ASHRAE 241-2023 Standard Testing for the Efficacy of the CerroZone Mini Air Purification Device at Reducing Aerosolized *MS2*

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ASHRAE 241-2023 Compliance:
This study was conducted in compliance with ASHRAE 241 and AHAM AC-5 along with 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 Mini air purification device to reduce the bacteriophage, MS2, per the ASHRAE 241-2023 test standard.

Background:
The CerroZone Mini 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 30m^3 bioaerosol test chamber. The species selected for this study was MS2, an ssRNA bacteriophage, that is a recognized surrogate for more dangerous pathogens. This study utilizes ASHRAE 241 and AHAM AC-5 testing guidelines to determine efficacy. Three separate bioaerosol test trials were performed at two different fan speeds for the CerroZone Mini device, as well as three control bioaerosol trials.

Methods:
The CerroZone Mini was sealed in a custom 30m^3 environmental test chamber for all testing. MS2 was aerosolized into the sealed test chamber, using a Collison 24-jet nebulizer. *MS2* was the microorganism used for all aerosol trials. Bioaerosol samples were taken, with AGI 30 glass impingers at multiple time points throughout each trial, using ASHRAE 241 and AHAM AC-5 testing parameters, 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 for each sampling time point. Chamber MS2 control trial data, or the natural decay rate of MS2, 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 Mini was able to reduce the MS2 bioaerosol by 2.16 +/- 0.18 and 3.04 +/- 0.09 net log reduction of on normal and high speed respectively in 60 minutes. The clean air delivery rate was calculated for this unit based off the CerroZone Mini trial data. It achieved an average CADR of 88.05 and 137.04 CFM on the normal and high speeds respectively in the 30 m^{3}chamber.

Conclusion:
The test device was capable of reducing the bioaerosol consistently and showed a log-linear reduction trend in the 30m^3 test chamber.

INTRODUCTION

This study was conducted to evaluate the efficacy of the CerroZone Mini air purification device's ability to eliminate viable pathogens from ambient air. The CerroZone is a Mini air purifier designed to reduce the viability of pathogens in medical facilities, classrooms, and other indoor spaces.

On June 24th, 2023, the new ASHRAE 241-2023 guidelines were released to establish a more uniform testing protocol for all air purification devices. This protocol standardized all components of bioaerosol testing for both in duct and standalone devices. This testing protocol establishes the minimum requirements needed to evaluate all production air purification devices adequately and effectively.

The ASHRAE standard includes guidelines for proper ventilation, infection risk management, laboratory testing requirements, the operation and maintenance of devices, as well as special requirements needed for residential and health care

facilities. With these new guidelines, testing must be done on all air purification devices that are certified as adhering to the ASHRAE 241 standard guidelines.

The test plan incorporated challenging the CerroZone device using the ASHRAE 241 and AHAM AC-5 protocols and requirements for a 30 m^3 test chamber. This report will focus on the efficacy of the CerroZone Mini air purification device. A picture of the Mini is shown in **Figure 1** below.

STUDY OVERVIEW

The effectiveness of the CerroZone Mini device was evaluated against a single aerosolized organism, MS2, an ssRNA virus. This allowed for a reasonable demonstration of the performance of the devices while operating in their intended manner. This study was done in accordance with ASHRAE 241, and AHAM AC-5 testing parameters.

This is one of two reports that detail the testing requirements and results for ASHRAE 241 and AHAM AC-5 testing of the CerroZone Mini. This report contains the bioaerosol testing method, data, and results, while the other report details the safety information required by ASHRAE 241 and AHAM testing guidelines. A test matrix can be found in **Figure 2** below.

Figure 1: CerroZone Mini air purifier.

TEST DEVICE DESCRIPTION

The CerroZone Mini 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 96 +/- 30 CFM on Speed 2 and 179 +/- 30 CFM on Speed 3 and exposes airborne pathogens to ozone and UVC light within the device.

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.

Trial	Run	Device	Device Fan Speed (ft^3/min)	Challenge Species (gram, description)	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	NA	NA	MS2 Bacteriophage (RNA Virus)	15597-B1	30.0	<1.0um	10^4-10^5	60	0, 2, 4, 8, 12, 16, 20, 30, 45, 60	AGI 30 Impingers	all samples in triplicate
2	Control											
3	Control											
5	Challenge	Cerrozone Mini	96 +/- 30	MS2 Bacteriophage (RNA Virus)	15597-B1	30.0	<1.0um	10^4-10^5	45	0, 2, 4, 8, 12, 16, 20, 30, 45, 60	AGI 30 Impingers	all samples in triplicate
6	Challenge											
7	Challenge											
10	Challenge	Cerrozone Mini	179 +/- 30	MS2 Bacteriophage (RNA Virus)	15597-B1	30.0	<1.0um	10^4-10^5	30	0, 2, 4, 8, 12, 16, 20, 30, 45, 60	AGI 30 Impingers	all samples in triplicate
11	Challenge											
12	Challenge											

Figure 2: Test Matrix for Bioaerosol Testing.

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.

The incorporation of negative pressure airflow allows for controlled purging, and a HEPA filter adds an additional layer of protection, inhibiting potential contamination. The 30m^3 testing chamber at ARE Labs fulfills both AHSRAE 241 and AHAM AC-5 requirements. **Figure 3** (right) shows the bioaerosol chamber used for all testing in this study. 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. A general flow diagram of the aerosol test system is shown in **Figure 4** below.

Bioaerosol Generation System

As per the AHAM AC-5 requirements, the Collison nebulizers are able to produce 0.05 um to 5 um particles from microbial suspensions using compressed air to generate aerosols. The nebulizer fluid is a mixture of the test microorganism, distilled water, phosphate buffer solution (PBS), and an antifoaming agent. A ceiling fan is used in the chamber to allow for homogenous mixing.

Figure 3: The 30 m^3 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.

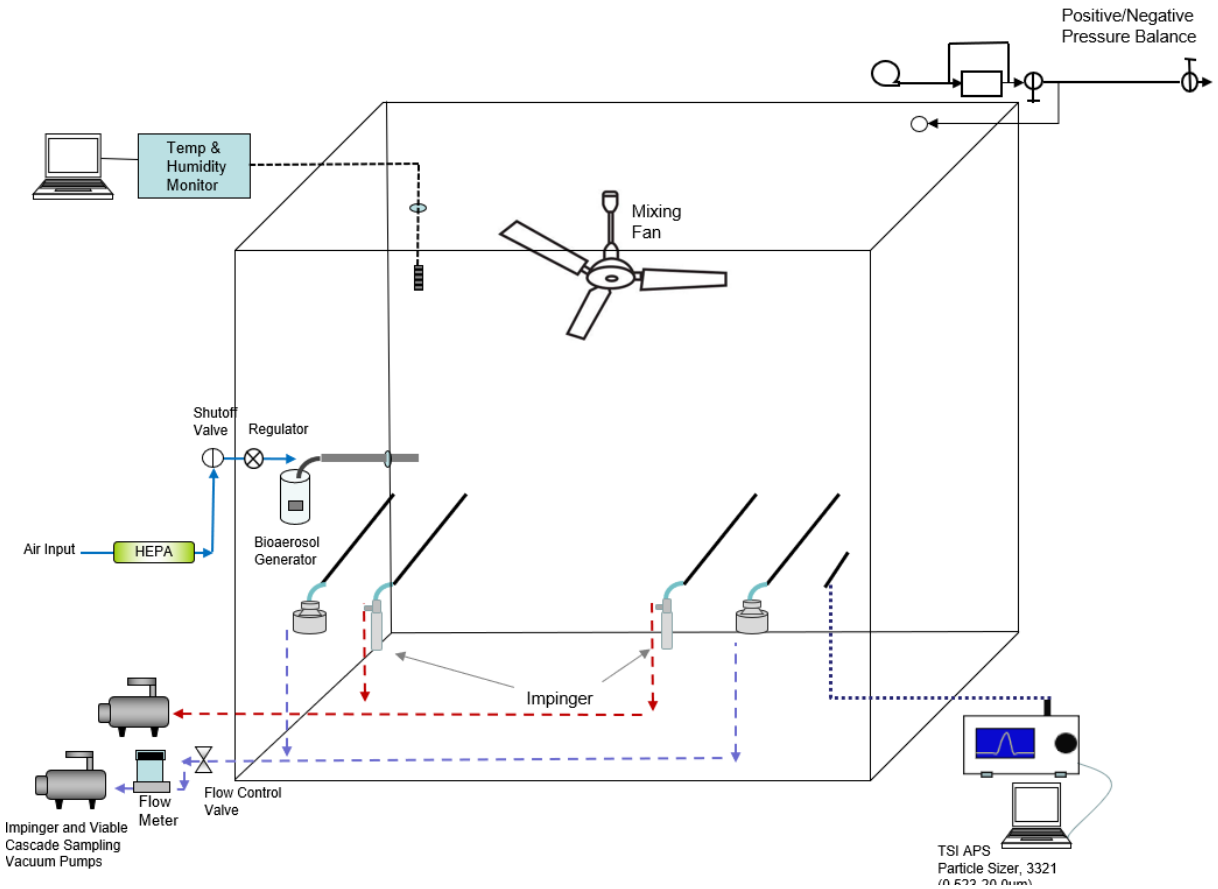

Figure 4: 30m^3 Environmental Test Chamber Flow Diagram. Chamber includes bioaerosol induction, multiple bioaerosol sampling ports, particle size monitoring, internal mixing fans, and temperature and humidity controls. Main system HEPA evacuation system not pictured.

A 24-Jet Collison (BGI Inc. Waltham MA), similar to the one shown in **Figure 5** below, was used during testing to introduce the properly sized particulates into the test chamber. The biologic was mixed with half PBS, half fresh Tryptic Soy Broth (TSB), both made with distilled water and 100uL of antifoam A concentrate. The aerosolization of bioaerosol was driven by dry, filtered house air. A pressure regulator allowed for control of disseminated particle size, use rate, and sheer force generated within the Collison nebulizer.

Prior to testing, the Collison nebulizer flow rate and use rate were checked 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 Collison nebulizer was flow characterized using a calibrated TSI model 4040 mass flow meter (TSI Inc., St Paul MN).

Figure 5. 6-Jet Collison nebulizer. Glass and 304 stainless steel construction, made by BGI Industries.

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. The mixing fans inside the chamber worked to ensure a homogenous air mixture inside the chamber. A picture of the AGI-30 is shown in **Figure 6** below.

The AGI-30 impinger vacuum source was maintained at a negative pressure of -18 inches of Hg during all characterization and test sampling to assure critical flow conditions. The AGI-30 impingers sample at a rate of 12.5 LPM impinger flows were characterized using a calibrated TSI model 4040 mass flow meter.

Figure 6: AGI-30 Impinger, Ace Glass Inc. Vineland NJ.

During testing with less resilient organisms and ones with larger particle sizes that fall out of the air more easily, sample collections were also obtained using a pair of viable cascade impactors. A viable cascade impactor (SKC Inc., Valley View, PA) is comprised of an inlet cone, precision-drilled 400-hole impactor stage, and a base that holds a standard-size agar plate (**Figure 7** below). A high flow pump pulls microorganisms in air through the holes (jets) at 30 liters per minute, where they are collected (impacted) directly onto the agar surface. This method is the most sensitive for detection of 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.

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.

ASHRAE 241 and AHAM AC-5 both have temperature and humidity requirements for temperature and humidity inside of the bioaerosol chamber during testing. The required range for humidity is 50% $\pm$ 10% while the temperature range is 73°F + 5° (23°C + 3°C). A picture of the controller is shown in **Figure 8** below.

Figure 8: AC Infinity Controller 69 Temperature and Humidity Controller.

Ion Monitoring Meter

The COM ion meter, Figure 9 below, measures ion concentrations in real time and was used during testing to ensure the ion concentrations were consistent inside the chamber. The ion meter measures ions using the Gerdien capacitor method and can detect positive and negative ions down to 10 per cubic centimeter.

Figure 9: COM 3200Pro II ion meter used for ion measurements of the 30m³ chamber.

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 10 above right.

Figure 10. 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.

CHAMBER VALIDATION

Validating a bioaerosol chamber is a crucial process to ensure its accuracy and reliability in maintaining controlled experiments. This involves thorough assessments to confirm that the chamber met the strict standards for conducting bioaerosol studies. Factors such as chamber homogeneity, ionization assessment, air exchange rates, and control stability are rigorously tested to ensure consistent and accurate results. Validation assures researchers that the chamber functions properly, enabling them to conduct reliable bioaerosol studies that contribute to informed decision-making in areas like indoor air quality and infectious disease research.

Homogeneity Validation

One key component of the chamber validation process is the bioaerosol homogeneity test. This test validates the homogeneity of the chamber, making sure that the atmosphere within the chamber is well mixed.

Six AGI-30 impingers were used for this chamber validation. The impingers were systematically rotated through all four impinger ports to generate a matrix of impinger tests against all ports. Each port was tested with each impinger a minimum of two times during this validation.

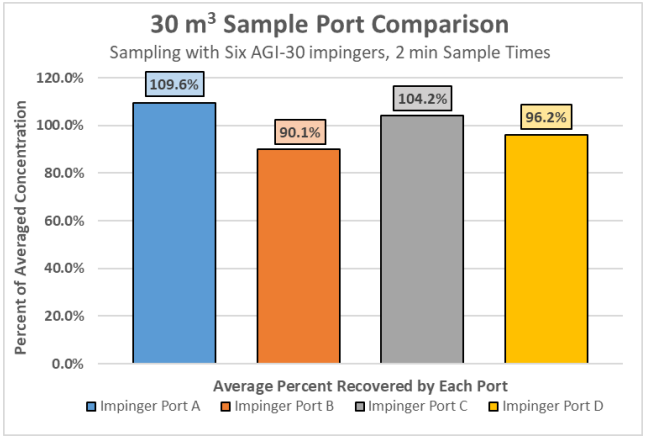

Figure 11: Impinger port-to-port comparison. Percent averages are calculated by taking the count for each port divided by the average plate count for the four ports.

These impinger samples were plated in triplicate by two technicians to reduce plating discrepancies. Each set of plate counts generated by each technician were compared to one another and a port-to-port comparison was created. This showed that each port of the 30m³ chamber produced a similar result to one another validating the chamber homogeneity during trials. A graphical representation of the average measured for each port is shown in **Figure 11** on the previous page.

Ionization Validation

To measure the baseline concentration of ions, present in the sealed 30 m³ chamber over 3 hours, a COM 3200 Pro II ion meter was used. The chamber had an average net ion concentration of -143.39 +/- 55.64 ions per cubic centimeter. Testing shows that the net ion concentration is essentially neutral in regard to the charge within the chamber. See ion data graph from trial in **Figure 12**. The total production of ions naturally occurring in the chamber is nominal.

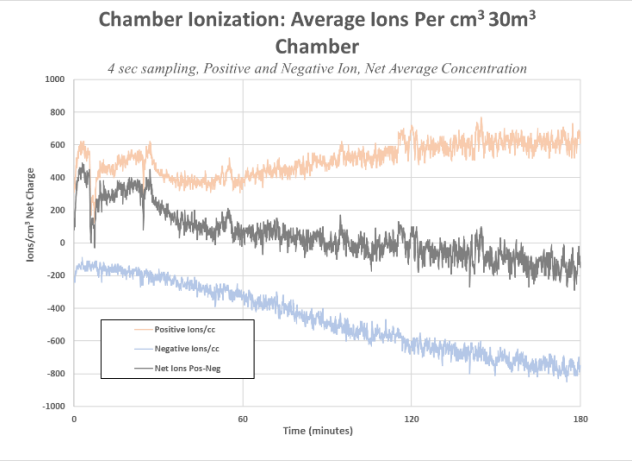

Figure 12. Total baseline level of ions present in the 30m³ chamber.

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 an AGI 30 impinger 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 can be employed in series to enhance collection efficiency.

The samples collected in the impingers are then carefully processed through serial dilution, plating, and enumeration in triplicate (see plating and enumeration section for more information). This meticulous analysis provides viable bioaerosol concentrations at each sampling point and contributes to accurate data interpretation.

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.

These control tests implement the ANSI/AHMA AC-5 2022 guidelines, ensuring a thorough and precise assessment of air cleaner performance in reducing airborne microbes. The methodical approach, from preparation to measurement and analysis, underscores the importance of consistent and accurate testing procedures.

BIOAEROSOL TESTING

Air Purifier Efficacy Evaluation Procedure

The process of evaluating the efficacy of air cleaners in reducing airborne microbial concentrations is similar to control test trials, but the test chamber contains the air purifier in operation. A suspension of test microbes is nebulized into the chamber air, and an initial measurement of the microbial concentration is taken before activating the air purifier.

Once the baseline is set, the air purifier is activated, with the operation time varying according to the specific characteristics of the unit. See **Figure 13**, at the bottom of the page, for an example sampling timeline. For air cleaners with higher Clean Air Delivery Rates (CADR), the operation time could be as brief as 10 minutes, while those with lower CADR might necessitate up to 60 minutes of operation. During the air cleaner's operation, air samples are systematically collected from the chamber at 4-minute intervals over a 20-minute duration. These samples are pivotal in assessing the air cleaner's effectiveness in reducing the microbial concentration. Depending on the capabilities of the air cleaner, supplementary samples can be obtained in 30 and 45 minutes, ensuring a minimum of five valid sampling points.

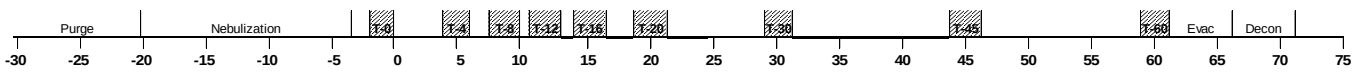

Figure 13: ASHRAE 241 Sampling Times for a 1 Hour Trial.

The collected samples undergo the following procedure: Serial dilution of the samples is followed by plating, and the viable bioaerosols are enumerated (see plating and enumeration section for more information regarding plating). This analysis yields the microbial concentration at each time point, providing a quantifiable measure of the air cleaner's performance. It's worth noting that, in cases where the microbial concentration becomes exceedingly low, an extension of the measurement duration beyond the originally planned 2-minute mark may be implemented, although this adjustment should be considered for its potential mathematical implications.

For air cleaners with exceptionally high CADR ratings, an alternative sampling approach is recommended. This entails obtaining air samples every 2 minutes over a 10-minute period during the air cleaner's operation. Additional sampling points can then be incorporated at 30-minute intervals, extending up to 30 minutes.

In adhering to the ASHRAE 241/AHAM protocol, the real-world efficacy of air cleaners across varying operating conditions and CADR levels can be established, thus producing more accurate conclusions regarding indoor air quality management.

Bioaerosol Challenge Particle Size Testing

Bioaerosol challenge 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 an Acer laptop computer, regressed, and plotted. A graphical representation of MS2 Particle Size Distribution can be found in **Figure 14** below.

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. The ASHRAE 241/AHAM guidelines for biological species selection provide several approved species that fill various biological testing niches such as viruses, mold, and both gram-positive and gram-negative bacterium. In this study the bacteriophage MS2 was used. MS2, is a ssRNA virus and is very commonly used for bioaerosol testing given its small size and hearty resilience to aerosolization and other disinfecting processes.

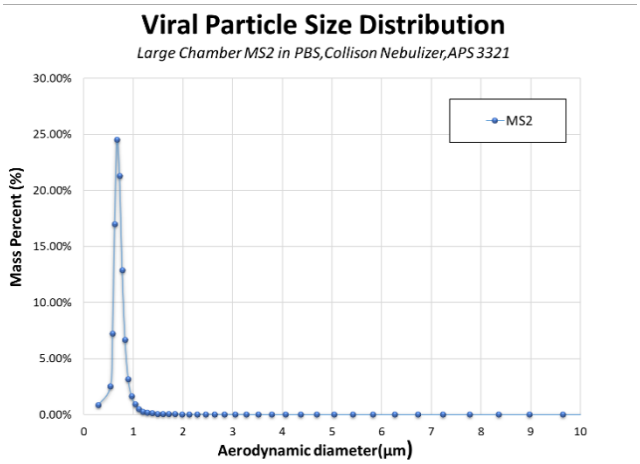

Figure 14: Aerodynamic Particle Size Distribution of the RNA virus MS2 in the test chamber. The MMAD for this viral species averaged approximately 0.7 µm.

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. If using a virus for testing the host organism is added to each tube to allow for viral replication and plaque formation.

The number of colonies or plaques that form on the plates is counted and used to calculate the original bacterial concentration in the liquid sample. The drop plate technique offers a practical and straightforward approach for quantifying bacterial populations, making it a fundamental tool in various research, clinical, and industrial settings for assessing microbial abundance and studying bacterial or viral growth dynamics.

Post-Testing Decontamination and Prep

After the completion of each testing session, a series of post-test actions were carried out to ensure the integrity and cleanliness of the testing environment. The interior of the test chamber underwent decontamination using a UV-C lamp or an appropriate disinfectant solution, such as 70% ethanol, bleach, or vaporous hydrogen peroxide (35%) to ensure the elimination of any residual bioaerosols in accordance with ANSI/AHAM AC-5-2022 guidelines (Section 5.1.14).

The chamber underwent a minimum of twenty minutes of air flow evacuation/purging to restore baseline particle concentration levels, as assessed by the APS. Special care was taken to ensure the thorough removal of any contaminants, with an emphasis on preventing residue buildup on surfaces and in the air. Adequate air exchanges were employed to facilitate the decontamination process, and this step was particularly rigorous when transitioning between different test microbes to mitigate cross-contamination risks.

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 are plotted showing log reduction in viable MS2 bioaerosol. All data is normalized with time zero enumerated concentrations. Subsequent samples are 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 A** for raw data for the control and challenge tests.

RESULTS

The CerroZone Mini, when tested at normal and high speed, achieved a net log reduction 2.16 +/- 0.18 and 3.04 +/- 0.09 respectively in 60 minutes. See **Figures 15 and 16** 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.

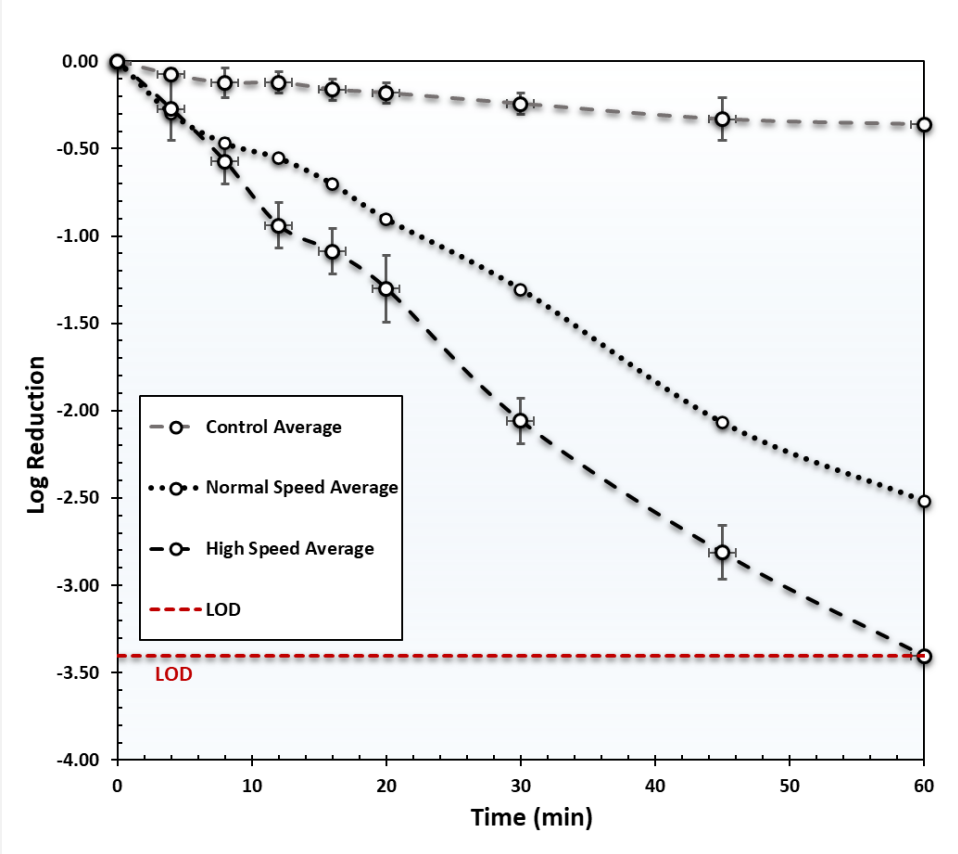

Figure 15: Log Reduction of Aerosolized *MS2* by the CerroZone Mini. Each line represents the average of three trials performed under the same conditions in the 30m³ chamber for statistical significance.

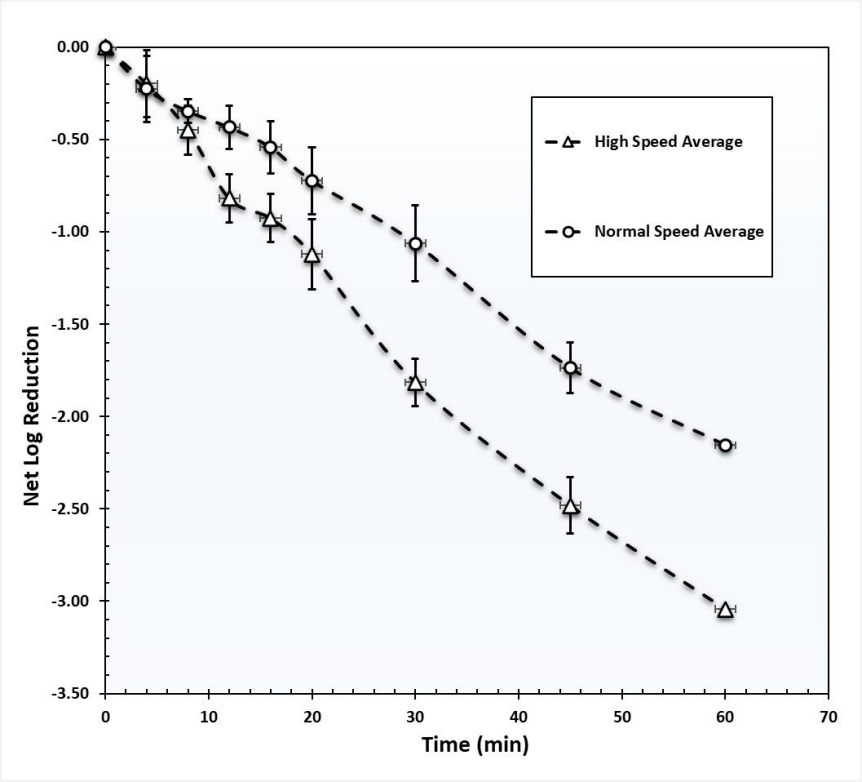

Figure 16: Net Log Reduction of Aerosolized *MS2* by the CerroZone Mini. Each line represents the average of three trials performed under the same conditions in the 30m³ chamber for statistical significance.

Bioaerosol Type	Species (description)	Trial Name	Reduction Type	Trial Time Elapsed (minutes) 4	8	12	16	20	30	45	60
Virus	*MS2* (RNA Virus)	*MS2 Mini Spd3-T1*	*Net Log*	-0.12	-0.37	-0.70	-0.78	-0.97	-1.79	-2.34	-2.94
			Net % Reduction	23.7%	57.6%	80.1%	83.4%	89.3%	98.4%	99.5%	99.88%
Virus	*MS2* (RNA Virus)	*MS2 Mini Spd3-T2*	*Net Log*	-0.07	-0.37	-0.96	-1.03	-1.33	-1.96	-2.65	-3.09
			Net % Reduction	15.4%	57.7%	89.0%	90.7%	95.4%	98.9%	99.8%	99.92%
Virus	*MS2* (RNA Virus)	*MS2 Mini Spd3-T3*	*Net Log*	-0.40	-0.60	-0.80	-0.97	-1.06	-1.70	-2.46	-3.10
			Net % Reduction	60.5%	75.0%	84.0%	89.2%	91.2%	98.0%	99.7%	99.92%
All Trial Averages +/- St. Dev.			***Net Log***	**-0.2 +/- 0.18**	**-0.45 +/- 0.13**	**-0.82 +/- 0.13**	**-0.93 +/- 0.13**	**-1.12 +/- 0.19**	**-1.82 +/- 0.13**	**-2.48 +/- 0.15**	**-3.04 +/- 0.09**
			Net % Reduction	**33.2% +/- 24%**	**63.4% +/- 10%**	**84.4% +/- 4.4%**	**87.8% +/- 3.9%**	**92% +/- 3.1%**	**98.4% +/- 0.4%**	**99.66% +/- 0.12%**	**99.91% +/- 0.02%**
Virus	MS2 (RNA Virus)	MS2 Mini Spd2-T1	*Net Log*	-0.22	-0.42	-0.55	-0.70	-0.76	-1.19	-1.85	-2.32
			Net % Reduction	40.0%	62.2%	72.0%	80.1%	82.8%	93.6%	98.6%	98.6%
Virus	MS2 (RNA Virus)	MS2 Mini Spd2-T2	*Net Log*	-0.05	-0.31	-0.32	-0.48	-0.53	-0.83	-1.58	-1.97
			Net % Reduction	10.6%	51.0%	52.2%	67.2%	70.2%	85.1%	97.4%	97.4%
Virus	MS2 (RNA Virus)	MS2 Mini Spd2-T3	*Net Log*	-0.41	-0.31	-0.43	-0.44	-0.88	-1.17	-1.78	-2.18
			Net % Reduction	61.0%	50.8%	62.8%	63.6%	86.8%	93.2%	98.3%	98.3%
All Trial Averages +/- St. Dev.			***Net Log***	**-0.23 +/- 0.18**	**-0.35 +/- 0.07**	**-0.43 +/- 0.12**	**-0.54 +/- 0.14**	**-0.72 +/- 0.18**	**-1.06 +/- 0.2**	**-1.74 +/- 0.14**	**-2.16 +/- 0.18**
			Net % Reduction	**37.2% +/- 25.3%**	**54.7% +/- 6.5%**	**62.3% +/- 9.9%**	**70.3% +/- 8.7%**	**79.9% +/- 8.7%**	**90.6% +/- 4.8%**	**98.1% +/- 0.6%**	**98.1% +/- 0.6%**

Figure 17: Executive Summary. Net log and associated percent reduction values for the CerroZone Mini at each timepoint.

Clean Air Delivery Rate Calculations (CADR)

The clean air delivery rate (CADR) was calculated for the CerroZone Mini at Normal and High fan speeds. The clean air delivery rate is the volume of air that has been purified of specific particles of interest, in this study, MS2 was the bioaerosol being assessed. This is calculated using the fraction of particles removed, multiplied by the volumetric flow rate, typically in cubic feet per minute (CFM) of the device.

For CADR calculations, the difference in slopes for the average of three control and test trials was calculated to determine the equivalent air exchange rate. The slope of the test trials was determined using the entire trial data of the natural log of the bioaerosol concentration reduction over time. The CADR was then calculated by multiplying the equivalent air exchange rate by the volume of the test chamber (30 m³). **Figure 18** shows a graphical example of the CADR calculations performed.

The CADR was calculated for each trial and averaged for a representative CADR. The CerroZone Mini on normal speed averaged 88.05 +/- 5.45 and on high 137.04 +/- 12.38 CADR in cubic feet per minute. A graphical summary of the results can be found in **Figure 19.**

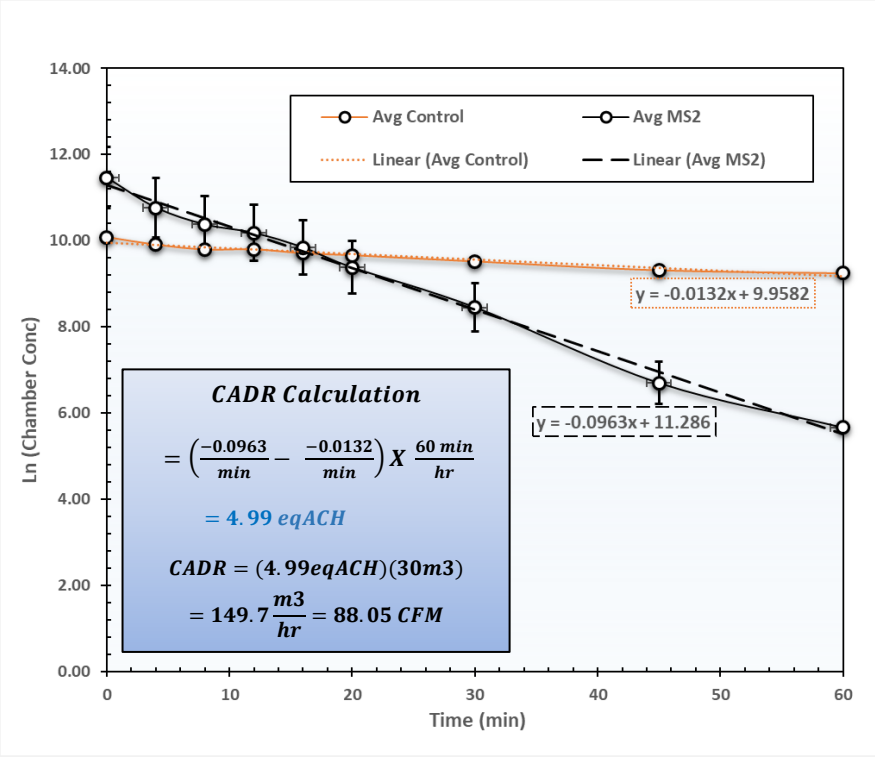

Figure 18: Graphical Method to compute Clean Air Delivery Rate from Actual Trial Test Data. The linear reduction slope of the natural log of bioaerosol chamber concentrations over time of both the natural decay and device decay were used to calculate the eqACH. The eqACH was multiplied by the volume of the test chamber to determine the the CADR .

The CADR calculated for the CerroZone Mini was 88.05 +/- 5.45 and on high 137.04 +/- 12.38 CADR in cubic feet per minute allows it to operate in several areas but is potentially limited by the number of people that are present based off on the stringent CADR requirements. However, the CADR and overall net log reduction shows its ability to reduce the viability of aerosolized viruses in each area.

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.

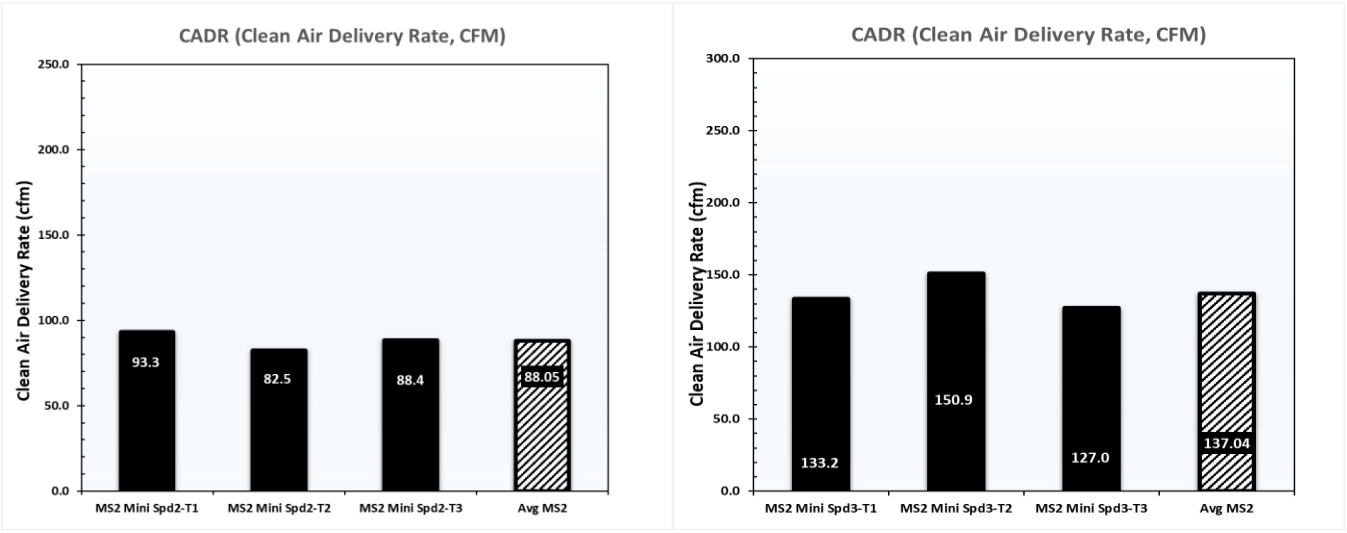

Figure 19: CADR for aerosolized *MS2* by the CerroZone Mini.

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Analytical Testing Facility

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

Project

10880.150.1

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 ASHRAE 241, AHAM AC-5, and 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:

9/27/2023
Richard Ludwick — Date
Study Director
ARE Labs, Inc.

Principal Investigator:

9/27/2023
Sean McLeod — Date
Staff Research Scientist
ARE Labs, Inc.

APPENDIX A: Bioaerosol Raw Data

Trial Information

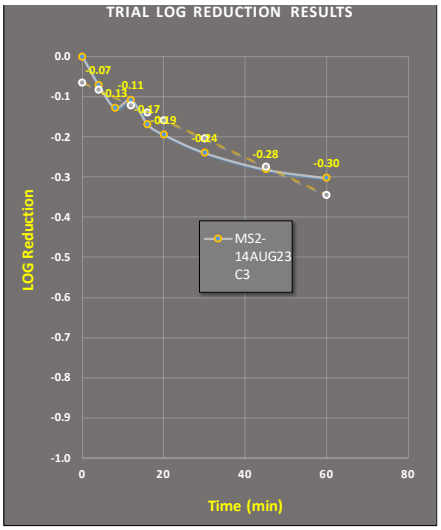
TEST DATE: Monday, August 14, 2023
TRIAL PERFORMED BY: SMM
TRIAL NUMBER: C1
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2-14AUG23 C3

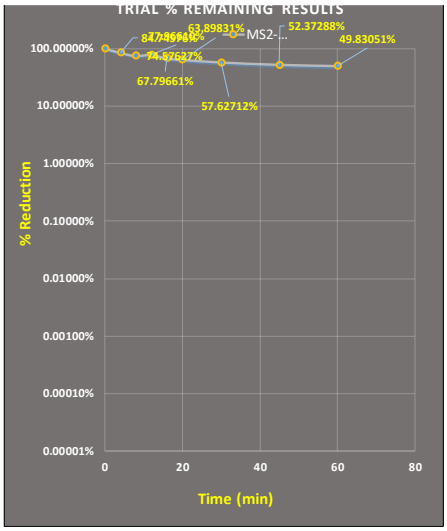
Device Information

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): 30
NEBULIZER CONDITIONS: Collison 24-Jet; approx. 10 min neb
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: HEPA Filters Capped





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	7.867E+03	6.667E+03	5.867E+03	6.133E+03	5.333E+03	5.027E+03	4.533E+03	4120.000	3920.000
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	3.33%					42.92%	21.05%	52.86%	
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**7.867E+03**	**6.667E+03**	**5.867E+03**	**6.133E+03**	**5.333E+03**	**5.027E+03**	**4.533E+03**	**4.120E+03**	**3.920E+03**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**84.7458%**	**74.5763%**	**77.9661%**	**67.7966%**	**63.8983%**	**57.6271%**	**52.3729%**	**49.8305%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**15.2542%**	**25.4237%**	**22.0339%**	**32.2034%**	**36.1017%**	**42.3729%**	**47.6271%**	**50.1695%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.07**	**-0.13**	**-0.11**	**-0.17**	**-0.19**	**-0.24**	**-0.28**	**-0.30**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1 **DILUTION RATIO (10^x)**	**-3**	**-2**	**-2**	**-2**	**-2**	**-2**	**-2**	**-2**	**-2**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
ENUMERATED PLATE COUNTS (# / drop)	1	10	13	5	5	7	6	7	
	1	9	4	10	6	5	8	10	
	1	6	5	8	9	12	5	4	
PLATE AVERAGE COUNT (# / drop)	1.00	8.33	7.33	7.67	6.67	8.00	6.33	7.00	
IMPINGER CONCENTRATION (cfu or pfu/ml)	10,000	8,333	7,333	7,667	6,667	8,000	6,333	7,000	
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**8.00E+03**	**6.67E+03**	**5.87E+03**	**6.13E+03**	**5.33E+03**	**6.40E+03**	**5.07E+03**	**5.60E+03**	
Dilution Range #1 **DILUTION RATIO (10^x)**	**-2**	**-1**	**-1**	**-1**	**-1**	**-1**	**-1**	**-1**	**-1**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
ENUMERATED PLATE COUNTS (# / drop)	5					47	46	36	54
	9					44	56	35	37
	15					46	48	28	56
PLATE AVERAGE COUNT (# / drop)	9.67					45.67	50.00	33.00	49.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	9,667					4,567	5,000	3,300	4,900
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**7.73E+03**					**3.65E+03**	**4.00E+03**	**2.64E+03**	**3.92E+03**

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

Trial Information

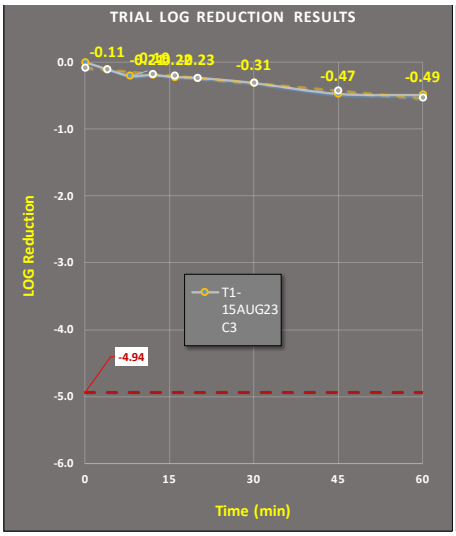
TEST DATE: Wednesday, August 16, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: C2
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): T1-15AUG23 C3

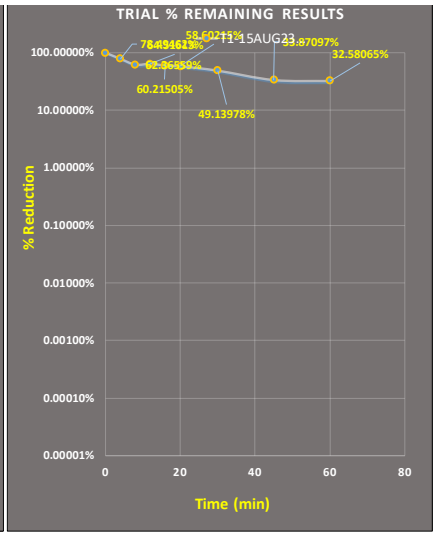
Device Information

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): 30
NEBULIZER CONDITIONS: Collison 24-Jet; approx. 10 min neb
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: HEPA Filters Uncapped





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.240E+04	9.733E+03	7.733E+03	8.000E+03	7.467E+03	7.267E+03	6.093E+03	4200.000	4040.000
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	67.14%	54.00%				63.75%	47.67%	42.50%	40.53%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.240E+04**	**9.733E+03**	**7.733E+03**	**8.000E+03**	**7.467E+03**	**7.267E+03**	**6.093E+03**	**4.200E+03**	**4.040E+03**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**78.4946%**	**62.3656%**	**64.5161%**	**60.2151%**	**58.6022%**	**49.1398%**	**33.8710%**	**32.5806%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**21.5054%**	**37.6344%**	**35.4839%**	**39.7849%**	**41.3978%**	**50.8602%**	**66.1290%**	**67.4194%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.11**	**-0.21**	**-0.19**	**-0.22**	**-0.23**	**-0.31**	**-0.47**	**-0.49**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
	IMPINGER FLOW RATE (lpm)	12.5	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**	**-2**	**-2**	**-2**	**-2**	**-2**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	1	1	7	7	7	16	8	5	7
		3	2	10	17	5	12	12	6	6
		3	2	12	6	16	12	10	9	6
	PLATE AVERAGE COUNT (# / drop)	2.33	1.67	9.67	10.00	9.33	13.33	10.00	6.67	6.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	23,333	16,667	9,667	10,000	9,333	13,333	10,000	6,667	6,333
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.87E+04**	**1.33E+04**	**7.73E+03**	**8.00E+03**	**7.47E+03**	**1.07E+04**	**8.00E+03**	**5.33E+03**	**5.07E+03**
Dilution Range #1	**DILUTION RATIO (10^x)**	**-2**	**-2**	**-1**	**-1**	**-1**	**-1**	**-1**	**-1**	**-1**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	8	5				46	46	47	45
		6	8				45	60	29	29
		9	10				54	51	39	39
	PLATE AVERAGE COUNT (# / drop)	7.67	7.67				48.33	52.33	38.33	37.67
	IMPINGER CONCENTRATION (cfu or pfu/ml)	7,667	7,667				4,833	5,233	3,833	3,767
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**6.13E+03**	**6.13E+03**				**3.87E+03**	**4.19E+03**	**3.07E+03**	**3.01E+03**

Figure 2A: *Control Trial 2 Bioaerosol Raw Data.*

Trial Information

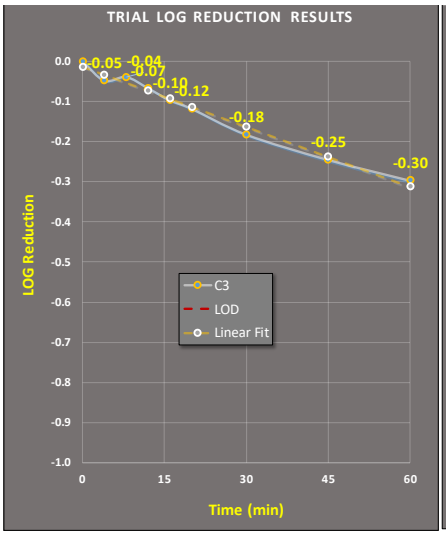
TEST DATE: Wednesday, August 16, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: C3
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): C3

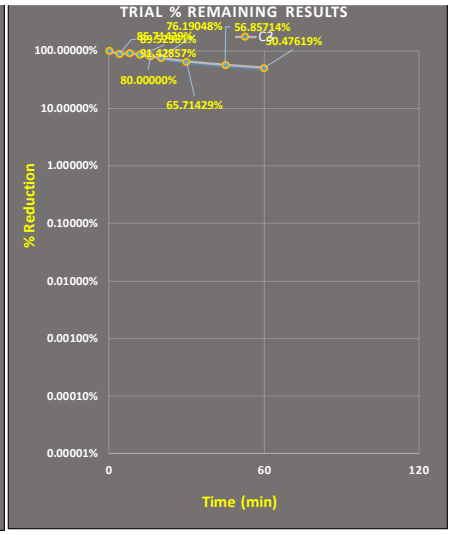
Device Information

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): 30
NEBULIZER CONDITIONS: Collison 24-Jet; approx. 10 min neb
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: HEPA Filters Uncapped





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.400E+05	1.253E+05	1.280E+05	1.200E+05	1.120E+05	1.067E+05	9.200E+04	79600.000	70666.667
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	9.09%		8.00%	50.00%	32.00%		67.31%	61.16%	52.78%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.400E+05**	**1.253E+05**	**1.280E+05**	**1.200E+05**	**1.120E+05**	**1.067E+05**	**9.200E+04**	**7.960E+04**	**7.067E+04**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**89.5238%**	**91.4286%**	**85.7143%**	**80.0000%**	**76.1905%**	**65.7143%**	**56.8571%**	**50.4762%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**10.4762%**	**8.5714%**	**14.2857%**	**20.0000%**	**23.8095%**	**34.2857%**	**43.1429%**	**49.5238%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.05**	**-0.04**	**-0.07**	**-0.10**	**-0.12**	**-0.18**	**-0.25**	**-0.30**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1 **DILUTION RATIO (10^x)**	**-4**	**-3**	**-4**	**-4**	**-4**	**-4**	**-3**	**-3**	**-3**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
ENUMERATED PLATE COUNTS (# / drop)	3	16	1	1	1		24	11	14
	1	15	2	2	2		15	12	12
	1	16	2	0	2		13	20	10
PLATE AVERAGE COUNT (# / drop)	1.67	15.67	1.67	1.00	1.67		17.33	14.33	12.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	166,667	156,667	166,667	100,000	166,667		173,333	143,333	120,000
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.33E+05**	**1.25E+05**	**1.33E+05**	**8.00E+04**	**1.33E+05**		**1.39E+05**	**1.15E+05**	**9.60E+04**
Dilution Range #1 **DILUTION RATIO (10^x)**	**-3**	**-2**	**-3**	**-3**	**-3**	**-3**	**-2**	**-2**	**-2**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
ENUMERATED PLATE COUNTS (# / drop)	25		20	15	9	20	43	42	58
	15		13	20	12	9	61	68	51
	15		13	25	13	11	66	57	61
PLATE AVERAGE COUNT (# / drop)	18.33		15.33	20.00	11.33	13.33	56.67	55.67	56.67
IMPINGER CONCENTRATION (cfu or pfu/ml)	183,333		153,333	200,000	113,333	133,333	56,667	55,667	56,667
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.47E+05**		**1.23E+05**	**1.60E+05**	**9.07E+04**	**1.07E+05**	**4.53E+04**	**4.45E+04**	**4.53E+04**

Figure 3A: *Control Trial 3 Bioaerosol Raw Data.*

Trial Information

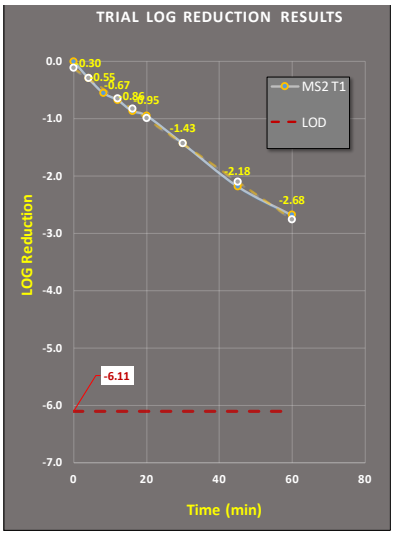
TEST DATE: Thursday, August 24, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T1
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T1

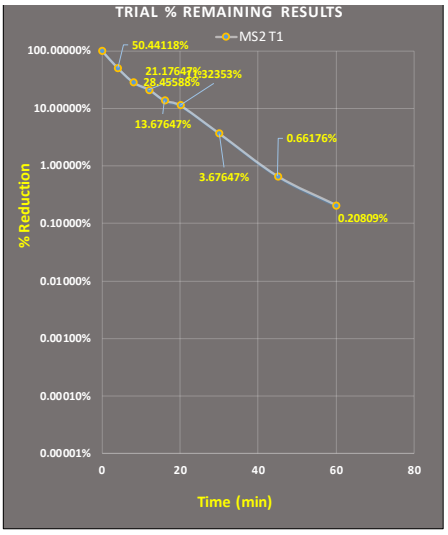
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mini
FAN SPEED (CFM): 110
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: Na

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
CHAMBER MIXING FAN: yes, Speed 6
TEMP (F): 74
RH (%): 50-60
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: Jeffs Frozen Stock Used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.813E+05	9.147E+04	5.160E+04	3.840E+04	2.480E+04	2.053E+04	6.667E+03	1200.000	377.333
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	30.00%	57.08%	24.09%						42.78%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.813E+05**	**9.147E+04**	**5.160E+04**	**3.840E+04**	**2.480E+04**	**2.053E+04**	**6.667E+03**	**1.200E+03**	**3.773E+02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**50.4412%**	**28.4559%**	**21.1765%**	**13.6765%**	**11.3235%**	**3.6765%**	**0.6618%**	**0.2081%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**49.5588%**	**71.5441%**	**78.8235%**	**86.3235%**	**88.6765%**	**96.3235%**	**99.3382%**	**99.7919%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.30**	**-0.55**	**-0.67**	**-0.86**	**-0.95**	**-1.43**	**-2.18**	**-2.68**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
DILUTION RATIO (10^x)	**-4**	**-3**	**-2**	**-2**	**-2**	**-2**	**-2**	**-2**	**-1**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	1	12	60	51	35	26	8		5
	4	17	51	40	26	30	8		6
	3	19	56	53	32	21	9		7
PLATE AVERAGE COUNT (# / drop)	2.67	16.00	55.67	48.00	31.00	25.67	8.33		6.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	266,667	160,000	55,667	48,000	31,000	25,667	8,333		600
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**2.13E+05**	**1.28E+05**	**4.45E+04**	**3.84E+04**	**2.48E+04**	**2.05E+04**	**6.67E+03**		**4.80E+02**
DILUTION RATIO (10^x)	**-3**	**-2**	**-3**	**-2**	**-2**	**-1**	**-1**	**-1**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	20	59	5					14	27
	18	73	8					18	36
	18	74	9					13	40
PLATE AVERAGE COUNT (# / drop)	18.67	68.67	7.33					15.00	34.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	186,667	68,667	73,333					1,500	343
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.49E+05**	**5.49E+04**	**5.87E+04**					**1.20E+03**	**2.75E+02**

Figure 4A: *CerroZone Mini Normal Speed T1 Bioaerosol Raw Data.*

Trial Information

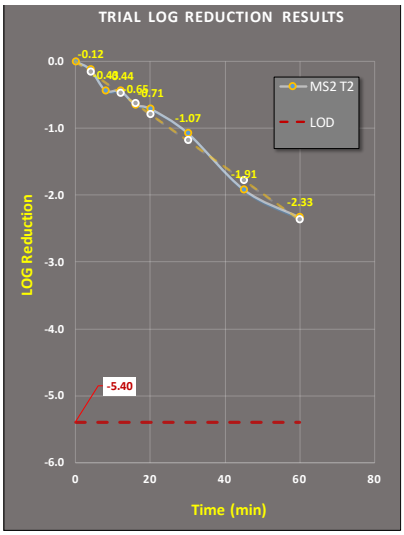
TEST DATE: Friday, August 25, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T2
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T2

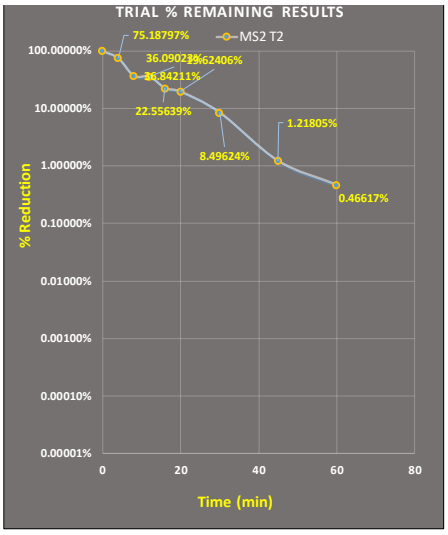
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mini
FAN SPEED (CFM): 110
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: Na

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
CHAMBER MIXING FAN: yes, Speed 6
TEMP (F): 74
RH (%): 50-60
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES Jeffs Frozen Stock Used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	3.547E+04	2.667E+04	1.307E+04	1.280E+04	8.000E+03	6.960E+03	3.013E+03	432.000	165.333
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	10.00%					55.00%		20.00%	
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**3.547E+04**	**2.667E+04**	**1.307E+04**	**1.280E+04**	**8.000E+03**	**6.960E+03**	**3.013E+03**	**4.320E+02**	**1.653E+02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**75.1880%**	**36.8421%**	**36.0902%**	**22.5564%**	**19.6241%**	**8.4962%**	**1.2180%**	**0.4662%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**24.8120%**	**63.1579%**	**63.9098%**	**77.4436%**	**80.3759%**	**91.5038%**	**98.7820%**	**99.5338%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.12**	**-0.43**	**-0.44**	**-0.65**	**-0.71**	**-1.07**	**-1.91**	**-2.33**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
DILUTION RATIO (10^x)	**-3**	**-3**	**-3**	**-3**	**-2**	**-2**	**-2**	**-1**	**-1**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	5	4			10	15		6	
	5	3			8	8		6	
	4	3			12	13		6	
PLATE AVERAGE COUNT (# / drop)	4.67	3.33			10.00	12.00		6.00	
IMPINGER CONCENTRATION (cfu or pfu/ml)	46,667	33,333			10,000	12,000		600	
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**3.73E+04**	**2.67E+04**			**8.00E+03**	**9.60E+03**		**4.80E+02**	
DILUTION RATIO (10^x)	**-2**	**-2**	**-2**	**-2**	**-1**	**-1**	**-1**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	47		17	16		49	38	57	16
	39		19	16		60	30	45	25
	40		13	16		53	45	42	21
PLATE AVERAGE COUNT (# / drop)	42.00		16.33	16.00		54.00	37.67	48.00	20.67
IMPINGER CONCENTRATION (cfu or pfu/ml)	42,000		16,333	16,000		5,400	3,767	480	207
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**3.36E+04**		**1.31E+04**	**1.28E+04**		**4.32E+03**	**3.01E+03**	**3.84E+02**	**1.65E+02**

Figure 5A: *CerroZone Mini Normal Speed T2 Bioaerosol Raw Data.*

Trial Information

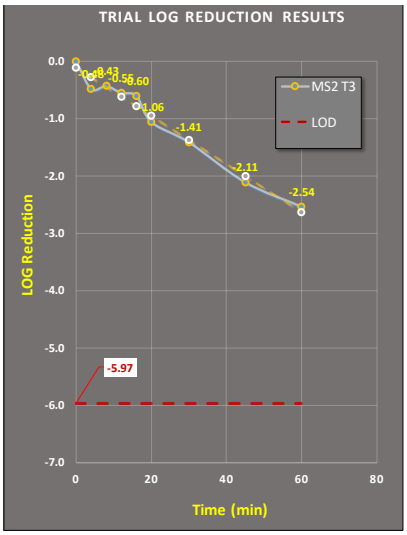
TEST DATE: Friday, August 25, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T3
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T3

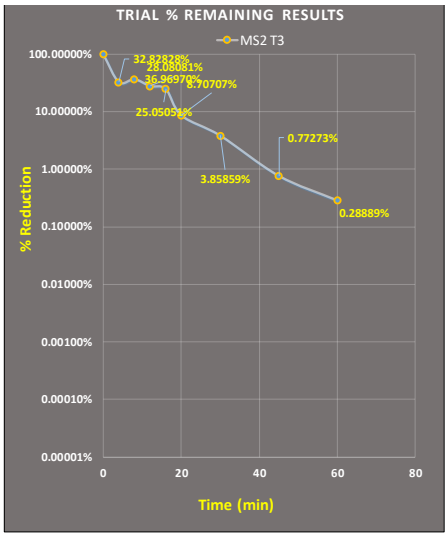
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mini
FAN SPEED (CFM): 110
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: Na

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
CHAMBER MIXING FAN: yes, Speed 6
TEMP (F): 74
RH (%): 50-60
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES Jeffs Frozen Stock Used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.320E+05	4.333E+04	4.880E+04	3.707E+04	3.307E+04	1.149E+04	5.093E+03	1020.000	381.333
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	58.57%	3.03%	17.00%			76.86%		43.88%	
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.320E+05**	**4.333E+04**	**4.880E+04**	**3.707E+04**	**3.307E+04**	**1.149E+04**	**5.093E+03**	**1.020E+03**	**3.813E+02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**32.8283%**	**36.9697%**	**28.0808%**	**25.0505%**	**8.7071%**	**3.8586%**	**0.7727%**	**0.2889%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**67.1717%**	**63.0303%**	**71.9192%**	**74.9495%**	**91.2929%**	**96.1414%**	**99.2273%**	**99.7111%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.48**	**-0.43**	**-0.55**	**-0.60**	**-1.06**	**-1.41**	**-2.11**	**-2.54**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
DILUTION RATIO (10^x)	**-4**	**-3**	**-3**	**-3**	**-2**	**-2**	**-2**	**-1**	**-1**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	4	8	9		42	20		15	
	1	4	9		42	20		14	
	2	4	2		40	30		20	
PLATE AVERAGE COUNT (# / drop)	2.33	5.33	6.67		41.33	23.33		16.33	
IMPINGER CONCENTRATION (cfu or pfu/ml)	233,333	53,333	66,667		41,333	23,333		1,633	
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.87E+05**	**4.27E+04**	**5.33E+04**		**3.31E+04**	**1.87E+04**		**1.31E+03**	
DILUTION RATIO (10^x)	**-3**	**-2**	**-2**	**-2**	**-1**	**-1**	**-1**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	8	70	54	38		49	52	109	44
	9	45	63	42		60	58	85	42
	12	50	49	59		53	81	81	57
PLATE AVERAGE COUNT (# / drop)	9.67	55.00	55.33	46.33		54.00	63.67	91.67	47.67
IMPINGER CONCENTRATION (cfu or pfu/ml)	96,667	55,000	55,333	46,333		5,400	6,367	917	477
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**7.73E+04**	**4.40E+04**	**4.43E+04**	**3.71E+04**		**4.32E+03**	**5.09E+03**	**7.33E+02**	**3.81E+02**

Figure 6A: *CerroZone Mini Normal Speed T3 Bioaerosol Raw Data.*

Trial Information

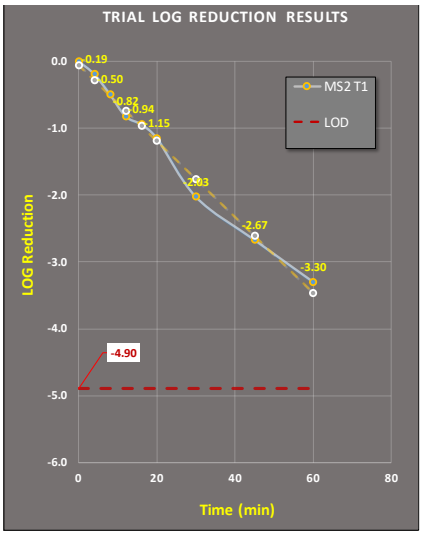
TEST DATE: Monday, August 21, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T1
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T1

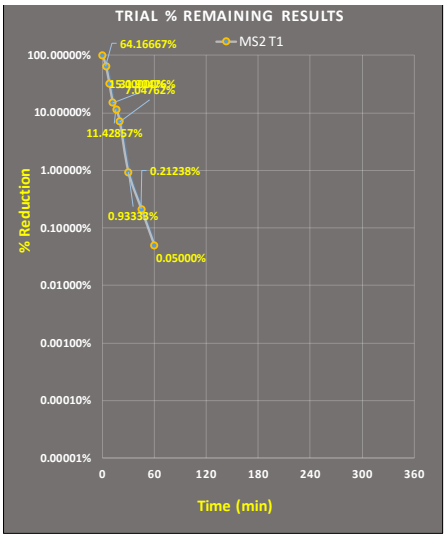
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mini
FAN SPEED (CFM): 200
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: Na

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
CHAMBER MIXING FAN: yes, Speed 6
TEMP (F): 74
RH (%): 50-60
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: Jeffs Frozen Stock Used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.120E+04	7.187E+03	3.573E+03	1.680E+03	1.280E+03	7.893E+02	1.045E+02	23.787	5.600
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	32.00%	68.54%	58.95%					53.77%	60.00%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.120E+04**	**7.187E+03**	**3.573E+03**	**1.680E+03**	**1.280E+03**	**7.893E+02**	**1.045E+02**	**2.379E+01**	**5.600E+00**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**64.1667%**	**31.9048%**	**15.0000%**	**11.4286%**	**7.0476%**	**0.9333%**	**0.2124%**	**0.0500%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**35.8333%**	**68.0952%**	**85.0000%**	**88.5714%**	**92.9524%**	**99.0667%**	**99.7876%**	**99.9500%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.19**	**-0.50**	**-0.82**	**-0.94**	**-1.15**	**-2.03**	**-2.67**	**-3.30**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	5.0	5.0	10.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1 **DILUTION RATIO (10^x)**	**-3**	**-1**	**-2**	**-1**	**-1**	**-1**	**0**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
ENUMERATED PLATE COUNTS (# / drop)	1	46	9	16	15	27	26	13	4
	2	44	4	25	20	17	26	20	6
	2	39	6	22	13	30	46	28	5
PLATE AVERAGE COUNT (# / drop)	1.67	43.00	6.33	21.00	16.00	24.67	32.67	20.33	5.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	16,667	4,300	6,333	2,100	1,600	2,467	327	203	50
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.33E+04**	**3.44E+03**	**5.07E+03**	**1.68E+03**	**1.28E+03**	**7.89E+02**	**1.05E+02**	**3.25E+01**	**8.00E+00**
Dilution Range #1 **DILUTION RATIO (10^x)**	**-2**	**-2**	**-1**	**0**	**-1**	**-1**	**0**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**500**	**500**	**500**
ENUMERATED PLATE COUNTS (# / drop)	10	10	36					47	8
	14	14	24						12
	10	17	18						
PLATE AVERAGE COUNT (# / drop)	11.33	13.67	26.00					47.00	10.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	11,333	13,667	2,600					94	20
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**9.07E+03**	**1.09E+04**	**2.08E+03**					**1.50E+01**	**3.20E+00**

Figure 7A: *CerroZone Mini High Speed T1 Bioaerosol Raw Data.*

Trial Information

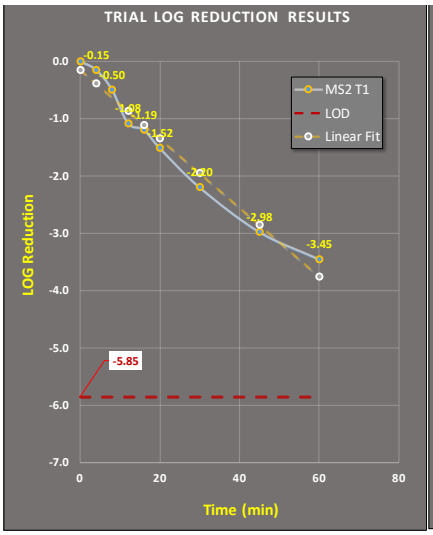
TEST DATE: Monday, August 21, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T2
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T1

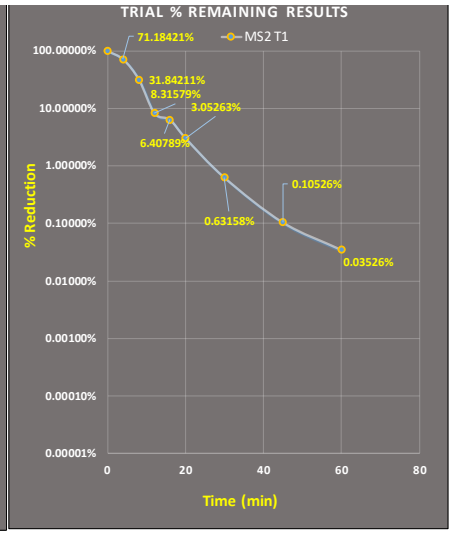
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mini
FAN SPEED (CFM): 200
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: Na

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
CHAMBER MIXING FAN: yes, Speed 6
TEMP (F): 74
RH (%): 50-60
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: Jeffs Frozen Stock Used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.013E+05	7.213E+04	3.227E+04	8.427E+03	6.493E+03	3.093E+03	6.400E+02	106.667	35.733
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		45.43%		37.95%	37.67%				
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.013E+05**	**7.213E+04**	**3.227E+04**	**8.427E+03**	**6.493E+03**	**3.093E+03**	**6.400E+02**	**1.067E+02**	**3.573E+01**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**71.1842%**	**31.8421%**	**8.3158%**	**6.4079%**	**3.0526%**	**0.6316%**	**0.1053%**	**0.0353%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**28.8158%**	**68.1579%**	**91.6842%**	**93.5921%**	**96.9474%**	**99.3684%**	**99.8947%**	**99.9647%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.15**	**-0.50**	**-1.08**	**-1.19**	**-1.52**	**-2.20**	**-2.98**	**-3.45**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	5.0	5.0	10.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
DILUTION RATIO (10^x)	**-4**	**-3**	**-2**	**-1**	**-1**	**-1**	**-1**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)		10	38	88	67			67	17
		17	42	96	57			57	20
		8	41	58	63			76	30
PLATE AVERAGE COUNT (# / drop)		11.67	40.33	80.67	62.33			66.67	22.33
IMPINGER CONCENTRATION (cfu or pfu/ml)		116,667	40,333	8,067	6,233			667	223
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		**9.33E+04**	**3.23E+04**	**6.45E+03**	**4.99E+03**			**1.07E+02**	**3.57E+01**
DILUTION RATIO (10^x)	**-3**	**-2**	**-1**	**-2**	**-2**	**-2**	**-2**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**500**	**500**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	17	58		12	10	15	1		
	13	67		13	8	6	2		
	8	66		14	12	8	3		
PLATE AVERAGE COUNT (# / drop)	12.67	63.67		13.00	10.00	9.67	2.00		
IMPINGER CONCENTRATION (cfu or pfu/ml)	126,667	63,667		13,000	10,000	9,667	2,000		
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.01E+05**	**5.09E+04**		**1.04E+04**	**8.00E+03**	**3.09E+03**	**6.40E+02**		

Figure 8A:. *CerroZone Mini High Speed T2 Bioaerosol Raw Data.*

Trial Information

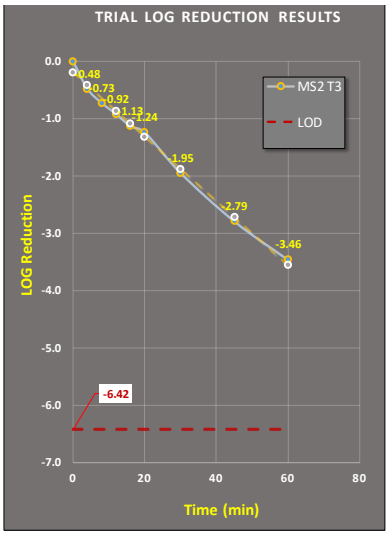
TEST DATE: Monday, August 21, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: T3
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T3

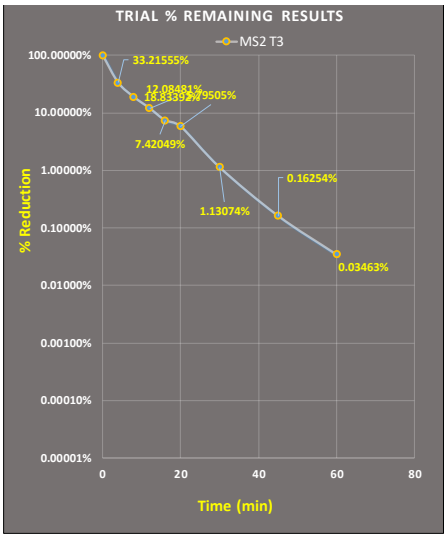
Device Information

MANUFACTURER: CerroZone
UNIT MODEL: Mini
FAN SPEED (CFM): 200
UNIT SERIAL #: NA
FITER ID #: NA
FILTER LOT #: Na

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
CHAMBER MIXING FAN: yes, Speed 6
TEMP (F): 74
RH (%): 50-60
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES: Jeffs Frozen Stock Used





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	3.773E+05	1.253E+05	7.107E+04	4.560E+04	2.800E+04	2.187E+04	4.267E+03	613.333	130.667
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	33.53%		38.48%					23.08%	55.88%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**3.773E+05**	**1.253E+05**	**7.107E+04**	**4.560E+04**	**2.800E+04**	**2.187E+04**	**4.267E+03**	**6.133E+02**	**1.307E+02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**33.2155%**	**18.8339%**	**12.0848%**	**7.4205%**	**5.7951%**	**1.1307%**	**0.1625%**	**0.0346%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**66.7845%**	**81.1661%**	**87.9152%**	**92.5795%**	**94.2049%**	**98.8693%**	**99.8375%**	**99.9654%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.48**	**-0.73**	**-0.92**	**-1.13**	**-1.24**	**-1.95**	**-2.79**	**-3.46**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
DILUTION RATIO (10^x)	**-4**	**-3**	**-2**	**-2**	**-2**	**-2**	**-2**	**-1**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	3	16	68	59	36	31		9	20
	6	16	65	57	33	28		8	21
	8	15	70	55	36	23		9	27
PLATE AVERAGE COUNT (# / drop)	5.67	15.67	67.67	57.00	35.00	27.33		8.67	22.67
IMPINGER CONCENTRATION (cfu or pfu/ml)	566,667	156,667	67,667	57,000	35,000	27,333		867	227
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**4.53E+05**	**1.25E+05**	**5.41E+04**	**4.56E+04**	**2.80E+04**	**2.19E+04**		**6.93E+02**	**1.81E+02**
DILUTION RATIO (10^x)	**-3**	**-2**	**-3**	**-1**	**-2**	**-2**	**-1**	**0**	**-1**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	33		12				50	66	
	36		10				50	58	2
	44		11				60	76	0
PLATE AVERAGE COUNT (# / drop)	37.67		11.00				53.33	66.67	1.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	376,667		110,000				5,333	667	100
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**3.01E+05**		**8.80E+04**				**4.27E+03**	**5.33E+02**	**8.00E+01**

Figure 9A: *CerroZone Mini High Speed T3 Bioaerosol Raw Data.*

Appendix B: 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:

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

ASHRAE 241-2023 Standard Safety Analysis of the CerroZone Mini Device

Sean McLeod [a], *Richard Ludwick* [a], *Jamie Balarashti* [a]

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

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Project # 10880.150.1.2

9/27/2023

ASHRAE 241-2023 Standard Safety Analysis of the CerroZone Mini Device

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[a] Aerosol Research and Engineering Laboratories Inc. Olathe KS

Report Info

Submitter:
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Testing Lab:
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Report History:
CerroZone Mini
Safety Monitoring
Project Number: 10880.150.1.2
Submitted: 22Sep23

Associated Report:
CerroZone Mini
Bioaerosol Efficacy Against MS2
Project Number: 10880.150.1.1
Submitted: 22Sep23

Keywords:
· *Bioaerosol Efficacy*
· *Safety Analysis*
· *ASHRAE 241-2023*

ASHRAE 241-2023 Compliance:
This study was conducted in compliance with ASHRAE 241 and AHAM AC-5 along with 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 three specific safety related items, per ASHRAE 241-2023, of the CerroZone Mini device while it was in normal operation. The four items that were measured were for the potential:
1) particulate generation from the device, (parts/meter cubed),
2) ozone production from the unit itself, (parts per billion), and
3) formaldehyde production rate from limonene (microgram/hour emitted).

Background:
The CerroZone Mini 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 30m^3 bioaerosol test chamber which housed the device. The safety testing included analysis of particulate generation, ozone, and formaldehyde production, and the sound level generated by the device. This study utilizes ASHRAE 241 testing parameters to determine safety. The system effectiveness testing information can be found in the associated **Report #10880.150.1.1.**

Methods:
The test device was placed into a sealed 30m^3 test chamber then switched on. Per ASHRAE 241 a standard safety analysis was performed by measuring the following:
1) particulate generation using a TSI Aerodynamic Particle Sizer (APS) Model 3321,
2) ozone production using a Teledyne Model 456L ozone monitor, and
3) formaldehyde production using an Interscan 4160.

Each safety item was tested individually, and the data was logged over the 4-hour test period while the device was in operation. Control trials (background) were performed to establish the background levels of particulates, ozone, and formaldehyde. The background control data was subtracted from the test data to yield a net value for each of the parameters that were monitored. Trials were performed in duplicate.

Results:
Safety tests were performed in duplicate, and the device did not exceed any target limits during testing. Formaldehyde emissions averaged -2.76 µg/hour, ozone levels did not exceed 5 parts per billion (ppb), and particulate matter counts did not exceed the ISO class 6 requirement.

Conclusion:
The safety test values for the CerroZone Mini air purifier were observed to be within target limits as stated in the ASHRAE 241 testing standard.

Introduction

On June 24th, 2023, the new ASHRAE 241-2023 guidelines were released to establish a more uniform testing protocol for all air purification devices. This protocol standardized all components of safety testing for both in-duct and standalone devices. This safety report details the testing and results of four specific safety tests, based on ASHRAE 241 guidance document requirements, performed on the CerroZone Mini device.

The ASHRAE standard includes guidelines for particulate, ozone, formaldehyde, and noise generation. With these new guidelines, testing must be done on all air purification devices that are listed as adhering to these ASHRAE 241 standards.

The test plan for the CerroZone's Mini device incorporated the applicable ASHRAE 241 safety requirements. The testing was performed in a 30m^3 test chamber. The test device is pictured in **Figure 1.**

Figure 1: **CerroZone Mini Test Device**

STUDY OVERVIEW

The safety of the CerroZone Mini device was evaluated against three different criteria to demonstrate safety of the device. This allowed for a reasonable demonstration and evaluation of the safety of the device while operating in the intended manner. This study was done in accordance with ASHRAE 241 standards. This guidance document details specific requirements for testing each of the criteria.

This is one of two reports that detail the test results for the ASHRAE 241 and AHAM AC-5 testing of the CerroZone Mini. This report presents the safety testing parameters, data, and results, while the other report details the bioaerosol efficacy testing results (**Report #10880.150.1.1**) by ASHRAE 241 and AHAM testing guidelines). The test matrix outlining the safety testing can be found in **Figure 2** below.

PRODUCT DESCRIPTION

The CerroZone Mini 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 96 +/- 30 CFM on Speed 2 and 179 +/- 30 CFM on Speed 3 and exposes airborne pathogens to ozone and UVC light within the device.

TEST CHAMBER VALIDATION

Validating a bioaerosol chamber is a crucial process to ensure its accuracy and reliability in maintaining controlled experiments. This involves thorough assessments to confirm that the chamber met the strict standards for conducting bioaerosol studies.

Factors such as chamber homogeneity, ionization assessment, air exchange rates, and control stability are rigorously tested to ensure consistent and accurate results. Validation assures researchers that the chamber functions properly, enabling them to conduct reliable bioaerosol studies that contribute to informed decision-making in areas like indoor air quality and infectious disease research.

Equipment used for Test Chamber Validation

Testing Chamber

The test chamber is the main component in safety testing used for controlled manipulation and testing of microorganisms. It allows for the introduction, sampling, and secure confinement of test analytes, 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.

Trial	Run	Device	Device Fan Speed (ft^3/min)	Test Species	Abbreviation	Chamber Size (m^3)	Detection Limit	Trial Time (hr)	Sampling Devices
1	Control	NA	NA	Ozone	O_3	30	0 ppb	4	Teledyne 465L
2	Control								
3	Challenge	Mini	96 +/- 30						
4	Challenge								
5	Challenge		179 +/- 30						
6	Challenge								
7	Control	NA	NA	Particulate	Particles greater than 0.3 µm	30	1 pt/L	4	TSI 3321 APS
8	Control								
9	Challenge	Mini	96 +/- 30						
10	Challenge								
11	Challenge		179 +/- 30						
12	Challenge								
13	Control	NA	NA	Formaldehyde	HCHO	30	0.2 ppb	4	Interscan 4160-1999b
14	Control								
15	Challenge	Mini	96 +/- 30						
16	Challenge								
17	Challenge		179 +/- 30						
18	Challenge								

Figure 2: **Test Matrix.**

Structurally, the chamber has dimensions of 30 ± 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 FRP material, the walls are easily cleaned. 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 12 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: The 30 m^3 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.

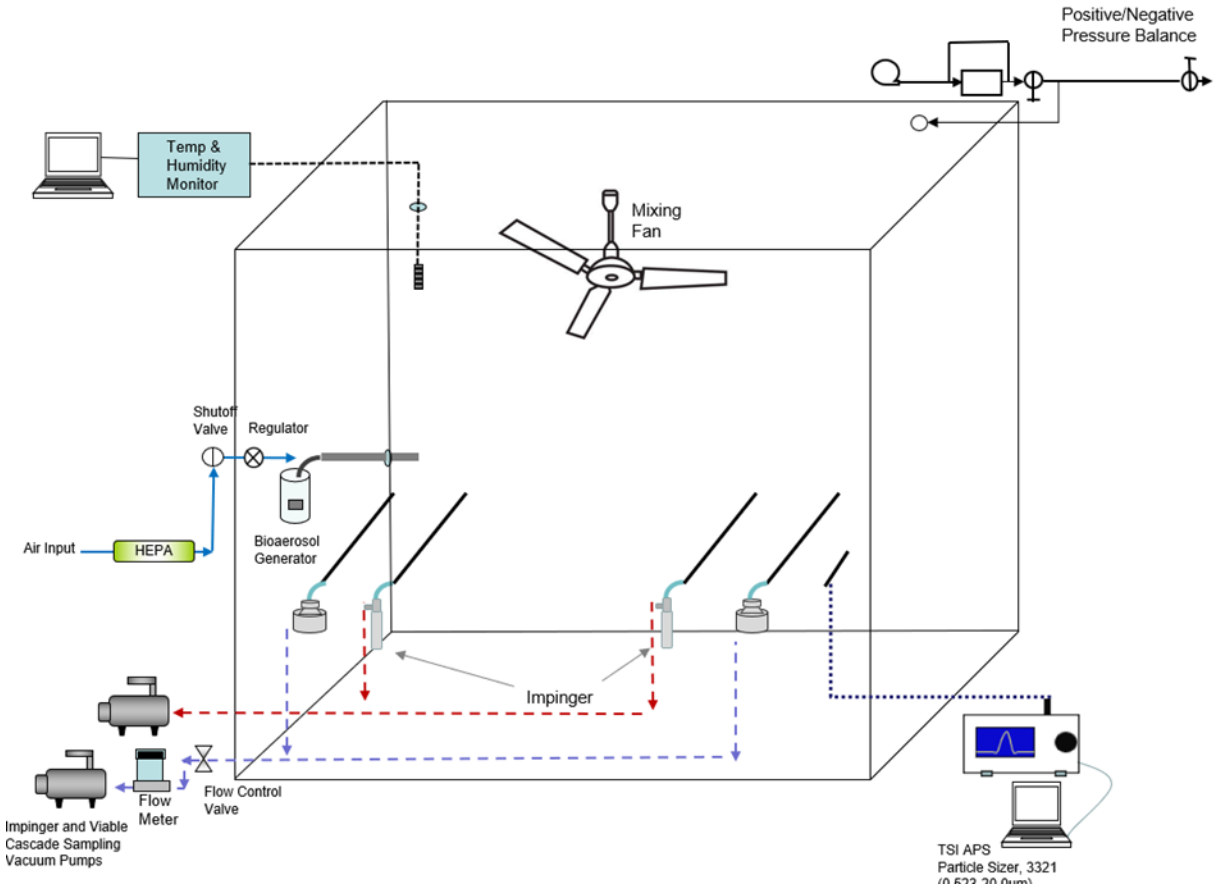


Figure 4: 30m^3 Environmental Test Chamber Flow Diagram. Chamber includes bioaerosol induction, multiple bioaerosol sampling ports, particle size monitoring, internal mixing fans, and temperature and humidity controls. Main system HEPA evacuation system not pictured.

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 3** shows the bioaerosol chamber used for all testing in this study. 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. A general flow diagram of the aerosol test system is shown in **Figure 4**.

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 30m³ bioaerosol chamber used for testing. Temperature and humidity control is essential for the stability of aerosolized micro-organisms during testing.

ASHRAE 241 and AHAM AC-5 both have temperature and humidity requirements for temperature and humidity inside of the bioaerosol chamber during testing. The required range for humidity is 50% ± 10% while the temperature range is 73°F + 5° (23°C + 3°C). A picture of the controller is shown in **Figure 5** below.

Figure 5: AC Infinity Controller 69 Temperature and Humidity Controller.

Ion Monitor

The COM ion meter, **Figure 6** below, measures ion concentrations in real time and was used during testing to ensure the ion concentrations were consistent inside the chamber.

Figure 6: COM 3200 Pro II ion meter used for ion measurements of the PA663 ionizer.

The ion meter measures ions using the Gerdien capacitor method and can detect positive and negative ions down to 10 per cubic centimeter. This was only used for the chamber validation aspect of the testing and not used during any portion of the safety testing as required for ASHRAE 241.

Testing Procedures

Bio-homogeneity – Impinger Tests

One key component of the chamber validation process is the bioaerosol homogeneity test. This test validates the homogeneity of the chamber, making sure that the atmosphere within the chamber is well mixed.

Six AGI-30 impingers were used for this chamber validation. The impingers were systematically rotated through all four impinger ports to generate a matrix of impinger tests against all ports. Each port was tested with each impinger a minimum of two times during this validation.

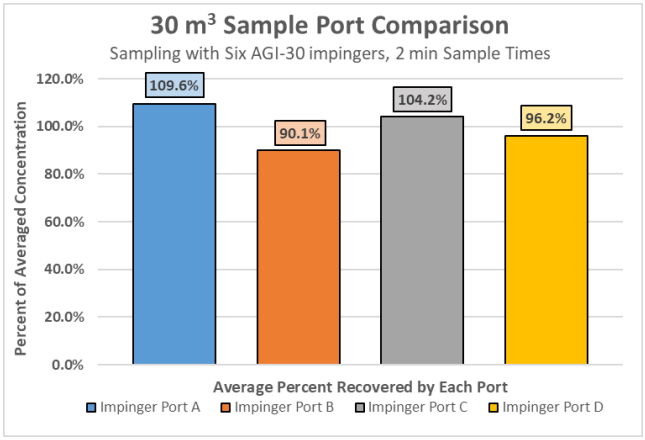


Figure 7: Impinger port-to-port comparison. Percent averages are calculated by taking the count for each port divided by the average plate count for the four ports.

These impinger samples were plated in triplicate by two technicians to reduce plating discrepancies. Each set of plate counts generated by each technician were compared to one another and a port-to-port comparison was created. This showed that each port of the 30m³ chamber produced a similar result to one another validating the chamber homogeneity during trials. A graphical representation of the average measured for each port is shown in **Figure 7**. While these results do not show gaseous homogeneity specifically, the homogeneity of a gas should be far more homogenous due to the much higher diffusion rate that gases have over aerosolized biologics.

Ionization Homogeneity Validation

To measure the baseline concentration of ions present in the sealed 30 m³ chamber over 4 hours, a COM 3200 Pro II ion meter was used. The chamber had an average net ion

concentration of -143.39 +/- 55.64 ions per cubic centimeter. Testing shows that the net ion concentration is essentially neutral in regard to the charge within the chamber. See ion data graph from trial in **Figure 8**. The total production of ions naturally occurring in the chamber is nominal.

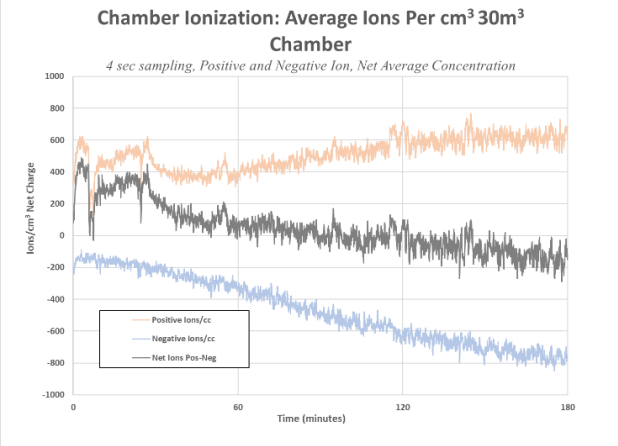


Figure 8. Total baseline level of ions measured in the 30m³ chamber.

These control tests implement the ANSI/AHAM AC-5 2022 guidelines, ensuring a thorough and precise assessment of air cleaner performance in reducing airborne microbes. The methodical approach, from preparation to measurement and analysis, underscores the importance of consistent and accurate testing procedures.

ASHRAE TESTING

Particulate Monitoring

Particulate Monitoring Instrumentation

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 testing. 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 9** below.

Figure 9. 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.

Particulate Test Methodology

Particulate Control Trials

In order to accurately assess the devices particulate output, the chamber, with no device, was measured first for aerosolized particulate concentrations. This allowed for a standard background to be established within the chamber. This was then used as comparison data for the device tested. The control test was run for 4 hours just as the test-trial duration. The chamber cannot exceed the ISO 5 Cleanliness Class based on Table 1 of ISO 14644-14. This table is provided in **Figure 10**.

ISO Class Number		Maximum allowable concentrations (particles/m3) for particles equal to and greater than the considered size					
		0.1 µm	0.2 µm	0.3 µm	0.5 µm	1 µm	5 µm
Cleanest	1	10					
	2	100	24	10			
	3	1,000	237	102	35		
	4	10,000	2,370	1,020	352	83	
(Class 100)	5	100,000	23,700	10,200	3,520	832	
(Class 1,000)	6	1,000,000	237,000	102,000	35,200	8,320	293
(Class 10,000)	7				352,000	83,200	2,930
(Class 100,000)	8				3,520,000	832,000	29,300
	9				35,200,000	8,320,000	293,000

ISO CLEANROOM

Figure 10. Particulate ISO classification particulate levels by particle size.

Device Test Trials

The device was placed into the test chamber and was subsequently turned on and monitored for a duration of 4 hours. This allowed for an adequate assessment of the total particulates generated by the device. This data was then compared to the control data to determine the net production of particulates produced by the device.

ASHRAE 241 Particulate Requirements

As stated in the ASHRAE 241 guidelines, the level of particulates produced by the device *"shall not exceed one cleanliness class greater than the empty test chamber or test duct as described in ISO 14644-14, Table 1."* Based on previous testing conducted and control trials run, the chamber at ARE Labs is classified as an ISO Class 5 Chamber. This in turn requires that the tested device needs to be classified at minimum as an ISO Class 6, based off of the ASHRAE 241 requirements. This might seem like a good classification method for device testing, but in actuality penalizes devices tested in less particulate filled chambers. For instance, if a chamber is class 4, the device can only expel 3,168 particles at 0.5 micron in order to pass while in a class 5 chamber the unit is allowed to expel 31,680 particles at the 0.5-micron level. Both are 10 times the previous, but the difference in number of particles allowed is significantly different.

Particulate Testing Results

After sampling the chamber with the CerroZone device in operation the average particle concentrations did not exceed the class 6 standard particulate levels inside the sealed test chamber. Results for particle monitoring are shown in Figure 11 below. The average total number of particles detected above the particle size range listed were used for concentrations. A total of 100, 1-minutes air samples were taken for statistical significance.

Mean Particle Concentration (pt/m^3)

Particle Size Range	Control 1	Control 2	Speed 2 Avg	Speed 3 Avg
≥0.3µm	9804.4	9481.2	46148.7	61837.8
≥0.5µm	109.5	110.1	22790.9	25058.6
≥ 1.0µm	7.6	7.0	807.1	547.2
≥ 5.0µm	0	0	8	0
ISO Classification	**5**	**5**	**6**	**6**

Figure 11. Particle Monitoring Results. The APS was used to monitor particle concentrations for a minimum of 100 samples twice with the device *off* and twice with the device *on*. These concentrations were used to classify the empty chamber and show the device does not raise the particle classification by greater than one level.

Particulate Testing Conclusion

The ASHRAE 241 standards provide a pass/fail criterion and given the device did not raise the classification of the test chamber, the device passed. The empty test chamber had particulate levels of the class 5 level, and the chamber with the device active was measured and did not exceed one cleanliness class greater than the test chamber. With the device in operation the chamber was ISO Class 6.

Ozone Monitoring

Ozone Monitoring Equipment

Model 465L Ozone Monitor

A Model 465L Ozone Monitor was used to measure chamber ozone concentrations during the device's safety test trial. The real-time ozone concentrations produced by the device were monitored and recorded automatically using specific software designed to capture these output readings. Sampling is performed every 20 seconds with this monitor allowing for multiple readings each minute of operation. The model 465L has a continuous flow rate of 0.8 liters per minute (LPM). A picture of the 465L Ozone Monitor is shown in Figure 12 below.

Ozone Monitoring Test Methodology

Ozone Control Test

In order to accurately assess the devices ozone production, the chamber, with no device, was measured first for background ozone concentrations. This allowed for a standard background to be established within the chamber. This was then used as comparison data for the tested device. The control test was run for 4 hours just like the official test duration. The chamber does not have an official level of ozone that it must be beneath in order for testing to occur.

Figure 12. The Teledyne Model 465L UV Photometric Ozone Monitor. This Ozone monitor can detect ozone levels as low as 0 ppb and up to 500 ppm while sampling at 0.8 L/min

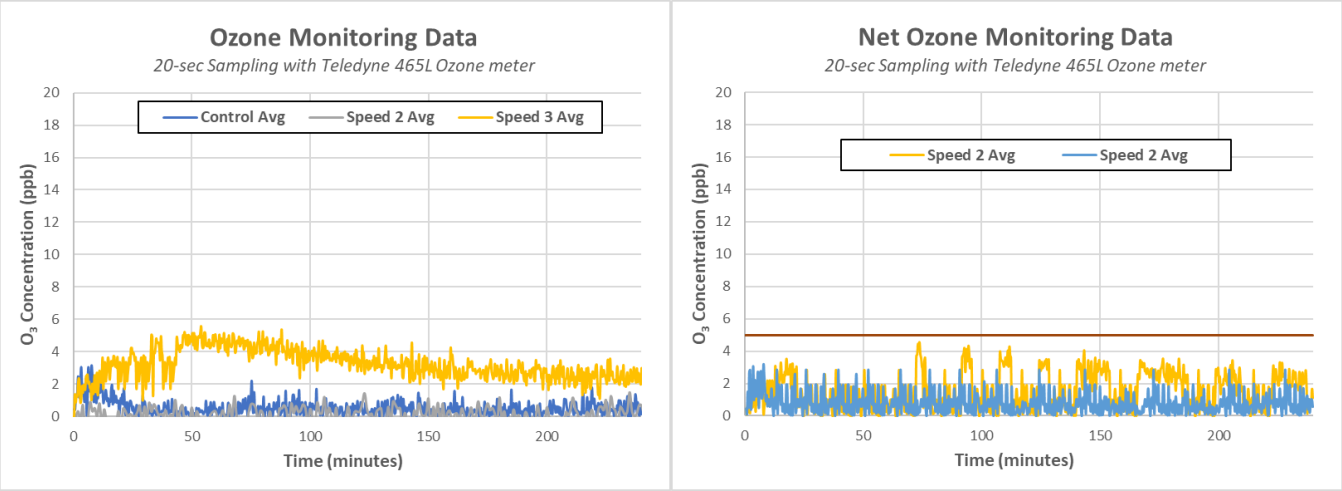


Figure 13. **Ozone Monitoring Results**. The ozone concentration over time for both tests with the test device *off* and both tests with the device *on*. 20-second sampling was performed with the Teledyne 465L ozone meter for a duration of 4-hours. Concentrations were normalized and the net ozone calculated by subtracting control measurements from trial data.

Ozone Test Trial

The device was placed into the test chamber and was subsequently turned on and monitored for a duration of 4 hours. This data was then compared to the control data to determine the net production of ozone produced by the device.

ASHRAE 241 Ozone Production Guidelines

The ASHRAE 241 requirements for ozone production are very low, only allowing for the tested device to produce <5ppb of ozone in 4 hours of testing.

Ozone Test Results

The CerroZone Mini was tested for the production of ozone which had to be below the required 5 ppb per ASHRAE safety testing. There was no detection of ozone above the 5-ppb limit after sampling every 20 seconds for 4 hours. Only producing a maximum of 4.89 ppb over 4 hours. This falls below the ASHRAE 241 Safety requirements. The graphical data for ozone production can be found in **Figure 13.**

Ozone Testing Conclusion

The CerroZone Mini was below the required ozone ppb production allowed by ASHRAE testing standard. This unit passed the ozone portion of the safety test.

Formaldehyde Safety Testing

Formaldehyde Monitoring Instrumentation

Interscan 4160-1999b Portable Formaldehyde Gas Detector

The Interscan 4160-1999b Formaldehyde Gas Detector is a portable sampling unit that gives a real-time digital readout of formaldehyde concentrations in parts per billion. It is highly sensitive and is capable of reading formaldehyde at concentrations as low as 0 ppb to 1999 ppb. This data was stored on a data logger and was exported to an excel file for further data analysis. A picture of the 465L Ozone Monitor is shown in **Figure 14**.

Figure 14. The Interscan 4160-1999b Portable Formaldehyde Gas Detector. This can detect formaldehyde concentrations from a range of 0-1999 ppb with a 0.2 ppb resolution.

Formaldehyde Monitoring Test Methodology

Formaldehyde Control Test

In order to accurately assess the devices formaldehyde production, the chamber, with no device, was measured first for background formaldehyde concentrations. This allowed for a standard background to be established within the chamber. This was then used as comparison data for the tested device. The control test was run for 4 hours just like the official test duration. Formaldehyde safety testing also included an additional control test with limonene. Limonene is a chemical commonly found in many household products including cleaning solutions and beauty products. Limonene is known to produce formaldehyde if exposed to ozone. This was vaporized instantaneously inside of the chamber in a specific concentration (25 µg/m³ or 4.5 ppb_v) and was used as a chemical input for formaldehyde production per ASHRAE 241 guidelines. This was also monitored for 4 hours to see natural limonene conversion to formaldehyde with the basal level of ozone within the chamber.

Device Formaldehyde Testing

The same concentration of limonene used for the control tests (25 µg/m^3 or 4.5 ppb$_v$) was aerosolized within the chamber containing the device. The device was then turned on and monitored for a duration of 4 hours. This allowed for an adequate assessment of the total concentration of formaldehyde conversion from limonene generated by the device. This data was then compared to the control data to determine the net production of formaldehyde produced by the device.

ASHRAE 241 Formaldehyde Guidelines

The ASHRAE 241 requirements for formaldehyde production are very low, only allowing for the tested device a formaldehyde emission rate of 50 µg/hr over the 4 hours of testing.

Emission rates are determined by the following equation provided by the ASHRAE Standard:

$$E = V\left(L_{off}C_{t=\Delta t} + \frac{C_{t=\Delta t} - C_{t=0}}{\Delta t}\right)$$

Where:
E= Emission rate, µg/hr
V= Volume of the Chamber in m^3
L_{off}= the first order loss rate for the chemical (which is 0 for formaldehyde)
$C_{t=\Delta t}$ = Concentration at the end of the trial in µg/m^3
$C_{t=0}$ = Concentration at the beginning of the trial in µg/m^3
Δt= total duration of the trial

Using this equation, the emission rate of formaldehyde was calculated.

Formaldehyde Test Results

Testing showed no production of formaldehyde from injection of limonene per ASHRAE guidelines (see **Figure 15**).

Trial ID	Limonene Starting Concentration (µg/m^3)	Test Duration (hours)	HCHO Emission Rate (µg/h)
Control 1	25	4	5.527
Control 2	25	4	4.606
Trial 1 Speed 2	25	4	0.921
Trial 2 Speed 2	25	4	2.763
Trial 1 Speed 3	25	4	3.685
Trial 2 Speed 3	25	4	1.842
		Net Emission Rate Speed 2	**-3.224**
		Net Emission Rate Speed 3	**-2.303**

Figure 15 Formaldehyde Emissions Results. Formaldehyde samples were taken every 10 seconds over each of the 4-hour trials. Two experiments were conducted with the device *off* and two with the device *on*. Similar emissions during device operation indicated no production of formaldehyde by the device.

Formaldehyde Test Conclusion

The emission rate for the Mini unit was calculated to be below the ASHRAE 241 requirement of 50 µg/hr. The total test was 4 hours meaning that the emission rate had to be below 200 cumulative µg/hr during the testing.

Summary of Results

The CerroZone Mini device passed all safety testing from the ASHRAE Standard 241. The formaldehyde measurements taken from the test chamber after limonene injection did not exceed the 50 µg/hour emission rate limit. Ozone levels in the chamber while the device was in operation did not exceed 5 ppb throughout the tests. Particulate monitoring of 4 different size ranges showed no increase in ISO classification greater than one level above the empty Class 5 chamber.

Analyte of Concern	Abbreviation	Test Method	Target	Results	Pass/Fail
Formaldehyde	HCHO	Formaldehyde shall be measured using any method described in ASTM D8407 [23] that has a detection limit better than 0.5 ppb$_v$ (0.6 µg/ m^3) for a 1-minute sample. Air change must be low enough to detect target emission rate with instrument detection limits.	Emission rate less than 50 µg/h	Speed 2 -3.224 µg/hr Average Net Emissions Speed 3 -2.303 µg/hr Average Net Emissions	Pass
Ozone	O_3	UL 2998-2020 or equivalent	<5 ppb	<5 ppb	Pass
Particulate matter count concentration (#/m3)	Particles greater than 0.3 µm	ISO 14644-14 [24] (duct testing requires isokinetic sampling)	Test results shall not exceed one cleanliness class greater than the empty test chamber or test duct as described in ISO 14644-14, Table 1. Empty chamber shall not measure higher than Class 5.	Did not exceed one ISO class greater then class 5	Pass

Figure 15 Executive Summary

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.

ASTM. 2021. ASTM D8407, *Standard Guide for Measurement Techniques of Formaldehyde in Air*. West Conshohocken, PA: ASTM International.

ISO. 2016. ISO 14644-14. *Cleanrooms and associated controlled environments—Part 14: Assessment of suitability for use of equipment by airborne particle concentration*. Geneva, Switzerland: International Organization for Standardization

UL. 2020. UL 2998, *Environmental Claim Validation Procedure (ECVP) for Zero Ozone Emissions from Air Cleaners.* Northbrook, IL: Underwriters Laboratories, LLC.

Analytical Testing Facility

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

Project

10880.150.1.2

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 ASHRAE 241, AHAM AC-5, and 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:

Richard Ludwick
Study Director
ARE Labs, Inc.

9/27/2023
Date

Principal Investigator:

Sean McLeod
Staff Research Scientist
ARE Labs, Inc.

9/27/2023
Date

APPENDIX A: Equipment Calibration

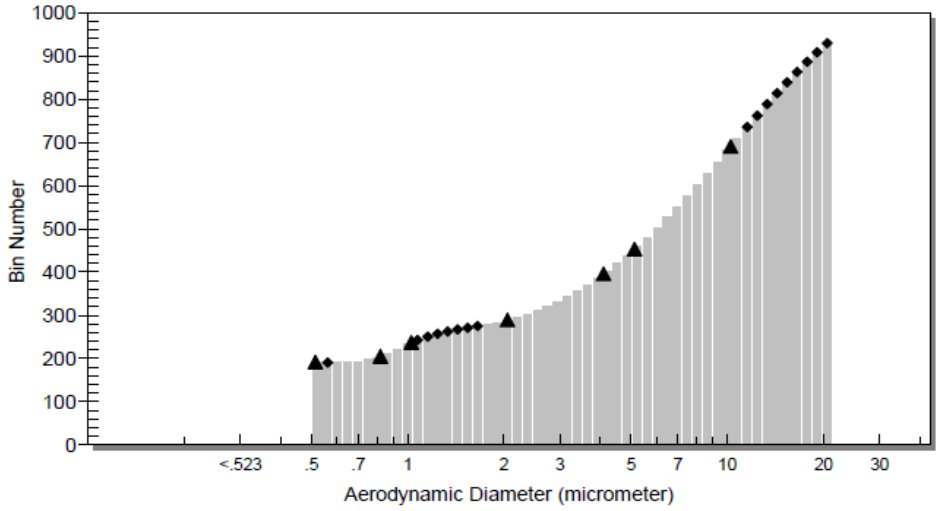
TSI
Particle Instrument Division
Mailing Address: P.O. Box 64394 St.Paul, MN 55164 USA
Shipping Address: 500 Cardigan Road Shoreview, MN 55126 USA
Phone: 1-800-677-2708 or (651)490-2833 FAX:(651)490-3860

Certificate of Calibration

TSI model 3321

Date of Calibration: August 01, 2023
Serial Number: 71043195

	Geometric Diameter (µm)	Accumulator Median Bin	Particle Density (g/ml)
Particle #1	0.5	192	1.05
Particle #2	0.8	205	1.05
Particle #3	1.0	237	1.05
Particle #4	2.0	289	1.05
Particle #5	4.0	396	1.05
Particle #6	5.0	453	1.05
Particle #7	10	691	1.05



TSI Incorporated does hereby certify that all materials, components, and workmanship used in the manufacture of this equipment are in strict accordance with the applicable specifications agreed upon by TSI and the customer and with all published specifications. All performance and acceptance tests were successfully conducted according to required specifications. TSI Incorporated certifies that the instruments used to calibrate this instrument are traceable to the National Institute of Standards and Technology (NIST), where applicable, and internal TSI calibration standards where NIST standards do not exist.

Calibrated by: _____

Figure 1A: APS calibration certificate.

CERTIFICATION OF CALIBRATION

This instrument has been calibrated using standards maintained at Teledyne API (9970 Carroll Canyon Raod, San Diego, CA 92131, USA), which are traceable to the United States National Institute of Standards and Technology. This calibration was performed to Teledyne API specifications and to the requirements of ISO 9001:2015. Supporting documentation relative to traceability is on file at this office, and is available for examination at Teledyne API upon request.

CERTIFICATION OF:

Model: 465L Rack MKS 6 Channel
Part Number: 060680500 Firmware Rev: B6
Serial Number: 2271

CAL DATE: 8/31/2018

CERTIFICATION LEVEL
RESTRICTED ☐ (see below)
INTERIM ☐
FINAL ☑

SALES ORDER NUMBER: 309035

CALIBRATION JOB INSTRUCTION NO:

AS RECEIVED CONDITION:

☑ Initial Calibration ☐ Item received in calibration ☐ Item is out of calibration

As found condition (test data): **INITIAL CALIBRATION**

CALIBRATION DATA: This certifies that the above referenced instrument meets or exceeds all design specifications. Testing has been performed using instruments calibrated by an independent party using a NIST-traceable SRP ozone analyzer for the assay of ozone as described in *40CFR50, Appendix D*. It is maintained as a certified transfer standard according to the guidelines described in EPA's Technical Assistance Documents: *Transfer Standards for the Calibration of Air Monitoring Analyzers for Ozone (EPA-600/4-79-056), September 1979, and Technical Assistance Document for the Calibration of Ambient Ozone Monitors (EPA-600/4-79-057), September 1979* for concentrations at or below 1 ppm. Instrument readings in excess of 1 ppm are extrapolated. This transfer standard is periodically verified against an NIST Standard Reference Photometer. Specific data are available upon request.

Environmental Conditions At Time Of Calibration: 65-75 °F, RH = 20-80%
If calibration is restricted, specify restriction: NO RESTRICTION

TEST DATA

Input (PPM)	Measured (PPM)	% Deviation
0.00	0.00	0.00%
0.25	0.25	-0.11%
0.50	0.50	0.02%
0.90	0.90	0.16%

TEST AND MEASUREMENT EQUIPMENT USED:

Model Number	Serial Number/Asset Number		Calibration Date	Calibretion Due
703E	SN: 225	EL#: 447	7/27/2018	7/27/2019

PERFORMED BY: 133 **DATE** 8/31/2018

APPROVED BY: Quality Representative **DATE** 8/31/18

06575G (DCN 7947) **7/2/18**

Figure 2A: Teledyne 465L factory calibration certificate.

IE MONITORING INSTRUMENTS
Industrial Environmental Monitoring Instruments, Inc.
7410 Worthington-Galena Rd
Worthington, OH 43085
Phone: (614) 436-4933
Website: www.ierents.com

Formaldehyde Monitor Calibration

Instrument: Interscan 4160 HCHO
Serial #: 22526

Date: 8/25/2023
Technician: Sam Shults

Calibration Data

	Standard	Reading
Zero Gas	Charcoal Filter	0 +/- 3 ppb
Span	682 ppb	683 ppb

Accuracy = +/- 5%

Calibration Standards

Standard	Serial#	Expires	Manufacturer
4348 ng/min HCHO Perm Tube	65536	3/21/2024	Kin-Tek
Bios ML-500 Calibrator	206586	9/29/2023	Bios International

Instrument must be calibrated and operated according to manufacturers specifications

Figure 3A: Interscan calibration certificate.