL, or cfu/g for dry powder.
- Collison 24 jet nebulizer use rate (R_{neb}) (volume of liquid generated by the nebulizer/time) at 28 psi air supply pressure = 1.0 mL/min.
- Collison 24 jet Generation time (t) = 20 or 30 minutes, test dependent.
- Chamber volume (V_c) = 15,993 Liters

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

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

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

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

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

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

AGI – 30 impinger or 47mm filter collection calculation:

- Viable aerosol concentration collection (C_a) = cfu or pfu/L of chamber air.
- Viable Impinger concentration collection (C_{Imp}) = cfu or pfu/mL from enumeration of impinger sample or filter sample.
- Impinger sample collection volume (I_{vol}) = 20 mL collection fluid/impinger, or extraction fluid for filter.
- AGI–30 impinger or filter sample flow rate (Q_{imp}) = 12.5 L/min.
- AGI–30 impinger or filter sample time (t) = 5 or 10 minutes, test dependent.

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

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

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

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