ASHRAE 241-2023 Standard Testing for the Efficacy of the Erlab Halo Device at Reducing Aerosolized *MS2*

Client: Erlab

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REPORT APPROVAL

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Report Info

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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 Erlab'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 Erlab Halo P device and its ability to reduce the bacteriophage, MS2, per the ASHRAE 241-2023 standard. The Erlab Halo is a multi-stage filtration device for use in various settings.

Background:

The Erlab Halo P is an air purification system using a proprietary filtration technology. Air is passed through a pre-filter then a HEPA filter for capture of pathogens and other particulates. All testing was conducted in a 30m³ bioaerosol test chamber. The species selected for this study was MS2, an ssRNA bacteriophage. This study utilizes ASHRAE 241 and AHAM AC-5 testing parameters to determine efficacy. Three separate bioaerosol test trials were performed with the device powered *on*, as well as three control bioaerosol trials with the device powered *off*.

Methods:

The Erlabs device was sealed into a custom 30m³ bioaerosol chamber for all tests. MS2 was aerosolized into the sealed 30m³ environmental bioaerosol 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 control trial data, or natural decay, was subtracted from the device trial data to yield the net log reduction attributable to the devices for each of the bioaerosol challenges.

Results:

The Erlab Halo P achieved an observed reduction of the MS2 bioaerosol by 3.06 +/- 0.15 net log after 60 minutes in the 30m³ test chamber . The clean air delivery rate was calculated for this unit based off the Erlab Halo P trial data, achieving an average clean air delivery rate (CADR) of 126.75 +/- 7.58 cubic feet per minute (CFM).

Conclusion:

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

Introduction

This study was conducted to evaluate the efficacy of the Erlab device at eliminating viable pathogens from ambient air. The Erlab Halo is a stationary air purifier designed to reduce the quantity of viable 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 moving forward.

The ASHRAE standard includes guidelines for proper ventilation, infection risk management, laboratory testing requirements, operation, and maintenance for 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 these ASHRAE 241 standards.

Following these guidelines, the test plan incorporated challenging the Erlab 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 Erlab Halo P device. A picture of the Halo P is shown in **Figure 1.**

Study Overview

The effectiveness of the Erlab Halo P 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 report of two that details the requirements for ASHRAE 241 and AHAM AC-5 testing. This report contains all of the bioaerosol testing parameters, data, and results, while the other report details the safety information required by ASHRAE 241 and AHAM testing guidelines. A test matrix outlining the testing can be found in **Figure 2**.

Test Device Description

The Erlab air purifier utilizes proprietary filtration technology. It consists of a pre-filter and HEPA 14 filter for purification of ambient air. The Erlab device had a measured air flow rate of 177 cubic feet per minute (CFM). The intended use for this device is constant operation in a room installed on the ceiling.

Figure 1: Erlabs Halo P air purification device.

Equipment

Bioaerosol Testing Chamber

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

Structurally, the chamber has dimensions of 30 $\pm$ 1.5 cubic meters, or approximately 1060 ft^3, with the width deliberately maintained within 85 to 100% of its length. This dimensional consistency ensures a uniform testing space, which allows for reliable experimentation.

Constructed from a non-porous material, the chamber's walls exhibit notable qualities. Beyond its physical attributes, this material emits minimal volatile organic compounds (VOCs), is non-reactive, non-reflective, and has a non-ionizing quenching nature. This creates an environment conducive to reliable and repeatable testing conditions.

Airtight integrity is monitored and controlled, within the chamber achieving a controlled air change rate (ACH) below 0.05, as per the benchmark set by ASTME 741. This characteristic provides the operator with the ability to isolate the testing environment, thus enhancing result reliability.

The chamber is designed to prevent external microbial contamination while maintaining internal atmospheric conditions. These features include an aseptic maintenance system, HEPA filtration, cross-contamination-free item transfer mechanisms, external power control, real-time observation facilitated by multiple viewing windows, and the capability to produce and evenly disperse aerosolized microbes.

Sampling ports, positioned approximately 48 inches from the floor and 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.

Trial	Run	Device	Device Fan Speed (ft^3/min)	Surrogate 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	104-105	60	0, 4, 8, 12, 16, 20. 30. 45, 60	TSI 3321 APS, Impingers	all samples in triplicate
2	Control											
3	Control											
4	Challenge	Halo P	177	MS2 Bacteriophage (RNA Virus)	15597-B1	30.0	<1.0um	10^4-10^5	60	0, 4, 8, 12, 16, 20. 30. 45, 60	TSI 3321 APS, Impingers	all samples in triplicate
5	Challenge											
6	Challenge											

Figure 2: Test Matrix for Bioaerosol Trials.

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**.

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

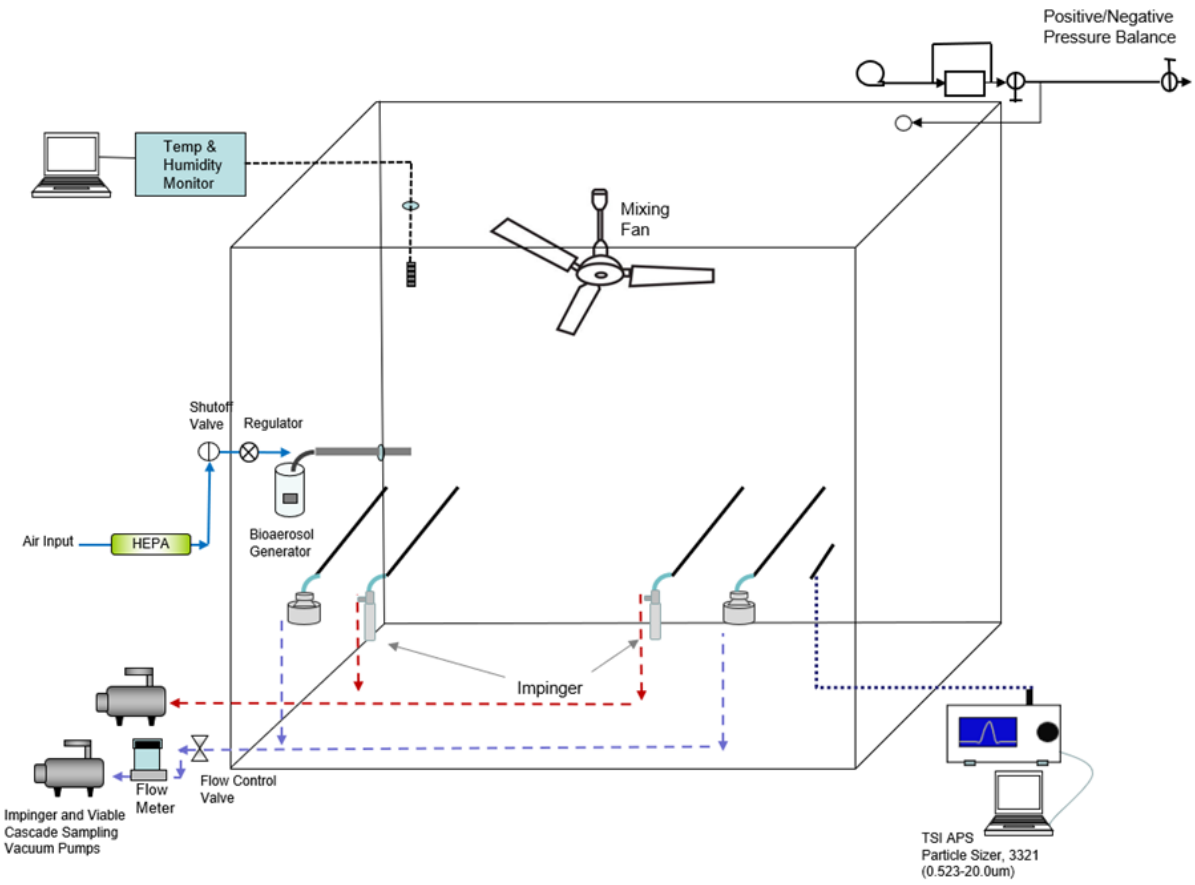

Figure 4: 30m³ Environmental Test Chamber Flow Diagram. Chamber includes bioaerosol induction, multiple bioaerosol sampling ports, particle size monitoring, internal mixing fan, and temperature and humidity controls. Main system HEPA evacuation system (not pictured).

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 bioaerosols 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 at 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**.

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 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 30m^3 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.

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

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.

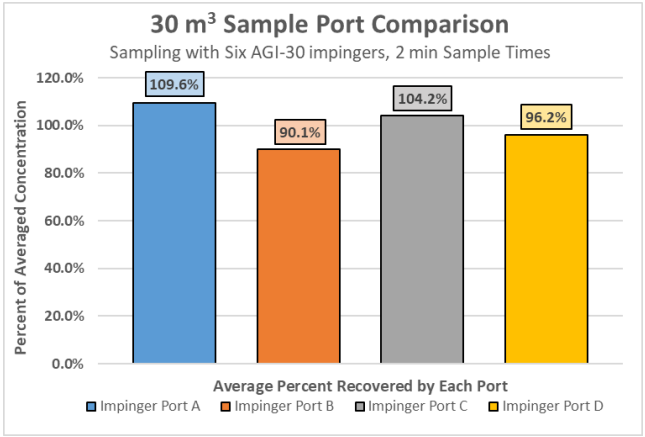

Figure 11: Impinger port-to-port comparison. Percent averages are calculated by taking the count for each port divided by the average plate count for the four ports.

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.

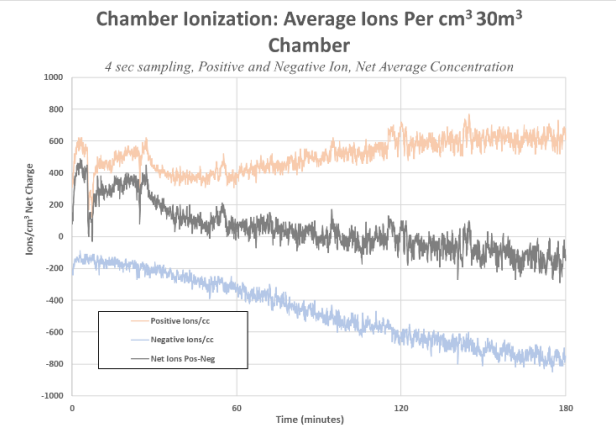

Figure 12. Total baseline level of ions detected in the 30m³ chamber.

Chamber Controls

Chamber controls involved assessing the natural decay rate of the test bioaerosol within the chamber over an hour without the air cleaner in operation. This time aligns with the intended operational testing time of the air cleaner, 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.

Testing

Air Cleaner Efficacy Evaluation Procedure

The process of evaluating the efficacy of air cleaners in reducing airborne microbial concentrations is similar to control tests, but the test chamber contains the air cleaner being tested. 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 cleaner.

Once the baseline is set, the air cleaner 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.

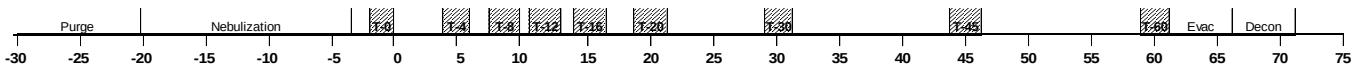

Figure 13: ASHRAE 241 Sampling Times for a 1 Hour Trial.

The collected air 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.

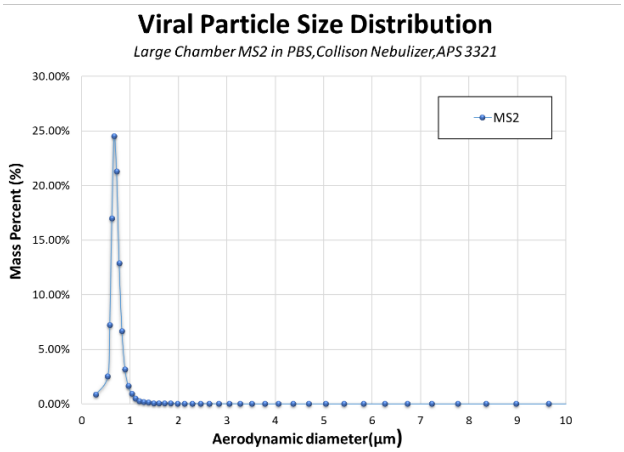

Figure 14: Aerodynamic Particle Size Distribution of the RNA virus MS2 in the test chamber. The MMAD for this viral species averaged approximately 0.7 µm.

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 assay 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 prior to plating.

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 graphed and plotted to show natural viability loss over time in the chamber. These control trials served as the basis for determining the reduction of the Erlab device at two different fan speeds over an hour trial, above the natural losses from the control runs. The control and trials are plotted showing log reduction in viable bioaerosol for MS2. All data is normalized with time zero enumerated concentrations. Subsequent samples are normalized and plotted to show the loss of viable bioaerosol over time. All raw data was recorded in a dedicated lab notebook, and analysis performed using Microsoft Excel.

Results

The Erlab Halo P on normal speed achieved a 3.06 +/- 0.15 net log reduction 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 30m^3 chamber under the same conditions per testing standard.

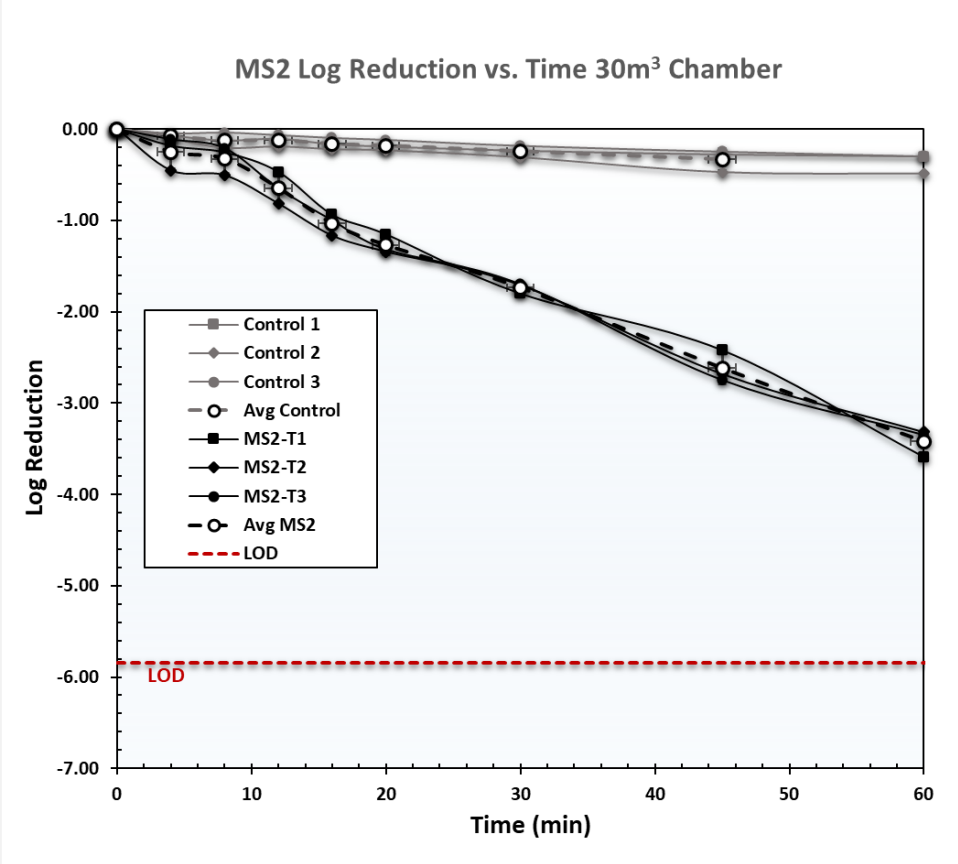

Figure 15: Log Reduction of Aerosolized *MS2* by the Erlab Halo P. Each line represents the average of three trials performed under the same conditions for statistical significance.

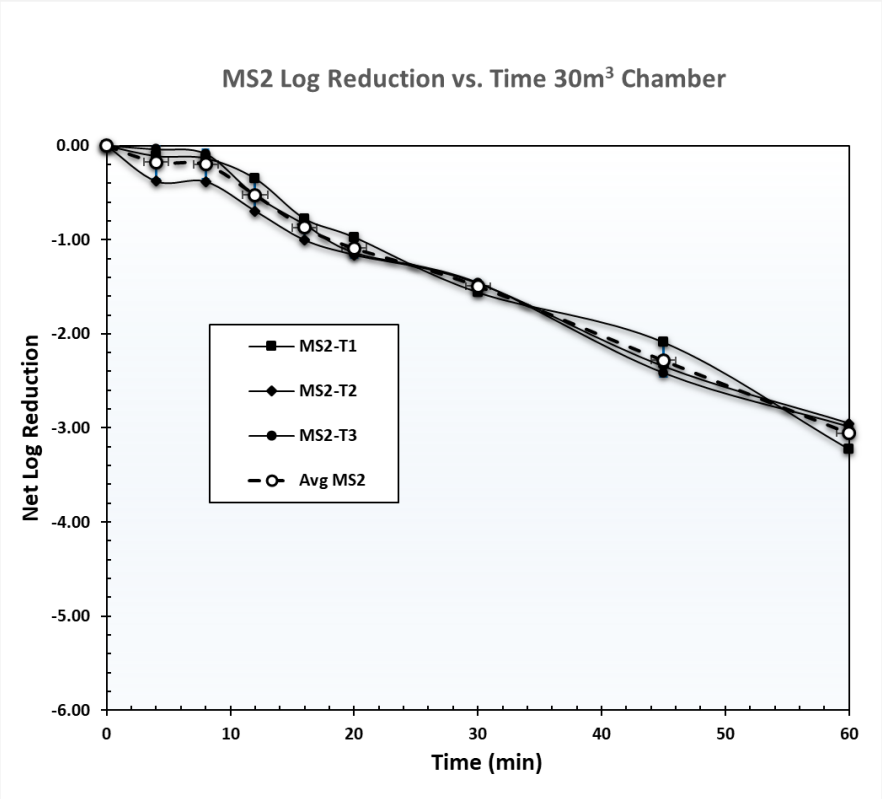

Figure 16: Net Log Reduction of Aerosolized *MS2* by the Erlab Halo P. Each line represents the average of three trials performed under the same conditions for statistical significance.

Bioaerosol Type	Species (description)	Reduction Type	Trial Time (minutes) 4	8	12	16	20	30	45	60
Virus	*MS2* (RNA Virus)	*Net Log Reduction*	-0.11	-0.13	-0.35	-0.78	-0.97	-1.56	-2.09	-3.23
		Net % Reduction	22.1176%	26.2480%	55.2152%	83.2712%	89.3310%	97.2395%	99.1858%	99.9408%
Virus	*MS2* (RNA Virus)	*Net Log Reduction*	-0.38	-0.38	-0.69	-1.00	-1.16	-1.46	-2.35	-2.95
		Net % Reduction	58.0540%	58.3897%	79.7601%	90.0695%	93.0811%	96.5647%	99.5496%	99.8886%
Virus	*MS2* (RNA Virus)	*Net Log Reduction*	-0.04	-0.09	-0.53	-0.84	-1.13	-1.46	-2.41	-2.99
		Net % Reduction	8.1912%	17.9184%	70.3308%	85.4539%	92.6668%	96.4969%	99.6148%	99.8968%
All Trial Averages +/- St. Dev.		***Net Log Reduction***	***-0.17 +/- 0.18***	***-0.2 +/- 0.16***	***-0.52 +/- 0.17***	***-0.87 +/- 0.12***	***-1.09 +/- 0.1***	***-1.49 +/- 0.06***	***-2.28 +/- 0.17***	***-3.06 +/- 0.15***
		Net % Reduction	*29.45% +/- 25.73%*	*34.19% +/- 21.37%*	*68.44% +/- 12.38%*	*86.26% +/- 3.47%*	*91.69% +/- 2.06%*	*96.77% +/- 0.41%*	*99.45% +/- 0.23%*	*99.91% +/- 0.03%*

Bioaerosol Type	Species (description)	Reduction Type	Trial Time (minutes) 4	8	12	16	20	30	45	60
Virus	MS2 (RNA Virus)	*Net Log Reduction*	-0.07	-0.13	-0.11	-0.17	-0.19	-0.24	-0.28	-0.30
		Net % Reduction	15.2542%	25.4237%	22.0339%	32.2034%	36.1017%	42.3729%	47.6271%	50.1695%
Virus	MS2 (RNA Virus)	*Net Log Reduction*	-0.11	-0.21	-0.19	-0.22	-0.23	-0.31	-0.47	-0.49
		Net % Reduction	21.5054%	37.6344%	35.4839%	39.7849%	41.3978%	50.8602%	66.1290%	67.4194%
Virus	MS2 (RNA Virus)	*Net Log Reduction*	-0.05	-0.04	-0.07	-0.10	-0.12	-0.18	-0.25	-0.30
		Net % Reduction	10.4762%	8.5714%	14.2857%	20.0000%	23.8095%	34.2857%	43.1429%	49.5238%
All Control Average +/- St. Dev.		***Net Log Reduction***	***-0.08 +/- 0.03***	***-0.12 +/- 0.08***	***-0.12 +/- 0.06***	***-0.16 +/- 0.06***	***-0.18 +/- 0.06***	***-0.24 +/- 0.06***	***-0.33 +/- 0.12***	***-0.36 +/- 0.11***
		Net % Reduction	*15.75% +/- 5.53%*	*23.88% +/- 14.59%*	*23.93% +/- 10.73%*	*30.66% +/- 9.98%*	*33.77% +/- 9.02%*	*42.51% +/- 8.29%*	*52.3% +/- 12.18%*	*55.7% +/- 10.15%*

Figure 17: Executive Summary. Net log and associated per cent reduction values for the Erlab Halo P at each timepoint.

Clean Air Delivery Rate Calculations (CADR)

The clean air delivery rate (CADR) was calculated for the Erlab Halo P 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 m^3). **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 Erlab Halo P on normal speed averaged 126.75 +/- 7.58 CADR in cubic feet per minute. A graphical summary of the results can be found in **Figure 19.**

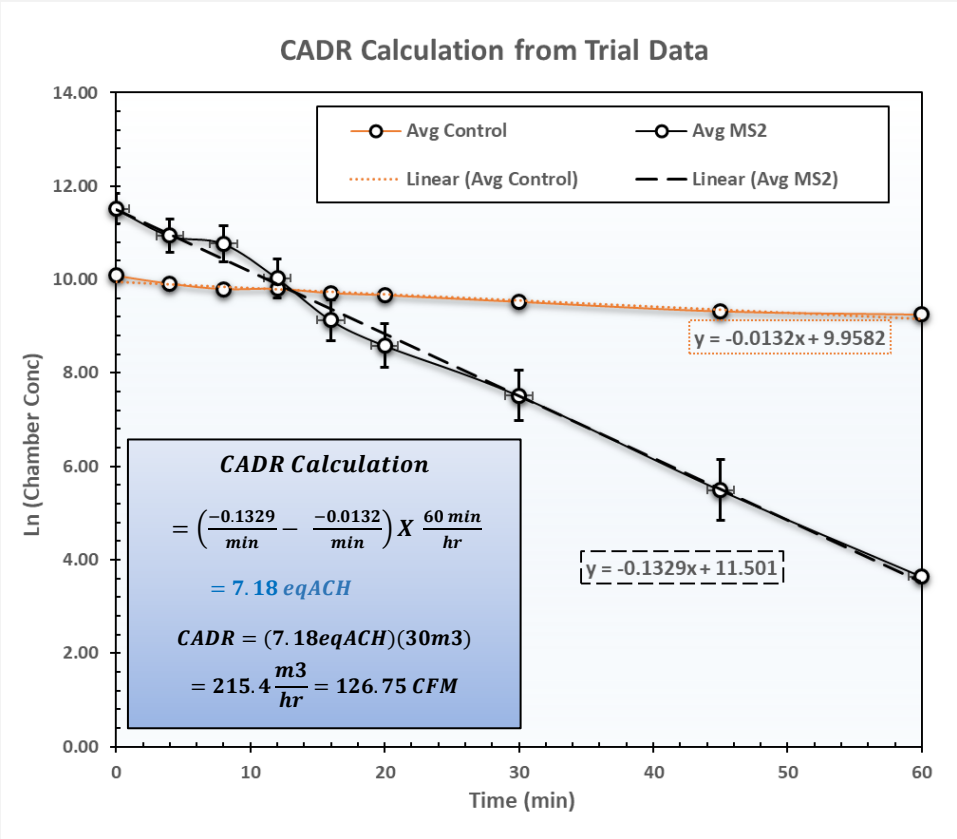

Figure 18: Graphical Method to compute Clean Air Delivery Rate from Actual Trial Test Data.

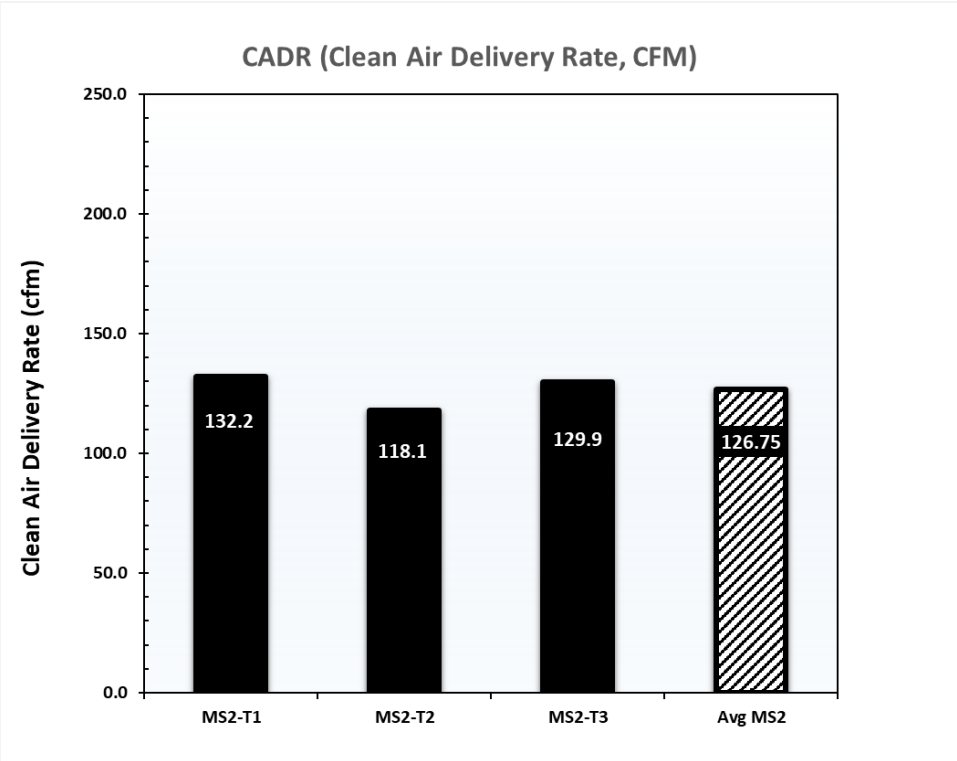

Figure 19: CADR for aerosolized *MS2* by the Erlab Halo P.

Conclusion

The ASHRAE 241 standards provides pass/fail criteria for any device using the testing protocols. These criteria are dependent on the area that the device is designed to operate in, the measured CADR, and how it equates to CFM/person in a given area. Most devices are designed to work in specifically sized spaces within various categories. Because of this, ARE Labs reports the CADR achieved by the test devices and does not make any determinations about the category that the device is designed to operate in.

The CADR calculated for the Erlab Halo P was 126.75 +/- 7.58 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.

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Analytical Testing Facility

Aerosol Research and Engineering Labs, Inc.
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Project

10903.20.1

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 Erlab's financial interests such as membership, employment, stock ownership, or other equity interest.

Study Director:

Richard Ludwick
Study Director
ARE Labs, Inc.

9/28/2023
Date

Principal Investigator:

Sean McLeod
Staff Research Scientist
ARE Labs, Inc.

9/28/2023
Date

APPENDIX A: Bioaerosol Raw Data

Trial Information

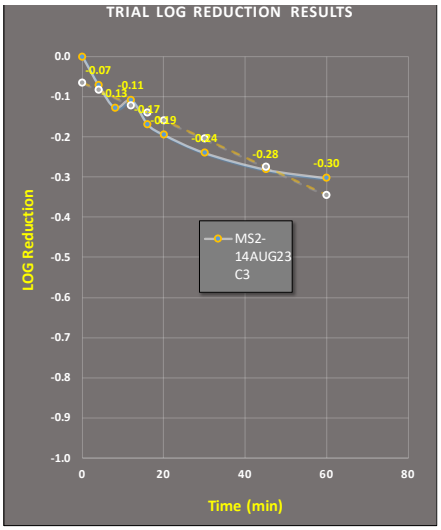
TEST DATE: Monday, August 14, 2023
TRIAL PERFORMED BY: SMM
TRIAL NUMBER: C1
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2-14AUG23 C3

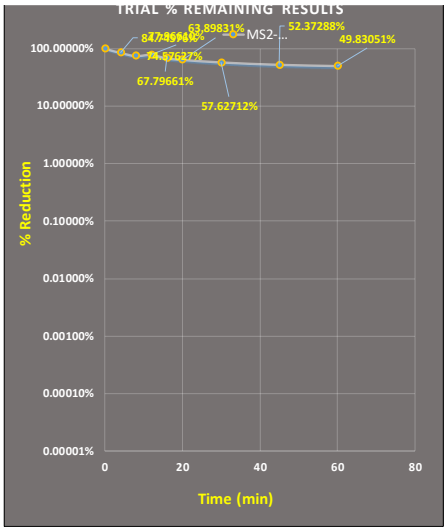
Device Information

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

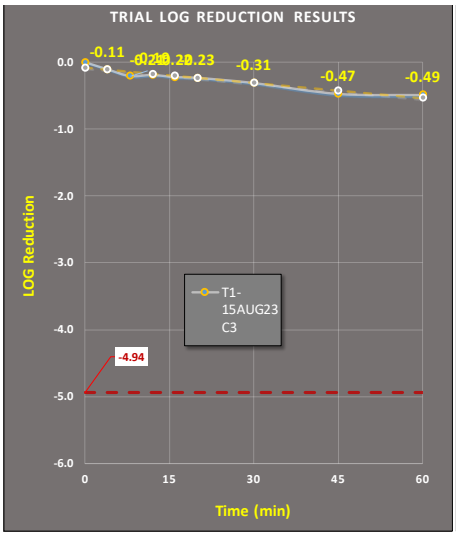
TEST DATE: Wednesday, August 16, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: C2
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): T1-15AUG23 C3

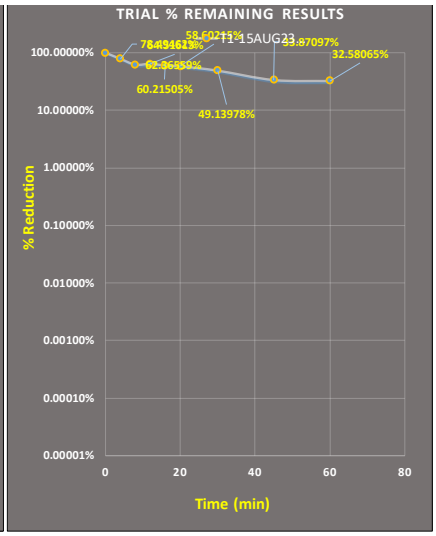
Device Information

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

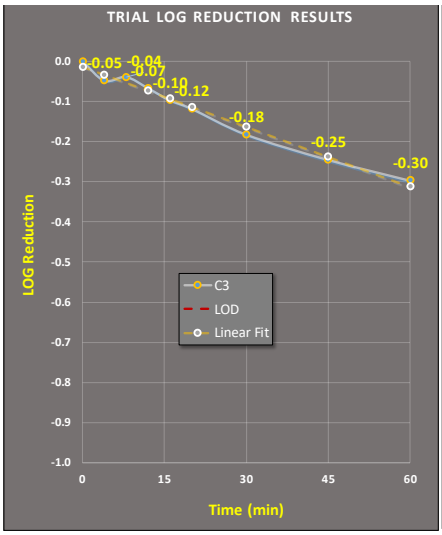
TEST DATE: Wednesday, August 16, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: C3
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): C3

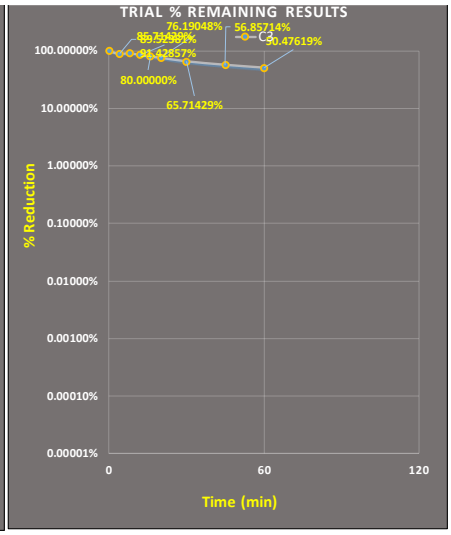
Device Information

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

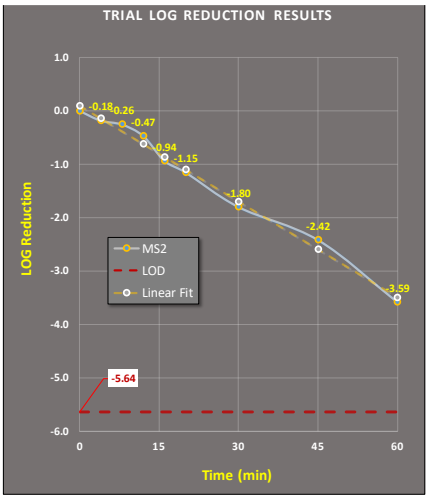
TEST DATE: Wednesday, August 23, 2023
TRIAL PERFORMED BY: SMM
TRIAL NUMBER: T1
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2

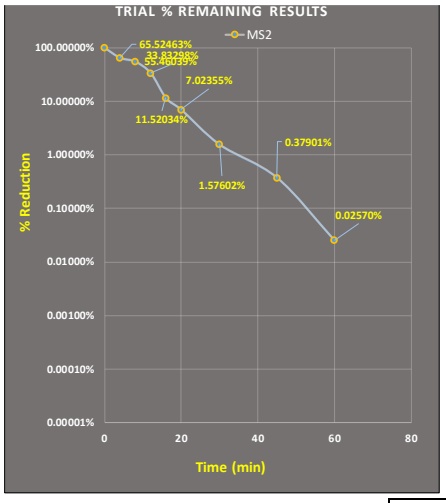
Device Information

MANUFACTURER: Erlab
UNIT MODEL: Halo P
FAN SPEED (CFM): 177
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 60 PSI
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES NA





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9	LOD
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60	LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	6.227E+04	4.080E+04	3.453E+04	2.107E+04	7.173E+03	4.373E+03	9.813E+02	236.000	16.000	1.422E-01
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)										
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	27.04%	38.95%	38.13%	24.44%	54.59%	4.76%	73.10%	82.00%		100.00%
VIABLE CONSISTENCY CHECKS (% agreement)										
IMP & VIABLE CROSS CHECK (% agreement)										
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**6.227E+04**	**4.080E+04**	**3.453E+04**	**2.107E+04**	**7.173E+03**	**4.373E+03**	**9.813E+02**	**2.360E+02**	**1.600E+01**	**0.1422**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**65.5246%**	**55.4604%**	**33.8330%**	**11.5203%**	**7.0236%**	**1.5760%**	**0.3790%**	**0.0257%**	**0.0002%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**34.4754%**	**44.5396%**	**66.1670%**	**88.4797%**	**92.9764%**	**98.4240%**	**99.6210%**	**99.9743%**	**99.9998%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.18**	**-0.26**	**-0.47**	**-0.94**	**-1.15**	**-1.80**	**-2.42**	**-3.59**	**-5.64**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60	LOD
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	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	5.0
IMPINGER FLOW RATE (lpm)	12.5	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**	**-1**	**-1**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**750**
Dilution Range #1 ENUMERATED PLATE COUNTS (# / drop)	12	8	6	5	9	7	18	6	2	1
	7	5	4	2	16	3	22	6	2	0
	8	6	6	2	12	6	18	3	2	0
PLATE AVERAGE COUNT (# / drop)	9.00	6.33	5.33	3.00	12.33	5.33	19.33	5.00	2.00	0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	90,000	63,333	53,333	30,000	12,333	5,333	1,933	500	20	0
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**7.20E+04**	**5.07E+04**	**4.27E+04**	**2.40E+04**	**9.87E+03**	**4.27E+03**	**1.55E+03**	**4.00E+02**	**1.60E+01**	**1.42E-01**
DILUTION RATIO (10^x)	**-2**	**-2**	**-2**	**-2**	**-1**	**-1**	**0**	**0**	**-1**	
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	
Dilution Range #1 ENUMERATED PLATE COUNTS (# / drop)	61	39	33	20	58	56	48	10		
	76	39	29	26	58	57	62	8		
	60	38	37	22	52	55	46	9		
PLATE AVERAGE COUNT (# / drop)	65.67	38.67	33.00	22.67	56.00	56.00	52.00	9.00		
IMPINGER CONCENTRATION (cfu or pfu/ml)	65,667	38,667	33,000	22,667	5,600	5,600	520	90		
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**5.25E+04**	**3.09E+04**	**2.64E+04**	**1.81E+04**	**4.48E+03**	**4.48E+03**	**4.16E+02**	**7.20E+01**		

Figure 4A: *Erlab Halo P Normal Speed T1 Bioaerosol Raw Data.*

Trial Information

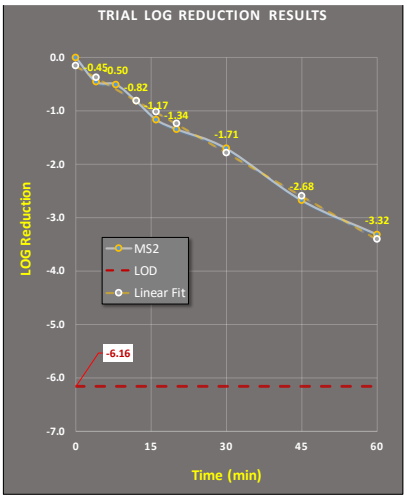
TEST DATE: Wednesday, August 23, 2023
TRIAL PERFORMED BY: SMM
TRIAL NUMBER: T2
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2

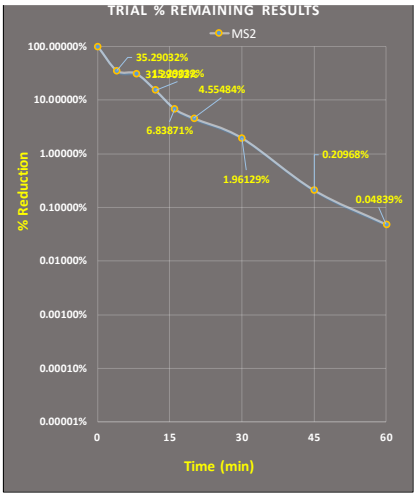
Device Information

MANUFACTURER: Erlab
UNIT MODEL: Halo P
FAN SPEED (CFM): 177
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 60 PSI
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES NA





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9	LOD
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60	LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	2.067E+05	7.293E+04	6.467E+04	3.160E+04	1.413E+04	9.413E+03	4.053E+03	433.333	100.000	1.422E-01
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)										
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	36.84%	48.06%	65.28%	30.71%		64.23%	48.00%	64.58%	50.00%	100.00%
VIABLE CONSISTENCY CHECKS (% agreement)										
IMP & VIABLE CROSS CHECK (% agreement)										
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**2.067E+05**	**7.293E+04**	**6.467E+04**	**3.160E+04**	**1.413E+04**	**9.413E+03**	**4.053E+03**	**4.333E+02**	**1.000E+02**	**0.1422**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**35.2903%**	**31.2903%**	**15.2903%**	**6.8387%**	**4.5548%**	**1.9613%**	**0.2097%**	**0.0484%**	**0.0001%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**64.7097%**	**68.7097%**	**84.7097%**	**93.1613%**	**95.4452%**	**98.0387%**	**99.7903%**	**99.9516%**	**99.9999%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.45**	**-0.50**	**-0.82**	**-1.17**	**-1.34**	**-1.71**	**-2.68**	**-3.32**	**-6.16**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60	LOD
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	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	5.0
	IMPINGER FLOW RATE (lpm)	12.5	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**	**-3**	**-3**	**-2**	**-2**	**-2**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**750**
	ENUMERATED PLATE COUNTS (# / drop)	1	12	11	3	20	20	6	10	8	1
		2	15	12	6	13	16	6	6	8	0
		3	9	13	5	20	16	8	8	9	0
	PLATE AVERAGE COUNT (# / drop)	2.00	12.00	12.00	4.67	17.67	17.33	6.67	8.00	8.33	0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	200,000	120,000	120,000	46,667	17,667	17,333	6,667	800	83	0
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.60E+05**	**9.60E+04**	**9.60E+04**	**3.73E+04**	**1.41E+04**	**1.39E+04**	**5.33E+03**	**6.40E+02**	**6.67E+01**	**1.42E-01**
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-2**	**-2**	**-2**	**-1**	**-1**	**-1**	**0**	**-1**	
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	
	ENUMERATED PLATE COUNTS (# / drop)	27	67	37	31		61	36	28	2	
		24	60	40	38		58	30	29	1	
		44	60	48	28		67	38	28	2	
	PLATE AVERAGE COUNT (# / drop)	31.67	62.33	41.67	32.33		62.00	34.67	28.33	1.67	
	IMPINGER CONCENTRATION (cfu or pfu/ml)	316,667	62,333	41,667	32,333		6,200	3,467	283	167	
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**2.53E+05**	**4.99E+04**	**3.33E+04**	**2.59E+04**		**4.96E+03**	**2.77E+03**	**2.27E+02**	**1.33E+02**	

Figure 5A: *Erlab Halo P Normal Speed T2 Bioaerosol Raw Data.*

Trial Information

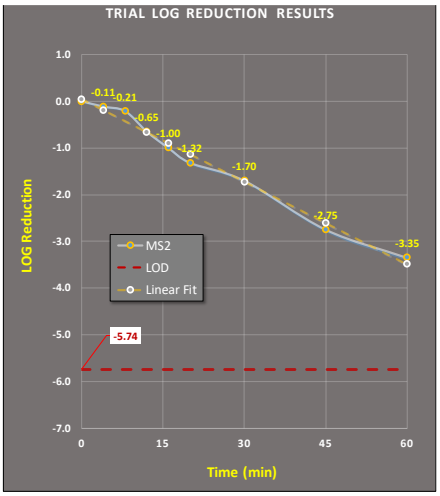
TEST DATE: Thursday, August 24, 2023
TRIAL PERFORMED BY: SMM
TRIAL NUMBER: T3
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2

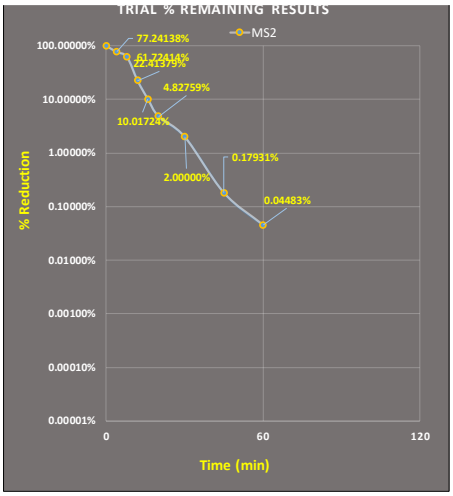
Device Information

MANUFACTURER: Erlab
UNIT MODEL: Halo P
FAN SPEED (CFM): 177
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 60 PSI
SAMPLING METHOD: Impinger
CHAMBER MIXING FAN: yes
TEMP (F): 74
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES NA





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	S9	LOD
SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60	LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOAEROSOL CONCENTRATION (cfu pfu/L Air)	7.733E+04	5.973E+04	4.773E+04	1.733E+04	7.747E+03	3.733E+03	1.547E+03	138.667	34.667	1.422E-01
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)										
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	47.37%	68.24%	67.41%	37.50%	54.75%	44.44%		26.67%		100.00%
VIABLE CONSISTENCY CHECKS (% agreement)										
IMP & VIABLE CROSS CHECK (% agreement)										
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**7.733E+04**	**5.973E+04**	**4.773E+04**	**1.733E+04**	**7.747E+03**	**3.733E+03**	**1.547E+03**	**1.387E+02**	**3.467E+01**	**0.1422**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**77.2414%**	**61.7241%**	**22.4138%**	**10.0172%**	**4.8276%**	**2.0000%**	**0.1793%**	**0.0448%**	**0.0002%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**22.7586%**	**38.2759%**	**77.5862%**	**89.9828%**	**95.1724%**	**98.0000%**	**99.8207%**	**99.9552%**	**99.9998%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.11**	**-0.21**	**-0.65**	**-1.00**	**-1.32**	**-1.70**	**-2.75**	**-3.35**	**-5.74**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30	45	60	LOD
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	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	5.0
IMPINGER FLOW RATE (lpm)	12.5	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**	**-1**	**-1**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**750**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	1	10	8	3	13	6	20	1	7	1
	1	13	7	2	15	8	18	4	3	0
	0	11	12	3	12	4	20	1	3	0
PLATE AVERAGE COUNT (# / drop)	0.67	11.33	9.00	2.67	13.33	6.00	19.33	2.00	4.33	0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	66,667	113,333	90,000	26,667	13,333	6,000	1,933	200	43	0
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**5.33E+04**	**9.07E+04**	**7.20E+04**	**2.13E+04**	**1.07E+04**	**4.80E+03**	**1.55E+03**	**1.60E+02**	**3.47E+01**	**1.42E-01**
DILUTION RATIO (10^x)	**-3**	**-2**	**-2**	**-2**	**-1**	**-1**	**0**	**0**	**-1**	
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	10	40	21	16	64	40		20		
	16	35	20	14	63	30		16		
	12	33	47	20	54	30		8		
PLATE AVERAGE COUNT (# / drop)	12.67	36.00	29.33	16.67	60.33	33.33		14.67		
IMPINGER CONCENTRATION (cfu or pfu/ml)	126,667	36,000	29,333	16,667	6,033	3,333		147		
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.01E+05**	**2.88E+04**	**2.35E+04**	**1.33E+04**	**4.83E+03**	**2.67E+03**		**1.17E+02**		

Figure 6A: *Erlab Halo P Normal 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:

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