ASHRAE 241-2023 Standard Testing for the Efficacy of the Agentis Brio 650 at Reducing Aerosolized *MS2*

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Report Info

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ASHRAE 241-2023 Compliance:
This study was conducted in compliance with ASHRAE 241 and 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 Agentis'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 Agentis Brio 650 for its ability to reduce the bacteriophage MS2, per the ASHRAE 241-2023 standard.

Background:

The Agentis Brio 650 is an air purifier developed with patented technology from the University of Washington and features a prefilter and an APART Collection Cartridge for capturing a wide array of particles, including viruses, smoke, and allergens. It integrates a specialized ozone catalyst to neutralize any ozone produced, ensuring the air released is clean and meets safety standards. This device is designed to be placed in a room in either a hospital, business, or residential home space.

All testing was conducted in a 30m^3 bioaerosol test chamber which housed the Brio 650 unit. The challenge organism specified by ASHRAE 241 standard and used for this study was the ssRNA bacteriophage MS2. This bacteriophage is a widely utilized and recognized surrogate for more dangerous pathogenic organisms like influenza and SARS-CoV-2. This study utilizes ASHRAE 241 and AHAM AC-5 testing parameters to determine efficacy. Three separate bioaerosol test trials were performed for the Agentis Brio 650 device.

Methods:

The Agentis Brio 650 was placed at the center of the 30m^3 chamber. MS2 was then aerosolized into the sealed 30m^3 environmental bioaerosol chamber, with the chamber containing the Agentis Brio 650, using a Collison 24-Jet Nebulizer. *MS2* was the microorganism used for all initial aerosol test trials. Previously prepared aliquots of *MS2* were used to keep a consistent concentration throughout all testing.

Bioaerosol samples were taken, with impingers, at multiple time points throughout each trial, using ASHRAE 241 and AHAM AC-5 testing parameters, in order to quantify the reduction rate, and hence 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 device for each of the bioaerosol challenges.

Results:

The standard Agentis Brio 650 device proved to be effective at reducing MS2. ASHRAE 241 focuses heavily on the reporting and calculation of the clean air delivery rate number or CADR. The CADR values were calculated using the net reduction slope multiplied by the chamber volume to get a CADR value. This value was then converted into CFM for a value of 406 CFM for t-0 to t-30 minutes. The Brio 650 showed a net log reduction of 4.73 for MS2 in 30 minutes of operation before hitting limits of detection. This equates to a 99.9981% reduction of viable MS2 in the 30m^3 test chamber.

Introduction

This study evaluated the effectiveness of the Agentis Brio 650 device in capturing and deactivating aerosolized MS2. The device was designed as a standalone unit that uses ionization, ozone, and a prefilter to remove and inactivate various pathogens from the air and surfaces when in operation.

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 bioaerosol testing components for in-duct and standalone devices and established the minimum requirements to evaluate all production air purification devices adequately and effectively.

The ASHRAE standard includes guidelines for proper ventilation, infection risk management, laboratory testing requirements, operation, device maintenance, and special requirements for residential and healthcare facilities. With

these new guidelines, testing must be done on all certified air purification devices that adhere to these ASHRAE 241 standards.

Following these guidelines, the test plan incorporated challenging the Agentis Brio 650 using the ASHRAE 241 and AHAM AC-5 protocols and requirements for a 30 m^3 test chamber. This report focuses on the device's efficacy at removing bioaerosols, specifically MS2.

Study Overview

The effectiveness of the Agentis Brio 650 device was evaluated against aerosolized MS2, an ssRNA virus. This allowed for a reasonable demonstration of the device's performance while operating in its intended manner. The study was performed following ASHRAE 241 and AHAM AC-5 testing parameters.

This is one of two reports that detail the requirements for ASHRAE 241 and AHAM AC-5 testing. This report contains all 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**.

Figure 1: Agentis Brio 650.

Test Device Description

The Agentis Brio 650, **Figure 1**, is an air purifier developed with patented technology from the University of Washington and features a prefilter and an APART Collection Cartridge for capturing a wide array of particles, including viruses, smoke, and allergens. It integrates a specialized ozone catalyst to neutralize any ozone produced, ensuring the air released is clean and meets safety standards. This device is designed to be placed in a room in either a hospital, business, or residential home space.

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 adhering 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, allowing 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 241. This feature allows the operator to isolate the testing environment, thus enhancing result reliability.

Bioaerosol Challenge Test Matrix

Trial Number	Test Device	Pathogenic Organism	Surrogate Species Used in Testing	ATCC #	Target Aerosol Particle Size (um)	Challenge Conc. (#/L)	Trial Length (min)	Sampling Time Points (min)	Sampling Equipment	Plating and Enumeration
1 2 3 4 5 6	Control Agentis Brio 650	*Influenza, Coronaviruses*	*MS2 Bacteriophage (RNA Virus)*	*15597-B1*	<1.0	10^4-10^6	45	0, 4, 8, 12, 16, 20, 30, 45	AGI-30 Impingers	All Samples Plated in Triplicate

Figure 2: Test Matrix for Bioaerosol Testing.

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. A programmable controller maintains the chamber's temperature and humidity within ASHRAE 241 limits.

Incorporating 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** 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.

Figure 3: The 30 m^3 bioaerosol testing chamber at ARE Labs adheres to AHAM AC-5 standards and ASHRAE 241 criteria.

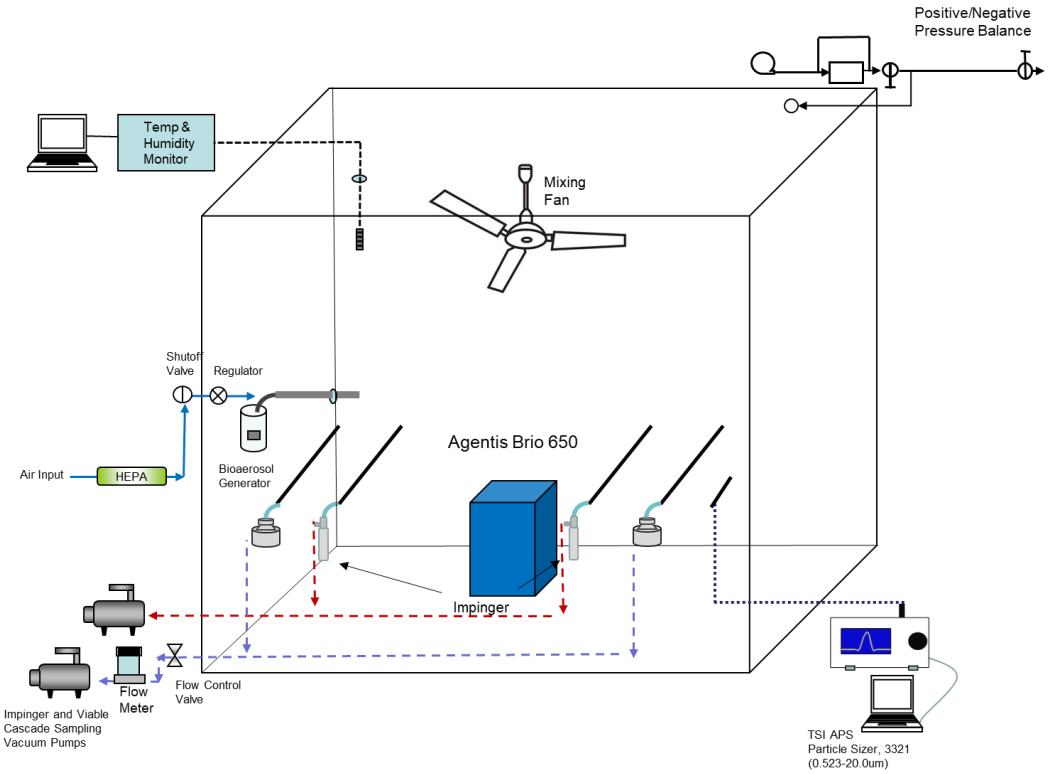

Figure 4: 30m^3 Environmental Test Chamber Flow Diagram. The chamber includes bioaerosol induction, multiple bioaerosol sampling ports, particle size monitoring, internal mixing fans, and temperature and humidity controls. The central system HEPA evacuation system is not pictured.

Bioaerosol Generation System

Per the AHAM AC-5 requirements, the Collison nebulizers can produce 0.05 µm to 5 µm 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.

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 and 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.

Before testing, the Collison nebulizer flow rate and use rate were checked using an air supply pressure of approximately 40-60 psi, producing 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. BGI Industries made the Glass and 304 stainless steel construction.

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 to better represent the entire room. The mixing fan inside the chamber ensured a homogenous air mixture. A picture of the AGI-30 is shown in **Figure 6**.

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

During all characterization and test sampling, the AGI-30 impinger vacuum source was maintained at a negative pressure of -18 inches of Hg to ensure critical flow conditions. The AGI-30 impingers sample at a 12.5 LPM impinger flow rate. The flow rate was measured using a calibrated TSI model 4040 mass flow meter.

Temperature and Humidity Monitor/Controller

The temperature and humidity within the chamber are monitored and controlled with an AC Infinity Controller 69. This controller allows for real-time monitoring and control of the temperature in the 30m^3 bioaerosol chamber used for testing. Temperature and humidity control is essential for the stability of aerosolized microorganisms during testing.

ASHRAE 241 and AHAM AC-5 both have temperature and humidity requirements for temperature and humidity inside 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 7** below.

Figure 7: AC Infinity Controller 69 Temperature and Humidity Controller.

Ion Monitor

The COM ion meter, **Figure 8** below, measured 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 8: COM 3200Pro II ion meter used for ion measurements of the PA663 ionizer.

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 9** below.

Figure 9. TSI Aerodynamic Particle Sizer (APS) model 3321 used to measure the total particle concentration and particle size distribution of the challenge bioaerosol. It has a 0.54-20.0 µm aerodynamic diameter range, with 1 particle/L detection limits.

Chamber Validation

Validating a bioaerosol chamber is crucial 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 appropriately, enabling them to conduct reliable bioaerosol studies that contribute to informed decision-making in indoor air quality and infectious disease research.

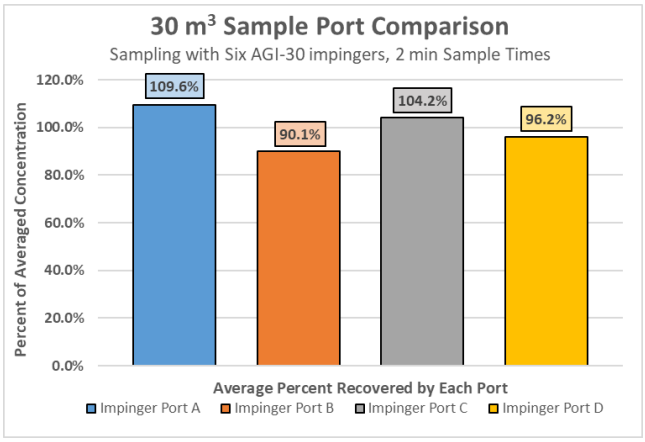

Figure 10: Impinger port-to-port comparison. Percent averages are calculated by dividing each port's count by the average plate count for the four ports.

Homogeneity

One key component of the chamber validation process is the bioaerosol homogeneity test. This test validates the chamber's homogeneity, ensuring 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.

Two technicians plated These impinger samples in triplicate to reduce plating discrepancies. Each set of plate counts generated by each technician was 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's homogeneity during trials. A graphical representation of the average measured for each port is shown in **Figure 10**.

Ionization Validation

To measure the baseline ion concentration, 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 regarding the charge within the chamber is essentially neutral. See the ion data graph from a trial in **Figure 11**. The total production of ions naturally occurring in the chamber is nominal.

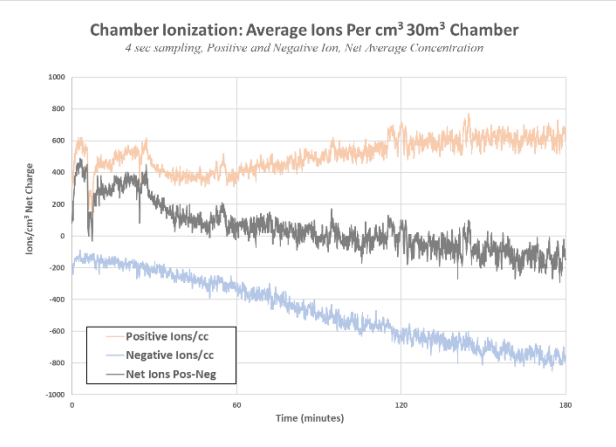

Figure 11. Total baseline level of ions present in the 30 m³ chamber.

Chamber Environmental Controls

Chamber controls involve assessing the natural decay rate of the microbes within the test chamber over a defined time without the air cleaner operation. The duration of this time aligns with the intended operational testing time of the air cleaner, with multiple sampling points set at intervals of twenty minutes to establish a robust natural decay curve.

Microbes are collected using an 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 are precisely defined. Multiple impingers can be employed in series to enhance collection efficiency if necessary.

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 12** at the bottom of this 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 collected from the chamber at 1-minute intervals over a 20-minute. These samples are pivotal in assessing the air cleaner's effectiveness in reducing 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.

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, quantifying the air cleaner's performance. It's worth noting that, in cases where the microbial concentration becomes exceedingly low, an extension of the sampling duration beyond the planned initial 2-minute sampling may be implemented. However, this adjustment should be considered for its potential mathematical implications.

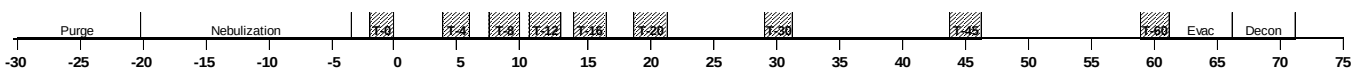

Figure 12: Standard ASHRAE 241 Sampling Times for a 1-Hour Trial. While these were the standard ASHRAE 241 (AHAM AC-5) sampling times, these trials were performed by sampling every minute for up to 10 minutes and then carried out for either 20 or 60 minutes.

An alternative sampling approach is recommended for air cleaners with exceptionally high CADR ratings. This entails obtaining air samples every minute over the relevant test period during the air cleaner's operation. Additional sampling points can then be incorporated at various intervals, extending to 60 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 13**.

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 AC-5 guidelines for biological species selection only require using MS2 Bacteriophage. The virus is an ssRNA bacteriophage commonly used for bioaerosol testing, given its small size and hearty resilience to aerosolization and other disinfecting processes.

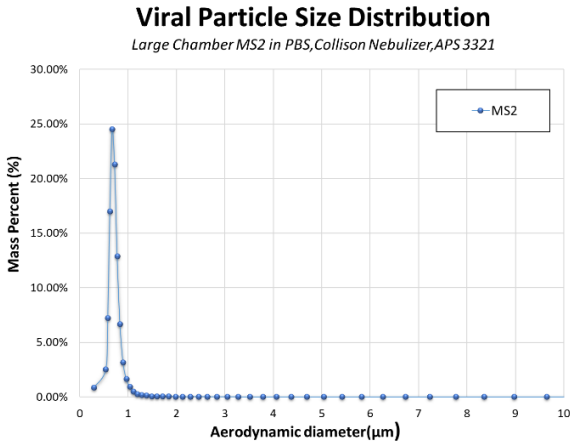

Figure 13: Aerodynamic Particle Size Distribution of the RNA virus, MS2, in the test chamber. The MMAD for this viral species averaged approximately 0.7 µm.

Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate. (Multiple drop samples for each dilution) A standard drop plate technique was used on tryptic soy agar plates.

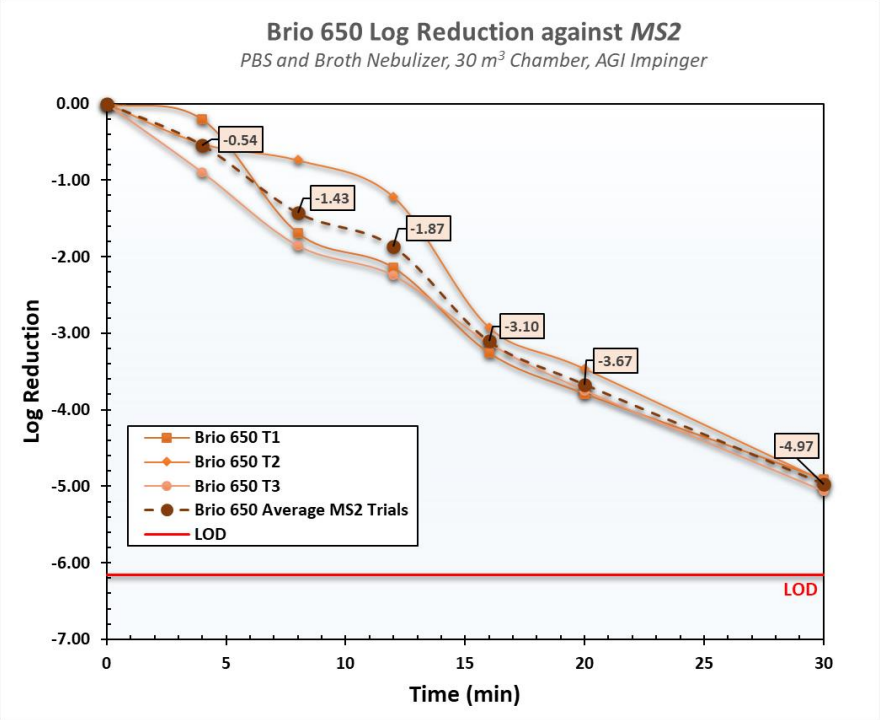

Figure 14: Log Reduction of Aerosolized *MS2* by the Brio 650.

The drop plate technique is widely used in microbiology to determine 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 a virus is used 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, straightforward approach to quantifying bacterial populations. It is 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 completing 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 following ANSI/AHAM AC-5-2022 guidelines (Section 5.1.14).

The chamber underwent at least twenty minutes of airflow evacuation/purging to restore baseline particle concentration levels, as the Aerosol Particle Spectrometer (APS) assessed. Special care was taken to ensure the thorough removal of contaminants, emphasizing the prevention of 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 were the basis for determining the reduction of both Agentis Brio 650 devices over an hour trial above the natural losses from the control runs. The control and trials are plotted, showing a 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 viability over time. CADR values were calculated using the graphical method shown in Figure 19 on page 10.

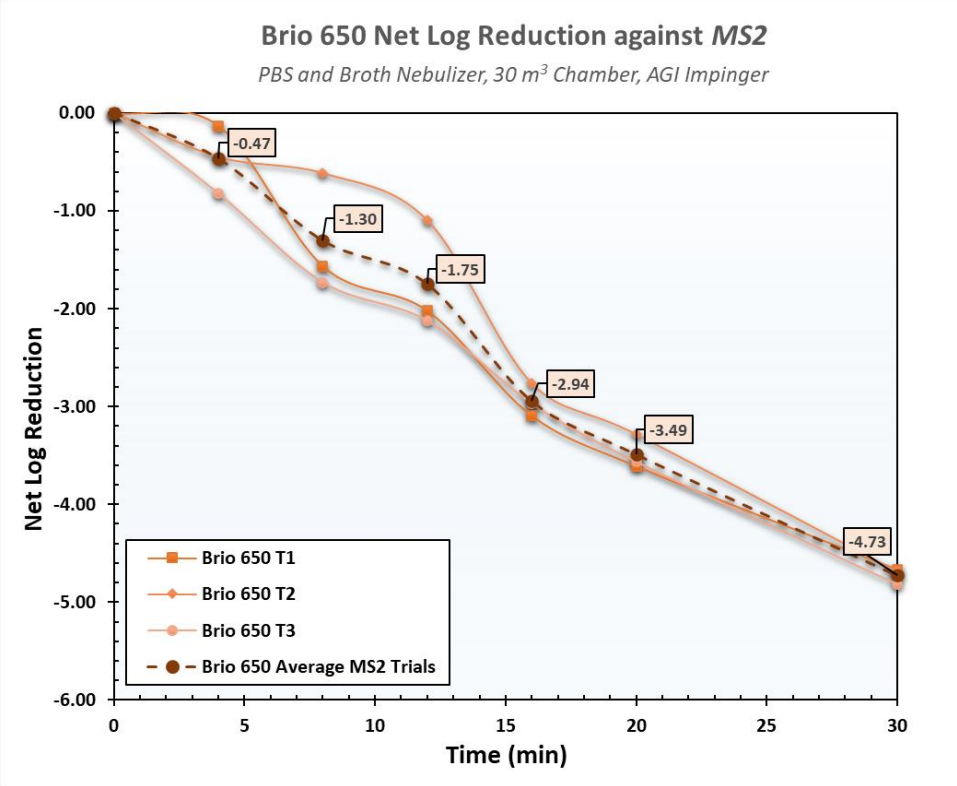

Figure 15: Net log Reduction of Aerosolized *MS2* by the Brio 650.

Agentis Brio 650 against MS2 Trial Summary Data

Bioaerosol Type	Species (description)	Reduction Type	Trial Time (minutes)					
			4	8	12	16	20	30
Virus	*MS2* (RNA Virus)	*Net Log Reduction*	-0.13	-1.57	-2.02	-3.09	-3.61	-4.67
		Net % Reduction	25.9413%	97.2892%	99.0480%	99.9196%	99.9755%	99.9978%
Virus	*MS2* (RNA Virus)	*Net Log Reduction*	-0.45	-0.61	-1.10	-2.77	-3.29	-4.69
		Net % Reduction	64.3421%	75.6741%	91.9932%	99.8282%	99.9481%	99.9979%
Virus	*MS2* (RNA Virus)	*Net Log Reduction*	-0.82	-1.73	-2.12	-2.97	-3.57	-4.82
		Net % Reduction	84.9095%	98.1422%	99.2473%	99.8925%	99.9729%	99.9985%
All Trial Averages +/- St. Dev.		***Net Log Reduction***	***-0.47 +/- 0.35***	***-1.3 +/- 0.6***	***-1.75 +/- 0.57***	***-2.94 +/- 0.17***	***-3.49 +/- 0.18***	***-4.73 +/- 0.08***
		Net % Reduction	*58.398% +/- 29.93%*	*90.369% +/- 12.733%*	*96.763% +/- 4.132%*	*99.88% +/- 0.047%*	*99.965% +/- 0.015%*	*99.9981% +/- 0.0003%*

Figure 16: Summary of the MS2 net log and associated percent reduction values for the Brio 650.

Results

The standard Brio 650 achieved an average of a 4.73 net log reduction in 30 minutes of operation; see **Figures 14 and 15** for a total graphical overview of both log and net log reduction. **Figure 16** presents a table summarizing the results. This is equivalent to a 99.9981% reduction.

Deviations and Acceptance Criteria

No deviations from the ASHRAE 241 protocol were used. All final endpoints were ≤0.30 standard deviations from the mean. Following ARE Lab's standard practices and 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.

Clean Air Delivery Rate Calculations (CADR)

The Clean Air Delivery Rate (CADR) is a measure of the efficiency of an air purifier at removing specific airborne pollutants from indoor air. As expressed in cubic feet per minute (CFM), CADR provides information on the volume of clean air an air purifier can deliver for various pollutants: tobacco smoke, pollen, dust, and biological pathogens. The CADR rating is determined through standardized testing procedures, and a higher CADR indicates a more effective air purifier in removing those contaminants from the air.

For CADR calculations, a linear fit trendline corresponding to a specific time interval was applied to both the trial and the control lines. The difference between these slopes was calculated to determine the equivalent air exchange rate. The time interval to calculate the linear regression slope of the test trials was determined using the t-0 and all time points through t-30 minutes. The CADR was then calculated by multiplying this equivalent air exchange rate by the volume of the test chamber (30 m^3).

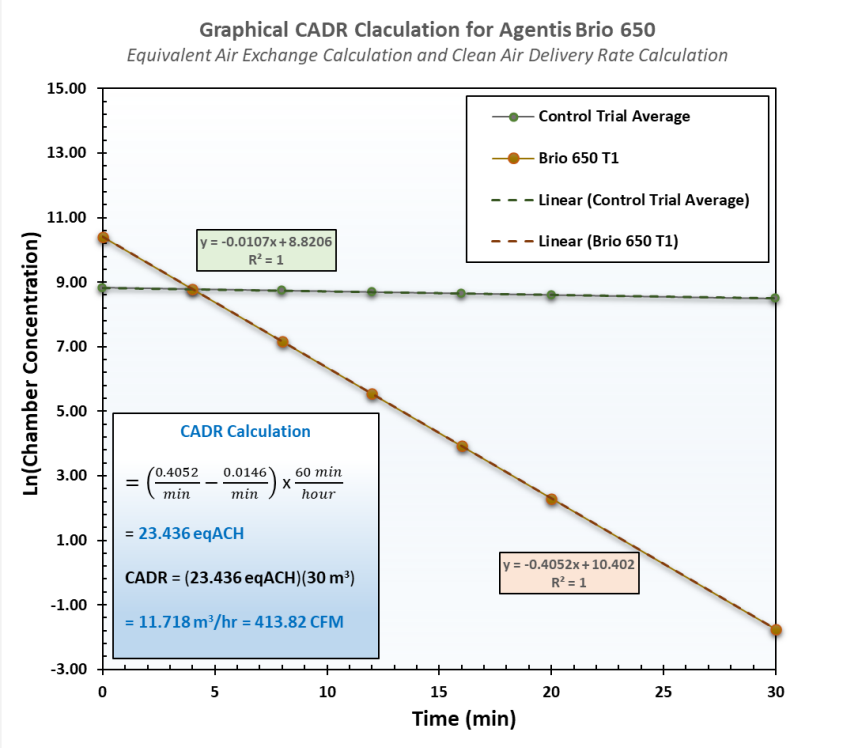

Figure 17: CADR Example Plot and Calculation for the Brio 650.

Calculated from 0-30 miunutes	
Trial	**CADR**
Brio 650 Trial 1	413.83
Brio 650 Trial 2	412.36
Brio 650 Trial 3	394.00
Average	**406.73**
STDEV	**11.05**

Figure 18: CADR summary for the Brio 650 showing the CADR calculated through a single time point against MS2.

CADR Result Summary

The CADR was analyzed across all trials with MS2 for a triplicate set. The Brio 650 Trial 1 against MS2 had the highest CADR at 413.83 CFM from t-0 to t-30, but all the trials averaged 406.73 CFM at that same time interval. A graphical visualization of the CADR calculation with actual trial results can be found in **Figure 17,** with a table breaking down the CADR at a single time point in **Figure 18**.

MS2 Results

The Brio 650 device performed well at removing viable MS2 from the air. It had a total reduction of 4.73 $\pm$ 0.08 net log, equaling a percent reduction of 99.9981%, from the air in 30 minutes of operation and averaged a CADR of 406.73 CFM over that same time frame. The initial calculated CADR from the device achieved roughly the device's baseline flow rate of approximately 500 CFM for MS2.

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Analytical Testing Facility

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

Project

10993.31

Study Director

Richard Ludwick
Aerosol Research and Engineering Laboratories

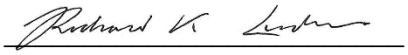
GLP Statement

We, the undersigned, certify that Aerosol Research and Engineering Laboratories conducted the work described herein in compliance with 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. has no affiliations with, or involvement in any capacity, with Agentis's financial interests such as membership, employment, stock ownership, or other equity interests.

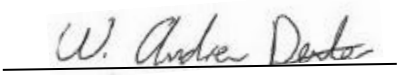
Study Director:



09/26/2024
Date

Richard Ludwick
Study Director
ARE Labs, Inc.

Principal Investigator:



09/26/2024
Date

W. Andrew Dexter M.S.
Staff Research Scientist
ARE Labs, Inc.

APPENDIX A: Bio Aerosol Raw Data

Trial Information

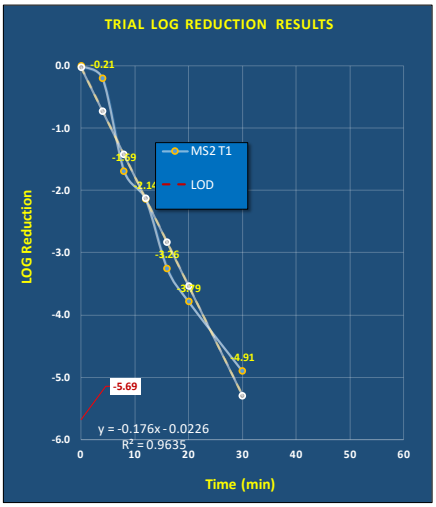
TEST DATE:	Friday, September 6, 2024
TRIAL PERFORMED BY:	WAD
TRIAL NUMBER:	T1
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T1

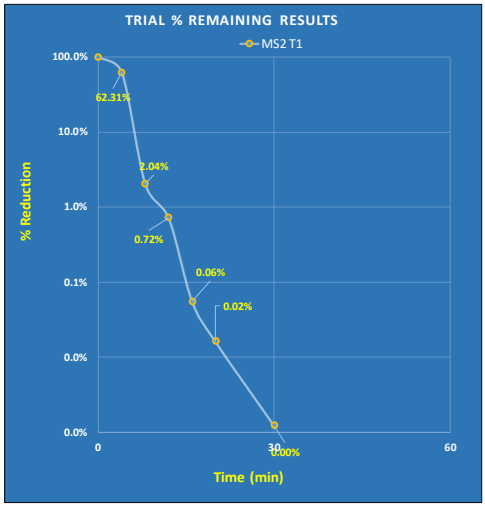
Device Information

MANUFACTURER:	Agentis
UNIT MODEL:	Brio 650
FAN SPEED (CFM):	High Speed
UNIT SERIAL #:	N/A
FITER ID #:	N/A
FILTER LOT #:	N/A

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	30
NEBULIZER CONDITIONS:	20 min nebulization, 10 min dwell
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	On for entire trial
TEMP (F):	75
RH (%):	59
OTHER INSTRUMENTS:	N/A
TRIAL COMMENTS/NOTES	Testing new voltage for electrostatic precipitator





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S7	S8			LOD
SAMPLE TIME (min)	0	4	8	12	16	20	30			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 BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	3.467E+04	2.160E+04	7.067E+02	2.493E+02	1.920E+01	5.600E+00	0.427	#DIV/0!	#DIV/0!	7.111E-02
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)										
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		52.73%	23.33%	13.00%						100.00%
VIABLE CONSISTENCY CHECKS (% agreement)										
IMP & VIABLE CROSS CHECK (% agreement)										
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**3.467E+04**	**2.160E+04**	**7.067E+02**	**2.493E+02**	**1.920E+01**	**5.600E+00**	**4.267E-01**			**0.0711**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**62.3077%**	**2.0385%**	**0.7192%**	**0.0554%**	**0.0162%**	**0.0012%**			**0.0002%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**37.6923%**	**97.9615%**	**99.2808%**	**99.9446%**	**99.9838%**	**99.9988%**			**99.9998%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.21**	**-1.69**	**-2.14**	**-3.26**	**-3.79**	**-4.91**			**-5.69**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30			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	5.0	5.0	5.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
DILUTION RATIO (10^x)	**-2**	**-3**	**-1**	**-1**	**-3**	**-2**	**0**	**-3**	**-3**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**500**	**100**	**100**	**750**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)		5	3	2						1
		5	11	2						0
		1	9	6						0
PLATE AVERAGE COUNT (# / drop)		3.67	7.67	3.33						0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)		36,667	767	333						0
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		2.93E+04	6.13E+02	2.67E+02						7.11E-02
DILUTION RATIO (10^x)	**-3**	**-2**	**-2**	**0**	**0**	**0**	**0**	**-2**	**-2**	
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**	**500**	**500**	**100**	**100**	
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	3	18	0	28	12	3	2			
	9	18	1	24		4	0			
	1	16	2	35			0			
PLATE AVERAGE COUNT (# / drop)	4.33	17.33	1.00	29.00	12.00	3.50	0.67			
IMPINGER CONCENTRATION (cfu or pfu/ml)	43,333	17,333	1,000	290	24	7	1			
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	3.47E+04	1.39E+04	8.00E+02	2.32E+02	1.92E+01	5.60E+00	4.27E-01			

Figure 1A: *Agentis Brio 650 Raw Data T1.*

Trial Information

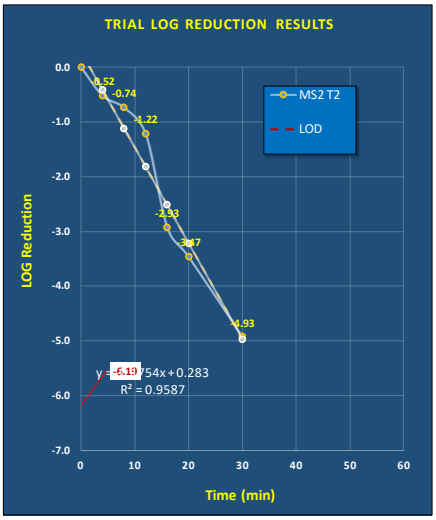
TEST DATE:	Friday, September 6, 2024
TRIAL PERFORMED BY:	WAD
TRIAL NUMBER:	T2
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T2

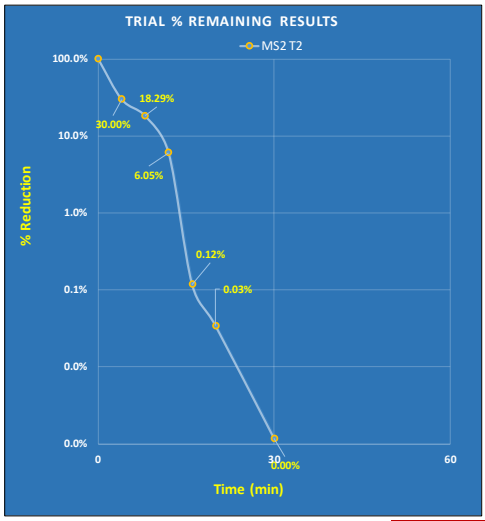
Device Information

MANUFACTURER:	Agentis
UNIT MODEL:	Brio 650
FAN SPEED (CFM):	High Speed
UNIT SERIAL #:	N/A
FITER ID #:	N/A
FILTER LOT #:	N/A

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	30
NEBULIZER CONDITIONS:	20 min nebulization, 10 min dwell
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	On for entire trial
TEMP (F):	72
RH (%):	60
OTHER INSTRUMENTS:	N/A
TRIAL COMMENTS/NOTES	Testing new voltage for electrostatic precipitator





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S7	S8			LOD
SAMPLE TIME (min)	0	4	8	12	16	20	30			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 BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.093E+05	3.280E+04	2.000E+04	6.613E+03	1.293E+02	3.733E+01	1.280	#DIV/0!	#DIV/0!	7.111E-02
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)										
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		36.00%			6.00%	25.00%				100.00%
VIABLE CONSISTENCY CHECKS (% agreement)										
IMP & VIABLE CROSS CHECK (% agreement)										
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.093E+05**	**3.280E+04**	**2.000E+04**	**6.613E+03**	**1.293E+02**	**3.733E+01**	**1.280E+00**			**0.0711**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**30.0000%**	**18.2927%**	**6.0488%**	**0.1183%**	**0.0341%**	**0.0012%**			**0.0001%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**70.0000%**	**81.7073%**	**93.9512%**	**99.8817%**	**99.9659%**	**99.9988%**			**99.9999%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.52**	**-0.74**	**-1.22**	**-2.93**	**-3.47**	**-4.93**			**-6.19**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30			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	5.0	5.0	5.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
DILUTION RATIO (10^x)	**-4**	**-3**	**-3**	**-1**	**-1**	**0**	**0**	**-3**	**-3**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**500**	**100**	**100**	**750**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)		7		80	1	12	2			1
		3		77	1	4	2			0
		5		91	3	0	2			0
PLATE AVERAGE COUNT (# / drop)		5.00		82.67	1.67	5.33	2.00			0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)		50,000		8,267	167	53	4			0
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		4.00E+04		6.61E+03	1.33E+02	42.67	1.28E+00			7.11E-02
DILUTION RATIO (10^x)	**-3**	**-2**	**-2**	**-2**	**0**	**0**	**-2**	**-2**	**-2**	
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**500**	**100**	**100**	**100**	
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	10	30	27		16	11				
	17	40	25		20	29				
	14	26	23		11					
PLATE AVERAGE COUNT (# / drop)	13.67	32.00	25.00		15.67	20.00				
IMPINGER CONCENTRATION (cfu or pfu/ml)	136,667	32,000	25,000		157	40				
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.09E+05	2.56E+04	2.00E+04		1.25E+02	3.20E+01				

Figure 2A: *Agentis Brio 650 Raw Data T2.*

Trial Information

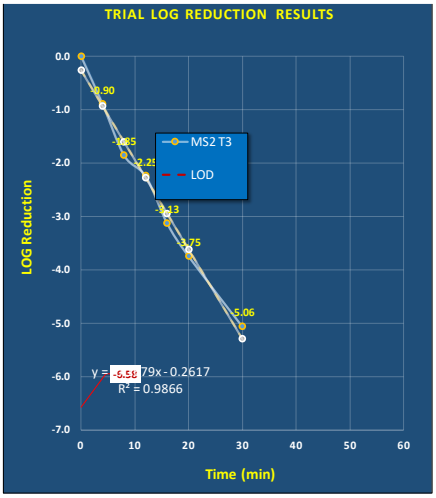
TEST DATE:	Friday, September 6, 2024
TRIAL PERFORMED BY:	WAD
TRIAL NUMBER:	T3
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T3

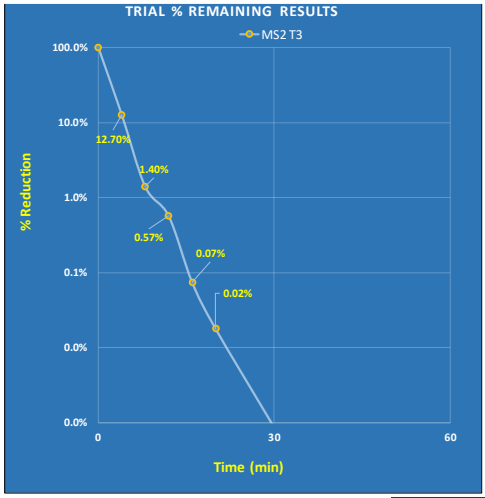
Device Information

MANUFACTURER:	Agentis
UNIT MODEL:	Brio 650
FAN SPEED (CFM):	High Speed
UNIT SERIAL #:	N/A
FITER ID #:	N/A
FILTER LOT #:	N/A

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	30
NEBULIZER CONDITIONS:	20 min nebulization, 10 min dwell
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	On for entire trial
TEMP (F):	70
RH (%):	58
OTHER INSTRUMENTS:	N/A
TRIAL COMMENTS/NOTES	Testing new voltage for electrostatic precipitator





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S7	S8			LOD
SAMPLE TIME (min)	0	4	8	12	16	20	30			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 BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	2.720E+05	3.453E+04	3.800E+03	1.547E+03	2.013E+02	4.853E+01	2.347	#DIV/0!	#DIV/0!	7.111E-02
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)										
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	43.08%	15.00%	10.00%	6.67%	8.86%	8.42%				100.00%
VIABLE CONSISTENCY CHECKS (% agreement)										
IMP & VIABLE CROSS CHECK (% agreement)										
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**2.720E+05**	**3.453E+04**	**3.800E+03**	**1.547E+03**	**2.013E+02**	**4.853E+01**	**2.347E+00**			**0.0711**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**12.6961%**	**1.3971%**	**0.5686%**	**0.0740%**	**0.0178%**	**0.0009%**			**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**87.3039%**	**98.6029%**	**99.4314%**	**99.9260%**	**99.9822%**	**99.9991%**			**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.90**	**-1.85**	**-2.25**	**-3.13**	**-3.75**	**-5.06**			**-6.58**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	4	8	12	16	20	30			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	5.0	5.0	5.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
DILUTION RATIO (10^X)	**-4**	**-3**	**-2**	**-2**	**0**	**0**	**0**	**-3**	**-3**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**500**	**100**	**100**	**750**
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	3	5	4	3	26	6	3			1
	4	6	5	2	20	6	3			0
	6	3	6	1	33	7	5			0
PLATE AVERAGE COUNT (# / drop)	4.33	4.67	5.00	2.00	26.33	6.33	3.67			0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	433,333	46,667	5,000	2,000	263	63	7			0
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	3.47E+05	3.73E+04	4.00E+03	1.60E+03	2.11E+02	50.67	2.35E+00			7.11E-02
DILUTION RATIO (10^X)	**-3**	**-2**	**-1**	**-1**	**0**	**0**	**-2**	**-2**	**-2**	
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**	**500**	**100**	**100**	**100**	
Dilution Range #1 — ENUMERATED PLATE COUNTS (# / drop)	31	36	35	15	120	22				
	26	43	40	22		36				
	17	40	60	19						
PLATE AVERAGE COUNT (# / drop)	24.67	39.67	45.00	18.67	120.00	29.00				
IMPINGER CONCENTRATION (cfu or pfu/ml)	246,667	39,667	4,500	1,867	240	58				
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.97E+05	3.17E+04	3.60E+03	1.49E+03	1.92E+02	4.64E+01				

Figure 3A: *Agentis Brio 650 Raw Data T3.*

Appendix B: Calculations

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

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

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

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

Plating and enumerating the biological to derive the dry powder concentration (Cp) concentration 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 the impinger or filter sample enumeration.
- Impinger sample collection volume (I_{vol}) = 20 mL collection fluid/impinger or extraction fluid for a filter.
- AGI–30 impingers or filter sample flow rate (Q_{imp}) = 12.5 L/min.
- AGI–30 impinger or filter sample time (t) = 5 or 10 minutes, test dependent.

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

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

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

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

The table below is based on the principle that, as the number of viable particles being impinged on a given plate increases, the probability of the next particle going into an "empty hole" decreases.
This can be corrected statistically by using the conversion formula of Feller [4]:

Pr = N [1/N + 1/N-1 + 1/N-2 + ... 1/N-r+1]

N is the number of holes (400) in the sampling head.
For easy use of this formula please refer to the table in chapter 17.2
For each colony count **r** a statistically corrected total count **Pr** can be easily seen in the table.