ASHRAE 241-2023 Standard Testing for the Efficacy of the CASPR Medik In-Duct Advanced Photocatalytic Oxidation Device at Reducing Aerosolized *MS2* Tested in a Partial HVAC Duct System

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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 CASPR'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 CASPR Medik device and its ability to reduce the bacteriophage, MS2, per the ASHRAE 241-2023 standard.

Background:

The Medik device is classified as a medical grade HVAC device that utilizes a cell made up of a cold plasma bulb and proprietary catalytic coated honeycomb. It is designed to be installed in the HVAC ductwork such that the cell is inserted into the air flow. The catalyst generates gaseous hydrogen peroxide for decontaminating both air and surfaces.

All testing was conducted in a 30m³ bioaerosol test chamber which housed the partial HVAC system that the devices were installed in. The species selected for this study was MS2, an ssRNA bacteriophage, that is a recognized surrogate for more dangerous pathogenic organisms like influenza. This study utilizes ASHRAE 241 and AHAM AC-5 testing parameters to determine efficacy. Three separate bioaerosol test trials were performed for each of the CASPR Medik devices.

Methods:

A partial HVAC system was constructed with installation points for the CASPR Medik unit with a blower rate set at 5 air changes per hour (5 ACH). MS2 was aerosolized into the sealed 30m³ environmental bioaerosol chamber, with the partial HVAC system containing the CASPR Medik , using a Collison 24-Jet Nebulizer. *MS2* was the microorganism used for all aerosol 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 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 standard CASPR Medik device proved to be effective at reducing MS2. It achieved a net log reduction of 3.57 +/- 0.32. While ASHRAE 241 does not specifically desire the reporting of net log reduction, this information provides a more robust analysis of the total efficacy of the device in addition to the V_{acs} value that they require for induct units. The overall V_{acs} and ε_{PR} values were calculated using the reduction rate multiplied by the chamber volume to get a V_{acs} value. This value was then plugged into equation 7-1 in ASHRAE 241 to obtain an ε_{PR} value. This value came out to be 176.9%.

Introduction

This study was conducted to evaluate the efficacy of the CASPR Medik device for its individual effectiveness at deactivating aerosolized MS2. The device is specifically designed to be installed into the ductwork of an already functioning HVAC system and utilizes a proprietary cold plasma bulb to generate gaseous hydrogen peroxide for reducing contamination in the air and on surfaces.

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 CASPR devices 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 CAPSR Medik device. A picture of the Medik is shown in **Figures 1** and **2**.

Study Overview

The effectiveness of the CASPR Medik 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 3**.

Test Device Description

The CASPR Medik is a medical-grade air purifier and surface decontamination device. This model houses a proprietary catalytic coated honeycomb and cold plasma bulb, producing a photocatalytic effect to produce gaseous hydrogen peroxide that reduces microbes in the air and on surfaces. Radiation from the bulb irradiates bacteria and viruses and produces a photocatalytic effect with its catalytic housing to produce hydrogen peroxide, further reducing microbes on surfaces. These devices are designed to be retrofitted into existing HVAC systems in either a hospital, business, or residential home. The ability of the CASPR Medik to utilize an already established HVAC system, with a fan and filter, greatly reduces the initial cost of implementing one of these systems.

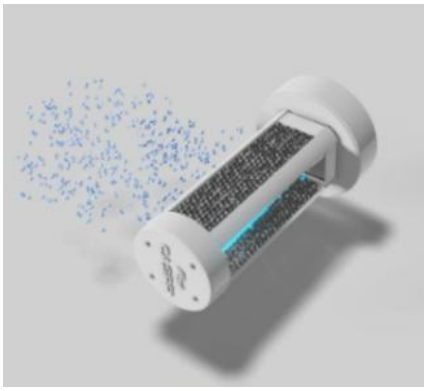

Figure 1: CASPR Medik.

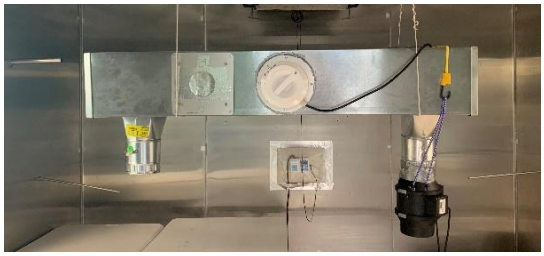

Figure 2: CASPR Medik – Installed in partial HVAC duct used for testing in the bioaerosol test chamber.

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.

Trial	Run	Device	Fan Speed (ACH)	Surrogate Species (description)	Pathogenic Species Represented	ATCC Ref #	Chamber Size (m3)	Target Particle Size (µm)	Challenge Conc. (#/L)	Trial Time (min)	Bioaerosol Sampling Time Points (min)	Sampling Devices	Plating and Enumeration
1 2 3	Challenge Challenge Challenge	CASPR Medik	5 ACH	MS2 Bacteriophage (RNA Virus)	Influenza, Coronaviruses	15597-B1	30	<1.0um	10^5-10^7	60	0, 2, 4, 8, 12, 16, 20, 30, 45, 60	TSI 3321 APS, Impingers	all samples in triplicate
1 2 3	Control Control Control	No Device	5 ACH	MS2 Bacteriophage (RNA Virus)	Influenza, Coronaviruses	15597-B1	30	<1.0um	10^5-10^7	60	0, 2, 4, 8, 12, 16, 20, 30, 45, 60	TSI 3321 APS, Impingers	all samples in triplicate

Figure 3: Test Matrix for Bioaerosol Testing.

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.

The incorporation of negative pressure airflow allows for controlled purging, and a HEPA filter adds an additional layer of protection, inhibiting potential contamination. The 30m^3 testing chamber at ARE Labs fulfills both AHSRAE 241 and AHAM AC-5 requirements. **Figure 4** 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 5**.

Figure 4: The 30 m^3 bioaerosol testing chamber at ARE Labs adheres to AHAM AC-5 standards and ASHRAE 241 criteria. The chamber is equipped with HEPA filtered air in/out, multiple bio aerosol sampling ports, decontamination, and pressure balance.

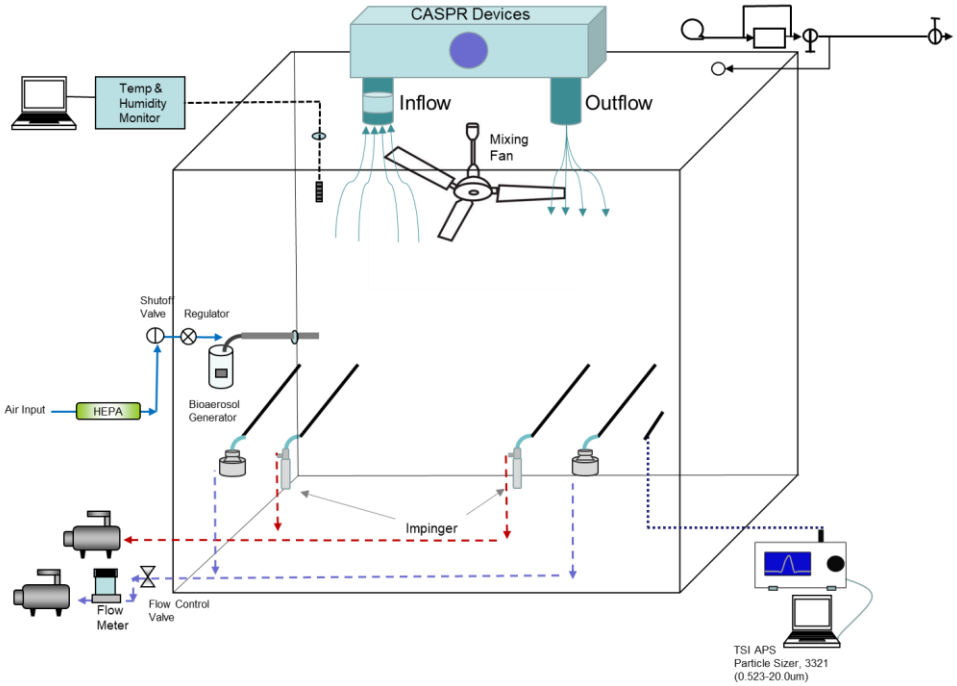

Figure 5: 30m^3 Environmental Test Chamber Flow Diagram. Chamber includes bioaerosol induction, multiple bioaerosol sampling ports, particle size monitoring, internal mixing fans, and temperature and humidity controls. Main system HEPA evacuation system not pictured.

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.

A 24-Jet Collison (BGI Inc. Waltham MA), similar to the one shown in **Figure 6** 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 6. 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 7** below.

Figure 7: AGI-30 Impinger, Ace Glass Inc. Vineland NJ.

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.

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 8** 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 8: 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% ± 10% while the temperature range is 73°F + 5° (23°C + 3°C). A picture of the controller is shown in **Figure 9** below.

Figure 9: AC Infinity Controller 69 Temperature and Humidity Controller.

Ion Monitor

The COM ion meter, **Figure 10** 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 10: 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 11** above.

Figure 11. 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.

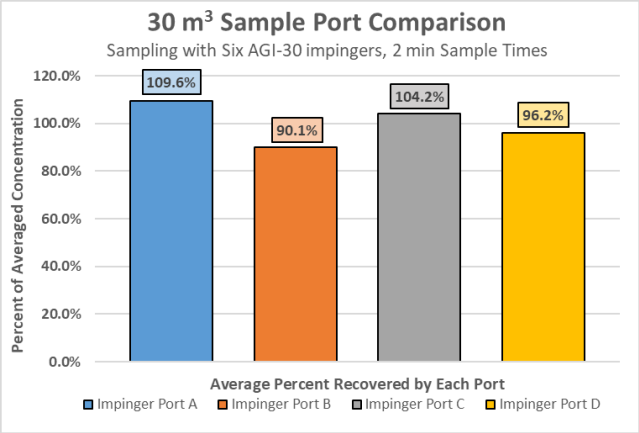

Figure 12: Impinger port-to-port comparison. Percent averages are calculated by taking the count for each port divided by the average plate count for the four ports.

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 12** on the previous page.

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 is essentially neutral in regard to the charge within the chamber. See ion data graph from trial in **Figure 13**. The total production of ions naturally occurring in the chamber is nominal.

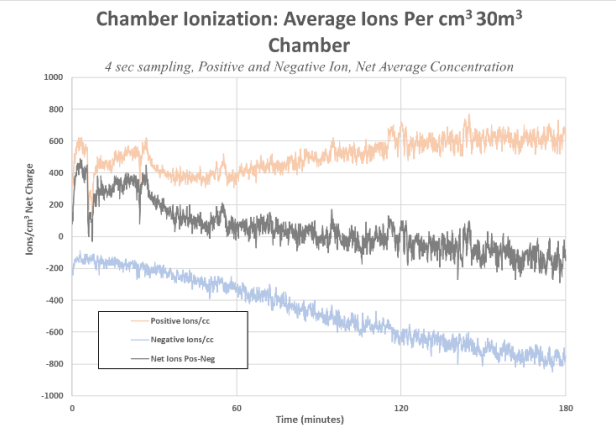

Figure 13. Total baseline level of ions present in the 30m³ chamber.

Chamber Environmental Controls

Chamber controls involve assessing the natural decay rate of the microbes within the test chamber over a defined time period without the air cleaner operation. The duration of this time period 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. 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 14**, 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.

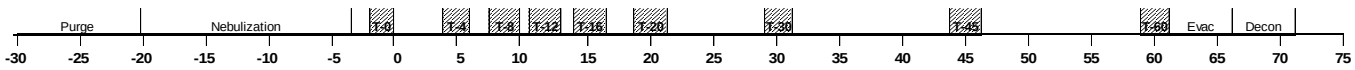

Figure 14: ASHRAE 241 Sampling Times for a 1 Hour Trial.

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 15** above.

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.

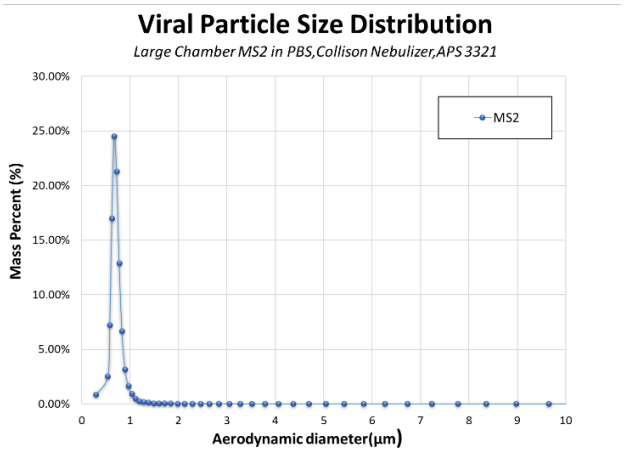

Figure 15: Aerodynamic Particle Size Distribution of the RNA virus MS2 in the test chamber. The MMAD for this viral species averaged approximately 0.7 µm.

Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate. (Multiple drop samples for each dilution) using a standard drop plate technique onto tryptic soy agar plates.

The drop plate technique is a widely utilized method in microbiology for determining bacterial or viral concentrations in liquid samples. In this technique, known volumes of the liquid sample are serially diluted, and each dilution is carefully dispensed onto solid agar plates. These plates provide a nutrient-rich environment that supports bacterial growth. Once the drops are evenly spread across the surface, the plates are incubated for 24-48 hours, depending on the species, then enumerated and recorded. If using a virus for testing the host organism is added to each tube to allow for viral replication and plaque formation.

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

Post-Testing Decontamination and Prep

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

The chamber underwent a minimum of twenty minutes of air flow evacuation/purging to restore baseline particle concentration levels, as assessed by the Aerosol Particle Spectrometer (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 both CASPR Medik devices 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 viability over time. CADR values were calculated using the graphical method shown in Figure 19 on page 10.

Results

The standard Medik achieved a 3.57 net log reduction see Figures 16 and 17 for a total graphical overview of both log and net log reduction. Figure 18 has a table summarizing the results seen in Figure 17.

Deviations and Acceptance Criteria

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

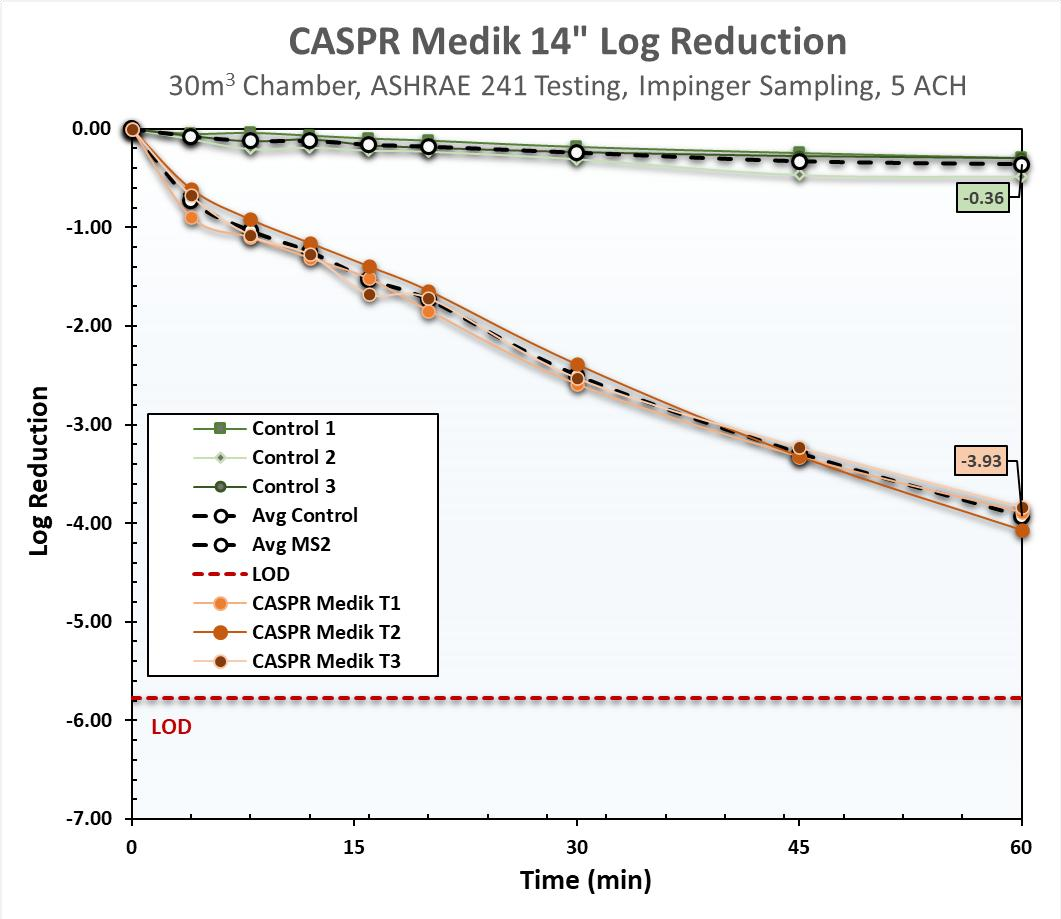

Figure 16: Log Reduction of Aerosolized *MS2* by the Medik.

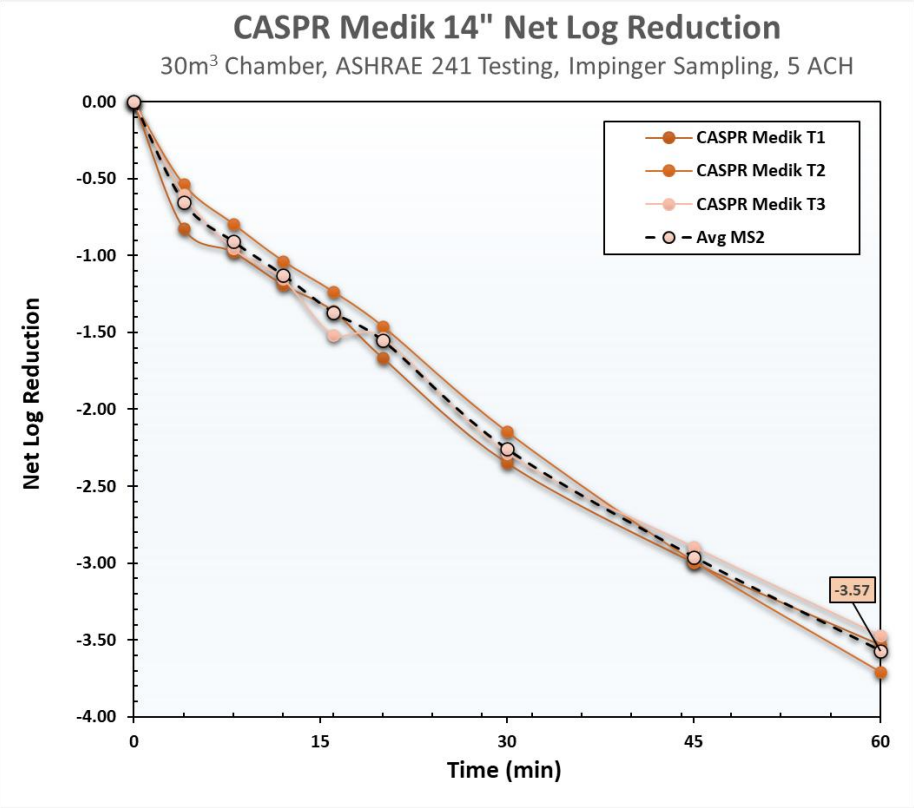

Figure 17: Net log Reduction of Aerosolized *MS2* by the Medik.

CASPR Medik 14" Executive Summary with Net Log Reduction

Bioaerosol Type	Species (description)	Reduction Type	Starting Concentration	Trial Time (minutes)							
				4	8	12	16	20	30	45	60
Virus	*MS2* (RNA Virus)	*Net Log Reduction*	*1.16E+05*	-0.82	-0.98	-1.19	-1.36	-1.66	-2.35	-2.99	-3.53
		Net % Reduction		84.9384%	89.4427%	93.5330%	95.6104%	97.8293%	99.5507%	99.8987%	99.9705%
Virus	*MS2* (RNA Virus)	*Net Log Reduction*	*1.64E+05*	-0.53	-0.79	-1.04	-1.23	-1.46	-2.15	-2.99	-3.71
		Net % Reduction		70.8019%	83.9555%	90.7916%	94.1600%	96.5327%	99.2860%	99.8977%	99.9805%
Virus	*MS2* (RNA Virus)	*Net Log Reduction*	*1.27E+05*	-0.60	-0.96	-1.15	-1.52	-1.53	-2.28	-2.90	-3.47
		Net % Reduction		75.0644%	88.9337%	92.9649%	96.9536%	97.0682%	99.4782%	99.8729%	99.9662%
All Trial Averages +/- St. Dev.		***Net Log Reduction***	***1.36E+05***	***-0.65 +/- 0.15***	***-0.91 +/- 0.1***	***-1.13 +/- 0.08***	***-1.37 +/- 0.14***	***-1.55 +/- 0.1***	***-2.26 +/- 0.1***	***-2.96 +/- 0.06***	***-3.57 +/- 0.12***
		Net % Reduction		*76.935% +/- 7.252%*	*87.444% +/- 3.032%*	*92.43% +/- 1.447%*	*95.575% +/- 1.397%*	*97.143% +/- 0.652%*	*99.4383% +/- 0.1368%*	*99.8898% +/- 0.0146%*	*99.9724% +/- 0.0073%*

Figure 18: Summary of the MS2 net log and associated percent reduction values for the Medik.

Conclusion

The ASHRAE 241 standard provides a method to calculate the aerosol reduction efficiency (ε_{PR}) of an in-duct air cleaner. The performance is calculated using the equivalent clean air flow rate (V_{ACS}) divided by the air flow rate through the duct system. For this test, the ε_{PR} was determined to be 176.9% for the CASPR Medik based on the provided ASHRAE 241 equations.

Calculations for the V_{ACS} and ε_{PR} can be found below in **Appendix B**. Both equations are used to calculate the V_{ACS} and then ε_{PR} per ASHRAE 241 Normative A Guidelines. However, ASHRAE 241 does not provide any guidelines for what any given in-duct air cleaner is required to achieve. A Table showing the final calculation values is provided in **Figure 19** below.

Medik

Trials	Average Control Slope	Trial Slope	V_{ACS} (CFM)	Flow Rate (CFM)	Absolute Value of εPR
T1	-0.0135	-0.159	-154.680	88.0	175.8%
T2	-0.0135	-0.163	-158.764	88.0	180.4%
T3	-0.0135	-0.158	-153.537	88.0	174.5%
Average	**-0.0135**	**-0.160**	**-155.661**	**88.0**	**176.9%**

Figure 19: V_{acs} and ε_{PR} values for the Medik.

References

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Analytical Testing Facility

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

Project

10926.20.1.1

Study Director

Richard Ludwick
Aerosol Research and Engineering Laboratories

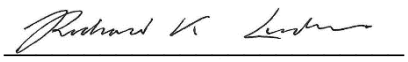
GLP Statement

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

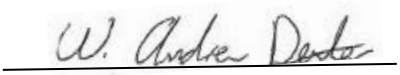
Study Director:



09/05/2023
Date

Richard Ludwick
Study Director
ARE Labs, Inc.

Principal Investigator:



09/05/2023
Date

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

APPENDIX A: Bio Aerosol Raw Data

Trial Information

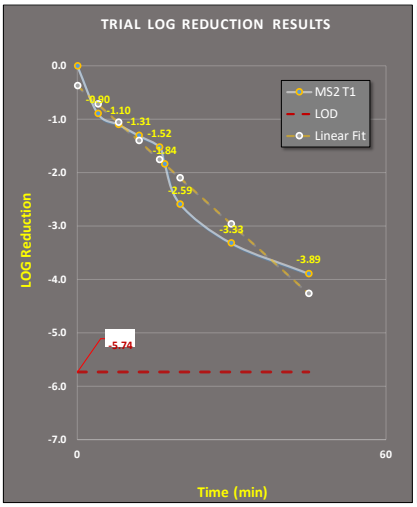
TEST DATE: Wednesday, July 19, 2023
TRIAL PERFORMED BY: WAD
TRIAL NUMBER: T1
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T1

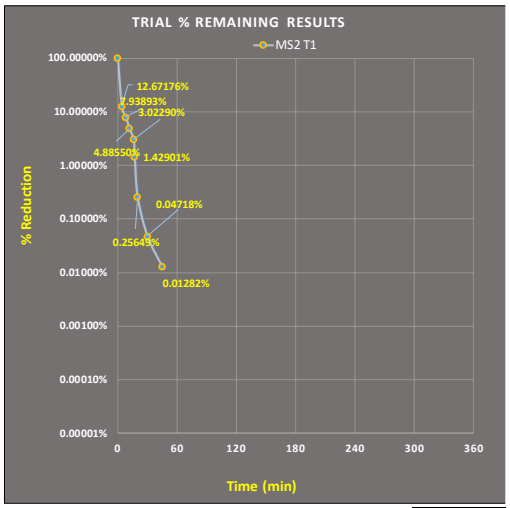
Device Information

MANUFACTURER: CASPR
UNIT MODEL: Medik Ozone Bulb
FAN SPEED (CFM): 5 ACH
UNIT SERIAL #:
FITER ID #:
FILTER LOT #:

General Testing Conditions (Can Be User Defined)

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





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S6	S7	S8	LOD
SAMPLE TIME (min)	0	4	8	12	16	17	20	30	45	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)	1.164E+05	1.476E+04	9.244E+03	5.689E+03	3.520E+03	1.664E+03	2.987E+02	54.933	14.933	2.133E-01
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)										
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	56.04%	6.98%				35.79%		52.86%		100.00%
VIABLE CONSISTENCY CHECKS (% agreement)										
IMP & VIABLE CROSS CHECK (% agreement)										
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.164E+05**	**1.476E+04**	**9.244E+03**	**5.689E+03**	**3.520E+03**	**1.664E+03**	**2.987E+02**	**5.493E+01**	**1.493E+01**	**0.2133**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**12.6718%**	**7.9389%**	**4.8855%**	**3.0229%**	**1.4290%**	**0.2565%**	**0.0472%**	**0.0128%**	**0.0002%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**87.3282%**	**92.0611%**	**95.1145%**	**96.9771%**	**98.5710%**	**99.7435%**	**99.9528%**	**99.9872%**	**99.9998%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.90**	**-1.10**	**-1.31**	**-1.52**	**-1.84**	**-2.59**	**-3.33**	**-3.89**	**-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)	3.0	3.0	3.0	3.0	5.0	5.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-3**	**-2**	**-2**	**-2**	**-1**	**-1**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	24	2	17	14	10	42	8	1	8	1
		35	3	14	8	15	37	15	5	4	0
		32	3	21	10	8	43	5	1	2	0
	PLATE AVERAGE COUNT (# / drop)	30.33	2.67	17.33	10.67	11.00	40.67	9.33	2.33	4.67	0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	303,333	26,667	17,333	10,667	11,000	4,067	933	233	47	1
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.62E+05**	**1.42E+04**	**9.24E+03**	**5.69E+03**	**3.52E+03**	**1.30E+03**	**2.99E+02**	**7.47E+01**	**1.49E+01**	**2.13E-01**
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-2**	**-2**	**0**	**-1**	**-1**	**-2**	**0**	**0**	
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	
	ENUMERATED PLATE COUNTS (# / drop)	2	18				65		15		
		1	28				53		9		
		1	40				72		9		
	PLATE AVERAGE COUNT (# / drop)	1.33	28.67				63.33		11.00		
	IMPINGER CONCENTRATION (cfu or pfu/ml)	133,333	28,667				6,333		110		
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**7.11E+04**	**1.53E+04**				**2.03E+03**		**3.52E+01**		

Figure 1A: *CASPR Medik at 5 ACH Bioaerosol Plates Raw Data.*

Trial Information

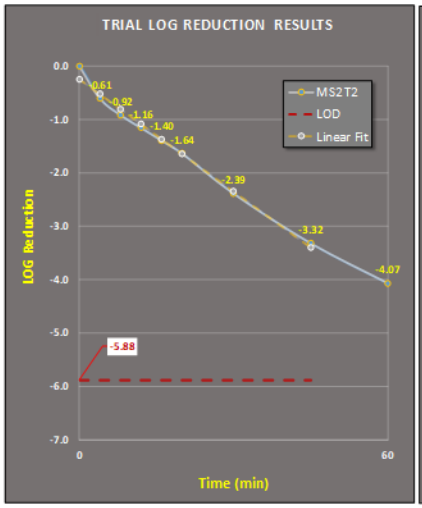
TEST DATE: Wednesday, July 19, 2023
TRIAL PERFORMED BY: WAD
TRIAL NUMBER: T2
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T2

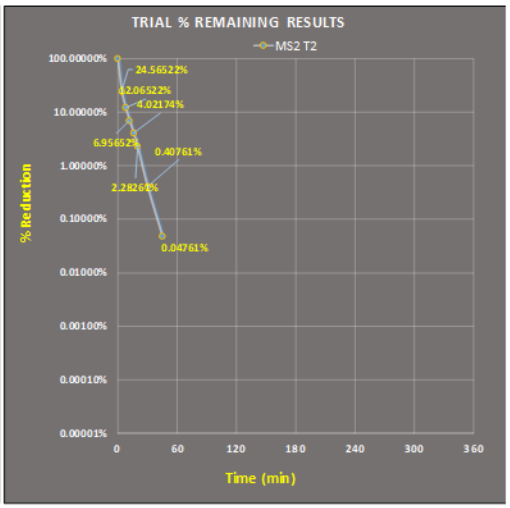
Device Information

MANUFACTURER: CASPR
UNIT MODEL: Medik Ozone Bulb
FAN SPEED (CFM): 5 ACH
UNIT SERIAL #:
FITER ID #:
FILTER LOT #:

General Testing Conditions (Can Be User Defined)

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





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 or pfu/L Air)	1.636E+05	4.018E+04	1.973E+04	1.138E+04	6.578E+03	3.733E+03	666.667	77.867	13.867	2.133E-01
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)										
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	58.46%	32.59%	53.95%	35.90%	52.00%		21.43%	17.50%		100.00%
VIABLE CONSISTENCY CHECKS (% agreement)										
IMP & VIABLE CROSS CHECK (% agreement)										
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.636E+05	4.018E+04	1.973E+04	1.138E+04	6.578E+03	3.733E+03	6.667E+02	7.787E+01	1.387E+01	0.2133
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	24.5652%	12.0652%	6.9565%	4.0217%	2.2826%	0.4076%	0.0476%	0.0085%	0.0001%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	75.4348%	87.9348%	93.0435%	95.9783%	97.7174%	99.5924%	99.9524%	99.9915%	99.9999%
LOG REDUCTION FROM T=0 (log_{10})	0.00	-0.61	-0.92	-1.16	-1.40	-1.64	-2.39	-3.32	-4.07	-5.88

Impinger Sampling Conditions

	S1	S2	S3	S4	S5	S6	S7	S8	S9	LOD
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)	3.0	3.0	3.0	3.0	3.0	5.0	5.0	5.0	5.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1										
DILUTION RATIO (10^n)	-3	-3	-3	-3	-3	-2	-2	0	0	0
DROPLET SIZE (µl)	100	100	100	100	100	100	100	100	100	500
ENUMERATED PLATE COUNTS (# / drop)	19	7	1	2	3	15	1	24	8	1
	13	15	4	1	1	13	1	22	2	0
	22	5	2	2	1	7	5	20	3	0
PLATE AVERAGE COUNT (# / drop)	18.00	9.00	2.33	1.67	1.67	11.67	2.33	22.00	4.33	0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	180,000	90,000	23,333	16,667	16,667	11,667	2,333	220	43	1
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	9.60E+04	4.80E+04	1.24E+04	8.89E+03	8.89E+03	3.73E+03	7.47E+02	7.04E+01	1.39E+01	2.13E-01
Dilution Range #1										
DILUTION RATIO (10^n)	-4	-2	-2	-2	-2	-1	-1	-1	-1	
DROPLET SIZE (µl)	100	100	100	100	100	100	100	100	100	
ENUMERATED PLATE COUNTS (# / drop)	8	73	45	31	7		20	2		
	2	53	61	22	16		20	1		
	3	56	46	25	1		15	5		
PLATE AVERAGE COUNT (# / drop)	4.33	60.67	50.67	26.00	8.00		18.33	2.67		
IMPINGER CONCENTRATION (cfu or pfu/ml)	433,333	60,667	50,667	26,000	8,000		1,833	267		
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	2.31E+05	3.24E+04	2.70E+04	1.39E+04	4.27E+03		5.87E+02	8.53E+01		

Figure 2A: *CASPR Medik at 5 ACH Bioaerosol Plates Raw Data.*

Trial Information

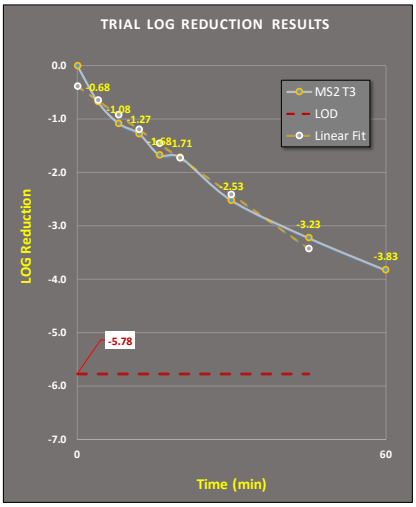
TEST DATE: Wednesday, July 19, 2023
TRIAL PERFORMED BY: WAD
TRIAL NUMBER: T3
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T3

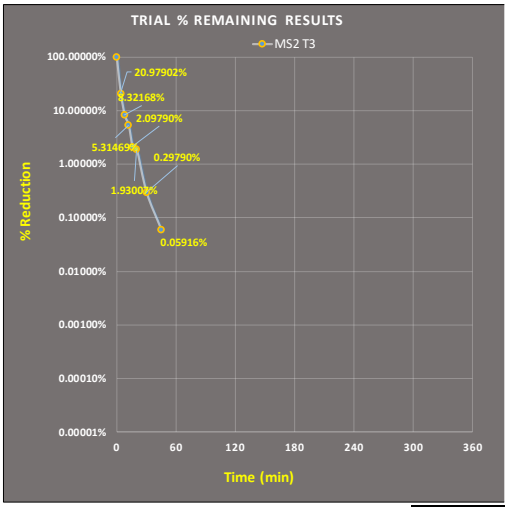
Device Information

MANUFACTURER: CASPR
UNIT MODEL: Medik Ozone Bulb
FAN SPEED (CFM): 5 ACH
UNIT SERIAL #:
FITER ID #:
FILTER LOT #:

General Testing Conditions (Can Be User Defined)

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





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)	1.271E+05	2.667E+04	1.058E+04	6.756E+03	2.667E+03	2.453E+03	378.667	75.200	18.667	2.133E-01
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)										
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	4.11%		1.67%				58.00%	1.41%	83.33%	100.00%
VIABLE CONSISTENCY CHECKS (% agreement)										
IMP & VIABLE CROSS CHECK (% agreement)										
CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.271E+05**	**2.667E+04**	**1.058E+04**	**6.756E+03**	**2.667E+03**	**2.453E+03**	**3.787E+02**	**7.520E+01**	**1.867E+01**	**0.2133**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**20.9790%**	**8.3217%**	**5.3147%**	**2.0979%**	**1.9301%**	**0.2979%**	**0.0592%**	**0.0147%**	**0.0002%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**79.0210%**	**91.6783%**	**94.6853%**	**97.9021%**	**98.0699%**	**99.7021%**	**99.9408%**	**99.9853%**	**99.9998%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.68**	**-1.08**	**-1.27**	**-1.68**	**-1.71**	**-2.53**	**-3.23**	**-3.83**	**-5.78**

Impinger Sampling Conditions

	S1	S2	S3	S4	S5	S6	S7	S8	S9	LOD
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)	3.0	3.0	3.0	3.0	3.0	5.0	5.0	5.0	5.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1 **DILUTION RATIO (10^x)**	**-3**	**-3**	**-3**	**-2**	**-2**	**-2**	**-2**	**0**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**500**
ENUMERATED PLATE COUNTS (# / drop)	20	2	1	12	6	9	0	25	5	1
	20	10	4	9	5	4	3	20	0	0
	33	3	1	17	4	10	2	26	0	0
PLATE AVERAGE COUNT (# / drop)	24.33	5.00	2.00	12.67	5.00	7.67	1.67	23.67	1.67	0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	243,333	50,000	20,000	12,667	5,000	7,667	1,667	237	17	1
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.30E+05**	**2.67E+04**	**1.07E+04**	**6.76E+03**	**2.67E+03**	**2.45E+03**	**5.33E+02**	**7.57E+01**	**5.33E+00**	**2.13E-01**
Dilution Range #1 **DILUTION RATIO (10^x)**	**-4**	**-1**	**-2**	**-1**	**-1**	**0**	**-1**	**-1**	**-1**	
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	
ENUMERATED PLATE COUNTS (# / drop)	1		19				9	4	1	
	3		23				7	3	0	
	3		17				5	0	2	
PLATE AVERAGE COUNT (# / drop)	2.33		19.67				7.00	2.33	1.00	
IMPINGER CONCENTRATION (cfu or pfu/ml)	233,333		19,667				700	233	100	
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.24E+05**		**1.05E+04**				**2.24E+02**	**7.47E+01**	**3.20E+01**	

Figure 3A: *CASPR Medik at 5 ACH Bioaerosol Plates Raw Data.*

Trial Information

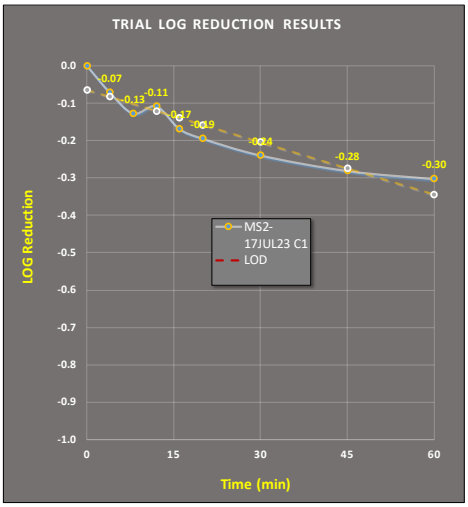
TEST DATE: Monday, July 17, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: C1
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2-17JUL23 C1

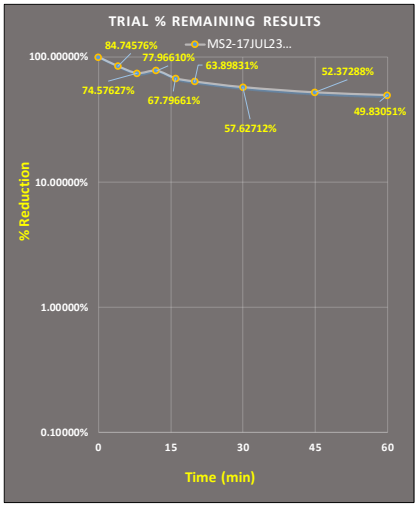
Device Information

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

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3): 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	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)	7.867E+03	6.667E+03	5.867E+03	6.133E+03	5.333E+03	5.027E+03	4.533E+03	4120.000	3920.000	1.422E-01
CHAMBER VIABLE BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)										
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	3.33%					42.92%	21.05%	52.86%		100.00%
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**	**0.1422**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**84.7458%**	**74.5763%**	**77.9661%**	**67.7966%**	**63.8983%**	**57.6271%**	**52.3729%**	**49.8305%**	**0.0018%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**15.2542%**	**25.4237%**	**22.0339%**	**32.2034%**	**36.1017%**	**42.3729%**	**47.6271%**	**50.1695%**	**99.9982%**
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**	**-4.74**

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 Range #1	**DILUTION RATIO (10^x)**	**-3**	**-2**	**-2**	**-2**	**-2**	**-2**	**-2**	**-2**	**-2**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**750**
	ENUMERATED PLATE COUNTS (# / drop)	1	10	13	5	5	7	6	7		1
		1	9	4	10	6	5	8	10		0
		1	6	5	8	9	12	5	4		0
	PLATE AVERAGE COUNT (# / drop)	1.00	8.33	7.33	7.67	6.67	8.00	6.33	7.00		0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	10,000	8,333	7,333	7,667	6,667	8,000	6,333	7,000		0
	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**		**1.42E-01**
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 4A: *MS2 Control 1 Bioaerosol Plates Raw Data.*

Trial Information

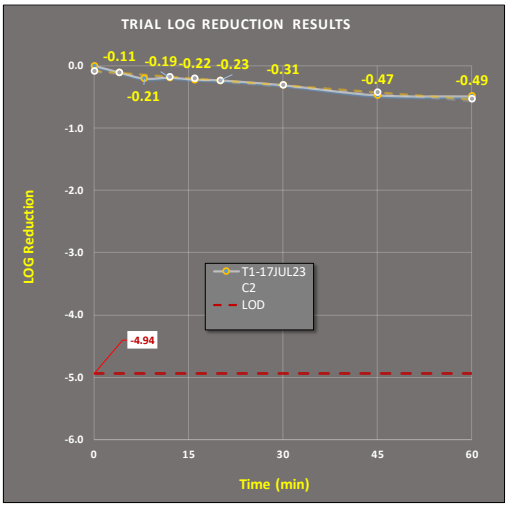
TEST DATE: Monday, July 17, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: C2
TEST ORGANSIM: T1
TRIAL NAME ID (GRAPHS/TABLES): T1-17JUL23 C2

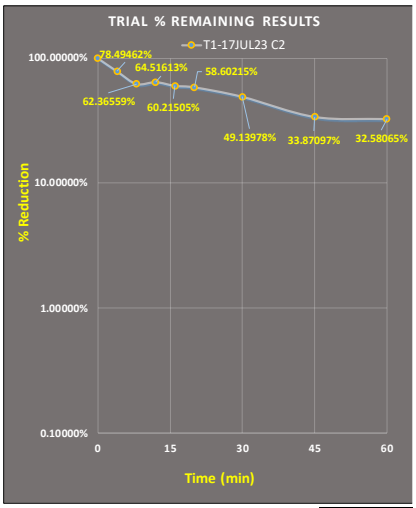
Device Information

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	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)	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	1.422E-01
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%	100.00%
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**	**0.1422**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**78.4946%**	**62.3656%**	**64.5161%**	**60.2151%**	**58.6022%**	**49.1398%**	**33.8710%**	**32.5806%**	**0.0011%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**21.5054%**	**37.6344%**	**35.4839%**	**39.7849%**	**41.3978%**	**50.8602%**	**66.1290%**	**67.4194%**	**99.9989%**
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**	**-4.94**

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)**	**-3**	**-3**	**-2**	**-2**	**-2**	**-2**	**-2**	**-2**	**-2**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**750**
	ENUMERATED PLATE COUNTS (# / drop)	1	1	7	7	7	16	8	5	7	1
		3	2	10	17	5	12	12	6	6	0
		3	2	12	6	16	12	10	9	6	0
	PLATE AVERAGE COUNT (# / drop)	2.33	1.67	9.67	10.00	9.33	13.33	10.00	6.67	6.33	0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	23,333	16,667	9,667	10,000	9,333	13,333	10,000	6,667	6,333	0
	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**	**1.42E-01**
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 5A: *MS2 Control 2 Bioaerosol Plates Raw Data.*

Trial Information

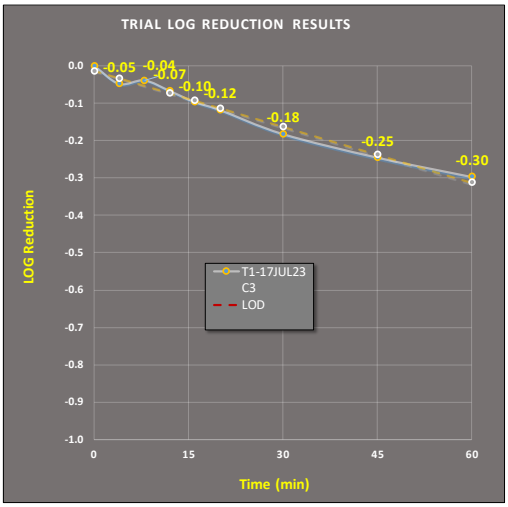
TEST DATE: Monday, July 17, 2023
TRIAL PERFORMED BY: ZT
TRIAL NUMBER: C3
TEST ORGANSIM: T1
TRIAL NAME ID (GRAPHS/TABLES): T1-17JUL23 C3

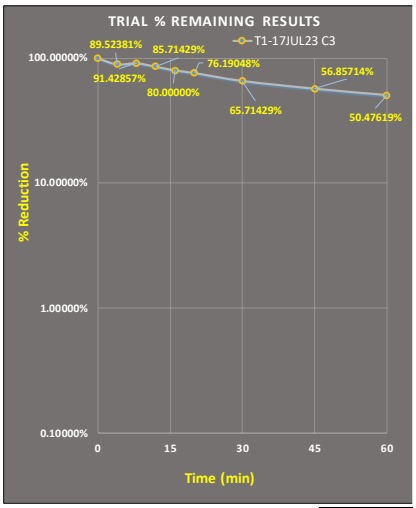
Device Information

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	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)	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	1.422E-01
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%	100.00%
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**	**0.1422**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**89.5238%**	**91.4286%**	**85.7143%**	**80.0000%**	**76.1905%**	**65.7143%**	**56.8571%**	**50.4762%**	**0.0001%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**10.4762%**	**8.5714%**	**14.2857%**	**20.0000%**	**23.8095%**	**34.2857%**	**43.1429%**	**49.5238%**	**99.9999%**
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**	**-5.99**

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**	**-4**	**-4**	**-4**	**-4**	**-3**	**-3**	**-3**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**750**
	ENUMERATED PLATE COUNTS (# / drop)	3	16	1	1	1		24	11	14	1
		1	15	2	2	2		15	12	12	0
		1	16	2	0	2		13	20	10	0
	PLATE AVERAGE COUNT (# / drop)	1.67	15.67	1.67	1.00	1.67		17.33	14.33	12.00	0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	166,667	156,667	166,667	100,000	166,667		173,333	143,333	120,000	0
	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**	**1.42E-01**
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 6A: *MS2 Control 3 Bioaerosol Plates Raw Data.*

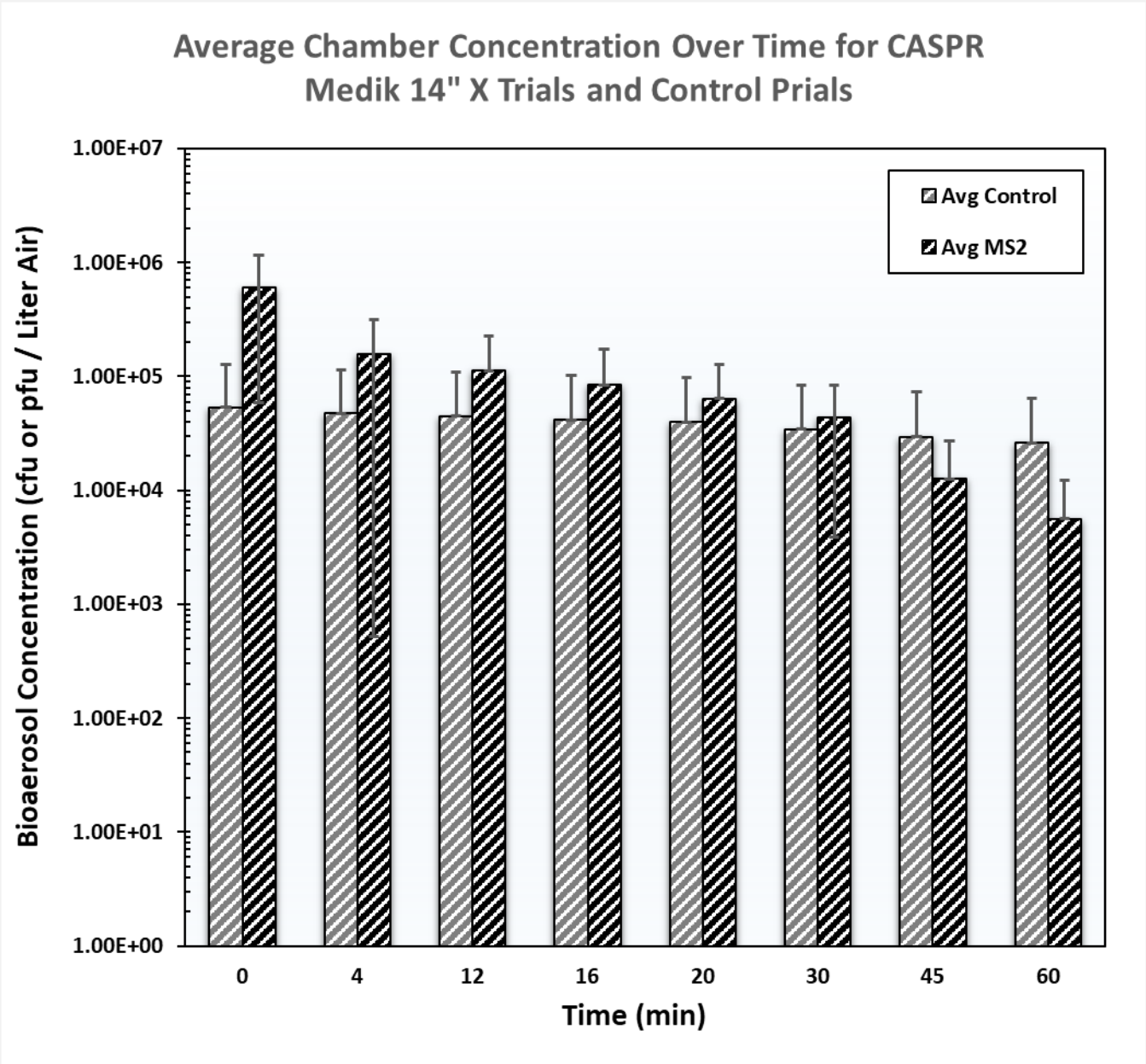


Figure 7A: *CASPR Medik 5 ACH Bioaerosol Plates Raw Data.*

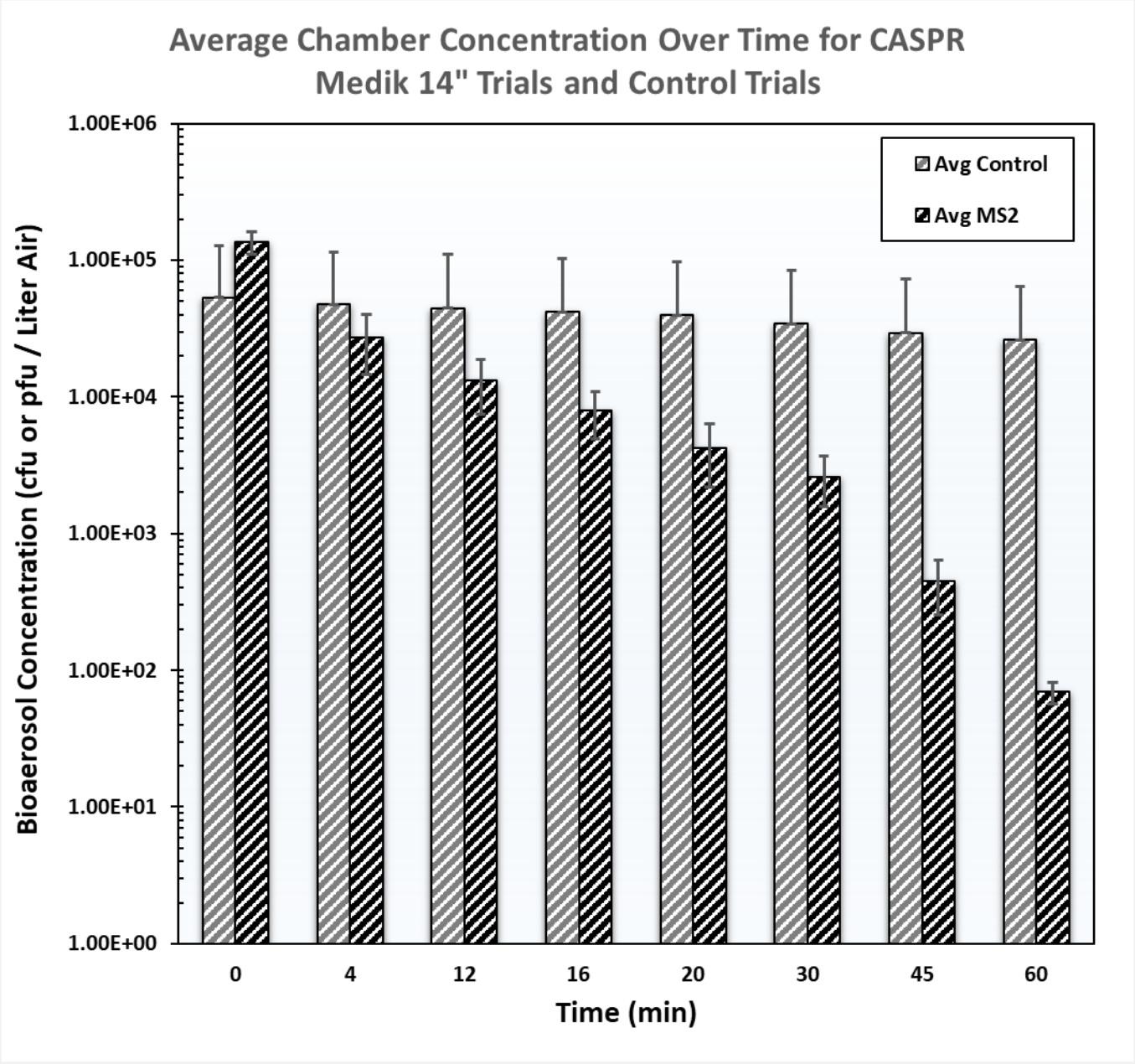


Figure 8A: *CASPR Medik at 10 ACH Bioaerosol Plates Raw Data.*

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:

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

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.

Air cleaning system equivalent clean airflow rate and equivalent clean airflow calculations

$$V_{ACS} = V(k_{td} - k_{nd})$$

where

V_{ACS} = *air cleaning* system *equivalent clean airflow* rate, cfm (L/s)
V = test chamber volume, ft^3 (L)
k_{td} = infectious microorganism decay rate with *air cleaning* system operating, $minute^{-1}$ (s^{-1})
k_{nd} = infectious microorganism decay rate without *air cleaning* system operating, $minute^{-1}$ (s^{-1})

$$V_{ACS} = \left[\frac{\varepsilon_{PR}}{100}\right] x\, V_{RC}$$

where

V_{ACS} = *air cleaning* system *equivalent clean airflow* rate due to the in-duct *air cleaning* system, cfm (L/s)
ε_{PR} = infectious aerosol reduction efficiency, determined in accordance with Section 7.3.1, Section 7.4.1.1, or Normative Appendix A, %
V_{RC} = recirculated airflow rate cleaned by the *air cleaning* system, cfm (L/s)