Efficacy of the P3000 PCO Device at Reducing Aerosolized MS2 Virus
(MS2 is used by ARE labs as a surrogate for SARS-CoV-2 and Influenza)

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Background: This in vitro study was designed to characterize the effectiveness of the Puraclenz P3000 device at reducing aerosolized MS2 virus from indoor air. The P3000 device is designed to reduce airborne viruses as well as bacterial and fungal spores. For this study, the P3000 was challenged using aerosolized MS2 bacteriophage. MS2 has historically been used as a surrogate for influenza, and is currently used by ARE labs as a surrogate for SARS CoV-2, the virus that causes COVID-19. Justification for using MS2 as a surrogate is based on the FDA guidance document; Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers during the Coronavirus Disease 2019 (COVID-19) Public Health Emergency. MS2, being of a more resistant class of organism, makes it a good candidate as a surrogate. The worldwide pandemic has infected millions. Consequently, methods of reducing airborne pathogens have come to the forefront of the public's attention. This analytical testing was done to evaluate the effectiveness of the P3000 at reducing aerosolized MS2 virus, in a 16m^3 stainless steel bioaerosol chamber, to demonstrate viral removal, over time, in an enclosed and controlled space. The study consisted of a total of three (3) live bioaerosol trials. In addition, an ion meter with a data logging feature was used to measure the ion levels in the chamber.

Methods: The MS2 virus was aerosolized into a sealed bioaerosol environmental chamber containing the P3000 device. AGI impingers were used to sample chamber bioaerosol concentrations. Samples were taken hourly from the chamber and used to quantify the viral reduction rate capability of the P3000 over time. All impinger samples were serially diluted, plated, and enumerated in triplicate to yield the viable bioaerosol concentrations at each sampling time point. Chamber control trial data was subtracted from the P3000 trial data to yield net LOG reduction in the MS2 concentration in the chamber for the bioaerosol challenges. In addition, a COM Systems ion meter was placed in the chamber. It was connected to a computer equipped with data logging software that recorded ion measurement data every minute.

Results: The P3000 demonstrated an MS2 net LOG reduction of 2.23, or 99.4%, in a 7-hour large (16m^3) chamber trial. This study did not differentiate between the various factors that had a biocidal effect on the MS2 virus. Rather, the net log reduction achieved by the device is most likely due to a combination of factors including; 1) the exposure to UV light, 2) the effects of the photo catalytic oxidation cell (PCO), and 3) the ions that were dispersed by the device. In a test trial, the average number of ions measured in the test chamber was approximately 400 ions/cm^3.

This study was conducted in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58. ARE Laboratories is an engineering and testing laboratory specializing in aerosol science.

Introduction

This study was conducted to evaluate the effectiveness of the P3000 air purification device at reducing aerosolized MS2 bacteriophage. The P3000 device is a photocatalytic oxidation (PCO) system. It is intended for use in medium to large sized offices, schools, retail stores, hospitality venues, doctor and dental offices, and laboratories. In addition, the P3000 contains a pre-filter to protect the device's optics and catalyst from airborne particulate that can cause fowling that may diminish the device's performance over time. The test plan was designed to challenge the P3000 and determine the rate at which it reduces aerosolized MS2 bacteriophage in a closed environmental chamber. Demonstrating the reduction in potentially hazardous organisms is key to determining efficacy of the device. A picture of the P3000 device is shown below in **Figure 1**.

Study Overview

The efficacy of the P3000 device was evaluated against the MS2 bacteriophage, a single-stranded, non-enveloped RNA virus. For more information about this organism please see the *Species Selection* section in the body of this report. Particulate testing was also performed with polydispersed latex microspheres ranging in size from 0.5 to 5 um. Testing was conducted with the MS2 virus to characterize a single P3000 unit with three (3) independent trials and one (1) control trial to determine the ability of the P3000 device to reduce viable bioaerosol concentrations from room air.

Figure 1*: P3000 device*

Bioaerosol Testing Chamber

A large (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 of 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 9.1 ft x 9.1 ft x 7 ft, with a displacement volume of 579 cubic feet. **Figure 2** shows the bioaerosol chamber used for all testing in this study.

The chamber is equipped with filtered HEPA inlets, a digital internal temperature and humidity monitor, external humidifiers for humidity control, a lighting system, multiple sampling ports, aerosol mixing fans, and a HEPA filtered exhaust system that are all operated by wireless remote control. The chamber is equipped with four 3/8-inch diameter stainless steel probe sampling ports. A 1-inch diameter port is used for bio-aerosol dissemination into the chamber using a Collison 24-jet nebulizer for the aerosolization of the bacteriophage.

A ¼ inch diameter probe was used for continuous aerosol particle size monitoring via a TSI Aerodynamic Particle Sizer (APS) model 3321. All sample and dissemination ports were inserted approximately 18 inches from the interior walls and at a height of approximately 40 inches from the floor of the chamber to avoid wall effects.

Figure 2*: Bioaerosol Test Chamber Exterior.*

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/purified 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 Dwyer Instruments Magnehelic gauge, with a range of 0.0 +/- 0.5 inch H_2O, was used to monitor and balance the system pressure during aerosol generation, aerosol purging, and testing cycles.

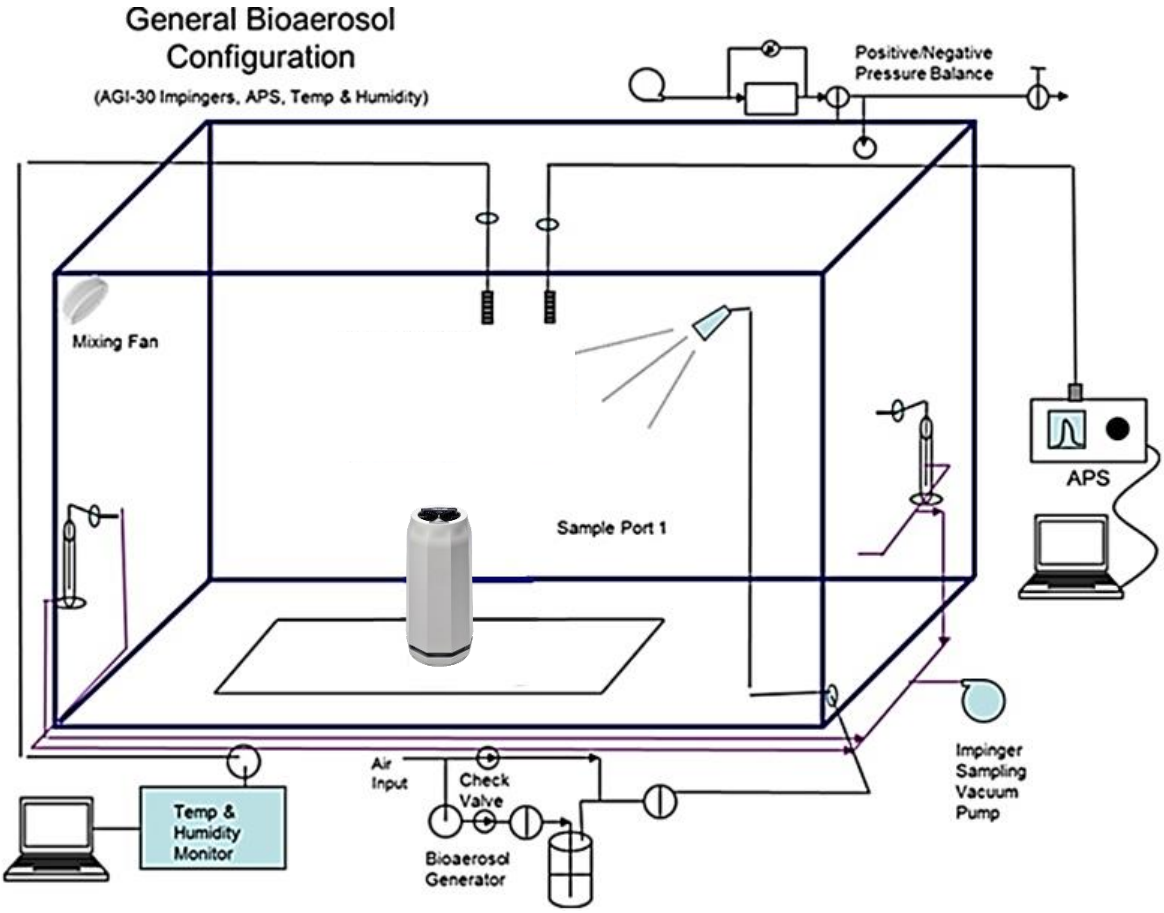

Figure 3: *Bio-Aerosol Test Chamber Flow Diagram.*

Bioaerosol Generation System

Test bioaerosols were disseminated using a Collison 24-jet nebulizer (BGI Inc., Waltham MA) driven by purified filtered house air supply. A pressure regulator allowed for control of the disseminated particle size, use rate, and sheer force generated within the Collison nebulizer.

Prior to testing, the Collison nebulizer flow and use rates were characterized using an air supply pressure of approximately 60 psi. This produced an output volumetric flow rate of 50-80 lpm 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 bio-aerosol collection of all biological aerosols to determine chamber concentration. 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 sample impingers were flow characterized using a calibrated TSI model 4040 mass flow meter.

Aerosol particle size distributions and count concentrations were measured in real-time through the duration of all control and P3000 trial runs using a model 3321 Aerodynamic Particle Sizer (APS) (TSI Inc., St Paul MN). The APS sampled for the entire duration of all trials (90 minutes) with 2-10 minute sampling intervals. A general flow diagram of the aerosol test system is shown above in **Figure 3** above.

Trial	Run	Device	Organism	Target Monodispersed Particle Size	Trial Time (min)	Sampling Period (min)	Sampling
C1	Control	P3000	*MS2 bacteriophage - RNA virus*	<1.0µm	420	0, 60, 120, 180, 240, 300, 360, 420	AGI Impingers
T1	Challenge						
T2	Challenge						
T3	Challenge						

Figure 4: *Bioaerosol Test Matrices for all trials*

Species Selection

Species selection is based on Biological Safety Level 1 (BSL1) surrogates for BSL2/BSL3 pathogenic organisms. MS2, a BSL1 organsim, is a viral RNA bacteriophage that is commonly used as a surrogate for the influenza virus and is used by ARE Labs as surrogate for SARS-CoV-2. MS2 is a single-stranded RNA virus with the average size of 25-30 nm and is similar to SARS CoV-2, which is also a single-stranded RNA virus with a size range of 60-140 nm. As mentioned, the ability of a device to reduce a surrogate of a pandemic causing organism is crucial. Minimizing the risk of infection will be the key point of all room air filtration devices. Although the FDA does not have an official surrogate for SARS-CoV2 to be used in bioaerosol studies, MS2 is widely accepted in a growing body of recently published scientific journals as a good surrogate for various human viruses such as SARS-CoV2 and influenza. However, based on FDA rules, efficacy claims for a specific organism would need to be tested with that particular organism.

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 enveloped viruses such, as coronaviruses, are the least resistant microorganisms to chemical kill mechanisms. **Figure 5** is a graphic representation, from the FDA document, that illustrates the most resistant to least resistance organisms.

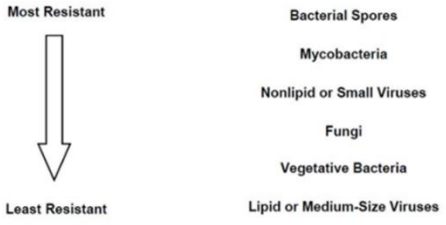

Figure 5: *FDA Graphic showing resistance to disinfection for various organisms.*

Viral Culture & Preparation

Pure strain viral seed stock were obtained from ATCC. Seed stock was grown 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 1×10^{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 1×10^{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 small drop plaque assay technique onto tryptic soy agar plates. The plated cultures were incubated for 24-48 hours then enumerated and recorded.

Bioaerosol Control Testing

To accurately assess the P3000 unit, test chamber pilot control trial was performed with the bioaerosol over a 420-minute period, without the device in operation, to characterize the biological challenge aerosol for particle size distribution, 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 P3000 challenge testing and verify that viable bioaerosol concentrations persisted above the required concentrations over the entire pilot control test period. During control runs, a single low velocity fan located in the corner of the bioaerosol test chamber was turned on for the duration of trial to ensure a homogenous aerosol concentration within the aerosol chamber. The mixing fan was used for all control runs and trials. The two impingers, used for bacteriophage collection, were pooled and mixed prior to plating and enumeration. A complete test matrix for

all bioaerosol trials can be found above in **Figure 4**. All control runs met ARE Labs specification.

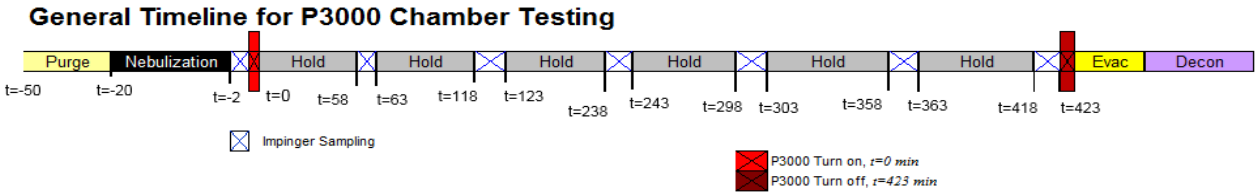

Figure 6: General *Trial Timeline for P3000 Decontamination Trials*

P3000 Testing

For each control and challenge test, the Collison nebulizer was filled with approximately 40 mL of biological stock and operated at 40 psi for a period of 20 minutes. For control and P3000 trials, the impingers were filled with 20 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection. The addition of Tween 80 was shown 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. Following the bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and P3000 test by sampling simultaneously with two AGI-30 impingers located at opposite corners of the chamber. The impinger samples were collected for 3 to 10 minutes at intervals of 60 minutes throughout the entire test period. **Figure 6,** seen above, illustrates the general timeline for each P3000 live bioaerosol challenge.

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

For testing, the P3000 unit was turned to its highest setting which correspond to a flow rate of ~174ft^3/min. It was switched on immediately following a time 0 baseline sample and operated for the entirety of the test (420 min). Subsequent impinger samples were taken at 60 minute intervals up to 420 minutes. The samples were plated and enumerated for viable concentrations to measure the effective viable bioaerosol reduction during operation of the P3000 device over time. All samples were plated in triplicate on tryptic soy agar media over a minimum of three serial ten-fold dilutions.

The plates were incubated for 24 hours then enumerated for viable plaque forming units (pfu). This data was used to calculate aerosol challenge concentrations in the chamber and measure the reduction of viable virus.

Post-Testing Decontamination and Prep

Following each test, the chamber was air flow evacuated/purged for a minimum of twenty minutes and analyzed with the APS for particle concentration decrease to baseline levels. 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 5% bleach bath for 20 minutes. The nebulizer and impingers were then submerged in a DI water bath, followed by spray rinsing 6x with DI water, then allowed to dry.

Data Analysis

Results from the control trials were graphed and plotted to show natural viability loss over time in the chamber. These control runs served as the basis to determine the time required for the P3000 to achieve a 4 log (99.99%) reduction in viable bioaerosol above the natural losses from the control runs. The control and trial runs are plotted showing log reduction in viable bioaerosol for MS2 virus. All data is normalized with time zero (*t=0* minutes) enumerated concentrations. Subsequent samples are normalized and plotted to show the loss of viability over time.

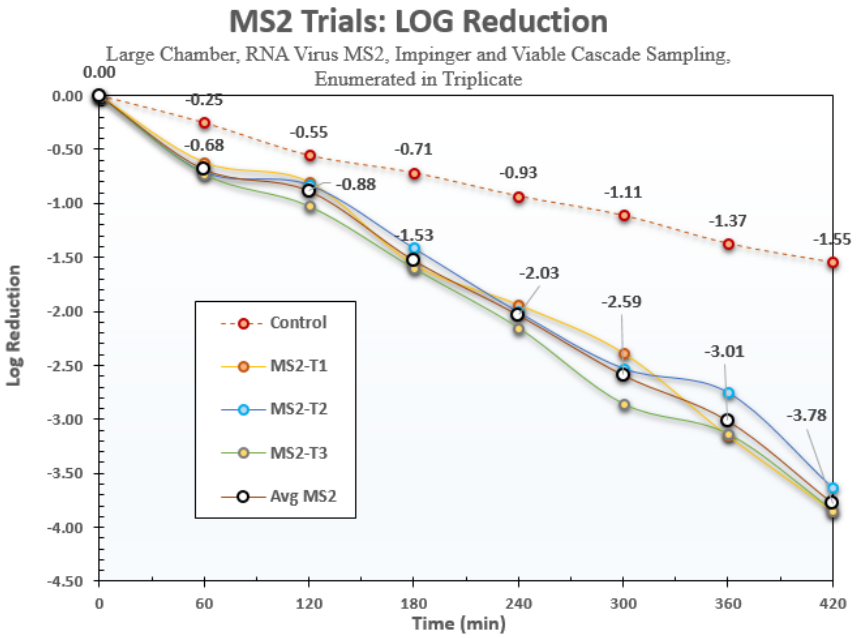

***Figure 7**: Net LOG Reduction vs. Time for all trials + Average*

Results

The device showed a steady log reduction of the MS2 virus concentration throughout the trials. At the 420-minute time point, there was an average 3.78 log reduction. This is represented graphically in **Figure 8** which plots the log reduction at each time point for each of the three trials and their average. This equates to an average 2.23 net log reduction with the natural decay taken into account. This data is presented in **Figure 7.**

Overall, the testing trials demonstrated the robust efficiency the P3000 device had for eliminating aerosolized MS2. The results also indicate that, in theory, the Puraclenz P3000 device could help prevent the spread of airborne infections, especially during flu season and pandemic events. A summary of all trial net log and net percent reductions are found below in **Figure 9.**

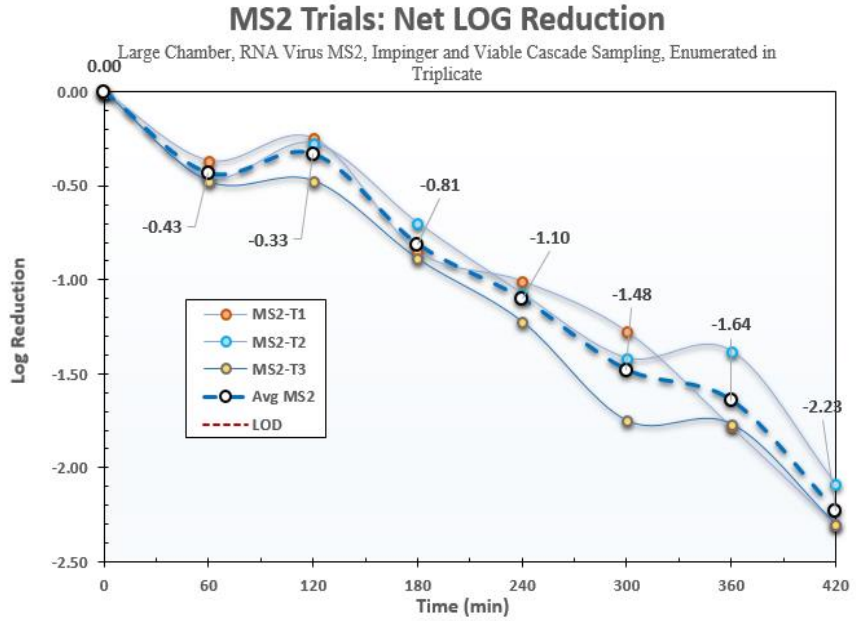

***Figure 8:** MS2 P3000 Log Reduction.*

Executive Summary Table

Average Net Reduction of MS2 By Puraclenz P3000 Device

Bioaerosol Type	Species	Surrogate	Trial ID		60min	120min	180min	240min	300min	360min	420min
Virus	*MS2 bacteriophage (ssRNA 25nm size)*	*Influenza, SARS-CoV-2*	*1*	*Net Log Reduction*	-0.37	-0.25	-0.85	-1.01	-1.27	-1.78	-2.30
				% Reduction	56.9%	43.4%	85.8%	90.1%	94.7%	98.3%	99.5%
Virus	*MS2 bacteriophage (ssRNA 25nm size)*	*Influenza, SARS-CoV-2*	*2*	*Net Log Reduction*	-0.46	-0.27	-0.70	-1.07	-1.42	-1.38	-2.09
				% Reduction	65.0%	46.7%	80.0%	91.6%	96.2%	95.8%	99.2%
Virus	*MS2 bacteriophage (ssRNA 25nm size)*	*Influenza, SARS-CoV-2*	*3*	*Net Log Reduction*	-0.48	-0.47	-0.89	-1.22	-1.75	-1.77	-2.30
				% Reduction	66.7%	66.4%	87.1%	94.0%	98.2%	98.3%	99.5%
Averages				**Average and St. dev**	**-0.43 +/- 0.06**	**-0.33 +/- 0.12**	**-0.81 +/- 0.1**	**-1.1 +/- 0.11**	**-1.48 +/- 0.24**	**-1.64 +/- 0.23**	**-2.23 +/- 0.12**
				Average and St. dev	**62.83% +/- 5.22%**	**52.15% +/- 12.42%**	**84.29% +/- 3.81%**	**91.91% +/- 1.97%**	**96.35% +/- 1.779%**	**97.48% +/- 1.442%**	**99.39% +/- 0.186%**

Figure 9: *Net LOG Reduction and Percent Reduction summary table for all trials*

References

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Analytical Testing Facility

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Project

10905.10

Study Director

Jamie Balarashti
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.

Study Director:

Jamie Balarashti
Study Director
ARE Labs Inc.

4/22/2021
Date

Principal Investigator:

Sean McLeod
Principal Investigator
ARE Labs, Inc.

4/22/2021
Date

Appendix A: Raw Data

Trial Information

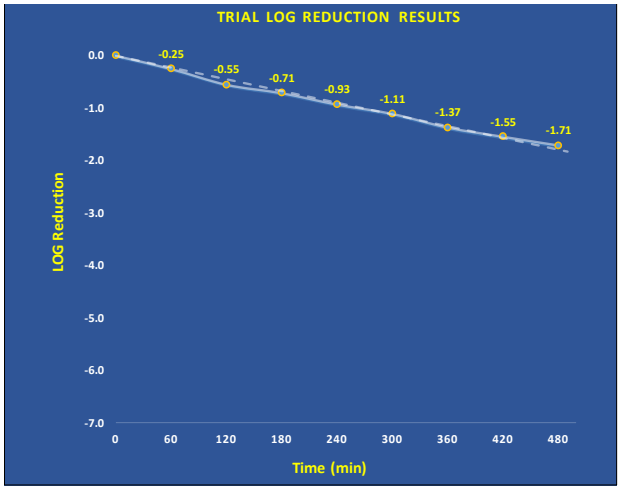
TEST DATE: Tuesday, January 5, 2021
TRIAL PERFORMED BY: SMM
TRIAL NUMBER: T1
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): Synexis MS2 Control with humidity

Device Information

MANUFACTURER: Puraclenz
UNIT MODEL: P3000
UNIT SERIAL #: n
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: Picarro, Interscan, Tiger
TRIAL COMMENTS/NOTES



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8
SAMPLING TIME (min)	0	60	120	180	240	300	360	420
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	4.800E+05	2.688E+05	1.344E+05	9.280E+04	5.632E+04	3.736E+04	20480.000	13680.000
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)								
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)					69.63%	44.33%	17.14%	29.00%
VIABLE CONSISTENCY CHECKS (% agreement)								
IMP & VIABLE CROSS CHECK (% agreement)								
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**4.800E+05**	**2.688E+05**	**1.344E+05**	**9.280E+04**	**5.632E+04**	**3.736E+04**	**2.048E+04**	**1.368E+04**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**56.0000%**	**28.0000%**	**19.3333%**	**11.7333%**	**7.7833%**	**4.2667%**	**2.8500%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**44.0000%**	**72.0000%**	**80.6667%**	**88.2667%**	**92.2167%**	**95.7333%**	**97.1500%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.25**	**-0.55**	**-0.71**	**-0.93**	**-1.11**	**-1.37**	**-1.55**

Impinger Sampling Conditions

	SAMPLING TIME (min)	0	60	120	180	240	300	360	420
	IMPINGER FILL VOL (ml)	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
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-3**	**-3**	**-3**	**-2**	**-2**	**-2**	**-2**
	DROPLET SIZE (µl)	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
	ENUMERATED PLATE COUNTS (# / drop)	1	44	20	15	41	45		19
		2	40	20	11	40	46	26	20
		3		22	16	47	39	29	16
		6		22	16	36	37	32	16
	PLATE AVERAGE COUNT (# / drop)	3.00	42.00	21.00	14.50	41.00	41.75	29.00	17.75
	IMPINGER CONCENTRATION (cfu or pfu/ml)	600,000	840,000	420,000	290,000	82,000	83,500	58,000	35,500
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	4.80E+05	2.69E+05	1.34E+05	9.28E+04	2.62E+04	2.67E+04	1.86E+04	1.14E+04
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-4**	**-2**	**-1**	**-3**	**-3**	**-3**	**-3**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**750**	**50**	**50**	**50**	**50**
	ENUMERATED PLATE COUNTS (# / drop)					12	11	3	3
						15	4	4	2
	PLATE AVERAGE COUNT (# / drop)					13.50	7.50	3.50	2.50
	IMPINGER CONCENTRATION (cfu or pfu/ml)					270,000	150,000	70,000	50,000
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)					8.64E+04	4.80E+04	2.24E+04	1.60E+04

Trial Information

TEST DATE: Tuesday, April 13, 2021
TRIAL PERFORMED BY: SMM
TRIAL NUMBER: T5
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T5

Device Information

MANUFACTURER: Puraclenz
UNIT MODEL: Device
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
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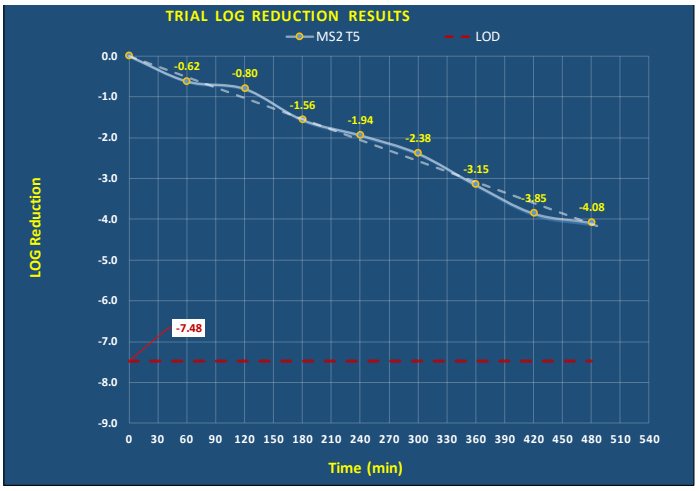
RH (%): 70
OTHER INSTRUMENTS: NA
TRIAL COMMENTS/NOTES NA



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S6	S7
SAMPLING TIME (min)	0	60	120	180	240	300	360	420
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	7.467E+04	1.803E+04	1.184E+04	2.048E+03	8.640E+02	3.093E+02	5.280E+01	1.056E+01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)								
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		10.11%	15.00%	25.45%			2.00%	35.00%
VIABLE CONSISTENCY CHECKS (% agreement)								
IMP & VIABLE CROSS CHECK (% agreement)								
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**7.467E+04**	**1.803E+04**	**1.184E+04**	**2.048E+03**	**8.640E+02**	**3.093E+02**	**5.280E+01**	**1.056E+01**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**24.1429%**	**15.8571%**	**2.7429%**	**1.1571%**	**0.4143%**	**0.0707%**	**0.0141%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**75.8571%**	**84.1429%**	**97.2571%**	**98.8429%**	**99.5857%**	**99.9293%**	**99.9859%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.62**	**-0.80**	**-1.56**	**-1.94**	**-2.38**	**-3.15**	**-3.85**

Impinger Sampling Conditions

	S1	S2	S3	S4	S5	S6	S6	S7
SAMPLING TIME (min)	0	60	120	180	240	300	360	420
IMPINGER FILL VOL (ml)	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	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

Dilution Range #1								
DILUTION RATIO (10^x)	**-3**	**-3**	**-3**	**-2**	**-1**	**-1**	**-1**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
ENUMERATED PLATE COUNTS (# / drop)	13	5	5	8	25	10	2	4
	14	6	3	6	25	10	1	4
	15	5	4	8	31	9	2	4
PLATE AVERAGE COUNT (# / drop)	14.00	5.33	4.00	7.33	27.00	9.67	1.67	4.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	140,000	53,333	40,000	7,333	2,700	967	167	40
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	7.47E+04	1.71E+04	1.28E+04	2.35E+03	8.64E+02	3.09E+02	5.33E+01	1.28E+01

Dilution Range #1								
DILUTION RATIO (10^x)	**-4**	**-2**	**-2**	**-1**	**0**		**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**		**100**	**500**
ENUMERATED PLATE COUNTS (# / drop)		60	31	46			10	13
		58	36	72			21	
		60	35	46			18	
PLATE AVERAGE COUNT (# / drop)		59.33	34.00	54.67			16.33	13.00
IMPINGER CONCENTRATION (cfu or pfu/ml)		59,333	34,000	5,467			163	26
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		1.90E+04	1.09E+04	1.75E+03			5.23E+01	8.32E+00

Trial Information

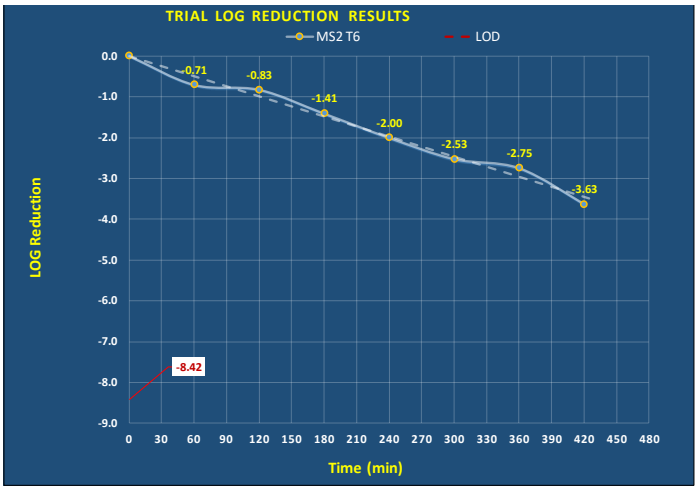
TEST DATE: Wednesday, April 21, 2021
TRIAL PERFORMED BY: SMM
TRIAL NUMBER: T6
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T6

Device Information

MANUFACTURER: Puraclenz
UNIT MODEL: Device
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: NA
TRIAL COMMENTS/NOTES NA



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5		S6	S7
SAMPLING TIME (min)	0	60	120	180	240	300	360	420
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	6.578E+05	1.291E+05	9.813E+04	2.549E+04	6.507E+03	1.963E+03	1.173E+03	1.536E+02
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)								
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	15.00%			29.29%				
VIABLE CONSISTENCY CHECKS (% agreement)								
IMP & VIABLE CROSS CHECK (% agreement)								
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**6.578E+05**	**1.291E+05**	**9.813E+04**	**2.549E+04**	**6.507E+03**	**1.963E+03**	**1.173E+03**	**1.536E+02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**19.6216%**	**14.9189%**	**3.8757%**	**0.9892%**	**0.2984%**	**0.1784%**	**0.0234%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**80.3784%**	**85.0811%**	**96.1243%**	**99.0108%**	**99.7016%**	**99.8216%**	**99.9766%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.71**	**-0.83**	**-1.41**	**-2.00**	**-2.53**	**-2.75**	**-3.63**

Impinger Sampling Conditions

	S1	S2	S3	S4	S5	S6	S7	S8
SAMPLING TIME (min)	0	60	120	180	240	300	360	420
IMPINGER FILL VOL (ml)	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	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

Dilution Range #1								
DILUTION RATIO (10^x)	**-4**	**-4**	**-4**	**-3**	**-2**	**-1**	**-1**	**-1**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
ENUMERATED PLATE COUNTS (# / drop)	10 10 14			8 10 10	20 18 23	61 60 63	41 35 34	
PLATE AVERAGE COUNT (# / drop)	11.33			9.33	20.33	61.33	36.67	
IMPINGER CONCENTRATION (cfu or pfu/ml)	1,133,333			93,333	20,333	6,133	3,667	
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	6.04E+05			2.99E+04	6.51E+03	1.96E+03	1.17E+03	

Dilution Range #1								
DILUTION RATIO (10^x)	**-5**	**-3**	**-3**	**-2**	**0**		**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**		**100**	**500**
ENUMERATED PLATE COUNTS (# / drop)	2 1 1	36 39 46	30 34 28	60 63 75				240
PLATE AVERAGE COUNT (# / drop)	1.33	40.33	30.67	66.00				240.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	1,333,333	403,333	306,667	66,000				480
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	7.11E+05	1.29E+05	9.81E+04	2.11E+04				1.54E+02

Trial Information

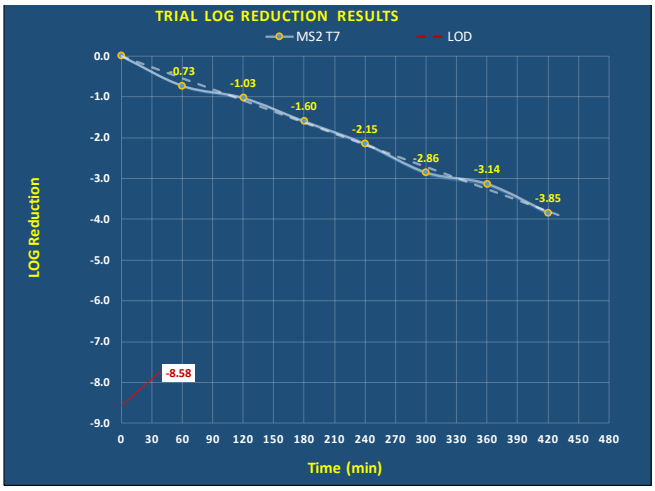
TEST DATE: Thursday, April 22, 2021
TRIAL PERFORMED BY: SMM
TRIAL NUMBER: T7
TEST ORGANSIM: MS2
TRIAL NAME ID (GRAPHS/TABLES): MS2 T7

Device Information

MANUFACTURER: Puraclenz
UNIT MODEL: Device
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: NA
TRIAL COMMENTS/NOTES NA



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5		S6	S7
SAMPLING TIME (min)	0	60	120	180	240	300	360	420
IMPINGER USED (y / n)	y	y	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	9.511E+05	1.776E+05	8.960E+04	2.373E+04	6.667E+03	1.317E+03	6.933E+02	1.355E+02
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)								
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	21.67%	24.74%	32.00%	56.45%	21.43%	45.63%		54.86%
VIABLE CONSISTENCY CHECKS (% agreement)								
IMP & VIABLE CROSS CHECK (% agreement)								
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**9.511E+05**	**1.776E+05**	**8.960E+04**	**2.373E+04**	**6.667E+03**	**1.317E+03**	**6.933E+02**	**1.355E+02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**18.6729%**	**9.4206%**	**2.4953%**	**0.7009%**	**0.1385%**	**0.0729%**	**0.0142%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**81.3271%**	**90.5794%**	**97.5047%**	**99.2991%**	**99.8615%**	**99.9271%**	**99.9858%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.73**	**-1.03**	**-1.60**	**-2.15**	**-2.86**	**-3.14**	**-3.85**

Impinger Sampling Conditions

	S1	S2	S3	S4	S5	S6	S7	S8
SAMPLING TIME (min)	0	60	120	180	240	300	360	420
IMPINGER FILL VOL (ml)	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	5.0	5.0	5.0	5.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

Dilution Range #1								
DILUTION RATIO (10^x)	**-4**	**-4**	**-4**	**-3**	**-3**	**-2**	**-1**	**-1**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
ENUMERATED PLATE COUNTS (# / drop)	15	5	3	10	1	6	23	11
	19	5	4	11	2	5	20	12
	13	9	3	10	4	5	22	12
PLATE AVERAGE COUNT (# / drop)	15.67	6.33	3.33	10.33	2.33	5.33	21.67	11.67
IMPINGER CONCENTRATION (cfu or pfu/ml)	1,566,667	633,333	333,333	103,333	23,333	5,333	2,167	1,167
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	8.36E+05	2.03E+05	1.07E+05	3.31E+04	7.47E+03	1.71E+03	6.93E+02	1.87E+02

Dilution Range #1								
DILUTION RATIO (10^x)	**-5**	**-3**	**-3**	**-2**	**-2**	**-1**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
ENUMERATED PLATE COUNTS (# / drop)	2	55	28	38	18	25		50
	1	46	22	46	20	30		58
	3	42	18	51	17	32		50
PLATE AVERAGE COUNT (# / drop)	2.00	47.67	22.67	45.00	18.33	29.00		52.67
IMPINGER CONCENTRATION (cfu or pfu/ml)	2,000,000	476,667	226,667	45,000	18,333	2,900		527
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.07E+06	1.53E+05	7.25E+04	1.44E+04	5.87E+03	9.28E+02		8.43E+01

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:

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