Study Summary Article

Efficacy of the P3000 System against Two Respirable Microorganisms:

Staphylococcus epidermidis and Aspergillus brasiliensis

Sean McLeod [a], Richard Ludwick [a], Jamie Balarashti [a]

[a] **Aerosol Research and Engineering Laboratories Inc. Olathe KS**

Abstract

Background: Due to the high rate of pulmonary disease occurrence, systems designed to reduce respirable bioaerosol transfer of pulmonary infections have been attracting significant attention. This in-vitro study characterized the efficacy of the P3000 air purification system at reducing respirable bioaerosol for two species of microorganisms from room air. The selected bacteria species, Methicillin Resistant *Staphylococcus epidermidis*, was chosen for its recognition as a surrogate species for other dangerous Gram-positive *Staphylococcus* species such as; Methicillin-Resistant *Staph. aureus* [MRSA]. In addition, the selected mold species *Aspergillus brasiliensis* can be considered a surrogate for other types of dangerous black molds such as *Stachybotrys chartarum* (Toxic Black Mold). This study was performed to demonstrate the efficacy of the device in a hermetically sealed test chamber in order to mimic real-world efficiency.

Methods: The microorganisms were aerosolized into a sealed environmental bioaerosol chamber containing the P3000 device using a Collison 24-Jet Nebulizer or dry powder feeder. All bioaerosols tested had a mass median aerodynamic diameter (MMAD) ranging from 0.7-4.0 µm (species dependent). Bioaerosol sampling was performed using impingers (Ace Glass, AGI-30) and viable cascade impactors (SKC BioStage) depending on the challenge species and concentrations. Bioaerosol samples were taken at multiple time points throughout each trial in order to quantify the reduction rate capability of the air purification device. Impinger samples were serially diluted, plated, incubated, and enumerated in triplicate to yield a viable bioaerosol concentration for each sampling time point. Chamber control trial data, or natural decay, was subtracted from the device trial data to yield net LOG reductions for each of the bioaerosol challenges. There were no deviations from protocol observed during trials.

Results: The P3000 air purifier was effective against both bacterial and fungal species. Results indicate *Staphylococcus epidermidis,* achieved a 2.63 +/- 0.13 net LOG reduction (99.76% reduction) in 420 minutes. When the P3000 was challenged with *Aspergillus brasiliensis*, it achieved a reduction of 1.31 +/- 0.14 net LOG or 95.1% of respirable spores. Real-time ion monitoring showed an average production of 500 negatively charged ions per cubic centimeter of ambient air measured 3 feet away from the device throughout testing.

Conclusion: The P3000 air purification device was shown to be effective at reducing the concentration of these microorganisms, in room air, by 95.1% with *A. brasiliensis* spores and 99.76% with *Staph. epidermidis*. Therefore, the P3000's unique ionization technology makes an effective air purifying system.

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

Introduction

This study was conducted to evaluate the efficacy of the P3000 air purification device at reducing two aerosolized respirable microorganisms. 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, veterinary clinics 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 bacteria and mold spores 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 effectiveness of the P3000 device was evaluated against a Gram positive bacteria and a mold spore. For more organism information, please see species selection section in the body of this report. Testing was conducted to characterize a single P3000 commercial unit against two microorganism species to demonstrate the capability of the P3000 device's unique PCO system's ability to reduce viable bioaerosol concentrations, therefore theoretically reducing chances of airborne infection.

Figure 1: The P3000 device is a photocatalytic oxidation (PCO) system

Test Device Description

The P3000 device uses photocatalytic oxidation, also known as PCO, technology to reduce pathogens in the environment. The PCO functions by exposing titanium oxide coated catalyst with UV light to produce positively and negatively charged ions that deactivate pathogens. A small pre-filter is located where air is pulled through the device to help prevent fowling of the catalyst or UV lamps. Ion monitoring was performed and it showed an average of approximately 500 negatively charged ions per cubic centimeter consistently throughout trials. The species and characterization of these ions was not analyzed during this test.

Bioaerosol Testing Chamber

A large sealed aerosol test chamber was used to replicate a potentially 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 6.9 ft, with a displacement volume of 579 cubic feet, or 16,000 liters. **Figure 2** shows the bioaerosol chamber used for all testing in this study.

Figure 2: Exterior picture of the Stainless Steel Bioaerosol Test Chamber used for all P3000 Testing. Chamber is equipped with HEPA in/out, multiple bioaerosol sampling ports, decontamination, temperature and humidity control, and pressure balance.

The chamber is equipped with filtered HEPA inlets, digital internal temperature and humidity monitors, heater and humidifier, lighting system, multiple sampling ports, aerosol mixing fans, and a HEPA filtered exhaust system that are operated with wireless remote control. 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 using a Collison 24-jet nebulizer or dry powder eductor for the aerosolization of the microorganisms.

All sample and dissemination ports are inserted approximately 18 inches from the interior walls of the chamber and at a height of approximately 40 inches from the floor to avoid wall effects. 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 and a HEPA filtered exhaust blower with a 500 ft^3/min rated flow capability for rapid evacuation of remaining bioaerosols. A Magnehelic gauge, with a range of 0.0 +/- 0.5 inch H_2O (Dwyer instruments, Michigan City IN), is used to monitor and balance the system pressure during aerosol generation, aerosol purging, and testing cycles.

General Large Chamber Bioaerosol Configuration

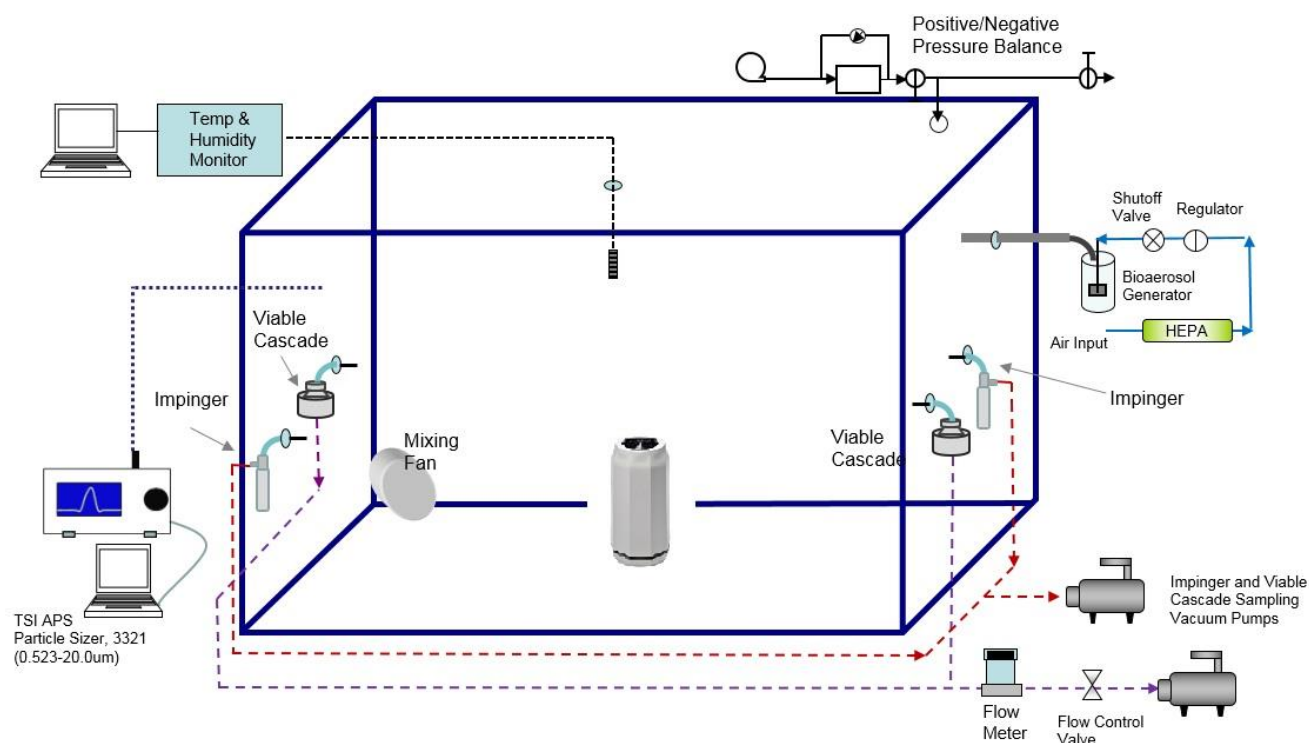### (AGI-30 Impingers, APS, Temp & Humidity)

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

Environmental Controls

For increased stability of bioaerosols, relative humidity inside the chamber is 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) **Figure 4**, with the exception of the *A. brasiliensis* spores which were aerosolized using a dry powder eductor. The aerosolization of bioaerosols were driven by purified filtered house air supply. 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 characterized using an air supply pressure of approximately 40-60 psi, which obtained 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).

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

Bioaerosol Sampling and Monitoring System

Two AGI impingers (Ace Glass Inc. Vineland NJ) were used for bioaerosol collection of all biological aerosols to determine chamber concentrations. The two AGI 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 5: 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.

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. A general flow diagram of the aerosol test system is shown above in **Figure 3**.

During testing with less resilient organisms, or those which 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) comprises an inlet cone, precision-drilled 400-hole impactor stage, and a base that holds a standard-size agar plate (**Figure 5**). A high flow pump pulls microorganisms in air through the holes (jets) at 30 liters per minute, where they are collected directly onto the agar surface. This method is the most sensitive for detection of organisms at low concentrations.

Vegetative Bacteria Challenges:

The vegetative bacteria organism used for this study was methicillin resistant *Staphylococcus epidermidis* (ATCC 12228). *Staphylococcus epidermidis* is a Gram-positive bacterium and BSL1 simulant for a wider range of medically significant pathogens, such as Methicillin Resistant *Staphylococcus aureus* (MRSA). These pathogens are most common in hospitals and can cause life-threatening infections if contracted.

Endospore Challenges:

Aspergillus brasiliensis (ATCC 16404), formerly known as A. *niger,* is one of the most common species of the genus *Aspergillus*. A. *brasiliensis* is routinely defined as a surrogate for various toxic black mold species such as *Stachybotrys Chartarum.* Mold is general is attributed to many respiratory problems found in infants, elderly and immunocompromised individuals. Purified A. *brasiliensis* spores were used in bulk dry powder form with an approximate concentration of 1×10^9 cfu/gram.

Vegetative Cells Culture & Preparation

Pure strain seed stocks were purchased from ATCC (American Type Culture Collection, Manassas VA). Working

stock cultures were prepared using aseptic techniques in a class 2 biological safety cabinet and followed standard preparation methodologies. Approximately 250mL of each biological stock was prepared in tryptic soy liquid broth media, and incubated for 24-48 hours with oxygen infusion (1cc/min) at 37°C. Biological stock concentrations were around 1×10^{10} cfu/ml.

Stock cultures were centrifuged for 10 minutes at 3000rpm in an LD-3 centrifuge in sterile 15mL conical tubes, growth media was removed, and the cells re-suspended in sterile PBS buffer for aerosolization. Aliquots of these suspensions were enumerated on tryptic soy agar plates (Hardy Diagnostics, Cincinnati OH) for viable counts and stock concentration calculation. For each organism, test working stocks were grown in sufficient volume to satisfy use quantities for all tests conducted using the same culture stock material.

Fungal Spore Culture & Preparation

A. brasiliensis fungal spores were obtained in purified bulk powder form at a concentration of 1×10^9 cfu/g. To verify the bulk powder spore concentration, an aliquot of weighed dry powder was prepared in suspension in PBS + 0.005% Tween 80 at a mass: volume ratio to obtain a concentration of 1×10^9 cfu/ml. This aliquoted spore suspension was plated prior to testing to verify concentration.

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 and enumerated and recorded.

When working with microorganisms at extremely low concentrations the viable cascade sampling was used. This method samples the chamber by pulling 30 liters per minute through the cascade device directly onto an agar plate. Enumeration of colonies grown depends on the concentration of the sample. Colony counts totaling up to 400 can then be adjusted using the positive conversion table.
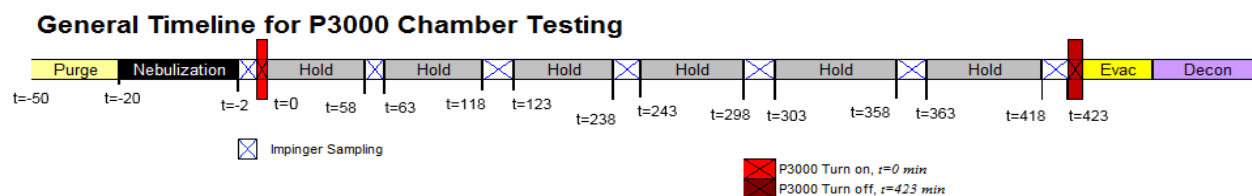
Figure 6: General Trial Timeline for P3000 Decontamination Trials.

This table 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 using the conversion formula of Feller, W (1950).

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 5% 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. A general trial timeline can be found in **Figure 6** above.

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 device to achieve at least 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 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

Methods Control:

To accurately assess the P3000 unit, test chamber pilot control trials were performed with all organisms over a 240-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 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 was turned off during P3000 decontamination trials. The two impingers used for bioaerosol collection were pooled and mixed prior to plating and enumeration

Methods: 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. Then, 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 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).

Biological Test Matrix

Trial	Run	Pathogenic Organism	Test Species (gram, description)	ATCC Ref	Target Monodispersed Particle Size	Challenge Conc. (#/L)	Trial Time (min)	Sample Time (min)	Sampling	Plating and Enumeration
1	Control	*Methicillin resistant staphylococcus aureus*	*Staphylococcus Epidermidis* (+, vegetative)	12228	2.5-3.0um	10^4-10^6	420	0, 60, 120, 180, 240, 300, 360, 420	Impingers and Viable Cascade	all samples in triplicate
2	Challenge									
3	Challenge									
4	Challenge									
5	Control	Toxic Black Molds (spore)	*Aspergillus brasiliensis* (mold, spore forming)	16404	<5.0um	10^4-10^6	180	0, 60, 120, 180	Impingers and Viable Cascade	all samples in triplicate
6	Challenge									
7	Challenge									
8	Challenge									

Figure 7: Test Matrix for the P3000 air purification system.

Following 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. AGI samples were collected for 2 to 10 minutes at intervals of 60 minutes throughout the entire test period. The biological test matrix can be found in **Figure 7**.

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.

The P3000 biological testing was done following GLP practices. The unit was turned on immediately following a time 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 P3000 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) or colony forming units (cfu) to calculate aerosol

challenge concentrations in the chamber and reduction of viable microorganisms.

Results

This study was performed to evaluate the P3000 device's efficacy at reducing bioaerosols, in a controlled bioaerosol test chamber. The variety of test organisms were chosen specifically for their ability to gauge a device's efficacy against the most commonly encountered microorganisms in room air.

The ions being produced by the device were logged periodically throughout testing to verify the production of approximately 500 negatively charged ions per cubic centimeter of ambient air. Consistent ion production is crucial in functionality of the device at reducing bioaerosols. The unique active approach to safer air could be pivotal in years to come.

When tested against the *Staphylococcus epidermidis*, the device showed a net LOG reduction of 2.63 +/- 0.13 in 420 minutes. When tested against *Aspergillus brasiliensis* the device achieved a net LOG reduction of 1.31 +/- 0.14 in 180 minutes. The highly electrostatic nature of spores lead to an increased natural decay and therefore limited the reduction efficiency resolution. Net LOG reduction data can be found in the graph **Figure 8** and the table in **Figure 9** below.
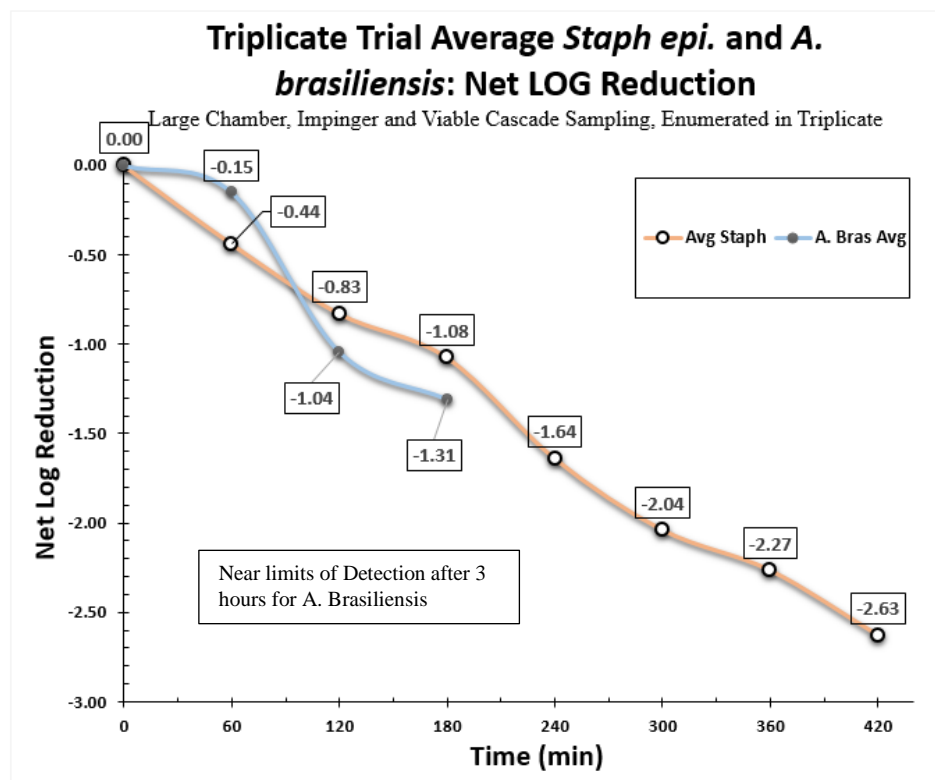
Figure 8: Net LOG Reduction for the P3000 for *Staph epi.* and *A. brasiliensis.*

Average % NET Reduction and NET LOG Reduction of Viable BioAerosols

Bioaerosol Type	Species (gram, description)	Number of Trials	Total Trial Time(minutes)	Data Type	Trial 1	Trial 2	Trial 3	Average
Bacterial	*Staphylococcus Epidermidis* (+, vegetative)	3	420	*Net Log Reduction*	-2.52	-2.77	-2.61	-2.63+/-0.13 99.8% +/- 0.07%
				Net % Reduction	99.7%	99.8%	99.8%	
Mold	*Aspergillus brasiliensis* (mold, spore forming)	3	180	*Net Log Reduction*	-1.44	-1.33	-1.16	-1.31+/- 0.14 95.1% +/- 1.67%
				Net % Reduction	96.4%	95.3%	93.1%	

Figure 9: Executive Summary.

Overall Study Conclusion

In conclusion, the device achieved net LOG reduction of all bioaerosols. There were no deviations from protocol observed throughout the trials. Reduction of a range of microorganisms should reduce the risks of contracting airborne illnesses when used as directed. All results are ≤0.30 standard deviation and data was quality checked for accuracy.

References

Feller, W. (1950). *An introduction to probability theory and its applications*. Wiley.

T. Reponen, K. Willeke, V. Ulevicius et al. *Techniques of Dispersion of Microorganisms in Air.* Aerosol Science and Technology. 27: 1997. pp. 405-421.

Ding and Wing. *Effects of Sampling Time on the Total Recovery rate of AGI-30 Impingers for E. coli*. Aerosol and Air Quality Research, Vol. 1, No. 1, 2001, pp. 31-36.

U.S. Department of Health and Human Services Food and Drug Administration. *Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency Guidance for Industry and Food and Drug Administration Staff*. March 2009

Analytical Testing Facility

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

Project

10905.30

Study Director

Jamie Balarashti
Aerosol Research and Engineering Laboratories
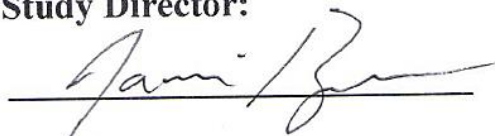
GLP Statement

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.

Conflict of Interest Statement

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

Study Director:
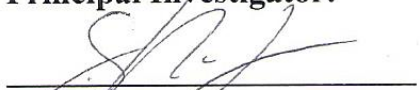
6/24/2021
Date

Jamie Balarashti
Study Director
ARE Labs Inc.

Principal Investigator:

6/24/2021
Date

Sean McLeod
Principal Investigator
ARE Labs, Inc.

Appendix A: LOG and Net LOG Reduction Graphs

A. Brasiliensis Trials: LOG Reduction

Large Chamber, Impinger and Viable Cascade Sampling, Enumerated in Triplicate
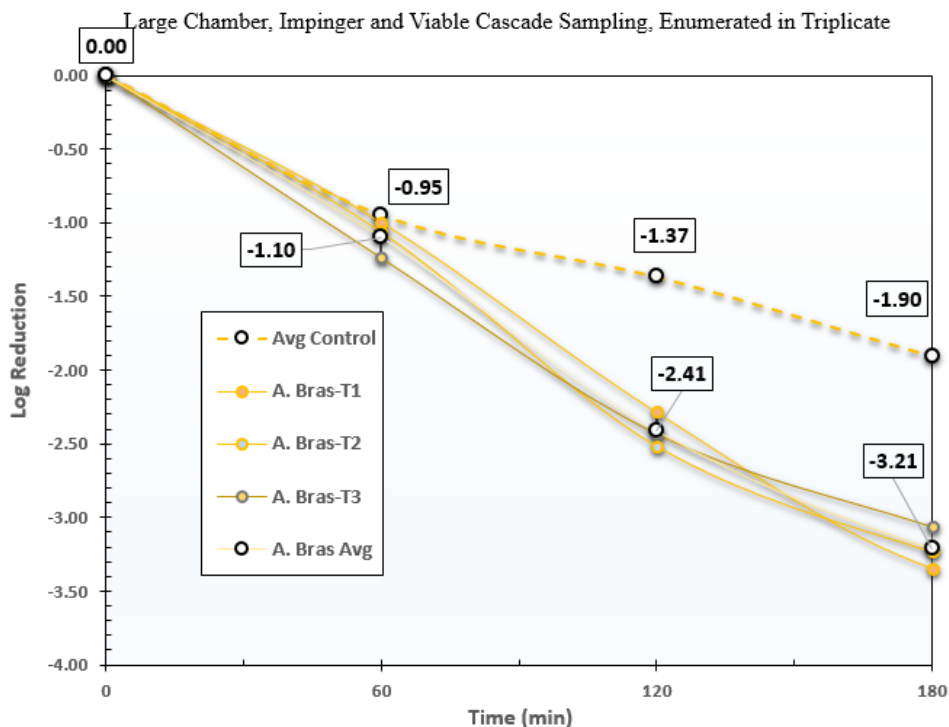
Figure 1A: *A.brasiliensis* P3000 LOG Reduction

A. Brasiliensis Trials: Net LOG Reduction

Large Chamber, Impinger and Viable Cascade Sampling, Enumerated in Triplicate

Figure 2A: *A.brasiliensis* P3000 Net LOG Reduction

Staph epidermidis Trials: LOG Reduction
Large Chamber, Impinger and Viable Cascade Sampling, Enumerated in Triplicate
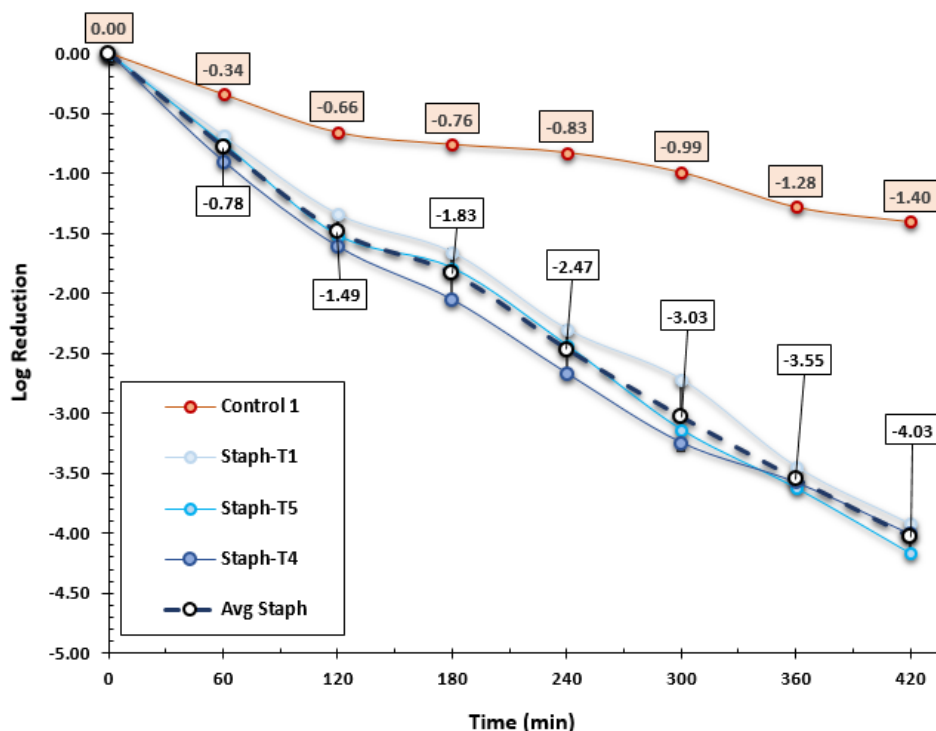
Figure 3A: Staph P3000 LOG Reduction

Staph epidermidis Trials: Net LOG Reduction
Large Chamber, Impinger and Viable Cascade Sampling, Enumerated in Triplicate
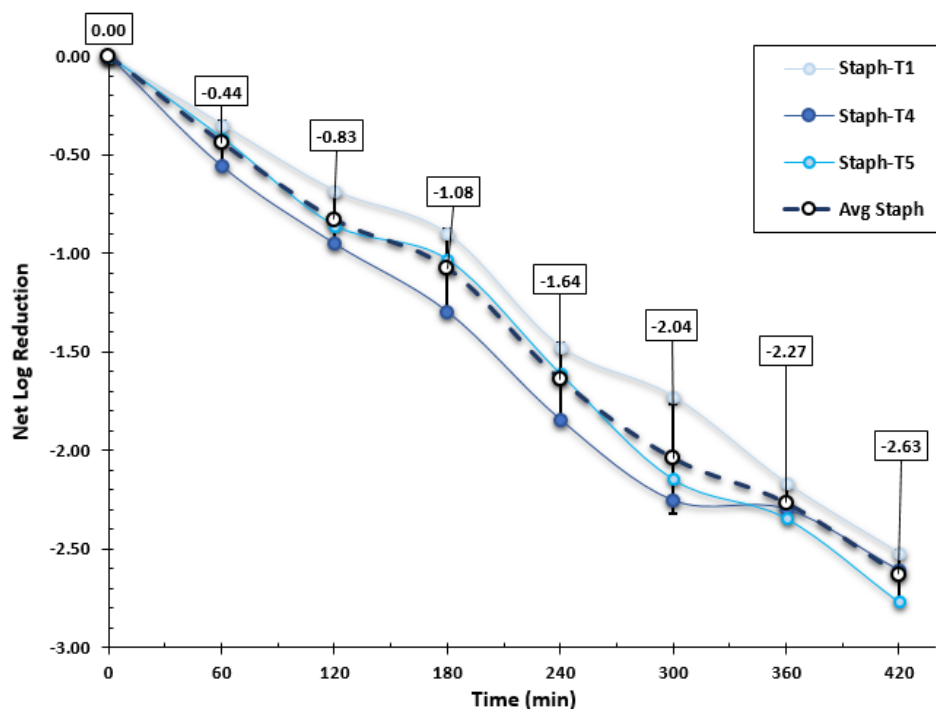
Figure 4A: Staph P3000 net LOG Reduction

Appendix B: Raw Data

Trial Information

TEST DATE:	Thursday, February 25, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	C1
TEST ORGANSIM:	Staph
TRIAL NAME ID (GRAPHS/TABLES):	Staph Control T1

Device Information

MANUFACTURER:	Puraclenz
UNIT MODEL:	P3000
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

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:	na
TRIAL COMMENTS/NOTES	na

TRIAL LOG REDUCTION RESULTS

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	y	y	y	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	3.213E+05	1.461E+05	70400.000	56000.000	48000.000	32746.667	16853.333	12693.333
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)								
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	● 0.83%	● 4.29%						
VIABLE CONSISTENCY CHECKS (% agreement)								
IMP & VIABLE CROSS CHECK (% agreement)								
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**3.213E+05**	**1.461E+05**	**7.040E+04**	**5.600E+04**	**4.800E+04**	**3.275E+04**	**1.685E+04**	**1.269E+04**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**45.4772%**	**21.9087%**	**17.4274%**	**14.9378%**	**10.1909%**	**5.2448%**	**3.9502%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**54.5228%**	**78.0913%**	**82.5726%**	**85.0622%**	**89.8091%**	**94.7552%**	**96.0498%**
LOG REDUCTION FROM T=0 ($\log_{10}$)	**0.00**	**-0.34**	**-0.66**	**-0.76**	**-0.83**	**-0.99**	**-1.28**	**-1.40**

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	10.0	10.0
			IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

		DILUTION RATIO (10^x)	**-4**	**-3**	**-3**	**-3**	**-3**	**-2**	**-2**	**-2**
		DROPLET SIZE (µl)	100	100	100	100	100	100	100	100
Dilution Range #1	ENUMERATED PLATE COUNTS (# / drop)		5	53	25	18	19	110	115	83
			5	44	19	17	14	100	107	78
			2	37			12	97	94	77
	PLATE AVERAGE COUNT (# / drop)		4.00	44.67	22.00	17.50	15.00	102.33	105.33	79.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)		400,000	446,667	220,000	175,000	150,000	102,333	105,333	79,333
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		3.20E+05	1.43E+05	7.04E+04	5.60E+04	4.80E+04	3.27E+04	1.69E+04	1.27E+04

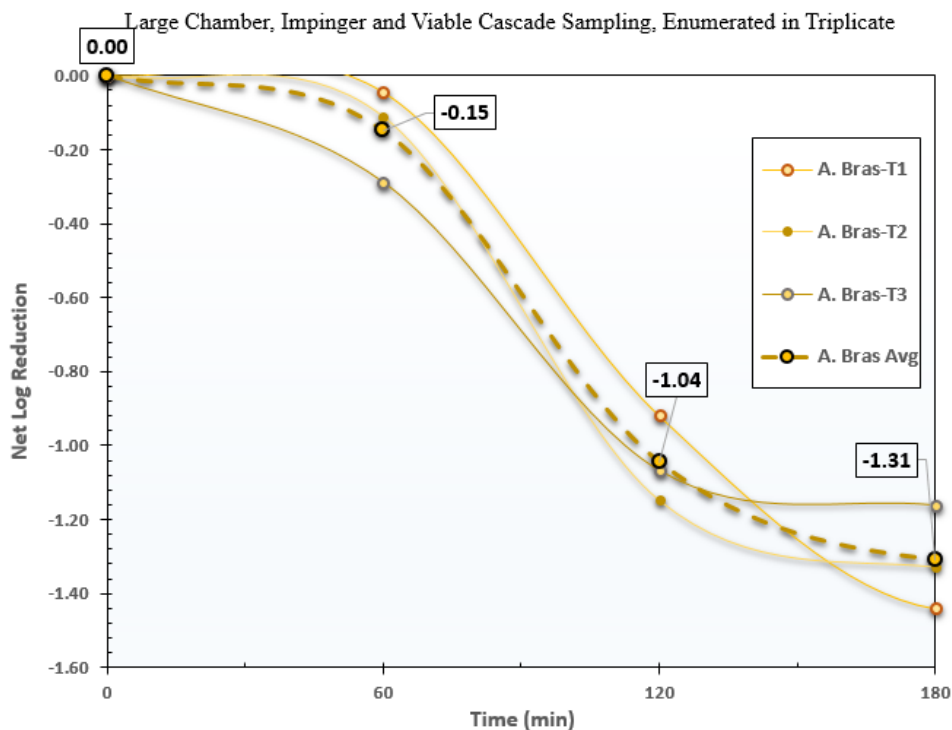|		**DILUTION RATIO (10^x)**	**-3**	**-4**	**-1**	**-1**				
		DROPLET SIZE (µl)	100	100	750	750				
Dilution Range #1	ENUMERATED PLATE COUNTS (# / drop)		48	6						
			37	6						
			36	2						
	PLATE AVERAGE COUNT (# / drop)		40.33	4.67						
	IMPINGER CONCENTRATION (cfu or pfu/ml)		403,333	466,667						
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		3.23E+05	1.49E+05						

Figure 1B: *S. epidermidis* Control

AEROSOL Research and Engineering Laboratories

Trial Information

TEST DATE:	Wednesday, May 26, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T1
TEST ORGANSIM:	Staph
TRIAL NAME ID (GRAPHS/TABLES):	Staph T1

Device Information

MANUFACTURER:	Puraclenz
UNIT MODEL:	P3000
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

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:	na
TRIAL COMMENTS/NOTES	na

TRIAL LOG REDUCTION RESULTS

— Staph T1

Graph plotting LOG Reduction vs Time (min): data points at 0.0, -0.69, -1.34, -1.66, -2.30, -2.72, -3.44, -3.92

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)	6.667E+05	1.365E+05	3.040E+04	1.461E+04	3.360E+03	1.280E+03	2.405E+02	8.000E+01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)								
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	🟢	27.27%						
VIABLE CONSISTENCY CHECKS (% agreement)								
IMP & VIABLE CROSS CHECK (% agreement)								
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	6.667E+05	1.365E+05	3.040E+04	1.461E+04	3.360E+03	1.280E+03	2.405E+02	8.000E+01
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	20.4800%	4.5600%	2.1920%	0.5040%	0.1920%	0.0361%	0.0120%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	79.5200%	95.4400%	97.8080%	99.4960%	99.8080%	99.9639%	99.9880%
LOG REDUCTION FROM T=0 ($\log_{10}$)	0.00	-0.69	-1.34	-1.66	-2.30	-2.72	-3.44	-3.92

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)	3.0	5.0	5.0	5.0	10.0	10.0	10.0	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	-3	-2	-2	-2	-1	0	0
DROPLET SIZE (µl)	100	100	100	100	100	100	100	100
ENUMERATED PLATE COUNTS (# / drop)	10	35	80	42	20	90	137	57
		48	70	44	18	80	155	47
	15	45	90	51	25	70	159	46
PLATE AVERAGE COUNT (# / drop)	12.50	42.67	80.00	45.67	21.00	80.00	150.33	50.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	1,250,000	426,667	80,000	45,667	21,000	8,000	1,503	500
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	6.67E+05	1.37E+05	2.56E+04	1.46E+04	3.36E+03	1.28E+03	2.41E+02	8.00E+01

Dilution Range #1

DILUTION RATIO (10^x)	-3	-2	-3	-1	0		0	0
DROPLET SIZE (µl)	100	100	100	100	500		100	500
ENUMERATED PLATE COUNTS (# / drop)			10					
			12					
			11					
PLATE AVERAGE COUNT (# / drop)			11.00					
IMPINGER CONCENTRATION (cfu or pfu/ml)			110,000					
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)			3.52E+04					

Figure 2B: *S. epidermidis* Trial 1

AEROSOL Research and Engineering Laboratories

Trial Information

TEST DATE:	Tuesday, June 15, 2021
TRIAL PERFORMED BY:	JCT
TRIAL NUMBER:	T2
TEST ORGANSIM:	Staph
TRIAL NAME ID (GRAPHS/TABLES):	Staph T2

Device Information

MANUFACTURER:	Puraclenz
UNIT MODEL:	P3000
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

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:	na
TRIAL COMMENTS/NOTES	na

TRIAL LOG REDUCTION RESULTS

Graph: Staph T2 — LOG Reduction vs Time (min). Data points: -0.90, -1.61, -2.06, -2.67, -3.25, -3.58, -4.01

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)	6.844E+05	8.587E+04	1.675E+04	6.027E+03	1.469E+03	3.893E+02	1.813E+02	6.693E+01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)								
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	36.17%	66.94%	8.54%	11.67%	3.21%	7.89%		52.35%
VIABLE CONSISTENCY CHECKS (% agreement)								
IMP & VIABLE CROSS CHECK (% agreement)								
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	6.844E+05	8.587E+04	1.675E+04	6.027E+03	1.469E+03	3.893E+02	1.813E+02	6.693E+01
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	12.5455%	2.4468%	0.8805%	0.2147%	0.0569%	0.0265%	0.0098%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	87.4545%	97.5532%	99.1195%	99.7853%	99.9431%	99.9735%	99.9902%
LOG REDUCTION FROM T=0 ($\log_{10}$)	0.00	-0.90	-1.61	-2.06	-2.67	-3.25	-3.58	-4.01

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	10.0	10.0	10.0	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	-3	-2	-2	-1	-1	-1	0
	DROPLET SIZE (µl)	100	100	100	100	100	100	100	100
	ENUMERATED PLATE COUNTS (# / drop)	13	38	40	19	99	32	11	24
		18	48	38	17	85	24	11	26
		16	35	86	17	87	20	12	31
	PLATE AVERAGE COUNT (# / drop)	15.67	40.33	54.67	17.67	90.33	25.33	11.33	27.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	1,566,667	403,333	54,667	17,667	9,033	2,533	1,133	270
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	8.36E+05	1.29E+05	1.75E+04	5.65E+03	1.45E+03	4.05E+02	1.81E+02	4.32E+01
Dilution Range #1	DILUTION RATIO (10^x)	-5	-4	-3	-3	-2	-2	0	-1
	DROPLET SIZE (µl)	100	100	100	100	100	100	500	100
	ENUMERATED PLATE COUNTS (# / drop)	1	1	5	3	10	2		4
		0	2	10	2	12	2		4
		2	1	0	1	6	3		9
	PLATE AVERAGE COUNT (# / drop)	1.00	1.33	5.00	2.00	9.33	2.33		5.67
	IMPINGER CONCENTRATION (cfu or pfu/ml)	1,000,000	133,333	50,000	20,000	9,333	2,333		567
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	5.33E+05	4.27E+04	1.60E+04	6.40E+03	1.49E+03	3.73E+02		9.07E+01

Figure 3B: *S. epidermidis* Trial 2

Trial Information

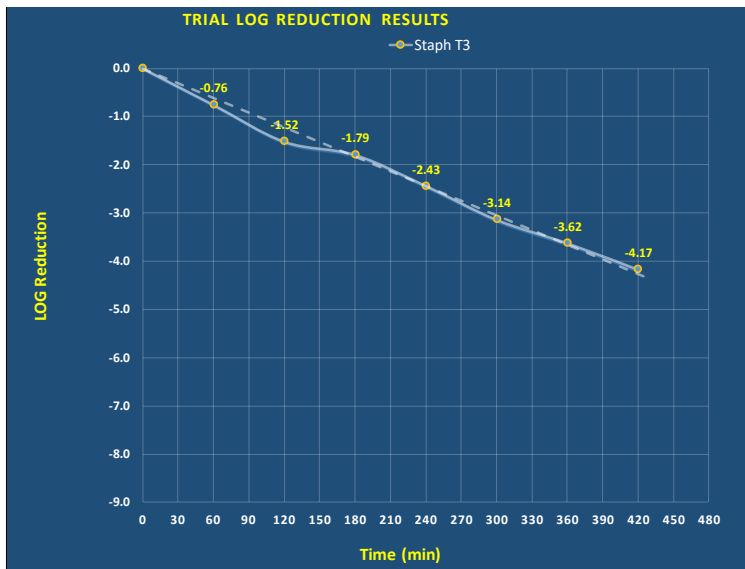|	
TEST DATE:	Wednesday, June 16, 2021
TRIAL PERFORMED BY:	JCT
TRIAL NUMBER:	T3
TEST ORGANSIM:	Staph
TRIAL NAME ID (GRAPHS/TABLES):	Staph T3

Device Information

MANUFACTURER:	Puraclenz
UNIT MODEL:	P3000
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

TEST CHAMBER VOLUME (m³):	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	

TRIAL LOG REDUCTION RESULTS

LOG Reduction vs Time (min)

Staph T3: 0.0, -0.76, -1.52, -1.79, -2.43, -3.14, -3.62, -4.17

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)	3.947E+05	6.933E+04	1.200E+04	6.400E+03	1.451E+03	2.880E+02	9.413E+01	2.672E+01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)								
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	22.40%	37.50%	20.00%	0.00%	24.52%	20.00%	31.90%	
VIABLE CONSISTENCY CHECKS (% agreement)								
IMP & VIABLE CROSS CHECK (% agreement)								
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	3.947E+05	6.933E+04	1.200E+04	6.400E+03	1.451E+03	2.880E+02	9.413E+01	2.672E+01
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	17.5676%	3.0405%	1.6216%	0.3676%	0.0730%	0.0239%	0.0068%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	82.4324%	96.9595%	98.3784%	99.6324%	99.9270%	99.9761%	99.9932%
LOG REDUCTION FROM T=0 (log₁₀)	0.00	-0.76	-1.52	-1.79	-2.43	-3.14	-3.62	-4.17

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)	3.0	5.0	5.0	5.0	10.0	10.0	10.0	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	-3	-3	-2	-2	-1	-1
DROPLET SIZE (µl)	100	100	100	100	100	100	100	100
ENUMERATED PLATE COUNTS (# / drop)	5	2	4	2	8	1	5	
	8	2	3	2	7	2	8	
	12	1	3	2	16	3	8	
PLATE AVERAGE COUNT (# / drop)	8.33	1.67	3.33	2.00	10.33	2.00	7.00	
IMPINGER CONCENTRATION (cfu or pfu/ml)	833,333	166,667	33,333	20,000	10,333	2,000	700	
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	4.44E+05	5.33E+04	1.07E+04	6.40E+03	1.65E+03	3.20E+02	1.12E+02	

Dilution Range #1

DILUTION RATIO (10^x)	-3	-3	-2	-2	-1	-1	0	0
DROPLET SIZE (µl)	100	100	100	100	100	100	100	500
ENUMERATED PLATE COUNTS (# / drop)	69	15	47	18	78	17	51	88
	65	37	49	22	81	16	51	79
	60	28	29	20	75	15	41	
PLATE AVERAGE COUNT (# / drop)	64.67	26.67	41.67	20.00	78.00	16.00	47.67	83.50
IMPINGER CONCENTRATION (cfu or pfu/ml)	646,667	266,667	41,667	20,000	7,800	1,600	477	167
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	3.45E+05	8.53E+04	1.33E+04	6.40E+03	1.25E+03	2.56E+02	7.63E+01	2.67E+01

Figure 4B: *S. epidermidis* Trial 3

Trial Information

TEST DATE:	Wednesday, February 12, 2020
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	C1
TEST ORGANSIM:	A. Bras
TRIAL NAME ID (GRAPHS/TABLES):	A. Bras C1

Device Information

MANUFACTURER:	Puraclenz
UNIT MODEL:	P3000
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

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:	na
TRIAL COMMENTS/NOTES	na

TRIAL LOG REDUCTION RESULTS

BIOAEROSOL Sample ID and Summary Data	S1	S5	S7	S8	S9
SAMPLING TIME (min)	0	60	120	180	240
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	2.267E+03	2.560E+02	97.600	28.320	8.533
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	● 30.00%		○ 33.64%	● 17.53%	
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**2.267E+03**	**2.560E+02**	**9.760E+01**	**2.832E+01**	**8.533E+00**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**11.2941%**	**4.3059%**	**1.2494%**	**0.3765%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**88.7059%**	**95.6941%**	**98.7506%**	**99.6235%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.95**	**-1.37**	**-1.90**	**-2.42**

Impinger Sampling Conditions

	SAMPLING TIME (min)	0	60	120	180	240
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	2.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5

	DILUTION RATIO (10^x)	-2	-1	-1	0	0
	DROPLET SIZE (µl)	100	100	100	100	500
Dilution Range #1	ENUMERATED PLATE COUNTS (# / drop)	4	7	4	8	17
		1	12	5	9	15
		2	5	2	7	8
	PLATE AVERAGE COUNT (# / drop)	2.33	8.00	3.67	8.00	13.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	2,333	800	367	80	27
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.87E+03	2.56E+02	1.17E+02	2.56E+01	8.53E+00

	DILUTION RATIO (10^x)	-3	0	0	0	-1
	DROPLET SIZE (µl)	100	750	100	500	750
Dilution Range #1	ENUMERATED PLATE COUNTS (# / drop)	1		30	39	
		0		20	58	
		0		23		
	PLATE AVERAGE COUNT (# / drop)	0.33		24.33	48.50	
	IMPINGER CONCENTRATION (cfu or pfu/ml)	3,333		243	97	
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	2.67E+03		7.79E+01	3.10E+01	

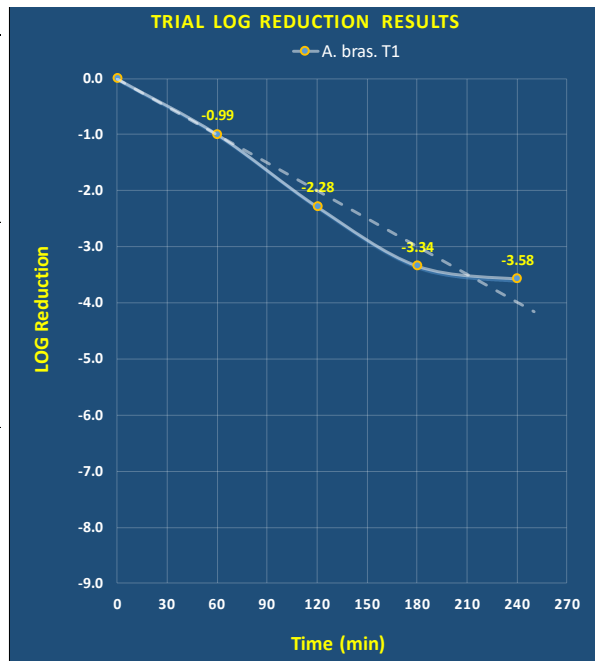**Figure 5B:** *A. brasiliensis* Control

Trial Information

TEST DATE:	Thursday, June 3, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T1
TEST ORGANSIM:	A. Brasiliensis
TRIAL NAME ID (GRAPHS/TABLES):	A. bras. T1

Device Information

MANUFACTURER:	Puraclenz
UNIT MODEL:	P3000
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

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:	na
TRIAL COMMENTS/NOTES	na

TRIAL LOG REDUCTION RESULTS

Graph: A. bras. T1 — LOG Reduction vs Time (min). Data points: -0.99, -2.28, -3.34, -3.58

BIOAEROSOL Sample ID and Summary Data

	S1	S2	S3	S4	S5
SAMPLING TIME (min)	0	60	120	180	240
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	9.867E+03	1.003E+03	5.120E+01	4.480E+00	2.613E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	● 15.00%			● 13.33%	● 4.00%
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	9.867E+03	1.003E+03	5.120E+01	4.480E+00	2.613E+00
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	10.1622%	0.5189%	0.0454%	0.0265%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	89.8378%	99.4811%	99.9546%	99.9735%
LOG REDUCTION FROM T=0 (log_{10})	0.00	-0.99	-2.28	-3.34	-3.58

Impinger Sampling Conditions

SAMPLING TIME (min)	0	60	120	180	240
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	10.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5

Dilution Range #1

DILUTION RATIO (10^x)	-2	-2	0	0	0
DROPLET SIZE (µl)	100	100	100	100	100
ENUMERATED PLATE COUNTS (# / drop)	26		20	2	2
	13		15	3	1
	12		13	4	2
PLATE AVERAGE COUNT (# / drop)	17.00		16.00	3.00	1.67
IMPINGER CONCENTRATION (cfu or pfu/ml)	17,000		160	30	17
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	9.07E+03		5.12E+01	4.80E+00	2.67E+00

Dilution Range #1

DILUTION RATIO (10^x)	-3	-1	0	0	0
DROPLET SIZE (µl)	100	100	500	500	500
ENUMERATED PLATE COUNTS (# / drop)	2	29		11	8
	2	30		15	
	2	35			
PLATE AVERAGE COUNT (# / drop)	2.00	31.33		13.00	8.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	20,000	3,133		26	16
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.07E+04	1.00E+03		4.16E+00	2.56E+00

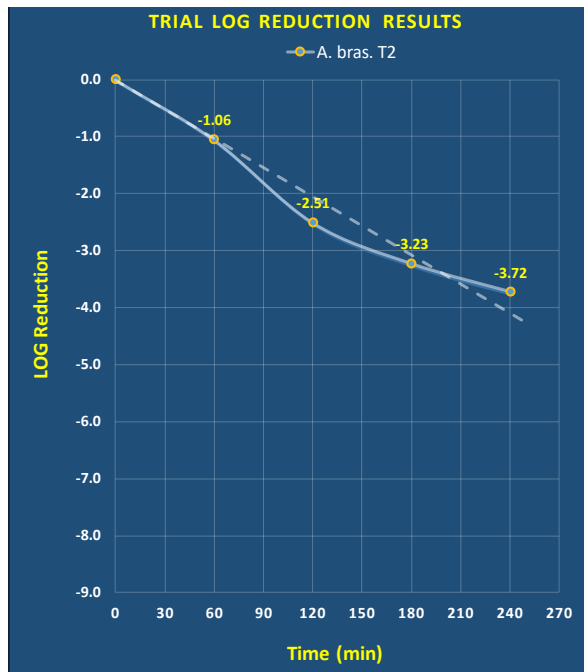**Figure 6B:** *A. brasiliensis* Trial 1

Trial Information

TEST DATE:	Thursday, June 3, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T2
TEST ORGANSIM:	A. Brasiliensis
TRIAL NAME ID (GRAPHS/TABLES):	A. bras. T2

Device Information

MANUFACTURER:	Puraclenz
UNIT MODEL:	P3000
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

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:	na
TRIAL COMMENTS/NOTES	na

TRIAL LOG REDUCTION RESULTS — A. bras. T2 (LOG Reduction vs Time (min)): -1.06, -2.51, -3.23, -3.72

BIOAEROSOL Sample ID and Summary Data

	S1	S2	S3	S4	S5
SAMPLING TIME (min)	0	60	120	180	240
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.244E+04	1.088E+03	3.813E+01	7.307E+00	2.347E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	25.00%		62.50%	42.53%	16.67%
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.244E+04	1.088E+03	3.813E+01	7.307E+00	2.347E+00
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	8.7429%	0.3064%	0.0587%	0.0189%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	91.2571%	99.6936%	99.9413%	99.9811%
LOG REDUCTION FROM T=0 (log_{10})	0.00	-1.06	-2.51	-3.23	-3.72

Impinger Sampling Conditions

		S1	S2	S3	S4	S5
	SAMPLING TIME (min)	0	60	120	180	240
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	10.0	10.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	DILUTION RATIO (10^x)	-2	-2	0	0	0
	DROPLET SIZE (µl)	100	100	100	100	100
	ENUMERATED PLATE COUNTS (# / drop)	37		15	2	2
		23		18	3	0
		20		19	5	2
	PLATE AVERAGE COUNT (# / drop)	26.67		17.33	3.33	1.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	26,667		173	33	13
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.42E+04		5.55E+01	5.33E+00	2.13E+00
Dilution Range #1	DILUTION RATIO (10^x)	-3	-1	0	0	0
	DROPLET SIZE (µl)	100	100	500	500	500
	ENUMERATED PLATE COUNTS (# / drop)	2	38	35	28	
		2	34	30	30	8
		2	30			
	PLATE AVERAGE COUNT (# / drop)	2.00	34.00	32.50	29.00	8.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	20,000	3,400	65	58	16
	CHAMBER BIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.07E+04	1.09E+03	2.08E+01	9.28E+00	2.56E+00

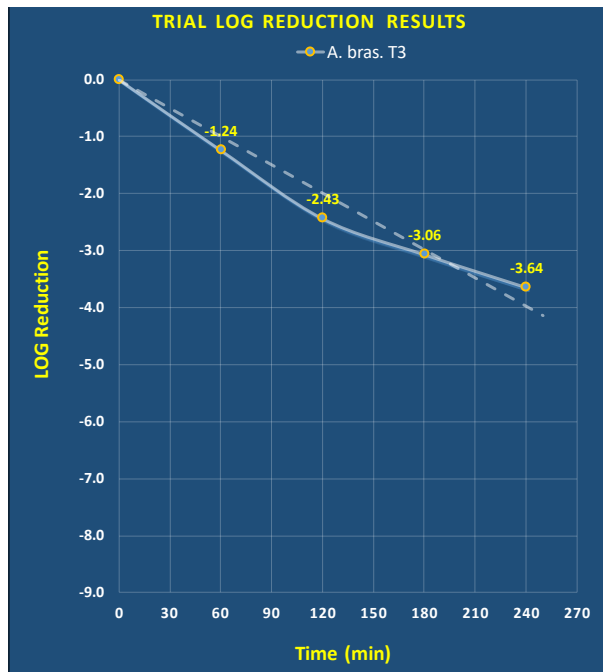**Figure 7B:** *A. brasiliensis* Trial 2

Trial Information

TEST DATE:	Thursday, June 3, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T3
TEST ORGANSIM:	A. Brasiliensis
TRIAL NAME ID (GRAPHS/TABLES):	A. bras. T3

Device Information

MANUFACTURER:	Puraclenz
UNIT MODEL:	P3000
FAN SPEED (CFM):	NA
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

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:	na
TRIAL COMMENTS/NOTES	na

TRIAL LOG REDUCTION RESULTS

BIOAEROSOL Sample ID and Summary Data

	S1	S2	S3	S4	S5
SAMPLING TIME (min)	0	60	120	180	240
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.093E+04	6.347E+02	4.037E+01	9.440E+00	2.507E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	🟢 24.29%	🟡 51.25%	🟡 35.43%		🟢 25.93%
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.093E+04	6.347E+02	4.037E+01	9.440E+00	2.507E+00
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	5.8049%	0.3693%	0.0863%	0.0229%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	94.1951%	99.6307%	99.9137%	99.9771%
LOG REDUCTION FROM T=0 (log_{10})	0.00	-1.24	-2.43	-3.06	-3.64

Impinger Sampling Conditions

		SAMPLING TIME (min)	0	60	120	180	240
		IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
		IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	10.0	10.0
		IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5
Dilution Range #1		**DILUTION RATIO (10^x)**	-2	-2	0	0	0
		DROPLET SIZE (µl)	100	100	100	100	100
		ENUMERATED PLATE COUNTS (# / drop)	15	1	14		1
			19	3	16		3
			19	4	16		0
		PLATE AVERAGE COUNT (# / drop)	17.67	2.67	15.33		1.33
		IMPINGER CONCENTRATION (cfu or pfu/ml)	17,667	2,667	153		13
		CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	9.42E+03	8.53E+02	4.91E+01		2.13E+00
Dilution Range #1		**DILUTION RATIO (10^x)**	-3	-1	0	0	0
		DROPLET SIZE (µl)	100	100	500	500	500
		ENUMERATED PLATE COUNTS (# / drop)	3	9	48	28	10
			3	11	51	31	8
			1	19			
		PLATE AVERAGE COUNT (# / drop)	2.33	13.00	49.50	29.50	9.00
		IMPINGER CONCENTRATION (cfu or pfu/ml)	23,333	1,300	99	59	18
		CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.24E+04	4.16E+02	3.17E+01	9.44E+00	2.88E+00

Figure 8B: *A. brasiliensis* Trial 3

Appendix C: 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.

$$y = 2.18464E\text{-}11x^6 - 2.59511E\text{-}08x^5 + 1.22612E\text{-}05x^4 - 2.91165E\text{-}03x^3 + 3.65959E\text{-}01x^2 - 2.16227E\text{+}01x + 5.39621E\text{+}02$$

$$R^2 = 9.99410E\text{-}01$$
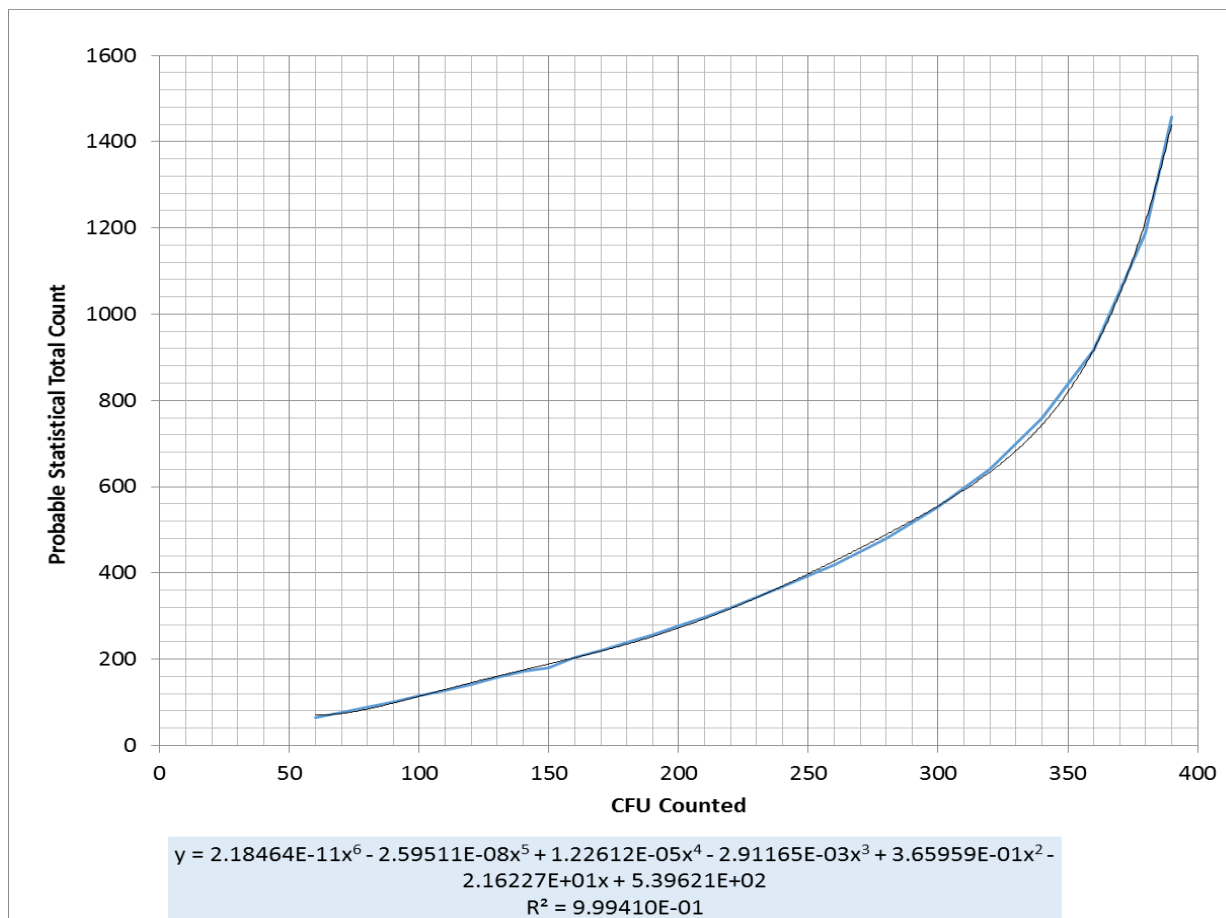
17.2 Positive hole conversion table for all MAS-100 air monitoring systems

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		