Third Party Efficacy Testing

Study Director

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Determination of the Single-pass Efficacy of PurePath Prototype Device for Reduction of Aerosolized *E. Coli*

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Background: This in-vitro study characterized the single-pass efficacy of the prototype PurePath Air Sterilizer against aerosolized *Escherichia* coli (E. *coli)*. The PurePath prototype device is a personal air purification device designed to be worn around the neck with clean air blowing into the user's facial area. The single-pass efficacy of the system was assessed using a single-pass bioaerosol challenge system with upstream and downstream sampling to assess the net reduction of bioaerosol passing through the PurePath device. A total of three (3) E. coli bioaerosol challenge trials were conducted to obtain percent and net LOG reduction of the bioaerosol. The study did not evaluate the efficacy of the device's capability to reduce infection rates when used in a real-world scenario and only represents viable E. coli bioaerosol reduction through the device itself.

Methods: *E. coli*(ATCC 15597) was aerosolized into a flow tube system via medical nebulizer, the PurePath device was adapted to allow in-line integration into the bioaerosol challenge system. Midget impingers sampled upstream and downstream of the PurePath device for the duration of the trials at a flow rate of 2.5 LPM. All impinger samples were serially diluted, plated and enumerated in triplicate to yield viable bioaerosol concentration upstream and downstream of the PurePath device in order to determine the single-pass reduction of viable bioaerosol with the device running. The PurePath device was tested on a low light intensity and high light intensity setting.

Results: The PurePath prototype device at a flow rate of 2.5 LPM challenged with aerosolized *E. coli* showed an average percent reduction of 57.06% +/- 5.2% which is equivalent to a 0.37 net LOG reduction on its low setting and an average percent reduction of 75.55% +/- 5.6% which is equivalent to a 0.62 LOG reduction on its high setting. Results are based on an average of triplicate trials.

Summary: These results show that the PurePath device did show a measurable reduction in viable bioaerosols equivalent on high and low settings at 75.55% and 57.06% with the high setting yielding more promising results. This study only determined the single-pass efficacy of the device and makes no claims regarding the real-world efficacy of the device.

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

Overview

This study was conducted to evaluate the effectiveness of the prototype PurePath air sterilizer device. This device is designed to be worn around the user's neck removing airborne contaminants and supplying a clean air stream to the user's facial area. The device utilizes LED UV sterilization technology to reduce viable airborne contaminants. The device has two settings for the LED light, a low intensity setting and a high intensity setting. It should be noted that this study only measured single-pass efficacy of the device. This study does not make any claims regarding the efficacy of this device in real-world use scenarios.

Testing was conducted in a custom stainless steel bioaerosol challenge system constructed at ARE Labs. The PurePath device's effectiveness was tested against aerosolized *Escherichia* coli *(E. coli)*. The efficacy of the device was assessed via an upstream and downstream sampling method to evaluate viable challenge bioaerosol concentration (cfu/L). Comparison of the upstream and downstream samples yielded the single-pass efficiency in terms of the percent and LOG reduction of the bioaerosol challenge. The preliminary effectiveness of the PurePath personal air sterilizer was evaluated against a single vegetative bacterium.

Testing was conducted to evaluate the single-pass reduction capabilities of the PurePath air sterilizer device on both settings against a single vegetative bacterium. A total of six (6) trials were conducted, three (3) on the low intensity, three (3) on the high intensity. A complete testing matrix is shown in **Figure 5**.

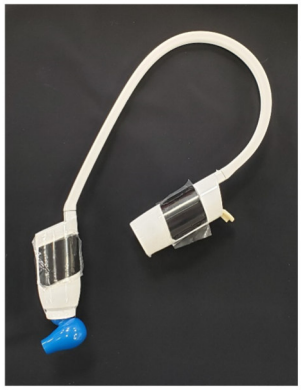
Device Picture	
Picture:	
Manufacturer:	PurePath
Type:	Air Sterilizer
Purification Method:	UV Light

Figure 1: *PurePath Personal Air Sterilizer Prototype Device*

PurePath Prototype Device

This device is intended to supply a clean stream of air to the user's facial area in order to reduce the risk of airborne infection. A picture of the PurePath device can be found in **Figure 1**.

The PurePath prototype personal air sterilizer device was shipped to ARE Labs from PurePath. The device was adapted the challenge system which was constructed of stainless steel sanitary. Adaptors for mating the PurePath device and ensuring an airtight seal was accomplished using PVC attached to the inlet and outlet of the device which silicone and fitted to the bioaerosol system.

Bioaerosol Testing System

A custom Bioaerosol testing system was constructed in order to conduct testing on the PurePath device. The test system was assembled using stainless steel sanitary fittings, impingers, medical nebulizer, HEPA filters and vacuum pump. **Figure 2** shows a picture of the challenge system in a BSL2 cabinet with major components labeled. A complete test system flow diagram can be found in **Figure 3,** on the following page.

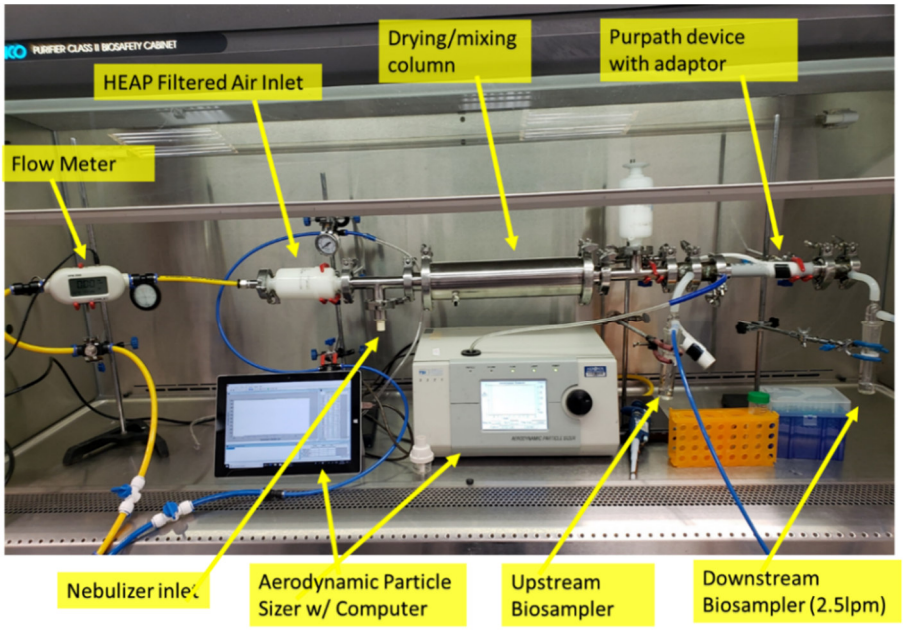


Figure 2*: Purepath Bioaerosol Challenge System*

Bioaerosol Challenge System

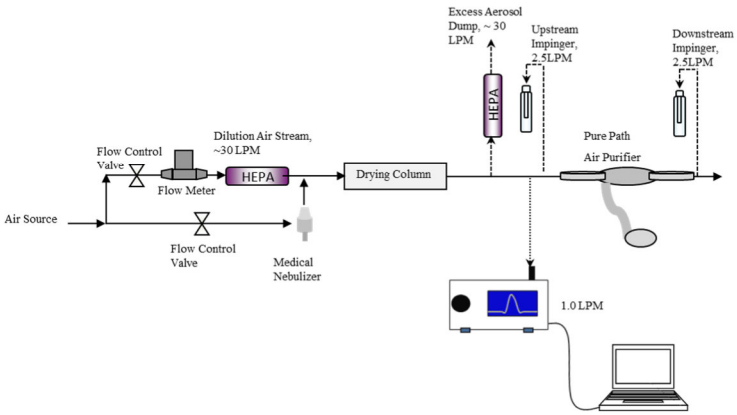


Figure 3: *Bio-Aerosol Challenge System and Flow Diagram.*

Bioaerosol Generation System

Test *E. coli* vegetative bioaerosols were disseminated using a medical nebulizer driven by HEPA filtered house air supply at 30 psi. A pressure regulator allowed for control of disseminated particle size, use rate and sheer force generated within the nebulizer.

Bioaerosol Sampling and Monitoring System

A pair of ChemGlass Midget Impingers (ChemGlass Life Sciences, Vineland, NJ) were used for bioaerosol sample collection of for all three (3) trials conducted. The impingers were filled with 5ml of Phosphate Buffered Saline (PBS) solution for collection of the vegetative bioaerosol. The impingers were then serially diluted and plated for direct enumeration of the viable bacteria.

The Impinger flow vacuum source was maintained using a valved Emerson 1/3 hp rotary vane vacuum pump (Emerson Electric, St. Louis, MO) equipped with a 0-30 inHg vacuum gauge (WIKA Instruments, Lawrenceville, GA). The pump was operated at a negative pressure of 18 inches of Hg during all characterization and test sampling to assure critical flow conditions. The ChemGlass Midget Impingers sample at a flow rate of 2.5 LPM. These were used in order to reduce the airflow through the test system.

Species Selection

The PurePath device was tested using a single vegetative bacteria as a simulant for a common pathogenic bacteria. *E. coli* (ATCC 15597). *E. coli* is a Gram-negative bacterium and BSL1 surrogate for infectious strains of *E. coli* that infects humans.

Vegetative Cells Culture & Preparation

Pure strain seed stocks were purchased from ATCC (American Type Culture Collection, Manassas VA). Working stock cultures were prepared using sterile techniques in a class 2 biological safety cabinet and followed standard preparation methodologies. Approximately 50ml of *E. coli* stock was prepared in tryptic soy liquid broth media, and incubated for 24 hours at 37°C. Biological stock concentrations were greater than 1 x 10^9 cfu/ml for *E. coli* stock solution using this method. Nebulizer stock solution was made by performing 1:9 dilution of the master stock in PBS.

Pure Path Testing Matrix

Trial	Run	Device Light Setting	Challenge Organism	PurePath Device Flow Rate (LPM)	Trial Time (minutes)	Sampling	Equipment
1 2 3	Challenge Challenge Challenge	Low	*E. Coli* (ATCC# 15597)	1.5	5	*Chem Glass CG-1820 Midget Impingers*	*Medical Nebulizer, TSI Aerodynamic Particle Sizer (APS)*
1 2 3	Challenge Challenge Challenge	High	*E. Coli* (ATCC# 15597)	1.5	5	*Chem Glass CG-1820 Midget Impingers*	*Medical Nebulizer, TSI Aerodynamic Particle Sizer (APS)*

Figure 5: *PurePath Test Matrix*

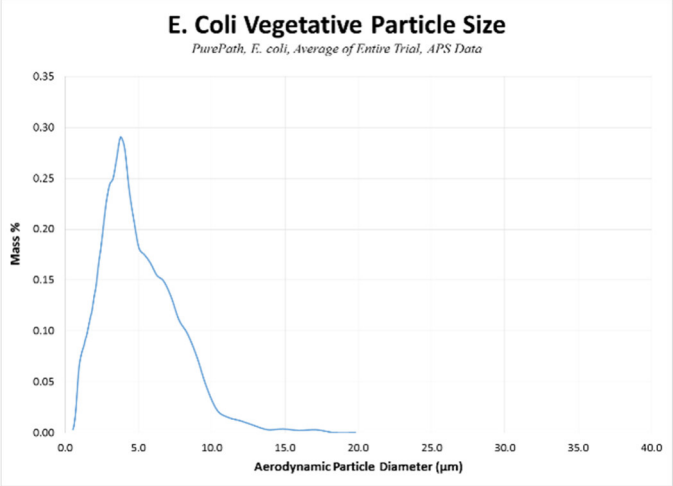


Figure 4*: E. coli Average Particle Size Distribution*

Bioaerosol Particle Size Data

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

Data was logged in real time to an Acer laptop computer, regressed, and plotted. Aerosol particle size distribution for E. coli during the test trials is shown above in **Figure 4**.

Testing Methods

The device was turned on for approximately two (2) minutes prior to the initiation of the test in order to ensure that the device was operating at full flow rate. Prior to Nebulization system flow was turned on and 30 LPM of dilution air was flowed through the system. A drying column was integrated into the system which combined with the dilution air in order to ensure the bioaerosol was dry. Upstream and Downstream sampling was performed using ChemGlass Midget Impingers to sample upstream and downstream which sample at 2.5 LPM.

A HEPA filtered excess air dump was integrated into the system in order to remove excess air from the system and ensure that the only flow through the device was the 2.5 LPM pulled from the downstream impinger. The nebulizer was then turned on and operated at a pressure of 30 psi. Air was allowed to run through the system for at least two minutes to ensure uniform concentration of bioaerosols within the test system. Upstream and Downstream Impingers were turned on and sampled for five minutes in order to assure adequate sample collection in the downstream impinger.

After testing the system HEPA filtered dilution air at 30 LPM was flowed through the system for 15-20 minutes in order to ensure that the system had no remaining Bioaerosols. Once this system purge was completed the PurePath device was decontaminated and plugged in to ensure the device was fully charged at the beginning of each trial.

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 hours and enumerated and recorded.

Post-Testing Decontamination and Prep

Following each trial the nebulizer was cleaned and filled with 35% Hydrogen Peroxide. The peroxide was nebulized for approximately fifteen minutes while 25 LPM of HEPA filtered air was run through the system. The nebulizer was then turned off and the dilution air continued to run through the system for an additional 15 minutes in order to ensure all hydrogen peroxide was removed from the system before beginning the next trial.

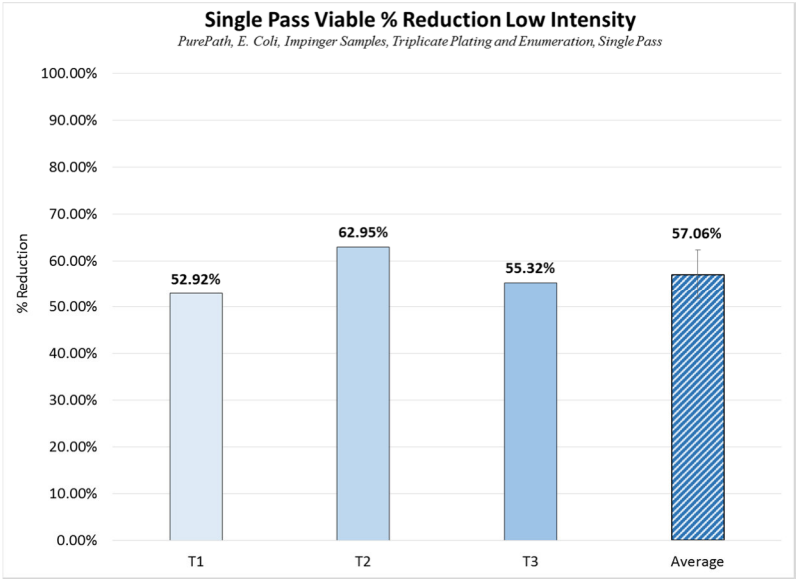


Figure 6: *PurePath Flow Device Testing Results for Low Light Intensity, % Reduction for all Trials & Group Average +/- Std. Deviation*

Results for Low Intensity Light

The PurePath prototype device at a flow rate of 2.5 LPM showed an average viable bioaerosol reduction of 57.06% +/- 5.2% which is equivalent to 0.37 LOG reduction. A graph showing representation of the percent reduction of the device can be found in **Figure 6** while LOG reduction results can be found in **Figure 7**.

When testing viable bioaerosols, some variation is to be expected. Results indicate variations of +/- 0.05 LOG which are within normal ranges of variation.

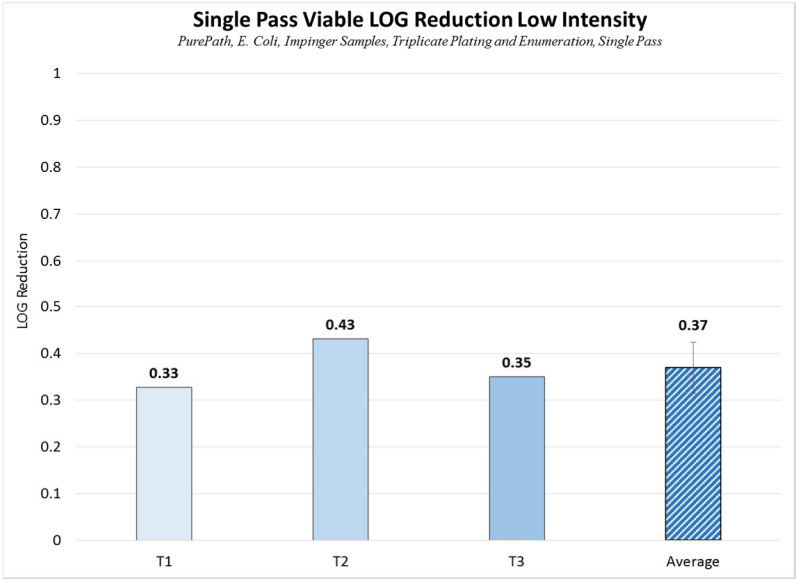


Figure 7: *PurePath Flow Device Testing Results for Low Light Intensity, LOG Reduction for all Trials & Group Average +/- Std. Deviation*

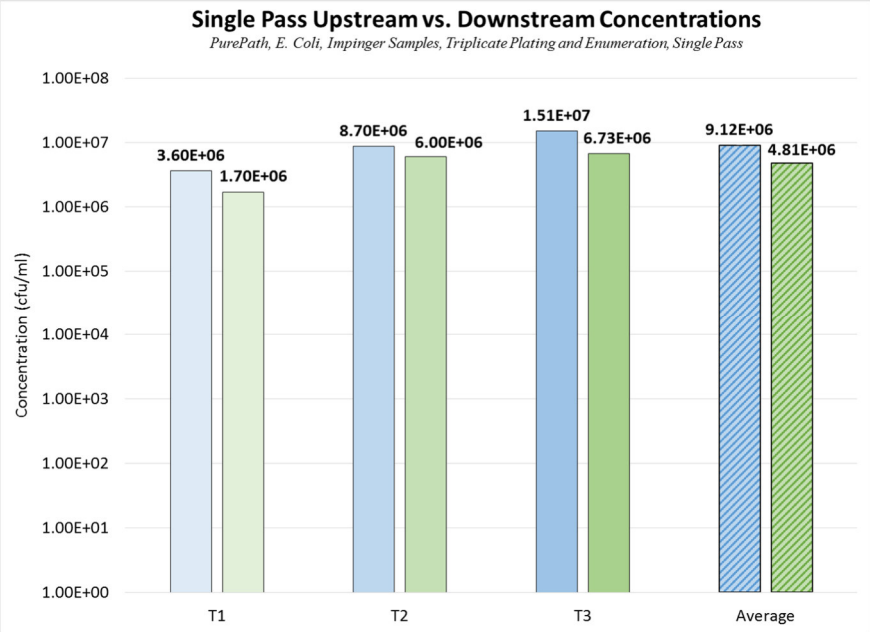


Figure 8: *PurePath Device Testing Result for Low Light Intensity, Upstream and Downstream Concentrations for all Trials & Overall Average +/- Std. Deviations*

The upstream concentrations ranged from 3.60E+06 to 1.51E+07 with an average of 9.12E+06 per trial whereas the downstream concentrations ranged from 1.70E+06 to 6.73E+06 with an average of 4.81E+06 per trial.

This reduction in concentration is indicative of the effect that the device had on the E. coli cultures during testing. Upstream and downstream concentrations are represented graphically in **Figure 8.** A summary table of the low intensity testing can be found in **Figure 9.**

Summary Data

Trial #	Upstream Conc.	Downstream Conc.	% Reduction	LOG Reduction
T1	3.60E+06	1.70E+06	52.92%	-0.33
T2	1.26E+07	4.65E+06	62.95%	-0.43
T3	1.51E+07	6.73E+06	55.32%	-0.35
Average	1.04E+07	4.36E+06	57.06%	-0.37
Std. Deviation	6.02E+06	2.53E+06	5.2%	0.05

Figure 9: *Summary Data for PurePath Air Sterilizer Prototype Device on Low Intensity*

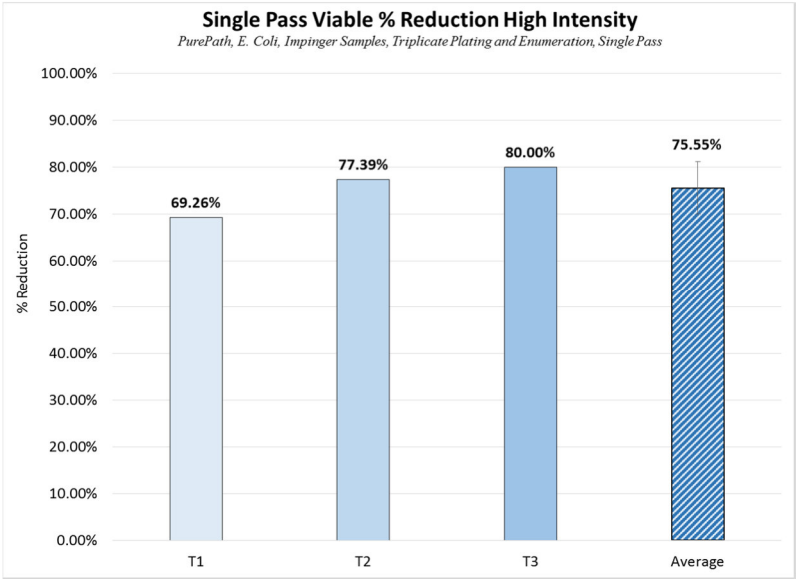


Figure 10: *PurePath Flow Device Testing Results for High Light Intensity, % Reduction for all Trials & Group Average +/- Std. Deviation*

Results for High Intensity Light

The PurePath prototype device at a flow rate of 2.5 LPM showed an average viable bioaerosol reduction of 75.55% +/- 5.6% which is equivalent to 0.62 LOG reduction. A graph showing representation of the percent reduction of the device can be found in **Figure 10** while LOG reduction results can be found in **Figure 11**.

The high intensity testing had some variation similar to the variation found in the low intensity testing. The high intensity testing had a variation of +/- 0.10 LOG which is also within a normal range of variation in bioaerosols. Upstream and downstream concentrations are represented graphically in **Figure 12. .** A summary table of the low intensity testing can be found in **Figure 13.**

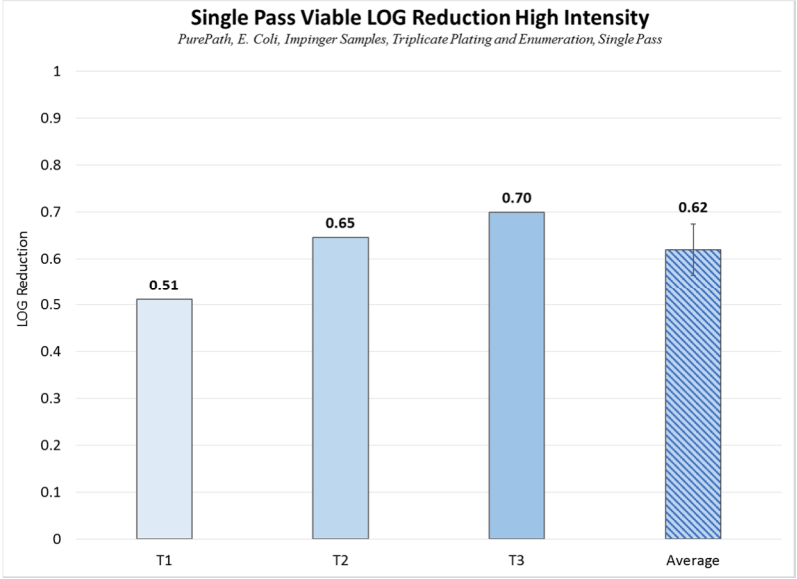


Figure 11: *PurePath Flow Device Testing Results for High Light Intensity, % Reduction for all Trials & Group Average +/- Std. Deviation*

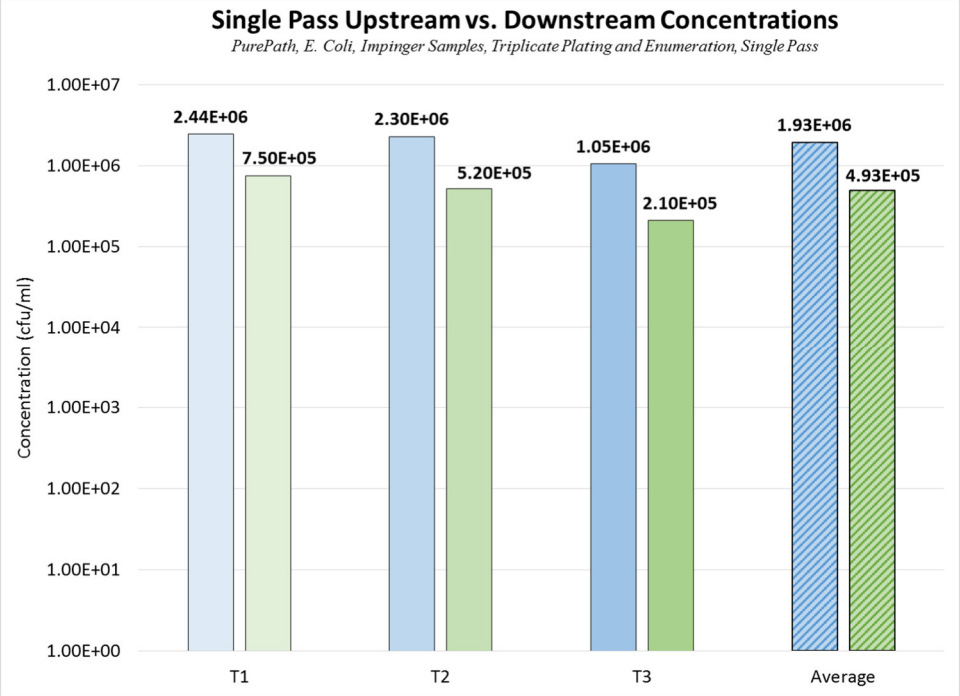


***Figure 12**: PurePath Flow Device Testing Results for High Light Intensity, Upstream and Downstream Concentrations for all Trials & Group Average +/- Std. Deviation*

The upstream concentrations ranged from 1.05E+06 to 2.44E+06 with an average of 1.93E+06 per trial whereas the downstream concentrations ranged from 2.10E+05 to 7.50E+05 with an average of 4.93E+05 per trial.

This reduction in concentration is indicative of the effect that the device had on the E. coli cultures during testing. Upstream and downstream concentrations are represented graphically in **Figure 12.** A summary table of the low intensity testing can be found in **Figure 13.**

Summary Data

Trial #	Upstream Conc.	Downstream Conc.	% Reduction	LOG Reduction
T1	2.44E+06	7.50E+05	69.26%	-0.51
T2	2.30E+06	5.20E+05	77.39%	-0.65
T3	1.05E+06	2.10E+05	80.00%	-0.70
Average	1.93E+06	4.93E+05	75.55%	-0.62
Std. Deviation	7.65E+05	2.71E+05	5.60%	0.10

***Figure 13:** Summary Data for PurePath Air Sterilizer Prototype Device on High Intensity*

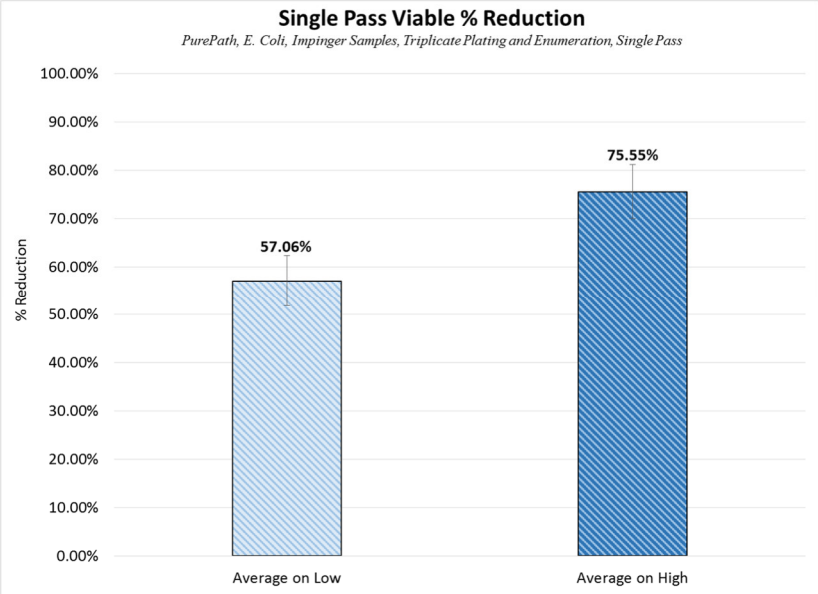


Figure 14: *PurePath Flow Device Testing Results, % Reduction for all Trials Group Averages*

Summary of Findings

Overall the PurePath Flow Device was more effective against E.coli on the high intensity light setting. The percent reduction group average raised from 57.06% +/- 5.2% on the low setting to 75.55%+/- 5.6% on the high setting. A comparison graph showing the group averages side by side can be found in **Figure 14.** The same trend followed as far a LOG reduction. The Device had an average 0.37 +/- 0.05 on the low setting and an average 0.62 +/- 0.10 on the high setting. A comparison graph of the two settings LOG reductions can be found in **Figure 15.**

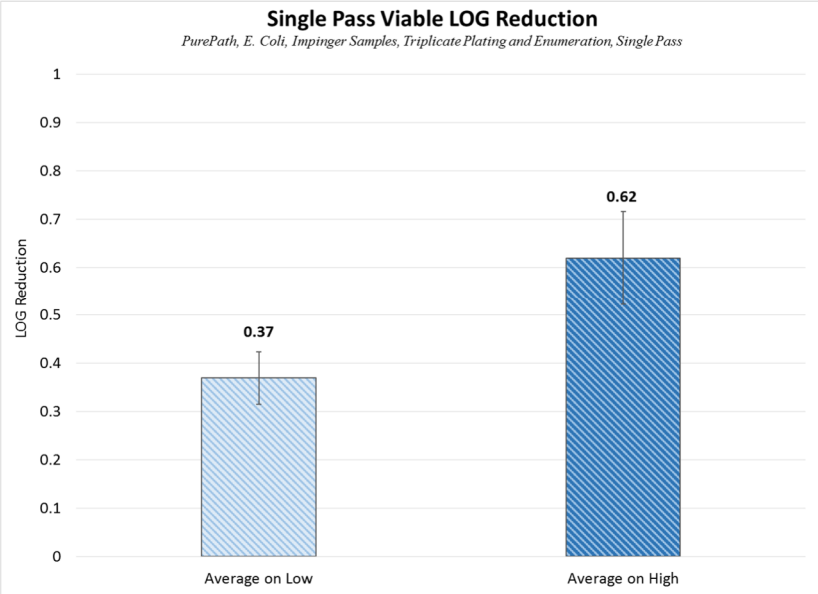


Figure 15: *PurePath Flow Device Testing Results, LOG Reduction for all Trials Group Averages*

References

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.

Analytical Testing Facility

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Project

10878.1

Study Director

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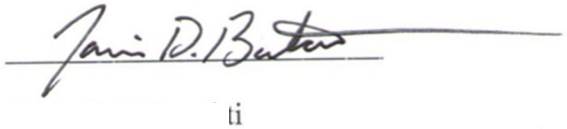
GLP Statement

We, the undersigned, herby certify that the work described herein was conducted by Aerosol Research and Engineering Laboratories in compliance with ARE Labs SOP's and general industry testing standards.

Study Director:



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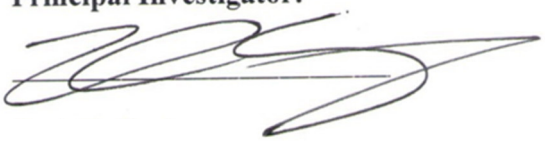
Date 10/16/2019
Study Director
ARE Labs, Inc.

03/02/2020
Date

Principal Investigator:



Date 10/16/2019
Principal Investigator

03/02/2020
Date

Appendix A: Calculations

CALCUALTIONS

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:

$$\text{Nebulizer: } V_P = \frac{C_s \cdot R_{neb}}{V_c} t$$

AGI impinger:

- 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}) = 5 mL collection fluid/impinger, or extraction fluid for filter.
- AGI impinger flow rate (Q_{imp}) = 2.5 L/min.
- AGI impinger time (t) = 5 or 10 minutes, test dependent.

For viable impinger or filter aerosol concentration collection (C_a) = cfu or pfu/L of chamber air:

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

The PurePath net reduction efficacy (expressed as %) is:

$$Efficiency = \frac{\mathrm{C_{downstream}}}{\mathrm{C_{upstream}}} \cdot 100$$

Appendix B: PuePath Raw Plate Enumerations Data

Trial 1 Data

Sample Id		Plate Dilution Factor	Plate Vol ul	Plate counts 1	2	3	4	Average Count	Average (cfu/ml)	Enumerated Concentrat
T1 Upstream	Impinger Data	4x	50	22	18	17	17	19	370	3.70E+06
		5x	50	2	3	0	2	2	35	3.50E+06
								Average		3.60E+06
T1 Downstream	Impinger Data	3x	50	42	41	42	43	42	840	8.40E+05
		4x	50	11	17	9	14	13	255	2.55E+06
								Average		1.70E+06

Trial 2 Data

Sample Id		Plate Dilution Factor	Plate Vol ul	Plate counts 1	2	3	4	Average Count	Average (cfu/ml)	Enumerated Concentrat
T1 Upstream	Impinger Data	4x	50	39	46	41	48	44	870	8.70E+06
								Average		8.70E+06
T1 Downstream	Impinger Data	4x	50	29	26	35	30	30	600	6.00E+06
								Average		6.00E+06

Trial 3 Data

Sample Id		Plate Dilution Factor	Plate Vol ul	Plate counts 1	2	3	4	Average Count	Average (cfu/ml)	Enumerated Concentrat
T1 Upstream	Impinger Data	4x	50	25	23	15	19	21	410	4.10E+06
		5x	50	11	12	17	12	13	260	2.60E+07
								Average		1.51E+07
T1 Downstream	Impinger Data	4x	50	20	10	17	22	17	345	3.45E+06
		5x	50	5	5	6	4	5	100	1.00E+07
								Average		6.73E+06