Efficacy of the Violett L Standalone Indoor Air Purifier Against Aerosolized MS2 Bacteriophage

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FDA Compliance:

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

Testing Lab:

Aerosol Research and Engineering Laboratories, Inc.
Project #: 10938.10

Conflict of Interest:

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

ABSTRACT

Purpose:

This study was done to test a prototype version of the Violett L for its efficacy at reducing a single microorganism, the bacteriophage MS2, from indoor room air.

Background:

This in-vitro study characterized the efficacy of Violett L, indoor air purifier, to reduce respirable bioaerosol levels for a single viral bioaerosol from the air in a 16m^3 stainless steel bioaerosol test chamber. The species selected for this study was the virus MS2, a bacteriophage, which is a recognized surrogate for more dangerous pathogenic organisms. MS2 is a non-enveloped ssRNA virus that is a common surrogate for influenza viruses and is a tentative surrogate for SARS-CoV-2.

Methods:

MS2 bacteriophage was aerosolized into a sealed 16m^3 environmental bioaerosol chamber, containing the Violett L, using a Collison 24-Jet Nebulizer. The MS2 bioaerosols had a mass median aerodynamic diameter (MMAD) averaging at 0.7µm (700nm). Bioaerosol samples were taken, with impingers, at multiple time points throughout each trial, 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 viable bioaerosol concentration for each sampling point. Chamber control trial data, or natural decay rate, was subtracted from the device trial data to yield the net log reduction for each of the bioaerosol challenges.

Results:

The device achieved an average log reduction of 6.45 +/- 0.28 within 30 minutes. This equates to a net log reduction of 6.21 +/- 0.28 (> 99.9999%) when accounting for control losses. These results show that the Violett L device is extremely effective at the rapid removal of viral bioaerosols from room air, achieving a 4 net log (99.99%) reduction within eighteen (18) minutes.

Conclusions:

In conclusion, the Violett L device achieved >4 net log reduction of MS2 bacteriophage bioaerosols within a short period of time by achieving a 6.21 (99.9999%) net log reduction within thirty (30) minutes. It is anticipated that such a reduction should reduce the likelihood of individuals being exposed to and contracting airborne infectious diseases in any enclosed environment, medical or otherwise.

Introduction

This study was conducted to evaluate the efficacy of the Violett L indoor air purifier, manufactured by Violett, at reducing aerosolized MS2 bacteriophage. The Violett L device is a free-standing air purifier that is equipped with a HEPA filter and UV-C emitters in a vortex reactor chamber. It is designed to reduce a broad range of gram-positive and gram-negative bacteria, RNA and DNA viruses, bacterial and fungal spores, and other airborne contaminants in indoor air. The Violett L is designed for use in medical facilities, dental offices, classrooms, eldercare facilities, offices, and other indoor spaces.

The test plan incorporated challenging the Violett L, in a closed environmental chamber, to determine the reduction rate of a single (1) viral bioaerosol, the MS2 bacteriophage. A picture of the Violett L device is shown in **Figure 1**.

Study Overview

Testing was conducted to characterize the reduction efficacy of a single Violett L unit against a single (1) aerosolized micro-organism; the MS2 bacteriophage which is an RNA virus. This will allow for a reasonable demonstration of the capability of the Violett L device to reduce viable bioaerosol concentrations and therefore, theoretically reducing the chances of exposure to airborne pathogens.

Previous R&D testing

Previous testing of Violett prototype devices have been conducted by ARE labs over the course of several months. Initial testing concluded that the devices had a high efficiency of bioaerosol removal from the 16m^3 bioaerosol chamber. Once this testing was complete, the testing proceeded to the Violett L device.

Test Device Description

The Violett L device is a standalone air purification system equipped with HEPA filtration and a photocatalytic vortex chamber equipped with UV-C emitters. The photocatalytic chamber amplifies the exposure wattage of the UV-C emitters by using a highly reflective material to coat the inside of the vortex. Air is channeled into this vortex through a filter and is exposed to this reflecting UV-C light. The Violett L has a large number of UV-C emitters which increases the decontamination efficacy.

An integrated High Efficiency Particulate Air (HEPA) filter removes respirable particles from the air inlet. The UV-C technology is intended to degrade any biological organisms that may have passed through the filters using high intensity UV-C emitters within the unit. The device is equipped with a 5-speed fan and is designed for large spaces. The device was placed in the center of the testing chamber for the duration of all the testing and was tested at the highest fan speed (speed 5).

Bioaerosol Testing Chamber

A 16m^3 sealed aerosol test chamber was used to replicate a contaminated room environment and to contain any potential release of aerosols into the surrounding environment.

The aerosol test chamber is constructed with 304 stainless steel and is equipped with three viewing windows and an air-tight lockable chamber door for system setup and general ingress and egress. The test chamber internal dimensions are 9.1ft x 9.1ft x 7ft, with a displacement volume of 580 cubic feet, or 16,000 liters. **Figure 2** shows the bioaerosol chamber used for all testing in this study.

Figure 1: Violett L Device: The Violett L device is a standalone air purification system equipped with HEPA filtration and a photocatalytic vortex chamber that uses UV-C emitters. The pictured device has a prototype enclosure with production working components.

Figure 2: Stainless Steel Bioaerosol Test Chamber used for all Violett L Testing. The chamber is equipped with HEPA filtered air (in/out), multiple bio aerosol sampling ports, decontamination, and pressure balance. Exterior picture.

The chamber is equipped with filtered HEPA inlets, digital internal temperature and humidity monitor, a heater and humidifier, lighting system, multiple sampling ports, aerosol mixing fans, and a HEPA filtered exhaust system that are operated with wireless remote control. For testing, the chamber is equipped with four 3/8-inch diameter stainless steel probes for aerosol sampling, a 1-inch diameter port for bio aerosol dissemination into the chamber. A Collison 24-jet nebulizer was used for the aerosolization of the microorganism tested.

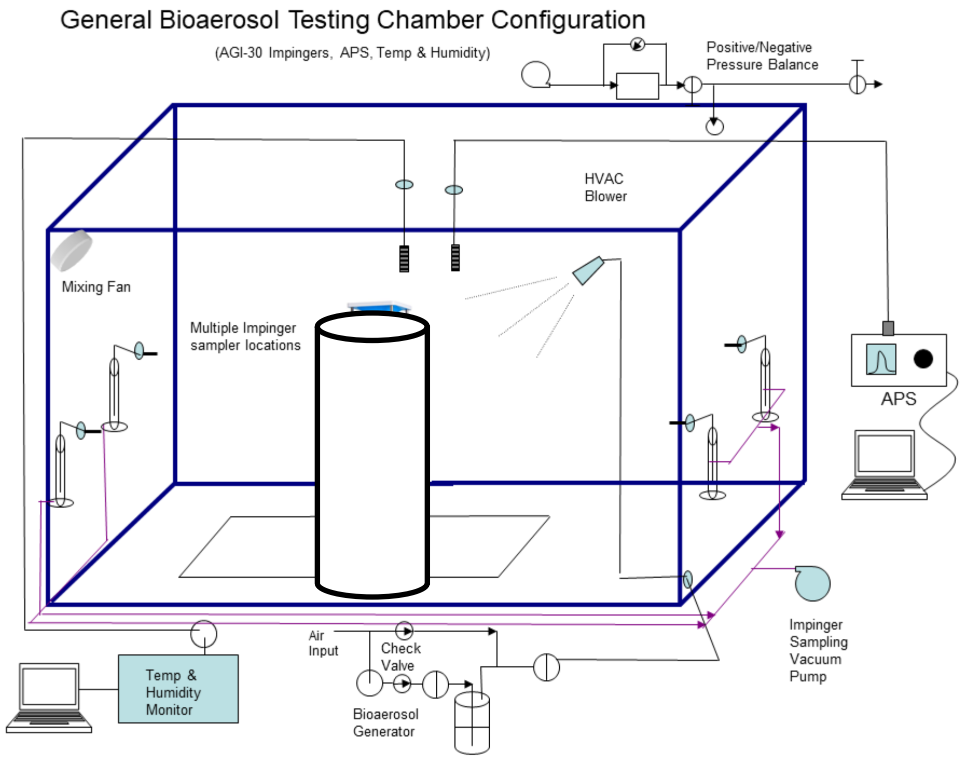

Figure 3: Bio-Aerosol Test Chamber Flow Diagram. Chamber includes bioaerosol induction, multiple bioaerosol sampling ports, particle size monitoring, internal mixing fans, and temperature and humidity controls. Main system HEPA evacuation system not pictured.

In order to avoid wall effects, all sample and dissemination ports were inserted approximately 18 inches in from the interior walls of the chamber and at a height of approximately 40 inches from the floor. The aerosol sampling and aerosol dissemination probes are stainless steel and bulk headed through the chamber walls to provide external remote access to the aerosol generator and samplers during testing.

The test chamber is equipped with two high-flow HEPA filters for the introduction of filtered room air into the test chamber during aerosol evacuation/purging of the system between test trials. A HEPA filtered exhaust blower, with a 500 ft^3/min rated flow capability, is used for rapid evacuation of remaining bioaerosols.

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 3**.

Environmental Controls

For increased stability of bioaerosols, the relative humidity inside the chamber was kept at 65% +/- 5% 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 74°F +/- 2°F

Bioaerosol Generation System

All test bioaerosols were disseminated using a Collison 24-jet nebulizer (BGI Inc. Waltham MA), like the one shown in **Figure 4**. The aerosolization of bioaerosols was driven by dry, filtered house air supply. A pressure regulator allows 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 characterized 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).

Bioaerosol Sampling and Monitoring System

Two AGI-30 impingers (Ace Glass Inc. Vineland NJ) were used for bioaerosol collection to determine chamber concentrations. The two AGI-30 Impingers were placed at opposite corners of the chamber in order to represent an entire room sample. The mixing fans inside the chamber worked to ensure a homogenous air mixture inside the chamber.

Figure 4. 6-Jet Collison nebulizer. Glass and 304 stainless steel construction, BGI Industries.

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

Figure 5. TSI Aerodynamic Particle Sizer (APS) model 3321 used to measure total particle concentration and particle size distribution of the challenge bioaerosol. Range 0.54-20.0 µm aerodynamic diameter, with 1 particle/L detection limits.

TSI Aerodynamic Particle Sizer

A TSI model 3321 Aerodynamic Particle Sizer (APS) (TSI Inc., Shoreview, MN) was used to measure aerosol concentrations and particle size 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 5**.

Species Selection

Due to safety concerns for bioaerosol testing, organism selection was based on Biological Safety Level 1 (BSL1) species which served as surrogates for more dangerous pathogenic (BSL2 & BSL3) organisms.

Viral Challenges:

MS2 bacteriophage is a viral single-stranded, non-enveloped RNA bacteriophage that has historically been used as a surrogate for influenza viruses. MS2 has also recently been used as a tentative surrogate for SARS-CoV-2 in numerous published bioaerosol studies.

The US FDA guidance document, *Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency*, states that lipid enveloped viruses, such as coronaviruses, are the least resistant microorganisms to germicidal chemicals. It is presumed that this susceptibility is similar for other chemical, physical, and catalytic methods of destruction.

MS2 is a non-enveloped virus, which makes it more resistant to disinfection than lipid viruses, and therefore, should represent a "worst case scenario" when compared to actual lipid-enveloped RNA viruses like SARS-CoV-2. **Figure 6** is a graphic from the FDA document, *COVID Sterilizers, Disinfectant Devices, and Air Purifiers Guidance*, demonstrating resistance to disinfection.

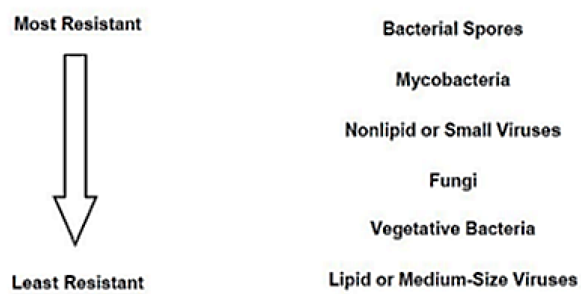

Figure 6: FDA graphic demonstrating general resistance to disinfection for various microorganisms. FDA, Guidance Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers during the Coronavirus Disease 2019 (COVID-19). Pg. 7. March 2009. SAR-CoV-2 (lipid or medium-Sized Virus), MS2 (non-lipid small virus).

For UV-C deactivation, MS2 is also a much more resilient organism than SARS-CoV-2. To achieve a 1 log deactivation of MS2 it takes 15.9 mJ/cm^2 of UV irradiation (Wilson et al 1992), coronaviruses such as SARS-CoV-2 takes 3.7mJ/cm^2 (Heßling et al 2020).

These results were obtained by investigations on many different coronaviruses, including SARS-CoV and MERS-CoV, but not SARS-CoV-2. Nevertheless, it can be assumed that they are also applicable for SARS-CoV-2 and all future mutations. RNA mutations might have a strong influence on the pathogenicity of a virus, but they do not result in larger structural differences, especially concerning the UV absorption properties of the RNA, which are the main cause for the antiviral effect of ultraviolet radiation.

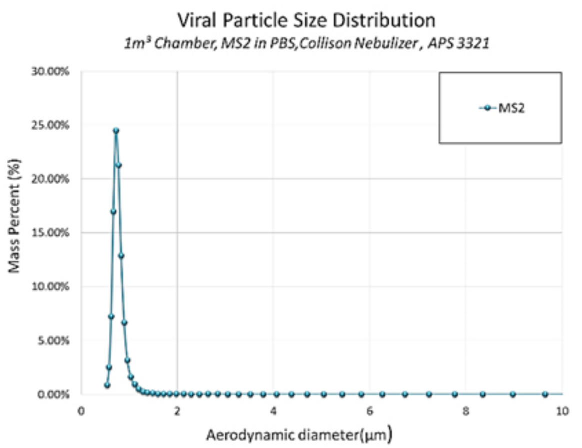

Figure 7: Aerodynamic Particle Size Distribution of RNA virus MS2 in the test chamber. MMAD for viral species averaged approximately 0.7 µm.

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.

The aerodynamic particle size distribution for all challenge bioaerosols is shown to be within the respirable range for regional alveolar tract deposition and show a low geometric standard deviation (GSD), indicating that a monodispersed aerosol was generated in the chamber for each of the challenge species. The aerodynamic particle size distribution for MS2 can be found in **Figure 7.**

While there may be variation in the particle size of a single virus to the dispersal size, shown in the previously mentioned graph, any virus that is encountered in nature is most likely suspended in a matrix solution. While viruses may be smaller than the particle size shown these aerosolized particles are far more representative of a real-life situation where the device would encounter these organisms.

Viral Culture & Preparation

Pure strain viral seed stock and host bacterium were obtained from ATCC. Host bacterium was grown in a similar fashion to vegetative cells in an appropriate liquid media. The liquid media was infected during the logarithmic growth cycle with the specific bacteriophage.

After an appropriate incubation time, the cells were lysed, and the cellular debris separated by centrifugation. MS2 stock yields were greater than 1 x 10^{11} plaque forming units per milliliter (pfu/mL) with a single amplification procedure. This stock MS2 viral solution was then diluted with PBS to approximately 1 x 10^{10} plaque forming units per milliliter (pfu/mL) for use in the Collision nebulizer.

Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate. (Multiple serial dilutions) using a standard spread plate assay technique onto tryptic soy agar plates. The plated cultures were incubated for 24-48 hours, depending on the species, then enumerated and recorded.

Post-Testing Decontamination and Prep

Following each test, the chamber was air flow evacuated/purged for a minimum of twenty minutes between tests and analyzed with the APS for particle concentration decrease to baseline levels between each test.

The chamber was decontaminated at the conclusion of the trials with aerosol/vaporous hydrogen peroxide (35%). The Collison nebulizer and impingers were cleaned at the conclusion of each day of testing by soaking in a 3% bleach bath for 20 minutes. The nebulizer and impingers were then submerged in a DI water bath, removed, and spray rinsed 6x with filtered DI water until use.

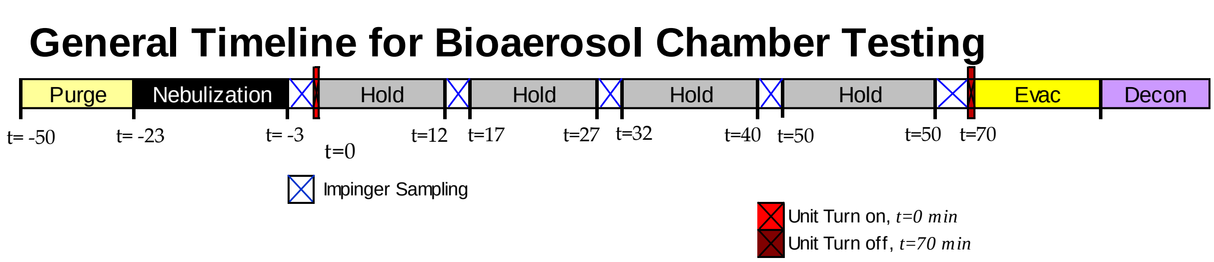

Figure 8: Sampling scheme for the Violett Violett L Air Purifier.

Bioaerosol Challenge Test Matrix

Trial	Challenge Organism	Surrogate for	ATCC #	Type	# of Trials	Sampling	Impinger Sample Times (min)
1	*MS2*	*Influenza & SARS-CoV-2*	*15597-B1*	ssRNA Virus	4	AGI Impingers	0, 5, 10, 15, 20, 30

Figure 9: Test Matrix for the Violett L Air Purifier.

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 to determine the time required for the Violett L device to achieve at least a 4 log (99.99%) reduction in viable bioaerosol above the natural losses from the control trials. Both the control and test trials are plotted showing the log reduction in viable bioaerosol for each organism. All data is normalized with time zero enumerated concentrations. Subsequent samples are normalized and plotted to show the loss of viability over time.

Methods: Bioaerosol Efficacy Testing

To accurately assess the Violett L unit, test chamber pilot control trials were performed with all organisms over a 90-minute time period to characterize the biological challenge aerosol delivery/collection efficiency, and viable concentration over time. Control testing was performed to provide baseline comparative data in order to assess the actual reduction from the Violett L challenge testing and verify that viable bioaerosol concentrations persisted above the required concentrations over the entire pilot control test period.

During the control trials, a single low velocity fan, located in the corner of the bioaerosol test chamber, was turned on for the duration of the trial to ensure a homogenous aerosol concentration within the aerosol chamber. The mixing fan was used for all control trials and was turned off during Violett L test trials. The two impingers used for bioaerosol collection were pooled and mixed prior to plating and enumeration. A complete test matrix for the bioaerosol trials can be found in **Figure 9** above.

For each control and challenge test, the Collison nebulizer was filled with approximately 40 mL of biological stock and operated at 50 psi for a period of 20 minutes. Then, the impingers were filled with 20 mL of sterilized PBS with an addition of 0.005% v/v Tween 80 for bioaerosol collection. The addition of Tween 80 was used in order to increase the impinger collection efficiency and de-agglomeration of all microorganisms. The chamber mixing fan was turned on during bioaerosol dissemination to assure a homogeneous bioaerosol concentration in the test chamber prior to taking the first impinger sample (T=0).

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and Violett L test by sampling simultaneously with two AGI-30 impingers located at opposite corners of the chamber. The samples were collected for 3 to 20 minutes at intervals of 15 or 30 minutes throughout the entire test period.

Collected impinger chamber samples were pooled and mixed at each sample interval for each test. Aliquots of impinger samples were collected and then used for plating. Impingers were rinsed 6x with sterile filtered water between each sampling interval and re-filled with sterile PBS using sterile graduated pipettes for sample collection.

For Violett L biological testing, the unit was turned on immediately following a time 0 (T=0) baseline sample and operated for the entirety of the test. Subsequent impinger samples were taken at various time points throughout the trial. These samples were enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the Violett L device over time.

All samples were plated in triplicate on tryptic soy agar media over a minimum 3 log dilution range. Plates were incubated for 24-48 hours and enumerated for viable plaque forming units (pfu) to calculate aerosol challenge concentrations in the chamber and reduction of viable microorganisms.

Results:

This study was performed to evaluate the Violett L device's efficacy at reduction of bioaerosols in a controlled test chamber. Reduction of viable bioaerosols by 4 logs, or 99.99%, is the minimum requirement for FDA approved use. The MS2 bacteriophage was selected specifically for determining the device's efficacy for reducing viral RNA bioaerosols.

The device showed an average log reduction of 6.45 +/- 0.28 within 30 minutes. This equates to a net log reduction of 6.21 +/- 0.28 (>99.9999%) when accounting for control losses. These results show that the Violett L device was extremely effective at rapidly removing viral bioaerosols, achieving a 4 net log (99.99%) reduction within eighteen (18) minutes. Log reduction data can be found in **Figure 10** net log reduction data can be found in **Figure 12.** A table summarizing all trial results can be found in **Figure 11**.

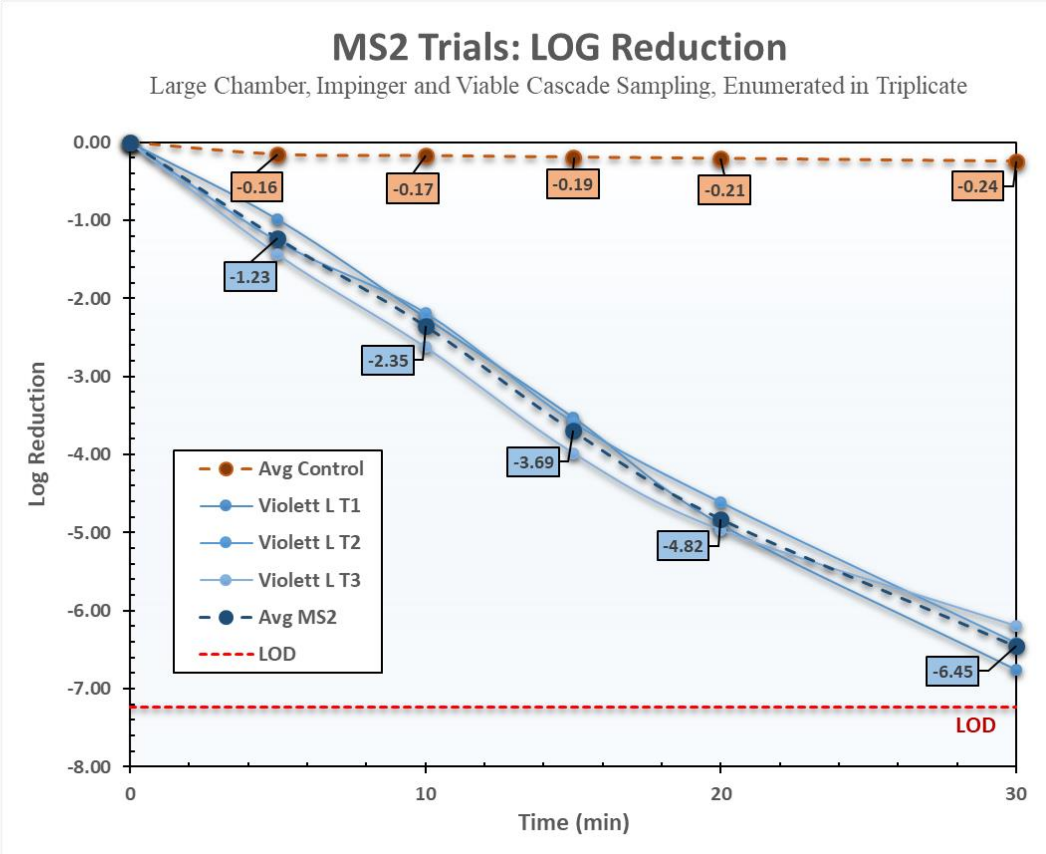

Figure 10: Log Reduction for the Violett L against MS2 bacteriophage bioaerosols.

Conclusion:

In conclusion, the Violett L device achieved >4 net log reduction of MS2 bacteriophage bioaerosols within a short period of time by achieving a 6.21 net log reduction within thirty (30) minutes.

It is anticipated that such a reduction should reduce the likelihood of individuals being exposed to and contracting airborne infectious diseases in any enclosed environment, medical or otherwise. A table summarizing all trial results can be found in **Figure 11**.

Deviations and Acceptance Criteria:

No deviations from the protocol were noted throughout the test trials. All final endpoints were ≤0.35 standard deviations from the mean. In accordance with ARE Labs' standard practices, and in compliance with GLPs, all data was verified for accuracy.

Violett L MS2 Trials Summary Data

Bioaerosol Type	Species (description)	Trial Name	Reduction Type	Trial Time (minutes)				
				5	10	15	20	30
Virus	*MS2* (RNA E. coli phage)	*Violett L T1*	*Net Log Reduction*	-0.83	-2.07	-3.33	-4.68	-6.51
			Net % Reduction	85.2183%	99.1463%	99.9532%	99.9979%	99.99997%
Virus	*MS2* (RNA E. coli phage)	*Violett L T2*	*Net Log Reduction*	-1.11	-2.01	-3.39	-4.40	-6.17
			Net % Reduction	92.2332%	99.0236%	99.9594%	99.9960%	99.9999%
Virus	*MS2* (RNA E. coli phage)	*Violett L T3*	*Net Log Reduction*	-1.28	-2.45	-3.79	-4.76	-5.95
			Net % Reduction	94.7767%	99.6451%	99.9837%	99.9983%	99.9999%
All Trial Averages			***Net Log Reduction***	**-1.07 +/- 0.23**	**-2.18 +/- 0.24**	**-3.5 +/- 0.25**	**-4.61 +/- 0.19**	**-6.21 +/- 0.28**
			Net % Reduction	***90.7427% +/- 4.9504%***	***99.2717% +/- 0.3292%***	***99.9654% +/- 0.0161%***	***99.9974% +/- 0.0012%***	***99.9999% +/- 0.00004%***

Figure 11: Executive Summary Data for Violett L device on highest fan speed setting when tested against MS2 bioaerosols.

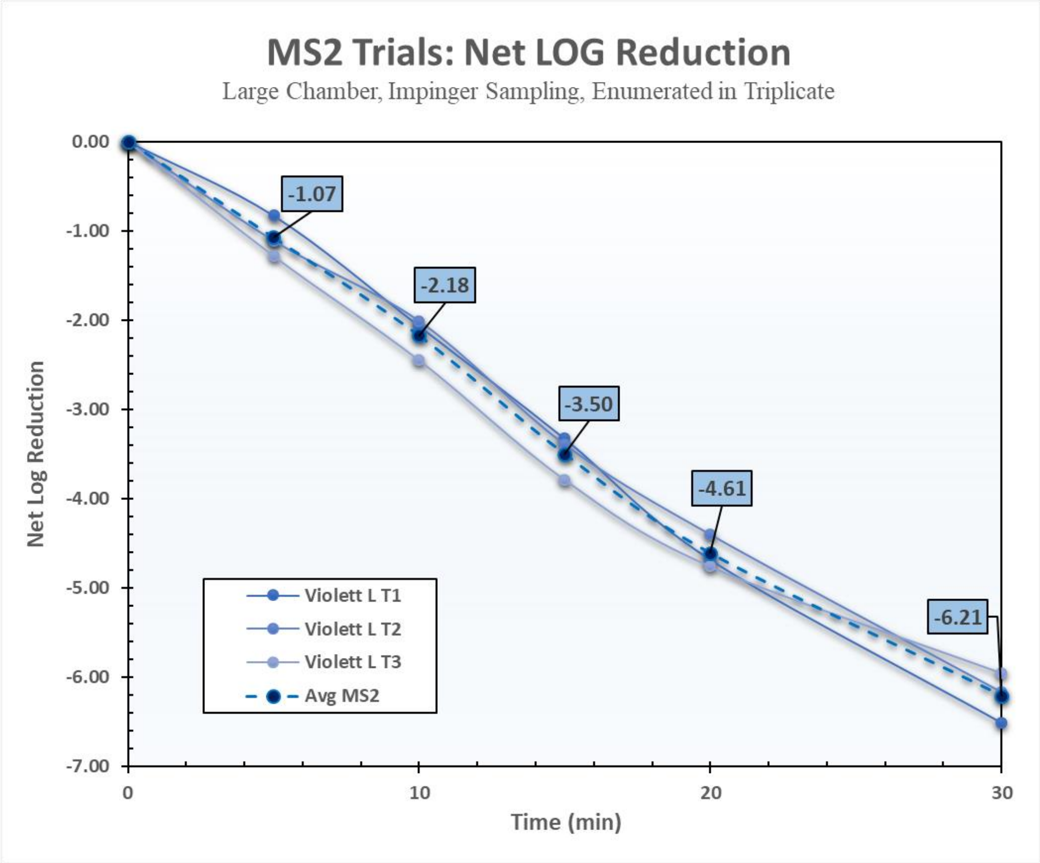

Figure 12: Net log Reduction for the Violett L on highest fan speed setting against MS2 bacteriophage bioaerosols.

Clean Air Delivery Rate (CADR):

In addition to testing, the clean air delivery rate (CADR) was calculated for the Violett L. The clean air delivery rate is the flow rate of air that has been purified of particles in a given size distribution. This is calculated using the fraction of particles for a given particle size distribution, multiplied by the flow rate (CFM) of the device.

For CADR calculations, the difference in slopes for the control and test trials was calculated to determine the equivalent air exchange rate. The slope of the test trials was determined using only the T-0 and T-20 time points due to low bioaerosol concentrations within the chamber that were close to limits of detection past this timepoint. The CADR was then calculated by multiplying the equivalent air exchange rate by the volume of the test chamber (16m^3). See **Figure 14** for an example of these calculations.

Clean Air Delivery Rate (CADR) Results:

The calculated CADR, based off trial data, suggests that Violett L device can achieve 28.23 equivalent air exchanges (eqACH) per hour. A table breakdown of these results is shown in **Figure 13**. While this number is only an estimation of the device's ability to remove viruses from a given volume, it does provide a general assessment of the device's performance in the bioaerosol test chamber.

The device is easily able to reach the 4-log reduction threshold with a CADR of 451.68 m^3 which translates to a 265.74 CADR in CFM (cubic feet per minute). These values illustrate an equivalent air exchange of 28.23 per hour, or the theoretical number of turnovers of clean air simulated by the device in a single hour.

CADR Summary Table

Trial Set	Device	eqACH	CADR (m^3)	CADR (CFM)
MS2 Average	Violett L	28.23	451.68	265.74

Figure 13: CADR and eqACH calculation table

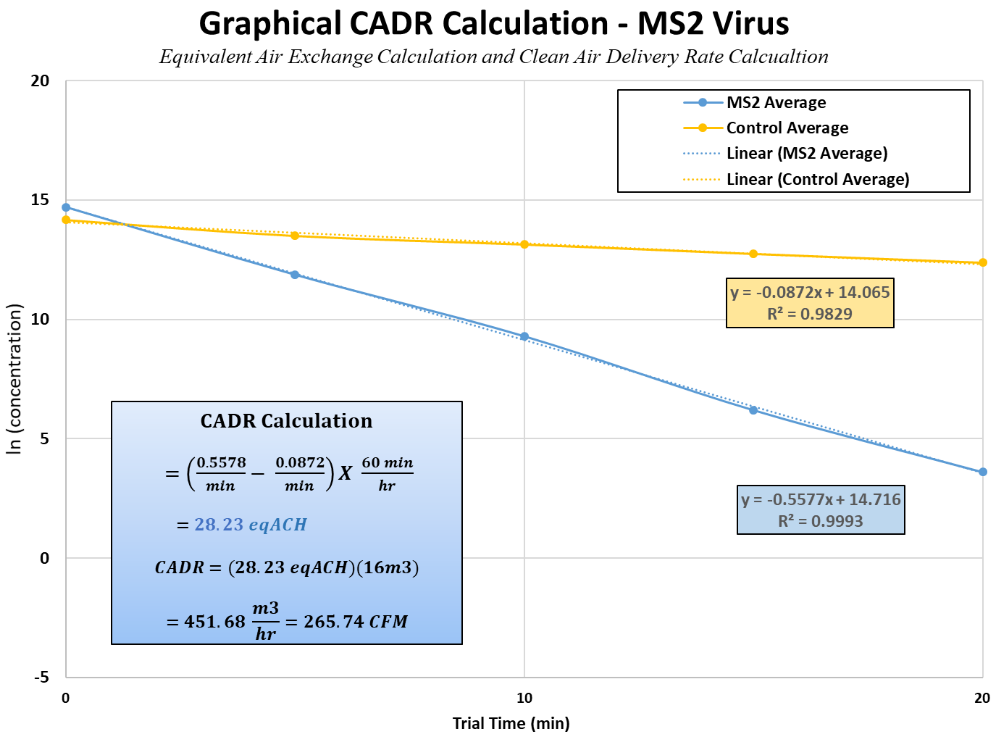

Figure 13: CADR and eqACH calculation example graph

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Analytical Testing Facility

Aerosol Research and Engineering Labs, Inc.
15320 S. Cornice Street
Olathe, KS 66062

Project #

10938.10

Study Director

Richard Ludwick
Aerosol Research and Engineering Laboratories

GLP Statement

We, the undersigned, herby certify that the work described herein was conducted by Aerosol Research and Engineering Laboratories in compliance with FDA 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 Violett's financial interests such as; membership, employment, stock ownership, or other equity interest.

Study Director:

Richard Ludwick — 11/22/2022
Richard Ludwick
Director of Operations
ARE Labs, Inc.
Date

Principal Investigator:

W. Andrew Dexter — 11/22/2022
W. Andrew Dexter M.S.
Staff Research Scientist
ARE Labs, Inc.
Date

APPENDIX A: Raw Data

Trial Information

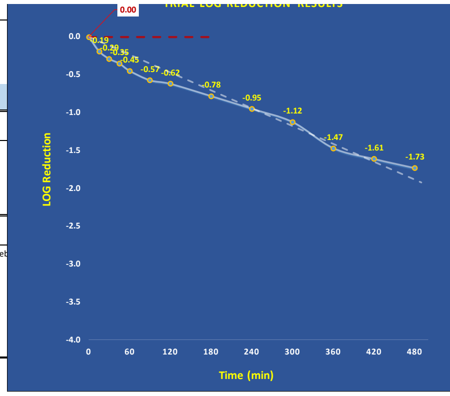
TEST DATE:	Friday, January 15th
TRIAL PERFORMED BY:	TD
TRIAL NUMBER:	Control T1
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	Control T1

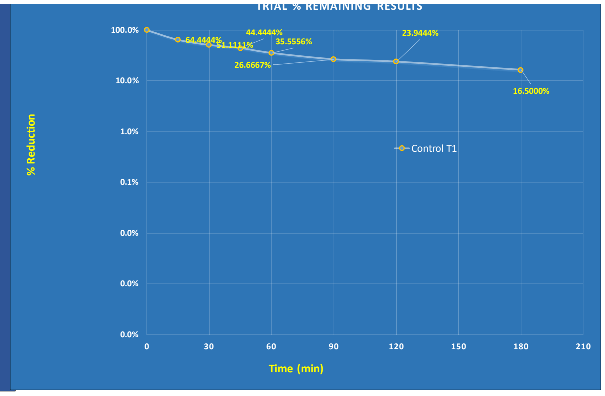
Device Information

MANUFACTURER:	Radic 8
UNIT MODEL:	VK Pro
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
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
SAMPLING TIME (min)	0	15	30	45	60	90	120	180	240	300	360	420	480	LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y	y	y	y	y	y	n
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.440E+06	9.280E+05	7.360E+05	6.400E+05	5.120E+05	3.840E+05	344800.000	237600.000	161600.000	108000.000	48800.000	35200.000	26666.667	
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)														
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)							34.23%	35.00%						
VIABLE CONSISTENCY CHECKS (% agreement)														
IMP & VIABLE CROSS CHECK (% agreement)														
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.440E+06	9.280E+05	7.360E+05	6.400E+05	5.120E+05	3.840E+05	3.448E+05	2.376E+05	1.616E+05	1.080E+05	4.880E+04	3.520E+04	2.667E+04	
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	64.4444%	51.1111%	44.4444%	35.5556%	26.6667%	23.9444%	16.5000%	11.2222%	7.5000%	3.3889%	2.4444%	1.8519%	
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	35.5556%	48.8889%	55.5556%	64.4444%	73.3333%	76.0556%	83.5000%	88.7778%	92.5000%	96.6111%	97.5556%	98.1481%	
LOG REDUCTION FROM T=0 (log_{10})	0.00	-0.19	-0.29	-0.35	-0.45	-0.57	-0.62	-0.78	-0.95	-1.12	-1.47	-1.61	-1.73	

Impinger Sampling Conditions

SAMPLING TIME (min)	0	15	30	45	60	90	120	180	240	300	360	420	480	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	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	10.0	10.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1														
DILUTION RATIO (10^x)	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-3	0
DROPLET SIZE (µl)	50	50	50	50	50	50	50	50	50	50	50	50	50	750
ENUMERATED PLATE COUNTS (# / drop)	12	10	12	10	8	6	6	6	2	2	3			1
	6	19	11	10	8	6	7	3	3	1	0			0
														0
PLATE AVERAGE COUNT (# / drop)	9.00	14.50	11.50	10.00	8.00	6.00	6.50	4.50	2.50	1.50	1.50			0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	1,800,000	2,900,000	2,300,000	2,000,000	1,600,000	1,200,000	1,300,000	900,000	500,000	300,000	300,000			0
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.44E+06	9.28E+05	7.36E+05	6.40E+05	5.12E+05	3.84E+05	4.16E+05	2.88E+05	1.60E+05	9.60E+04	4.80E+04			1.42E-01
Dilution Range #1														
DILUTION RATIO (10^x)	-3	-4	-2	-1	-3	-3	-3	-3	-3	-3	-3	-3	-3	-1
DROPLET SIZE (µl)	50	100	100	100	100	100	50	50	50	50	50	50	50	750
ENUMERATED PLATE COUNTS (# / drop)							47	34	33	21	17			1
							42	30	27	20	17	12	9	0
							42	30	23	18	14	11	9	0
							40	23	19	16	14	10	7	
PLATE AVERAGE COUNT (# / drop)							42.75	29.25	25.50	18.75	15.50	11.00	8.33	0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)							855,000	585,000	510,000	375,000	310,000	220,000	166,667	4
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)							2.74E+05	1.87E+05	1.63E+05	1.20E+05	4.96E+04	3.52E+04	2.67E+04	1.42E+00

Viable Cascade Sampling Conditions **Statistical Correction Applied Automatically for counts>60

SAMPLING TIME (min)	0	15	30	45	60	90	120	180		LOD
Viable Cascade #1										
VIABLE CASCADE SAMPLING TIME (min)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	10.0		10.0
VIABLE CASCADE FLOW RATE (lpm)	30	30	30	30	30	30	30	30		30
ENUMERATED PLATE COUNTS (# / plate)										1
STATISTICALLY CORRECTED PLATE COUNTS (# / plate)										1
PLATE AVERAGE COUNT (# / plate)										1.00
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)										0.003
Viable Cascade #2										
VIABLE CASCADE SAMPLING TIME (min)	0.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0		10.0
VIABLE CASCADE FLOW RATE (lpm)	30	30	30	30	30	30	30	30		30
ENUMERATED PLATE COUNTS (# / plate)										1
										0
STATISTICALLY CORRECTED PLATE COUNTS (# / plate)										1
										0
PLATE AVERAGE COUNT (# / plate)										0.50
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)										0.002

Figure 1A: Raw Plate Counts for MS2 Control Trial.

Trial Information

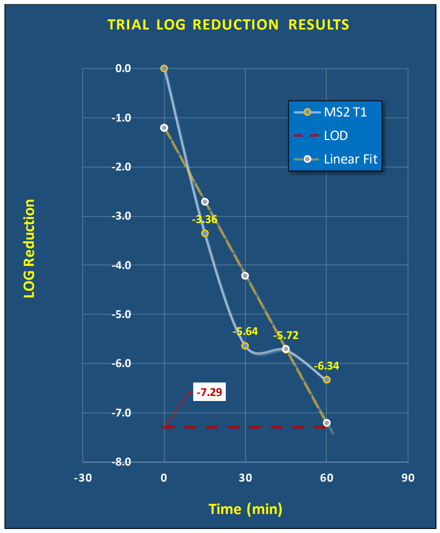
TEST DATE:	Tuesday, November 15, 2022
TRIAL PERFORMED BY:	WAD
TRIAL NUMBER:	T1
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T1

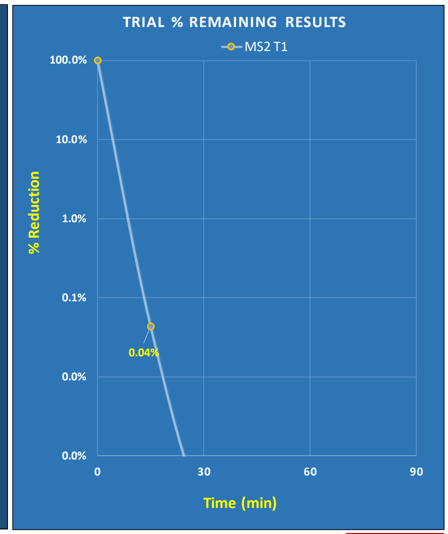
Device Information

MANUFACTURER:	Violett
UNIT MODEL:	Standalone
FAN SPEED (CFM):	650
UNIT SERIAL #:	
FITER ID #:	
FILTER LOT #:	

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	70
OTHER INSTRUMENTS:	Picarro, Interscan, Tiger
TRIAL COMMENTS/NOTES	





BIOAEROSOL Sample ID and Summary Data

	S1	S2	S3	S4	S5	S6	S7	S8	LOD
SAMPLE TIME (min)	0	15	30	45	60				LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	n	n	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	2.791E+06	1.211E+03	6.400E+00	5.333E+00	1.280E+00	#DIV/0!			1.422E-01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	19.54%	10.83%		18.18%	40.00%				100.00%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**2.791E+06**	**1.211E+03**	**6.400E+00**	**5.333E+00**	**1.280E+00**				**0.1422**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**0.0434%**	**0.0002%**	**0.0002%**	**0.0000%**				**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**99.9566%**	**99.9998%**	**99.9998%**	**100.0000%**				**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-3.36**	**-5.64**	**-5.72**	**-6.34**				**-7.29**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	15	30	45	60	0	0	0	LOD
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	10.0	10.0	5.0	10.0	10.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

Dilution Range #1

DILUTION RATIO (10^x)	**-5**	**-2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**750**
ENUMERATED PLATE COUNTS (# / drop)	5	6	2	6	2				1
	5	3	2	2	1				0
	4	3	2	3	0				0
PLATE AVERAGE COUNT (# / drop)	4.67	4.00	2.00	3.67	1.00				0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	4,666,667	4,000	20	37	10				0
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	2.49E+06	1.28E+03	6.40E+00	5.87E+00	1.60E+00				1.42E-01

Dilution Range #1

DILUTION RATIO (10^x)	**-4**	**-1**	**-5**	**0**	**0**	**0**	**0**	**0**	
DROPLET SIZE (µl)	**100**	**100**	**100**	**500**	**500**	**750**	**750**	**750**	
ENUMERATED PLATE COUNTS (# / drop)	67	32		15	3				
	53	42							
	54	33							
PLATE AVERAGE COUNT (# / drop)	58.00	35.67		15.00	3.00				
IMPINGER CONCENTRATION (cfu or pfu/ml)	5,800,000	3,567		30	6				
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	3.09E+06	1.14E+03		4.80E+00	9.60E-01				

Figure 2A: Raw Plate Counts for MS2 Trial 1.

Trial Information

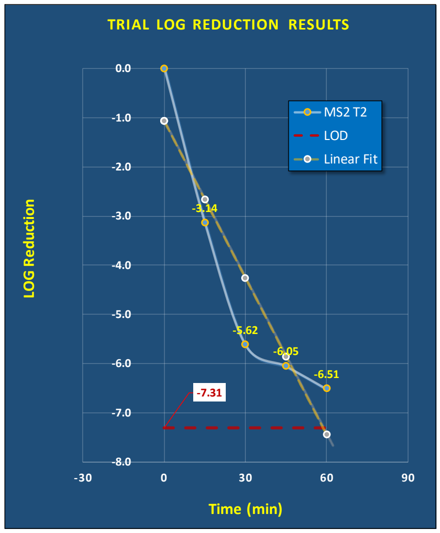
TEST DATE:	Tuesday, November 15, 2022
TRIAL PERFORMED BY:	WAD
TRIAL NUMBER:	T2
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T2

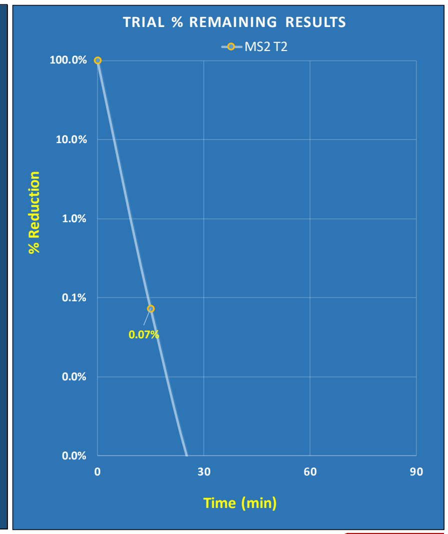
Device Information

MANUFACTURER:	Violett
UNIT MODEL:	Standalone
FAN SPEED (CFM):	650
UNIT SERIAL #:	
FITER ID #:	
FILTER LOT #:	

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	70
OTHER INSTRUMENTS:	Picarro, Interscan, Tiger
TRIAL COMMENTS/NOTES	





BIOAEROSOL Sample ID and Summary Data

	S1	S2	S3	S4	S5	S6	S7	S8	LOD
SAMPLE TIME (min)	0	15	30	45	60				LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	n	n	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	2.880E+06	2.101E+03	6.933E+00	2.560E+00	8.800E-01	#DIV/0!			1.422E-01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	20.00%			40.00%					100.00%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**2.880E+06**	**2.101E+03**	**6.933E+00**	**2.560E+00**	**8.800E-01**				**0.1422**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**0.0730%**	**0.0002%**	**0.0001%**	**0.0000%**				**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**99.9270%**	**99.9998%**	**99.9999%**	**100.0000%**				**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-3.14**	**-5.62**	**-6.05**	**-6.51**				**-7.31**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	15	30	45	60	0	0	0	LOD
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	10.0	10.0	20.0	5.0	10.0	10.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

Dilution Range #1

DILUTION RATIO (10^x)	**-5**	**-1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**	**100**	**100**	**100**	**750**
ENUMERATED PLATE COUNTS (# / drop)	7	65	4	2	6				1
	7	76	7	3	5				0
	4	56	2	1					0
PLATE AVERAGE COUNT (# / drop)	6.00	65.67	4.33	2.00	5.50				0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	6,000,000	6,567	43	20	11				0
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	3.20E+06	2.10E+03	6.93E+00	3.20E+00	8.80E-01				1.42E-01

Dilution Range #1

DILUTION RATIO (10^x)	**-4**	**-1**	**-5**	**0**	**0**	**0**	**0**	**0**	
DROPLET SIZE (µl)	**100**	**100**	**100**	**500**	**500**	**750**	**750**	**750**	
ENUMERATED PLATE COUNTS (# / drop)	47			6					
	57								
	40								
PLATE AVERAGE COUNT (# / drop)	48.00			6.00					
IMPINGER CONCENTRATION (cfu or pfu/ml)	4,800,000			12					
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	2.56E+06			1.92E+00					

Figure 3A: Raw Plate Counts for MS2 Trial 2.

Trial Information

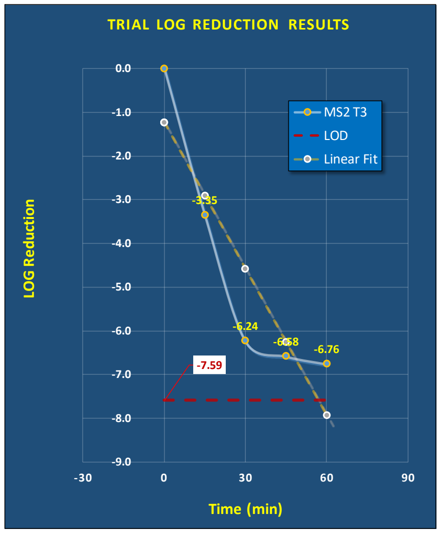
TEST DATE:	Tuesday, November 15, 2022
TRIAL PERFORMED BY:	WAD
TRIAL NUMBER:	T3
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T3

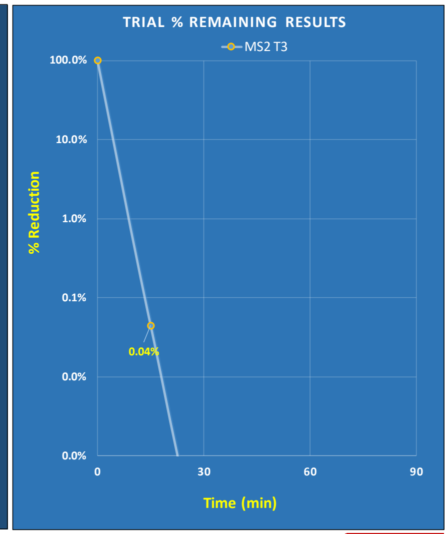
Device Information

MANUFACTURER:	Violett
UNIT MODEL:	Standalone
FAN SPEED (CFM):	650
UNIT SERIAL #:	
FITER ID #:	
FILTER LOT #:	

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascade
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	70
OTHER INSTRUMENTS:	Picarro, Interscan, Tiger
TRIAL COMMENTS/NOTES	





BIOAEROSOL Sample ID and Summary Data

	S1	S2	S3	S4	S5	S6	S7	S8	LOD
SAMPLE TIME (min)	0	15	30	45	60				LOD
IMPINGER USED (y / n)	y	y	y	y	y	y	n	n	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	5.511E+06	2.464E+03	3.200E+00	1.440E+00	9.600E-01	#DIV/0!			1.422E-01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		0.86%		20.00%					100.00%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**5.511E+06**	**2.464E+03**	**3.200E+00**	**1.440E+00**	**9.600E-01**				**0.1422**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**0.0447%**	**0.0001%**	**0.0000%**	**0.0000%**				**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**99.9553%**	**99.9999%**	**100.0000%**	**100.0000%**				**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-3.35**	**-6.24**	**-6.58**	**-6.76**				**-7.59**

Impinger Sampling Conditions

		S1	S2	S3	S4	S5	S6	S7	S8	LOD
	SAMPLE TIME (min)	0	15	30	45	60	0	0	0	LOD
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	10.0	10.0	20.0	5.0	10.0	10.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-5**	**-2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Dilution Range #1	**DROPLET SIZE (µl)**	**100**	**100**	**100**	**100**	**500**	**100**	**100**	**100**	**750**
Dilution Range #1	ENUMERATED PLATE COUNTS (# / drop)	15	8	1	0	5				1
Dilution Range #1		8	8	1	1	7				0
Dilution Range #1		8	7	4	2					0
Dilution Range #1	PLATE AVERAGE COUNT (# / drop)	10.33	7.67	2.00	1.00	6.00				0.33
Dilution Range #1	IMPINGER CONCENTRATION (cfu or pfu/ml)	10,333,333	7,667	20	10	12				0
Dilution Range #1	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	5.51E+06	2.45E+03	3.20E+00	1.60E+00	9.60E-01				1.42E-01
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-1**	**-5**	**0**	**0**	**0**	**0**	**0**	
Dilution Range #1	**DROPLET SIZE (µl)**	**100**	**100**	**100**	**500**	**500**	**750**	**750**	**750**	
Dilution Range #1	ENUMERATED PLATE COUNTS (# / drop)		88		4					
Dilution Range #1			62							
Dilution Range #1			82							
Dilution Range #1	PLATE AVERAGE COUNT (# / drop)		77.33		4.00					
Dilution Range #1	IMPINGER CONCENTRATION (cfu or pfu/ml)		7,733		8					
Dilution Range #1	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		2.47E+03		1.28E+00					

Figure 4A: Raw Plate Counts for MS2 Trial 3.

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:

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

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.

17.2 Positive hole conversion table for all MAS-100 air monitoring systems

r = number of colony forming units counted on 100 mm petri dish   Pr = probable statistical total count

r	Pr	r	Pr	R	Pr	R	Pr	R	Pr	r	Pr	R	Pr	R	Pr
1	1	51	54	101	116	151	189	201	279	251	394	301	557	351	836
2	2	52	56	102	118	152	191	202	281	252	397	302	561	352	844
3	3	53	57	103	119	153	193	203	283	253	400	303	565	353	853
4	4	54	58	104	120	154	194	204	285	254	402	304	569	354	861
5	5	55	59	105	122	155	196	205	287	255	405	305	573	355	870
6	6	56	60	106	123	156	197	206	289	256	408	306	578	356	879
7	7	57	61	107	124	157	199	207	291	257	411	307	582	357	888
8	8	58	63	108	126	158	201	208	293	258	413	308	586	358	897
9	9	59	64	109	127	159	202	209	295	259	416	309	591	359	907
10	10	60	65	110	128	160	204	210	297	260	419	310	595	360	917
11	11	61	66	111	130	161	206	211	299	261	422	311	599	361	927
12	12	62	67	112	131	162	207	212	301	262	425	312	604	362	937
13	13	63	68	113	133	163	209	213	304	263	428	313	608	363	947
14	14	64	70	114	134	164	211	214	306	264	431	314	613	364	958
15	15	65	71	115	135	165	212	215	308	265	433	315	618	365	969
16	16	66	72	116	137	166	214	216	310	266	436	316	622	366	981
17	17	67	73	117	138	167	216	217	312	267	439	317	627	367	992
18	18	68	74	118	140	168	218	218	314	268	442	318	632	368	1005
19	19	69	76	119	141	169	219	219	317	269	445	319	637	369	1017
20	20	70	77	120	142	170	221	220	319	270	449	320	642	370	1030
21	22	71	78	121	144	171	223	221	321	271	452	321	647	371	1043
22	23	72	79	122	145	172	224	222	323	272	455	322	652	372	1057
23	24	73	80	123	147	173	226	223	325	273	458	323	657	373	1071
24	25	74	82	124	148	174	228	224	328	274	461	324	662	374	1086
25	26	75	83	125	150	175	230	225	330	275	464	325	667	375	1102
26	27	76	84	126	151	176	232	226	332	276	467	326	673	376	1118
27	28	77	85	127	153	177	233	227	335	277	471	327	678	377	1134
28	29	78	87	128	154	178	235	228	337	278	474	328	684	378	1152
29	30	79	88	129	156	179	237	229	339	279	477	329	689	379	1170
30	31	80	89	130	157	180	239	230	342	280	480	330	695	380	1189
31	32	81	90	131	158	181	241	231	344	281	484	331	701	381	1209
32	33	82	92	132	160	182	242	232	346	282	487	332	706	382	1230
33	34	83	93	133	161	183	244	233	349	283	491	333	712	383	1252
34	35	84	94	134	163	184	246	234	351	284	494	334	718	384	1276
35	37	85	95	135	164	185	248	235	353	285	497	335	724	385	1301
36	38	86	97	136	166	186	250	236	356	286	501	336	730	386	1327
37	39	87	98	137	167	187	252	237	358	287	504	337	737	387	1356
38	40	88	99	138	169	188	254	238	361	288	508	338	743	388	1387
39	41	89	101	139	171	189	255	239	363	289	511	339	749	389	1420
40	42	90	102	140	172	190	257	240	366	290	515	340	756	390	1456
41	43	91	103	141	174	191	259	241	368	291	519	341	763	391	1496
42	44	92	104	142	175	192	261	242	371	292	522	342	769	392	1541
43	45	93	106	143	177	193	263	243	373	293	526	343	776	393	1591
44	47	94	107	144	178	194	265	244	376	294	530	344	783	394	1648
45	48	95	108	145	180	195	267	245	378	295	534	345	791	395	1715
46	49	96	110	146	181	196	269	246	381	296	537	346	798	396	1795
47	50	97	111	147	183	197	271	247	384	297	541	347	805	397	1895
48	51	98	112	148	185	198	273	248	386	298	545	348	813	398	2028
49	52	99	114	149	186	199	275	249	389	299	549	349	820	399	2228
50	53	100	115	150	188	200	277	250	391	300	553	350	828		