SCUVA™

Results of Independent Testing of SCUVA Efficacy

Conducted by

Aerosol Research and Engineering Laboratories

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SCUVA™

Efficacy of SCUVA Prototype Against Aerosolized Phix-174 In a Large Chamber

TESTING LAB
Aerosol Research and Engineering Laboratories

PURPOSE
To determine the efficacy of SCUVA C100 (prototype name during testing: Ecosense LUVA 100) at removing aerosolized Phix-174 bacteriophage, a commonly used surrogate for DNA-based virus species.

METHODOLOGY
Phix-174 was aerosolized within a sealed test chamber also containing the LUVA 100 unit. One control trial was conducted with the unit turned off to provide baseline comparative data. Three challenge trials were conducted in which the unit was turned on and in continuous operation for the entire 3-hour duration.

RESULTS
Efficiency results indicate it would take the unit just under 3 minutes to reach 99% reduction of the Phix-174 bacteriophage in a 100 cubic foot space.

- CONTINUE READING FOR COMPLETE RESULTS -

Efficacy of the EcoSense LUVA 100 against Aerosolized PhiX-174 in a Large Chamber

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Background: This in vitro study was to determine the efficacy of the EcoSense LUVA 100 at removing aerosolized PhiX-174 bacteriophage. This EcoSense device is designed to reduce airborne bacteria, viruses, and fungal spores in order to decrease infection rates from airborne pathogens. For this study, the EcoSense LUVA 100 was challenged using aerosolized PhiX-174 bacteriophage, which has been historically used as a surrogate for DNA based virus species such as herpes simplex and smallpox. These are both pathogenic viruses to humans and cause a variety of symptoms for whoever is infected. The study consisted of a total of three (3) live bioaerosol challenge trials, and a single (1) bioaerosol control run.

Methods: PhiX-174 bacteriophage was aerosolized into a hermetically sealed environmental bioaerosol chamber containing the EcoSense LUVA 100. AGI impinger samples were taken from the chamber in order to quantify the reduction speed and capabilities of the EcoSense device. AGI impingers were used to sample chamber bioaerosol concentrations. All impinger samples were serially diluted, plated and enumerated in triplicate to yield viable bioaerosol concentration at each sample point and time. The chamber control trial data was subtracted from the EcoSense trial data to yield net LOG reduction in the chamber for the bioaerosol challenges.

Results: When tested against the PhiX-174 bacteriophage, the EcoSense device showed a consistent net LOG reduction throughout the testing. The average net LOG reduction went from 1.93 at the 15-minute time point down to 4.88 at the 60-minute time point. A net LOG reduction of this magnitude over 60 minutes indicates the efficacy and speed of this device against the PhiX-174 bacteriophage. With this efficiency, the time it takes to reach 99% reduction in a 100 cubic foot space would be just under 3 minutes.

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 EcoSense LUVA 100 at reducing aerosolized PhiX-174 bacteriophage. The EcoSense LUVA 100 is an air purification system intended for use in medium to large sized rooms. The unit was used at full capacity for the duration of the trials.

The EcoSense LUVA 100 uses a chamber of UV lights as a mechanism of reduction against bioaerosols, such as PhiX-174. A fan draws air through the device, where the biological agent is exposed to UV lights which damage the DNA or RNA (species-dependent) beyond repair. The test plan incorporated challenging the EcoSense device in a closed environmental chamber to determine the destruction rate of PhiX-174 bacteriophage by the EcoSense LUVA 100. A picture of the EcoSense LUVA 100 is shown in **Figure 1,** on the following page.

Study Overview

The effectiveness of the EcoSense device was evaluated against a single stranded, non-enveloped DNA virus which was PhiX-174 bacteriophage. For more information on the PhiX-174 bacteriophage, please see species selection section below.

Testing was conducted to characterize a single EcoSense unit against PhiX-174 with triplicate (3) independent trials as well as a single (1) control trial to demonstrate the capability of the EcoSense device to reduce viable bioaerosol concentrations; therefore theoretically reducing chances of airborne infection. This study does not make any claims regarding the efficacy of this device at reducing airborne infections.

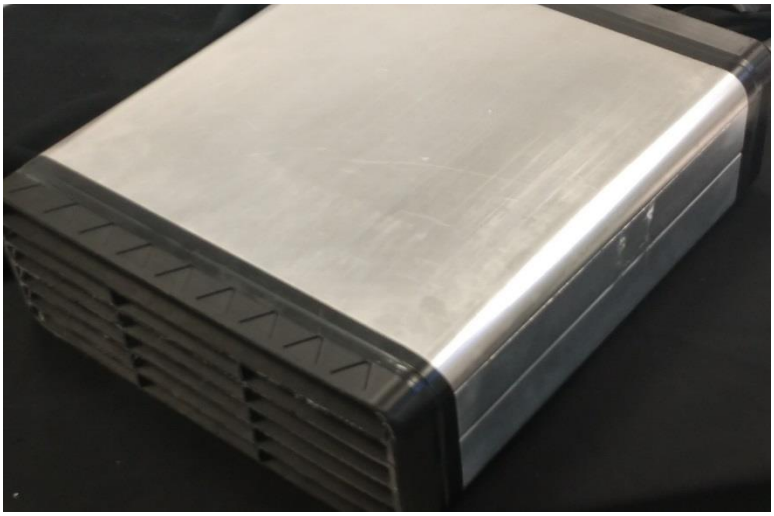

Figure 1: *EcoSense LUVA 100*

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 7 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.

The chamber is equipped with filtered HEPA inlets, digital internal temperature and humidity monitor, external humidifiers (for humidity control), 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 was 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 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 (TSI Inc., St Paul MN). All sample and dissemination ports were inserted approximately 18 inches from the interior walls of the chamber to avoid wall effects 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.

Figure 2: *Bioaerosol Test Chamber Exterior*

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) was used to monitor and balance the system pressure during aerosol generation, aerosol purge and testing cycles.

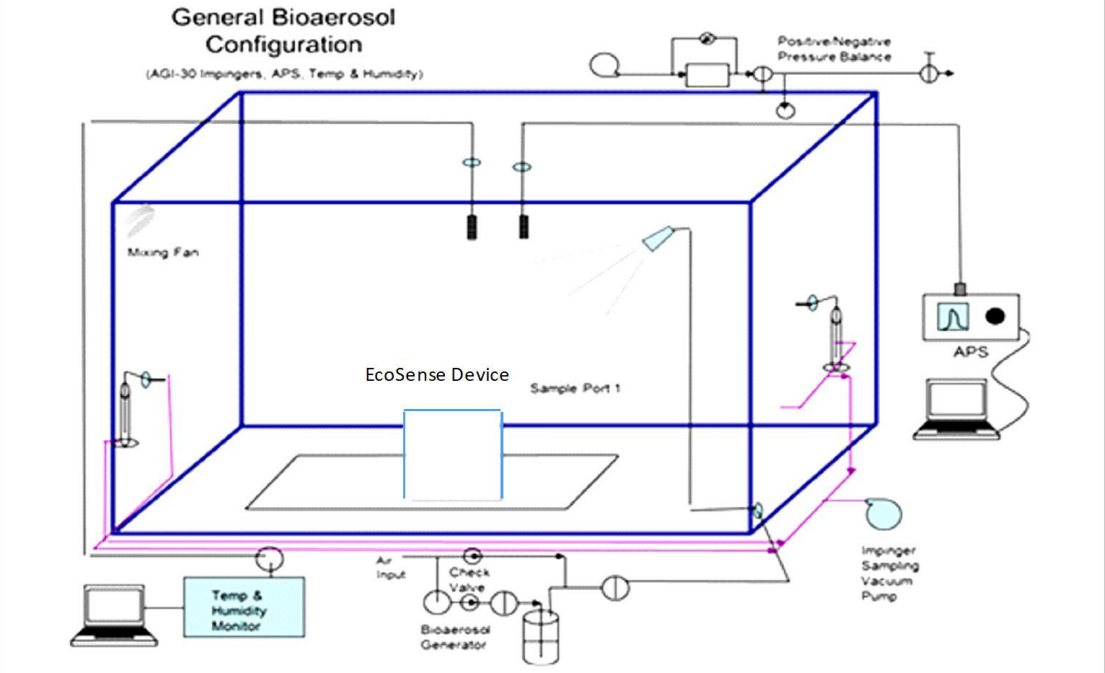

Figure 3: *Bio-Aerosol Test Chamber Flow Diagram*

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 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 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.

Bioaerosol Sampling and Monitoring System

Two AGI 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 EcoSense trial runs using a Model 3321 Aerodynamic Particle Sizer (APS). The APS sampled for the entire duration of all trials with 1 minute sampling intervals. A general flow diagram of the aerosol test system is shown above in **Figure 3** above.

Species Selection

Species selection is based on Biological Safety Level 1 (BSL1) surrogates for BSL3 pathogenic organisms. PhiX-174 is a viral, single stranded, non-enveloped, DNA bacteriophage traditionally used as a surrogate for virus species such as herpes simplex and smallpox. Moreover, according to Misstear and Gill (2010), the UV dose to inactivation PhiX-174 by 90% was determined to be 2.5 mJ/cm^2, which is similar to the UV dosage to inactivate SARS CoV-2 (Patterson *et al*, 2020).

Viral Culture & Preparation

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

Biological Test Matrix

Trial	Run	Pathogenic Organism	Surrogate Species (gram, description)	ATCC Ref	Target Monodispersed Particle Size	Challenge Conc. (#/L)	Trial Time (min)	Sample Time (min)	Sampling	Plating and Enumeration
1	Control	*Herpes simplex and smallpox*	*PhiX-174 (E. coli phage)*	13706-B1	<1.0 µm	10^4-10^6	180	0, 15, 30, 60	APS, Impingers	all samples in triplicate
2	Challenge									
3	Challenge									
4	Challenge									

Figure 4: *Bioaerosol Test Matrices for all Trials*

PhiX-174 bacteriophage. After an appropriate incubation time the cells were lysed and the cellular debris separated by centrifugation. PhiX-174 stock yields were greater than 1 x 10^9 plaque forming units per milliliter (pfu/mL) with a double amplification procedure. This stock PhiX-174 viral solution was used undiluted, at approximately 1 x 10^9 plaque forming units per milliliter (pfu/mL), for use in the Collision nebulizer

Plating and Enumeration

Impinger and stock PhiX-174 bacteriophage 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 and enumerated and recorded.

Bioaerosol Control Testing

To accurately assess the EcoSense unit, test chamber pilot control trials were performed with PhiX-174 bacteriophage over a 180-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 EcoSense 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 and trial runs during EcoSense decontamination trials. The two impingers used for bacteriophage were pooled and mixed prior to plating and enumeration. A complete test matrix for the bioaerosol trials can be found above in **Figure 4**.

EcoSense Testing

For each control and challenge test, the Collison nebulizer was filled with approximately 40 mL of biological stock and operated at 60 psi for a period of 20 minutes. For control and EcoSense 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 PhiX-174 bacteriophage dissemination to assure a homogeneous bioaerosol concentration in the test chamber prior to taking the first impinger sample

Following bioaerosol generation, baseline PhiX-174 concentrations were established for each pilot control and LUVA 100 test by sampling simultaneously with two AGI-30 impingers, located at opposite corners of the chamber. AGI samples were collected for 2 to 20 minutes at intervals of 30 or 60 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.

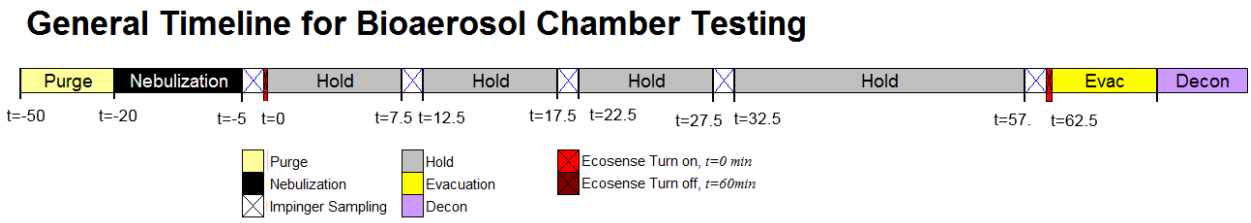

***Figure 5**: General Timeline for Bioaerosol Chamber Testing*

For EcoSense biological testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the test (3 hours). Subsequent impinger samples were taken at 0, 15, 30, 60 and samples enumerated for viable concentration to measure the effective viable PhiX-174 bacteriophage reduction during operation of the EcoSense LUVA 100 over time. A diagram of the general bioaerosol chamber testing can be found in **Figure 5**.

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

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 after the device was removed 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.

Bioaerosol Particle Size Data

Aerosol particle size distributions were measured with the APS throughout the trials. The APS has a dynamic measurement range of 0.5 to 20 µm and was programmed to take consecutive real time one-minute aerosol samples throughout the duration of each aerosol trial.

The particle size distribution for PhiX-174 bioaerosols are shown to be within the respirable range for alveolar region tract lung deposition and show a low geometric standard deviation (GSD) indicating a monodispersed aerosol was generated into the test chamber.

Data Analysis

Results from the control trial were graphed and plotted to show natural viability loss over time in the chamber. The control run served as the basis to determine the time required for the EcoSense LUVA 100 to reduce viable bioaerosol above the natural losses from the control runs.

The control and trial runs were serially diluted, plated in triplicate, enumerated and then plotted showing log reduction in viable bioaerosol for each organism. 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.

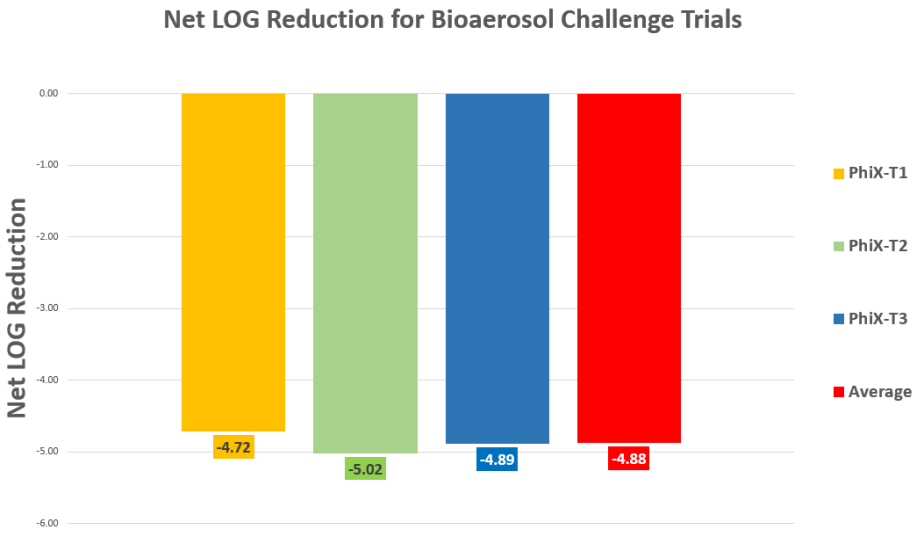

Figure 6: *PhiX-174 EcoSense LUVA 100 Total Tet LOG Reduction after 60 Minutes*

Results

When tested against the PhiX-174 bacteriophage, the device showed a consistent net log reduction throughout the duration of the trials. The total net LOG reduction average at the last time point for the three trials was 4.88 LOG. A net LOG reduction of 4.0 is equivalent to a 99.99% reduction. A graphic displaying the net log reduction for each trial as well as an average for all of the trials can be found in **Figure 6.**

Results indicate that at 15 minutes, the average net LOG reduction was -1.93 (+/- 0.38), and when extrapolated, would demonstrate -2.0 net LOG (99%) reduction at 15 minutes and 32 seconds. Testing was performed in a 16 m^3 chamber and it can be calculated that a net 2 LOG reduction (99%) of PhiX-184 could be achieved in a 100 ft^3 enclosed environment in 2.75 minutes by the LUVA 100 device.

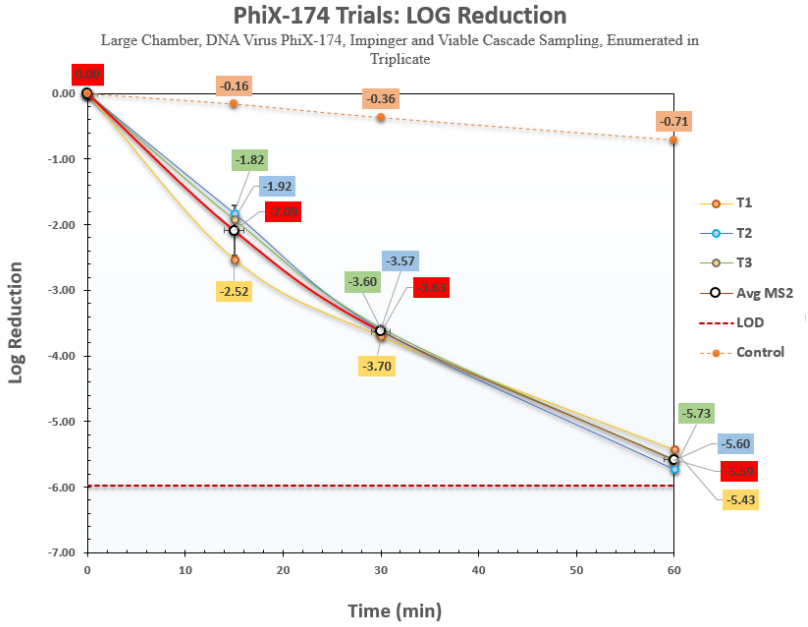

Figure 7: *PhiX-174 EcoSense LOG Reduction All Trials*

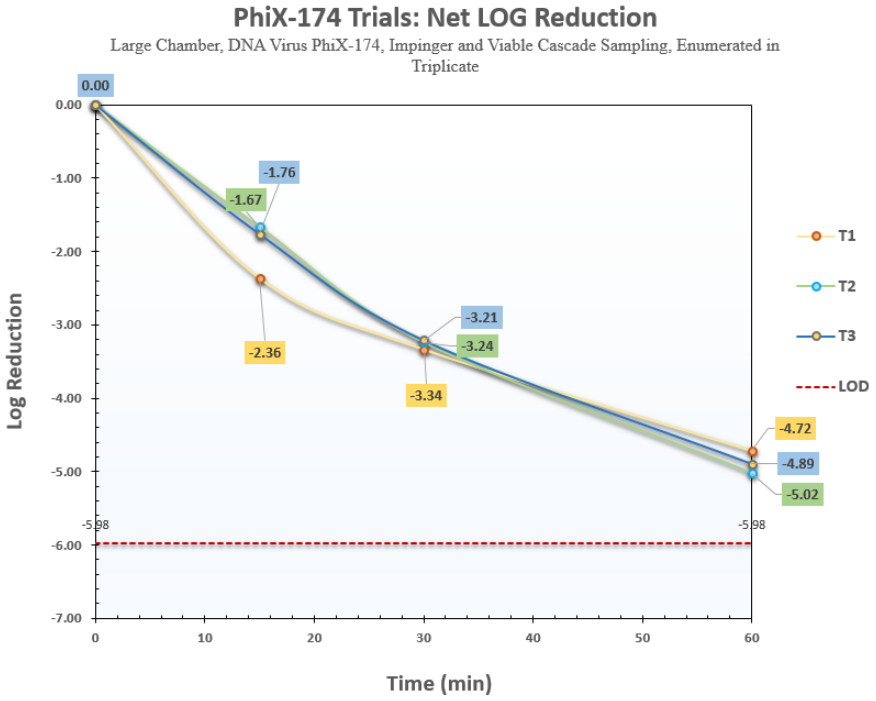

Figure 8: *PhiX-174 EcoSense Net LOG Reduction All Trials*

Summary of Results

When tested against the PhiX-174 bacteriophage, the device showed consistent reduction throughout each trial. By the 60-minute time point, results demonstrated an average 5.59 LOG reduction which equates to an average 4.88 net LOG reduction. LOG reduction results can be found in **Figure 7**, Net LOG reduction results can be found in **Figure 8**.

At 15 minutes the device had an average net LOG reduction of 1.93 LOG or 99% reduction of viable bioaerosol. At the 30-minute time point there is over a LOG and a half reduction down to an average of 3.63 LOG. All trials were run out to 60 minutes. These results indicate that, in theory, the EcoSense LUVA 100 would help prevent the spread of airborne infection. Due to the similarity in UV sensitivity, there could be similar reductions in SARS CoV-2 as well with the same device. A table showing the results in net LOG reduction can be found in **Figure 9**.

Average Net LOG and Percent Reduction of PhiX-174 By Ecosens

Bioaerosol Type	Species	Trial ID		15min	30min	60min
Virus	*PhiX-174 (DNA E.coli Phage)*	*1*	*Net Log Reduction*	-2.36	-3.34	-4.72
			% Reduction	99.70%	99.98%	99.9996%
Virus	*PhiX-174 (DNA E.coli Phage)*	*2*	*Net Log Reduction*	-1.67	-3.24	-5.02
			% Reduction	98.50%	99.98%	99.9998%
Virus	*PhiX-174 (DNA E.coli Phage)*	*3*	*Net Log Reduction*	-1.76	-3.21	-4.89
			% Reduction	98.80%	99.97%	99.9998%
Averages			**Average and St. dev**	**-1.93 +/- 0.38**	**-3.26 +/- 0.07**	**-4.88 +/- 0.15**
			Average and St. dev	**99% +/- 0.62%**	**99.98% +/- 0.003%**	**99.9997% +/- 0.0001%**

Figure 9*: Net Log and Percent Reduction Summary Table*

References

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.

Flint et al. *Principles of Virology.* Principles of Virology (ASM). Chapter 2 Virological Methods. Vol. 2. 2008.

P Hyman et al. *Practical Methods for Determining Phage Growth Parameters*. Bacteriophages: Methods and Protocols, Vol. 1*: Isolation, Characterization and Interactions.* vol. 501. 2009. pp. 175-201.

Mazzocco et al. *Enumeration of Bacteriophages Using the Small Drop Plaque Assay System.* Bacteriophages: Methods and Protocols, Vol. 1: Isolation, Characterization and Interactions. vol. 501. 2009. pp. 81-95.

D. Misstear, L. Gill. (2012). The inactivation of phages MS2, PhiX174, and PR772 using UV and solar photocatalysis. *Journal of Photochemistry and Photobiology B: Biology 107,* 1-8.

E. Patterson, T. Prince, E. R Anderson, *et al*. *The Journal of Infectious Diseases*, Volume 222, Issue 9, 1 November 2020, Pages 1462–1467.

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

Analytical Testing Facility

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Project

10902.10

Study Director

Dr. Michael Hornback
Aerosol Research and Engineering Laboratories

GLP Statement

We, the undersigned, hereby certify that the work described herein was conducted by Aerosol Research and Engineering Laboratories in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

Study Director:

11/30/2020
Date

Dr. Michael Hornback
Study Director
ARE Labs, Inc.

Principal Investigator:

11/30/2020
Date

Sean McLeod
Principal Investigator
ARE Labs, Inc.

Appendix A: Calculations

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:

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

Appendix B: Raw Data

Trial Information

TEST DATE:	Wednesday, November 18, 2020
TRIAL PERFORMED BY:	Sean
TRIAL NUMBER:	Con-T1
TEST ORGANSIM:	PhiX-174
TRIAL NAME ID (GRAPHS/TABLES):	Control T1

Device Information

MANUFACTURER:	EcoSense
UNIT MODEL:	Device 2
UNIT SERIAL #:	2
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger & Cascader
CHAMBER MIXING FAN:	yes
TEMP (F):	73
RH (%):	40
OTHER INSTRUMENTS:	
TRIAL COMMENTS/NOTES	Older Control

BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4
SAMPLING TIME (min)	**0**	**15**	**30**	**60**
IMPINGER USED (y / n)	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.307E+05	9.067E+04	5.653E+04	2.560E+04
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)				
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	**4.00%**			
VIABLE CONSISTENCY CHECKS (% agreement)				
IMP & VIABLE CROSS CHECK (% agreement)				
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.307E+05**	**9.067E+04**	**5.653E+04**	**2.560E+04**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**69.3878%**	**43.2653%**	**19.5918%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**30.6122%**	**56.7347%**	**80.4082%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.16**	**-0.36**	**-0.71**

Impinger Sampling Conditions

	S1	S2	S3	S4
SAMPLING TIME (min)	**0**	**15**	**30**	**60**
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	5.0	5.0	5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5

Dilution Range #1					
	DILUTION RATIO (10^x)	**-4**	**-3**	**-3**	**-3**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	3 1 1	20 35 30	15 20 18	7 8 9
	PLATE AVERAGE COUNT (# / drop)	1.67	28.33	17.67	8.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	166,667	283,333	176,667	80,000
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.33E+05**	**9.07E+04**	**5.65E+04**	**2.56E+04**

Dilution Range #1					
	DILUTION RATIO (10^x)	**-3**	**-4**	**-2**	**-1**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	16 14 18			
	PLATE AVERAGE COUNT (# / drop)	16.00			
	IMPINGER CONCENTRATION (cfu or pfu/ml)	160,000			
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.28E+05**			

Trial Information

TEST DATE:	Wednesday, November 18, 2020
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T1
TEST ORGANSIM:	PhiX-174
TRIAL NAME ID (GRAPHS/TABLES):	PhiX T1

Device Information

MANUFACTURER:	Ecosense
UNIT MODEL:	Device 2
UNIT SERIAL #:	2
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	36
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	NA

BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4
SAMPLING TIME (min)	**0**	**15**	**30**	**60**
IMPINGER USED (y / n)	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.067E+04	3.200E+01	2.133E+00	4.000E-02
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)				
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)				
VIABLE CONSISTENCY CHECKS (% agreement)				
IMP & VIABLE CROSS CHECK (% agreement)				
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.067E+04**	**3.200E+01**	**2.133E+00**	**4.000E-02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**0.3000%**	**0.0200%**	**0.0004%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**99.7000%**	**99.9800%**	**99.9996%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-2.52**	**-3.70**	**-5.43**

Impinger Sampling Conditions

	S1	S2	S3	S4
SAMPLING TIME (min)	**0**	**15**	**30**	**60**
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	5.0	5.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5

Dilution Range #1					
	DILUTION RATIO (10^x)	**-3**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**4000**
	ENUMERATED PLATE COUNTS (# / drop)	2 1 1	1 2 0	1 1 0	1
	PLATE AVERAGE COUNT (# / drop)	1.33	1.00	0.67	1.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	13,333	100	7	0
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.07E+04	3.20E+01	2.13E+00	4.00E-02

Trial Information

TEST DATE:	Wednesday, November 18, 2020
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T2
TEST ORGANSIM:	PhiX-174
TRIAL NAME ID (GRAPHS/TABLES):	PhiX T2

Device Information

MANUFACTURER:	Ecosense
UNIT MODEL:	Device 2
UNIT SERIAL #:	2
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	36
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	NA

BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4
SAMPLING TIME (min)	**0**	**15**	**30**	**60**
IMPINGER USED (y / n)	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	2.133E+04	3.200E+02	5.333E+00	4.000E-02
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)				
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)				
VIABLE CONSISTENCY CHECKS (% agreement)				
IMP & VIABLE CROSS CHECK (% agreement)				
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**2.133E+04**	**3.200E+02**	**5.333E+00**	**4.000E-02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**1.5000%**	**0.0250%**	**0.0002%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**98.5000%**	**99.9750%**	**99.9998%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.82**	**-3.60**	**-5.73**

Impinger Sampling Conditions

SAMPLING TIME (min)	**0**	**15**	**30**	**60**
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	5.0	5.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5

Dilution Range #1					
	DILUTION RATIO (10^x)	**-3**	**-2**	**-1**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**1000**	**1000**
	ENUMERATED PLATE COUNTS (# / drop)	2 3 3	1 2 0	1 2 2	1 0 0 0
	PLATE AVERAGE COUNT (# / drop)	2.67	1.00	1.67	0.25
	IMPINGER CONCENTRATION (cfu or pfu/ml)	26,667	1,000	17	0
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	2.13E+04	3.20E+02	5.33E+00	4.00E-02

Trial Information

TEST DATE:	Monday, November 23, 2020
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T3
TEST ORGANSIM:	PhiX
TRIAL NAME ID (GRAPHS/TABLES):	PhiX T3

Device Information

MANUFACTURER:	Ecosense
UNIT MODEL:	Device 2
UNIT SERIAL #:	2
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	yes
TEMP (F):	70
RH (%):	36
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	NA

BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4
SAMPLING TIME (min)	**0**	**15**	**30**	**60**
IMPINGER USED (y / n)	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	8.000E+03	9.600E+01	2.133E+00	2.000E-02
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)				
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)				
VIABLE CONSISTENCY CHECKS (% agreement)				
IMP & VIABLE CROSS CHECK (% agreement)				
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**8.000E+03**	**9.600E+01**	**2.133E+00**	**2.000E-02**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**1.2000%**	**0.0267%**	**0.0003%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**98.8000%**	**99.9733%**	**99.9998%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.92**	**-3.57**	**-5.60**

Impinger Sampling Conditions

	S1	S2	S3	S4
SAMPLING TIME (min)	**0**	**15**	**30**	**60**
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	5.0	5.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5

Dilution Range #1					
	DILUTION RATIO (10^x)	**-3**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**2000**
	ENUMERATED PLATE COUNTS (# / drop)	2 1 0	5 3 1	1 0 1	1 0 0 0
	PLATE AVERAGE COUNT (# / drop)	1.00	3.00	0.67	0.25
	IMPINGER CONCENTRATION (cfu or pfu/ml)	10,000	300	7	0
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	8.00E+03	9.60E+01	2.13E+00	2.00E-02

SCUVA™

Efficacy of the SCUVA 10,000 Against Aerosolized PhiX-174

TESTING LAB
Aerosol Research and Engineering Laboratories

PURPOSE
To determine the efficacy of SCUVA G10k (prototype name during testing: SCUVA 10,000) at reducing airborne bacteria, viruses, and fungal spores in order to decrease infection rates from airborne pathogens. For this study, SCUVA 10,000 was challenged, using aerosolized Phix-174 bacteriophage, a commonly used surrogate for DNA-based virus species.

METHODOLOGY
For this study, Phix-174 was aerosolized within a sealed test chamber also containing the SCUVA 10,000 unit. One control trial was conducted with the unit turned off to provide baseline comparative data. Three challenge trials were conducted in which the unit was turned on and in continuous operation for the entire 3-hour duration.

RESULTS
Within the 579 cu. ft. test chamber, the SCUVA 10,000 unit was able to achieve a net percent reduction of PhiX-174 by 99.99% in under 8 minutes.

- CONTINUE READING FOR COMPLETE RESULTS -

Efficacy of the SCUVA 10,000 against Aerosolized PhiX-174

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

Background: This in vitro study was to determine the efficacy of the SCUVA 10,000 at removing aerosolized PhiX-174 bacteriophage. This device is designed to reduce airborne bacteria, viruses, and fungal spores in order to decrease infection rates from airborne pathogens. For this study, the SCUVA 10,000 was challenged using aerosolized PhiX-174 bacteriophage, primarily due to the similarity in UV dose required to achieve one LOG inactivation of PhiX-174 and SARS-CoV-2. The study consisted of a total of three (3) live bioaerosol challenge trials, and a single (1) bioaerosol control run.

Methods: PhiX-174 bacteriophage was aerosolized into a hermetically sealed environmental bioaerosol chamber containing the SCUVA 10,000 device. AGI impinger samples were used to sample chamber bioaerosol concentrations from the chamber in order to quantify the reduction speed and capabilities of the device. All impinger samples were serially diluted, plated and enumerated in triplicate to yield viable bioaerosol concentration at each sample point and time. The chamber control trial data was subtracted from the trial data to yield net LOG reduction in the chamber for the bioaerosol challenges.

Results: When tested against the PhiX-174 bacteriophage, the device demonstrated a consistent net LOG reduction throughout the testing, and in a very short time. The average net LOG reduction went from 2.89 at the 4-minute time point, down to 4.70 at the 10-minute time point. A net LOG reduction of this magnitude at 10 minutes indicates excellent efficacy and speed of this device against the PhiX-174 bacteriophage. Given an average air flow rate of 600 ft^3/min, this device could be used for very large room and/or buildings.

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 SCUVA 10,000 (10K) at reducing aerosolized PhiX-174 bacteriophage. The SCUVA 10K is an air purification system intended for use in large sized rooms or buildings. The unit was used at full capacity for the duration of the trials.

The SCUVA 10K uses a chamber of UV lights as a mechanism of reduction against bioaerosols, such as PhiX-174. A fan draws air through the device, where the biological agent is exposed to UV lights, which damage the DNA or RNA (species-dependent) beyond repair. The test plan incorporated challenging the device in a closed environmental chamber to determine the destruction rate of PhiX-174 bacteriophage by the SCUVA 10K. A picture of the SCUVA 10K is shown in **Figure 1,** on the following page.

Study Overview

The effectiveness of the device was evaluated against a single stranded, non-enveloped DNA virus which was PhiX-174 bacteriophage. For more information on the PhiX-174 bacteriophage, please see species selection section below.

Testing was conducted to characterize a single unit against PhiX-174 with triplicate (3) independent trials as well as a single (1) control trial to demonstrate the capability of the device to reduce viable bioaerosol concentrations; therefore theoretically reducing chances of airborne infection. This study does not make any claims regarding the efficacy of this device at reducing airborne infections.

Figure 1: *SCUVA 10,000 Device*

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 7 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.

The chamber is equipped with filtered HEPA inlets, digital internal temperature and humidity monitor, external humidifiers (for humidity control), 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 was equipped with four 3/8-inch diameter stainless steel probes for aerosol sampling, a 1-inch diameter port for bioaerosol 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 (TSI Inc., St Paul MN). All sample and dissemination ports were inserted approximately 18 inches from the interior walls of the chamber to avoid wall effects 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.

Figure 2: *Bioaerosol Test Chamber Exterior*

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) was used to monitor and balance the system pressure during aerosol generation, aerosol purge and testing cycles.

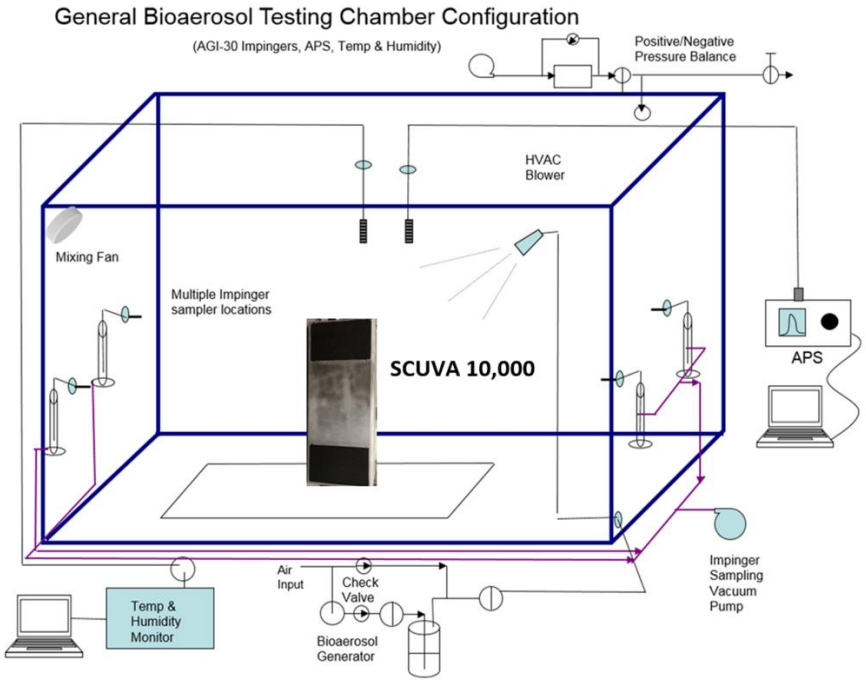


Figure 3: *Bioaerosol Test Chamber Flow Diagram*

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 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 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.

Bioaerosol Sampling and Monitoring System

Two AGI 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 trial runs using a Model 3321 Aerodynamic Particle Sizer (APS). The APS sampled for the entire duration of all trials with 1 minute sampling intervals. A general flow diagram of the aerosol test system is shown above in **Figure 3** above.

Species Selection

Species selection is based on Biological Safety Level 1 (BSL1) surrogates for BSL3 pathogenic organisms. PhiX-174 is a viral, single stranded, non-enveloped, DNA bacteriophage traditionally used as a surrogate for virus species such as herpes simplex and smallpox. However, for this study, PhiX-174 was chosen as a surrogate for SARS-CoV-2 based on their similar UV inactivation rates. According to Malayeri et al, who compiled a list of UV irradiation dosages for microorganisms found in peer-reviewed literature, the UV dosage to inactivate PhiX-174 by 90% ranged between 1.6 to 7.1 mJ/cm^2 (average 3.0 mJ/cm^2), which is similar to the results of a recent study that demonstrated 5.0 mJ/cm^2 of UV-C was required inactivate 90% of SARS-CoV-2 (Patterson 2020).

Biological Test Matrix

Trial	Run	Pathogenic Organism	Surrogate Species (gram, description)	ATCC Ref	Target Monodispersed Particle Size	Challenge Conc. (#/L)	Trial Time (min)	Sample Time (min)	Sampling	Plating and Enumeration
1	Control	*Herpes simplex* and *Smallpox*	*Phi X 174 (E. coli phage)*	13706-B1	<1.0um	10^4-10^6	10	0, 2, 4, 6, 8, 10	APS, Impingers	all samples in triplicate
2	Challenge									
3	Challenge									
4	Challenge									

Figure 4: *Bioaerosol Test Matrices for all Trials*

Viral Culture & Preparation

Pure strain viral seed stock and host bacterium were obtained from ATCC. Host bacterium was grown in a similar fashion to the vegetative cells in an appropriate liquid media. The liquid media was infected during the logarithmic growth cycle with the PhiX-174 bacteriophage. After an appropriate incubation time, the cells were lysed and the cellular debris separated by centrifugation. PhiX-174 stock yields were greater than 1 x 10^9 plaque forming units per milliliter (pfu/mL) with a double amplification procedure. This stock PhiX-174 viral solution was used undiluted, at approximately 1 x 10^9 plaque forming units per milliliter (pfu/mL), for use in the Collision nebulizer

Plating and Enumeration

Impinger and stock PhiX-174 bacteriophage 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 and enumerated and recorded.

Bioaerosol Control Testing

To accurately assess the unit, test chamber pilot control trials were performed with PhiX-174 bacteriophage over a 10-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 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 and trial runs during decontamination trials. The two impingers used for bacteriophage were pooled and mixed prior to plating and enumeration. A complete test matrix for the bioaerosol trials can be found above in **Figure 4**.

SCUVA 10,000 Testing

For each control and challenge test, the Collison nebulizer was filled with approximately 40 mL of biological stock and operated at 60 psi for a period of 20 minutes. For control and challenge 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 PhiX-174 bacteriophage dissemination to assure a homogeneous bioaerosol concentration in the test chamber prior to taking the first impinger sample.

Following bioaerosol generation, baseline PhiX-174 concentrations were established for each pilot control and SCUVA 10K test by sampling simultaneously with two AGI-30 impingers, located at opposite corners of the chamber. AGI samples were collected for 2 minute intervals in order to capture the efficacy and speed of the device.

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 biological testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the test (3 hours). Subsequent impinger samples were taken at 0, 15, 30, 60 and samples enumerated for viable concentration to measure the effective viable PhiX-174 bacteriophage reduction during operation of the SCUVA 10K over time. A diagram of the general bioaerosol chamber testing can be found in **Figure 5**.

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

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 after the device was removed 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.

Bioaerosol Particle Size Data

Aerosol particle size distributions were measured with the APS throughout the trials. The APS has a dynamic measurement range of 0.5 to 20 µm and was programmed to take consecutive real time one-minute aerosol samples throughout the duration of each aerosol trial.

The particle size distribution for PhiX-174 bioaerosols are shown to be within the respirable range for alveolar region tract lung deposition and show a low geometric standard deviation (GSD) indicating a monodispersed aerosol was generated into the test chamber.

Data Analysis

Results from the control trial were graphed and plotted to show natural viability loss over time in the chamber. The control run served as the basis to determine the time required for the SCUVA 10K to reduce viable bioaerosol above the natural losses from the control runs.

The control and trial runs were serially diluted, plated in triplicate, enumerated and then plotted showing LOG reduction in viable bioaerosol for each organism. 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.

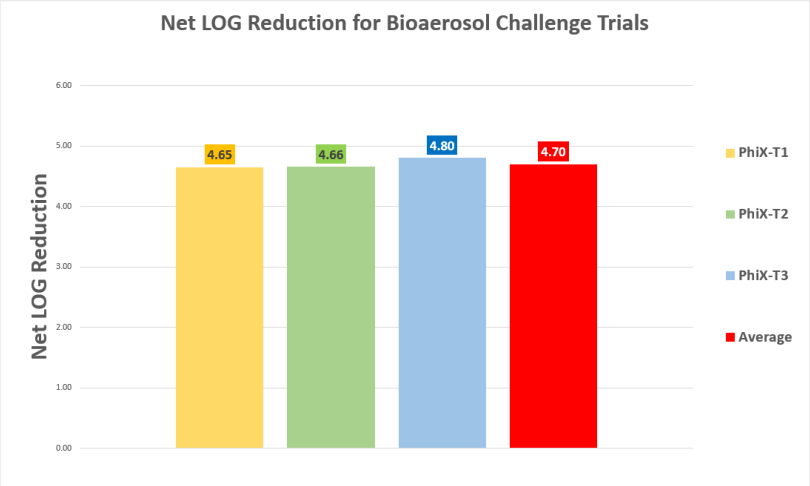


Figure 5: *PhiX-174 SCUVA 10K Total Net LOG Reduction after 10 Minutes*

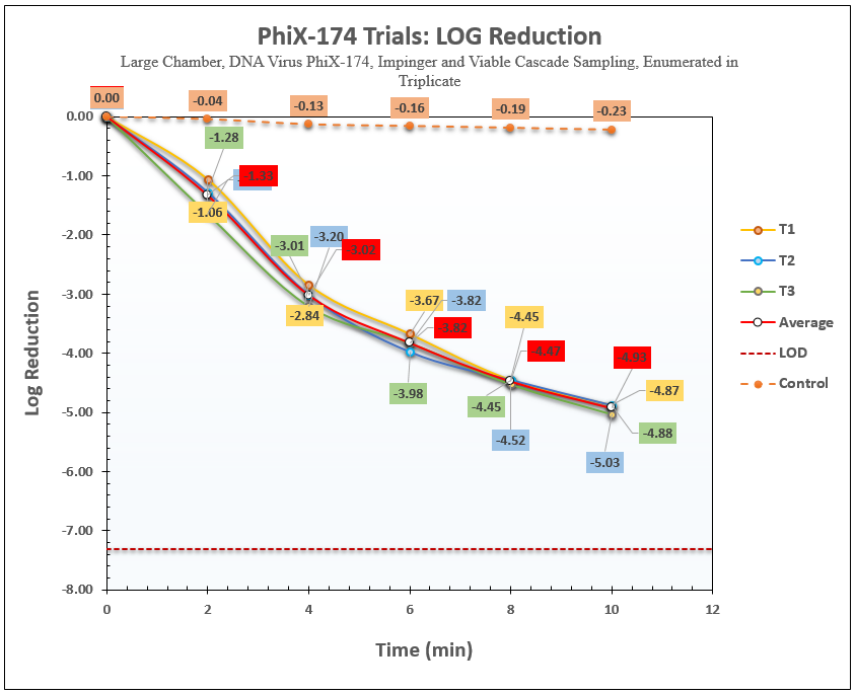


Figure 6: *PhiX-174 LOG Reduction All Trials*

Results

When tested against the PhiX-174 bacteriophage, the device showed a consistent net log reduction throughout the duration of the trials. The total net LOG reduction average at the last time point for the three trials was 4.70 LOG. A net LOG reduction of 4.0 is equivalent to a 99.99% reduction. A graphic displaying the net LOG reduction for each trial as well as an average for all of the trials can be found in **Figure 5.**

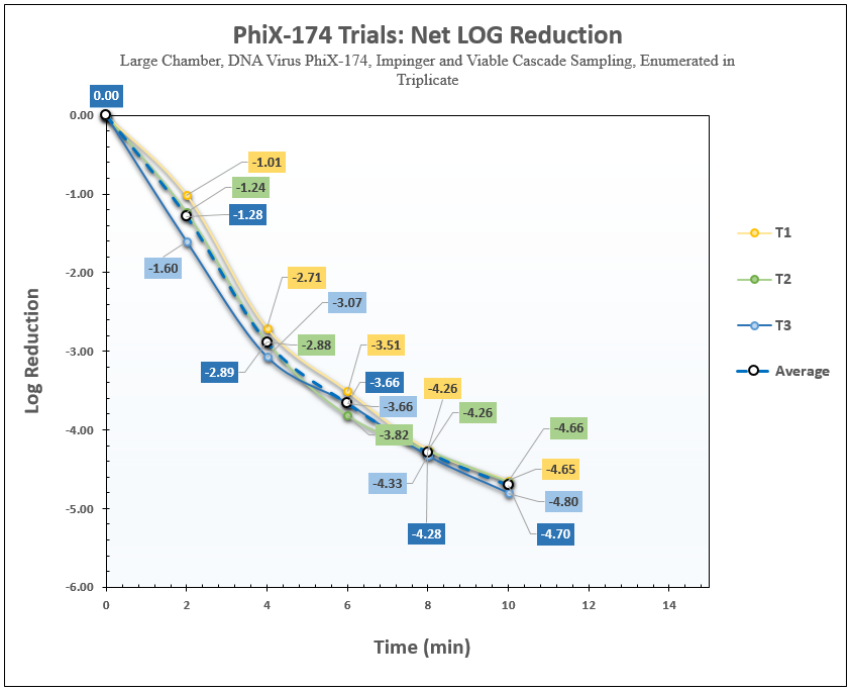


Figure 7: *PhiX-174 Net LOG Reduction All Trials*

Results indicate that at 4 minutes, the average net LOG reduction was 2.89 (+/- 0.18). Testing was performed in a 579 ft^3 chamber and the SCUVA 10K device achieved a net percent reduction of PhiX-174 by 99.99% in under 8 minutes. With the high airflow of the device, use could be ideally focused on HVAC systems in office buildings.

Summary of Results

When tested against the PhiX-174 bacteriophage, the device showed consistent reduction throughout each trial. By the 10-minute time point, results demonstrated an average 4.93 LOG reduction, which equates to an average 4.70 net LOG reduction. LOG reduction results can be found in **Figure 6**, Net LOG reduction results can be found in **Figure 7**.

At two minutes, the device had an average net LOG reduction of 1.28 LOG, or 94% reduction of viable bioaerosol. At the 4-minute time point, there was over an additional LOG and a half reduction, down to an average of 2.89 LOG. All trials were run out to 10 minutes. A table showing the results in net LOG reduction can be found in **Figure 8**.

These results indicate that, in theory, the SCUVA 10K would help prevent the spread of airborne infection. Due to the similarity in UV sensitivity, there could be similar reductions in SARS-CoV-2 as well with the same device, though any claims specific to SARS-CoV-2 would need to be tested with SARS-CoV-2 in a biosafety level 3 facility. Regardless, the SCUVA 10,000 device was highly efficient at reducing PhiX-174 in a very short amount of time

Average Net LOG and Percent Reduction of PhiX-174 by the SCUVA 10,000 Device

Bioaerosol Type	Species	Trial ID		2min	4min	6min	10min
Virus	*PhiX-174 (DNA E.coli Phage)*	*1*	*Net Log Reduction*	-1.01	-2.71	-3.51	-4.65
			% Reduction	91.25%	99.86%	99.9786%	99.9987%
Virus	*PhiX-174 (DNA E.coli Phage)*	*2*	*Net Log Reduction*	-1.24	-2.88	-3.82	-4.66
			% Reduction	94.74%	99.90%	99.9895%	99.9987%
Virus	*PhiX-174 (DNA E.coli Phage)*	*3*	*Net Log Reduction*	-1.60	-3.07	-3.66	-4.80
			% Reduction	97.75%	99.94%	99.9850%	99.9991%
Averages			**Average and St. dev**	**-1.28 +/- 0.3**	**-2.89 +/- 0.18**	**-3.66 +/- 0.15**	**-4.7 +/- 0.09**
			Average and St. dev	**94.579% +/- 3.25%**	**99.9% +/- 0.041%**	**99.9843% +/- 0.0055%**	**99.9988% +/- 0.0002%**

***Figure 8**: Net LOG and Percent Reduction Summary Table*

References

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.

Flint et al. *Principles of Virology.* Principles of Virology (ASM). Chapter 2 Virological Methods. Vol. 2. 2008.

P Hyman et al. *Practical Methods for Determining Phage Growth Parameters*. Bacteriophages: Methods and Protocols, Vol. 1*: Isolation, Characterization and Interactions.* vol. 501. 2009. pp. 175-201.

Mazzocco et al. *Enumeration of Bacteriophages Using the Small Drop Plaque Assay System.* Bacteriophages: Methods and Protocols, Vol. 1: Isolation, Characterization and Interactions. vol. 501. 2009. pp. 81-95.

A. Malayeri, M. Mohseni, and B. Cairns. (2016). Fluence (UV Dose) Required to Achieve Incremental Log Inactivation of Bacteria, Protozoa, Viruses and Algae. IUVA News. 18. 4-6.

E. Patterson, T. Prince, E. R Anderson, et al. *The Journal of Infectious Diseases*, Volume 222, Issue 9, 1 November 2020, Pages 1462–1467.

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

Analytical Testing Facility

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

Project

10902.30

Study Director

Michael Hornback, Ph.D.
Aerosol Research and Engineering Laboratories

GLP Statement

We, the undersigned, hereby certify that the work described herein was conducted by Aerosol Research and Engineering Laboratories in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

Study Director:

Dr. Michael Hornback
Study Director
ARE Labs, Inc.

1/19/2021
Date

Principal Investigator:

Sean McLeod
Principal Investigator
ARE Labs, Inc.

1/19/2021
Date

Appendix A: Calculations

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:

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

Appendix B: Raw Data

Trial Information

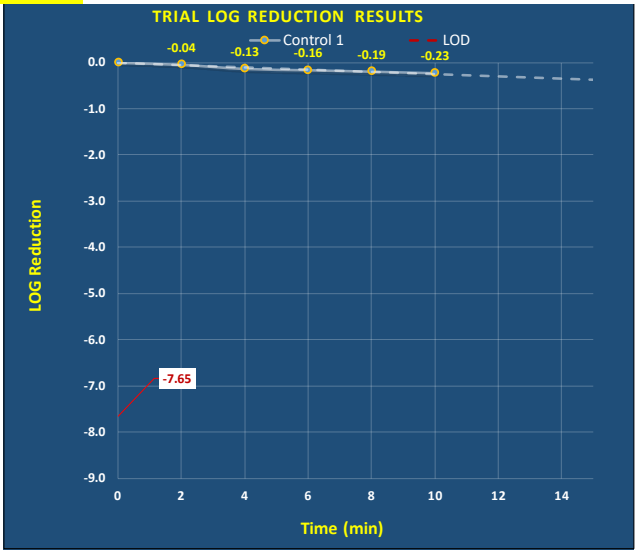
TEST DATE:	Thursday, January 7, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	C1
TEST ORGANSIM:	PhiX
TRIAL NAME ID (GRAPHS/TABLES):	Control 1

Device Information

MANUFACTURER:	Ecosense
UNIT MODEL:	SCUVA 10000
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	70
OTHER INSTRUMENTS:	NA
TRIAL COMMENTS/NOTES	Humidity over 60% mixing fan only during neb



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6
SAMPLING TIME (min)	0	2	4	6	8	10
IMPINGER USED (y / n)	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	y	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.120E+05	1.013E+05	8.267E+04	7.733E+04	7.200E+04	6.667E+04
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)						
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	60.00%					
VIABLE CONSISTENCY CHECKS (% agreement)						
IMP & VIABLE CROSS CHECK (% agreement)						
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.120E+05**	**1.013E+05**	**8.267E+04**	**7.733E+04**	**7.200E+04**	**6.667E+04**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**90.4762%**	**73.8095%**	**69.0476%**	**64.2857%**	**59.5238%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**9.5238%**	**26.1905%**	**30.9524%**	**35.7143%**	**40.4762%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.04**	**-0.13**	**-0.16**	**-0.19**	**-0.23**

Impinger Sampling Conditions

SAMPLING TIME (min)	0	2	4	6	8	10
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5

Dilution Range #1							
DILUTION RATIO (10^x)	**-4**	**-3**	**-3**	**-3**	**-3**	**-3**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**
ENUMERATED PLATE COUNTS (# / drop)	3 2 1	13 12 13	10 11 10	9 9 11	10 8 9	11 6 8
PLATE AVERAGE COUNT (# / drop)	2.00	12.67	10.33	9.67	9.00	8.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	200,000	126,667	103,333	96,667	90,000	83,333
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.60E+05	1.01E+05	8.27E+04	7.73E+04	7.20E+04	6.67E+04

Dilution Range #1							
DILUTION RATIO (10^x)	**-3**	**-4**	**-2**	**-1**	**-3**	**-1**
DROPLET SIZE (µl)	**100**	**100**	**100**	**750**	**100**	**750**
ENUMERATED PLATE COUNTS (# / drop)	7 8 9					
PLATE AVERAGE COUNT (# / drop)	8.00					
IMPINGER CONCENTRATION (cfu or pfu/ml)	80,000					
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	6.40E+04					

Trial Information

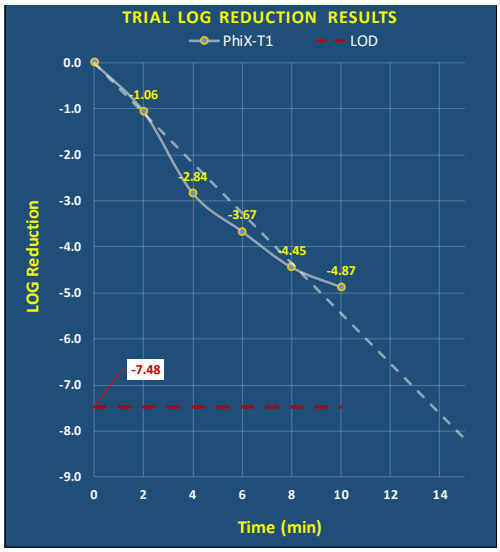
TEST DATE:	Thursday, January 14, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T1
TEST ORGANSIM:	PHIX-174
TRIAL NAME ID (GRAPHS/TABLES):	PhiX-T1

Device Information

MANUFACTURER:	NA
UNIT MODEL:	SCUVA 10000
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
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
SAMPLING TIME (min)	0	2	4	6	8	10
IMPINGER USED (y / n)	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	7.467E+04	6.533E+03	1.080E+02	1.600E+01	2.667E+00	1.000E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)						
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	13.33%	77.50%	65.00%			
VIABLE CONSISTENCY CHECKS (% agreement)						
IMP & VIABLE CROSS CHECK (% agreement)						
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**7.467E+04**	**6.533E+03**	**1.080E+02**	**1.600E+01**	**2.667E+00**	**1.000E+00**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**8.7500%**	**0.1446%**	**0.0214%**	**0.0036%**	**0.0013%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**91.2500%**	**99.8554%**	**99.9786%**	**99.9964%**	**99.9987%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.06**	**-2.84**	**-3.67**	**-4.45**	**-4.87**

Impinger Sampling Conditions

	S1	S2	S3	S4	S5	S6
SAMPLING TIME (min)	0	2	4	6	8	10
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5

		S1	S2	S3	S4	S5	S6
Dilution Range #1	**DILUTION RATIO (10^x)**	**-3**	**-3**	**0**	**0**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**600**	**800**
	ENUMERATED PLATE COUNTS (# / drop)	10 9 7	2 2 0	8 8 5	2	2	1
	PLATE AVERAGE COUNT (# / drop)	8.67	1.33	7.00	2.00	2.00	1.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	86,667	13,333	70	20	3	1
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	6.93E+04	1.07E+04	5.60E+01	1.60E+01	2.67E+00	1.00E+00

		S1	S2	S3	S4	S5	S6
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-2**	**-1**	**-1**	**-1**	**-1**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**750**	**750**	**750**
	ENUMERATED PLATE COUNTS (# / drop)	1 2 0	3 3 3	2			
	PLATE AVERAGE COUNT (# / drop)	1.00	3.00	2.00			
	IMPINGER CONCENTRATION (cfu or pfu/ml)	100,000	3,000	200			
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	8.00E+04	2.40E+03	1.60E+02			

Trial Information

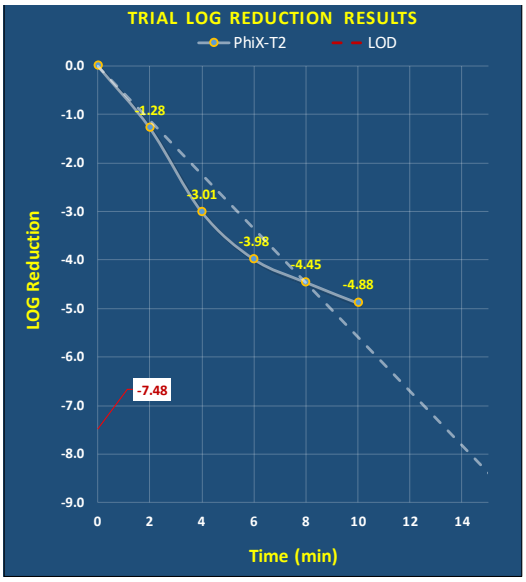
TEST DATE:	Thursday, January 14, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T2
TEST ORGANSIM:	PHIX-174
TRIAL NAME ID (GRAPHS/TABLES):	PhiX-T2

Device Information

MANUFACTURER:	NA
UNIT MODEL:	SCUVA 10000
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
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
SAMPLING TIME (min)	0	2	4	6	8	10
IMPINGER USED (y / n)	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	7.600E+04	4.000E+03	7.467E+01	8.000E+00	2.667E+00	1.000E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)						
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	10.00%	0.00%				
VIABLE CONSISTENCY CHECKS (% agreement)						
IMP & VIABLE CROSS CHECK (% agreement)						
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**7.600E+04**	**4.000E+03**	**7.467E+01**	**8.000E+00**	**2.667E+00**	**1.000E+00**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**5.2632%**	**0.0982%**	**0.0105%**	**0.0035%**	**0.0013%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**94.7368%**	**99.9018%**	**99.9895%**	**99.9965%**	**99.9987%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.28**	**-3.01**	**-3.98**	**-4.45**	**-4.88**

Impinger Sampling Conditions

SAMPLING TIME (min)	0	2	4	6	8	10
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5

Dilution Range #1							
	DILUTION RATIO (10^x)	**-3**	**-2**	**0**	**0**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**600**	**800**
	ENUMERATED PLATE COUNTS (# / drop)	12 10 5	1 6 8	9 10 9	1	2	1
	PLATE AVERAGE COUNT (# / drop)	9.00	5.00	9.33	1.00	2.00	1.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	90,000	5,000	93	10	3	1
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	7.20E+04	4.00E+03	7.47E+01	8.00E+00	2.67E+00	1.00E+00

Dilution Range #1							
	DILUTION RATIO (10^x)	**-4**	**-3**	**-2**	**-1**	**-1**	**-1**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**750**	**750**	**750**
	ENUMERATED PLATE COUNTS (# / drop)	2 0	1 0				
	PLATE AVERAGE COUNT (# / drop)	1.00	0.50				
	IMPINGER CONCENTRATION (cfu or pfu/ml)	100,000	5,000				
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	8.00E+04	4.00E+03				

Trial Information

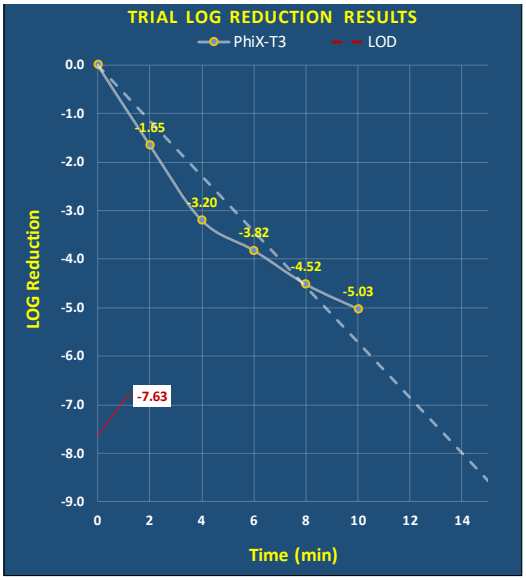
TEST DATE:	Thursday, January 14, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T3
TEST ORGANSIM:	PHIX-174
TRIAL NAME ID (GRAPHS/TABLES):	PhiX-T3

Device Information

MANUFACTURER:	NA
UNIT MODEL:	SCUVA 10000
UNIT SERIAL #:	NA
FITER ID #:	NA
FILTER LOT #:	NA

General Testing Conditions

NEBULIZER CONDITIONS:	Collison 24-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
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
SAMPLING TIME (min)	0	2	4	6	8	10
IMPINGER USED (y / n)	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.067E+05	2.400E+03	6.667E+01	1.600E+01	3.200E+00	1.000E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)						
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		20.00%	33.33%			
VIABLE CONSISTENCY CHECKS (% agreement)						
IMP & VIABLE CROSS CHECK (% agreement)						
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.067E+05**	**2.400E+03**	**6.667E+01**	**1.600E+01**	**3.200E+00**	**1.000E+00**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**2.2500%**	**0.0625%**	**0.0150%**	**0.0030%**	**0.0009%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**97.7500%**	**99.9375%**	**99.9850%**	**99.9970%**	**99.9991%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.65**	**-3.20**	**-3.82**	**-4.52**	**-5.03**

Impinger Sampling Conditions

SAMPLING TIME (min)	0	2	4	6	8	10
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5

Dilution Range #1						
DILUTION RATIO (10^x)	**-3**	**-2**	**0**	**0**	**0**	**0**
DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**	**800**
ENUMERATED PLATE COUNTS (# / drop)	11 14 15	3 2 3	12 8 10	2	2	1
PLATE AVERAGE COUNT (# / drop)	13.33	2.67	10.00	2.00	2.00	1.00
IMPINGER CONCENTRATION (cfu or pfu/ml)	133,333	2,667	100	20	4	1
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.07E+05	2.13E+03	8.00E+01	1.60E+01	3.20E+00	1.00E+00

Dilution Range #1						
DILUTION RATIO (10^x)	**-4**	**-3**	**-1**	**-1**	**-1**	**-1**
DROPLET SIZE (µl)	**100**	**100**	**100**	**750**	**750**	**750**
ENUMERATED PLATE COUNTS (# / drop)		1 0 0	1 1 0			
PLATE AVERAGE COUNT (# / drop)		0.33	0.67			
IMPINGER CONCENTRATION (cfu or pfu/ml)		3,333	67			
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		2.67E+03	5.33E+01			