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Characterization of pureAir 3000+ and HEPA+ Devices in Aerosol Deactivation of SARS-CoV-2

Final Report

FOR:

GreenTech Environmental, LLC

Kim Wilson, Director of Communications
6118 Kingsport Highway
Johnson City, Tennessee 37615

MRIGlobal Project No. 311757.01.001

October 29, 2021

Preface

This Final Report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311757.01.001, "Characterization of pureAir 3000+ and HEPA+ Devices in Aerosol Deactivation of SARS-CoV-2." The experimental phase of this task was initiated by MRIGlobal on September 9, 2021 and ended on September 14, 2021.

The test was performed by Rick Tuttle and Kristy Solocinski, Ph.D. They were assisted by Jacob Wilkinson and Briana Cox. The project was managed by William Sosna.

All operations pertaining to this study were performed according to Standard Operating Procedures or approved laboratory procedures of MRIGlobal.

All study records are stored at MRIGlobal.

Sincerely,

MRIGLOBAL

Rick Tuttle
Principal Scientist
Life Sciences Division

Approved:

Claire R. Croutch, Ph.D.
Portfolio Director
Medical Research

October 29, 2021

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Section 1.
Objective:

The objective of this project was to measure the efficacy of the Client's pureAir 3000+ air purifier ("Test Device 1") and HEPA+ air purifier ("Test Device 2") "Test Devices" in elimination of aerosolized SARS-CoV-2 in controlled tests conducted at MRIGlobal. Each Test Device was tested independently in a primary aerosol containment system within a Class III biological safety cabinet. MRIGlobal characterized the Test Devices to evaluate the log reduction effectiveness against an enveloped virus (SARS-CoV-2 Washington State Isolate Strain or USA-WA1/2020).

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor's Representative

Kim Wilson
Director of Communications
6118 Kingsport Highway
Johnson City, TN 37615

2.2 Testing Laboratories

MRIGlobal
425 Dr. Martin Luther King Jr. Blvd.
Kansas City, Missouri 64110
Phone: (816) 753-7600
Fax: (816) 753-8823

2.3 Personnel Responsibilities

2.3.1 Study Director—MRIGlobal

Rick Tuttle
Phone: (816) 753-7600, ext. 5752
email: rtuttle@mriglobal.org

2.3.2 Task Lead—MRIGlobal

Kristy Solocinski, Ph.D.
Phone: (816) 753-7600, ext. 5280
email: ksolocinski@mriglobal.org

2.3.3 Analysts—MRIGlobal

Jacob Wilkinson
Phone: (816) 753-7600, ext. 5379
email: jwilkinson@mriglobal.org

Brianna Cox
Phone: (816) 326-5414
email: bcox@mriglobal.org

Section 3.
Test Materials

3.1 Test Units

pureAir 3000+ air purifier
HEPA+ air purifier

3.2 Cell and Viral Growth Media

DMEM/F12 (Serum-free media)
Vendor: Gibco
Lot No.: 2323161
Expiration date: 6/22

Growth Media – 5% FBS (fetal bovine serum)

Lot No.: 20210902CHA
Expiration date: 12/21

3.3 Challenge Virus

Severe Acute Respiratory Syndrome-related Coronavirus-2 (SARS-CoV-2)
Strain: USA-WA1/2020
Vendor: BEI Resources
Lot: 202010401KS-B
Passage: 10

3.4 Cell Host

Vero E6 Cells
Vendor: ATCC
Cat: CRL 1586
Passage No.: +13

Section 4.
Test System

MRIGlobal utilized the USA-WA1/2020 strain of the virus, acquired from BEI Resources (NR-52281). The virus was propagated in Vero E6 cells (ATCC CRL-1586).Vero E6 cells were cultured in growth media consisting of Dulbecco's Modified Eagle Medium/F12 (DMEM/F12) supplemented with 5% FBS (Fetal Bovine Serum), and PSN (penicillin, streptomycin, and neomycin). MRIGlobal designed and fabricated the SARS-CoV-2 aerosol test system for the evaluation of multi pass air recirculation purifiers. The aerosol system is equipped with aerosol generation and sampling systems and calibrated digital flow controllers and meters.

Section 5.
Study Design

Aerosol testing was performed using an aerosol test system fabricated out of Plexiglas. The test system was housed in the Class III Biosafety Cabinet for all conducted tests. The aerosol containment system has internal dimensions of 2.5ft high × 3.5ft wide × 1.5ft deep, with a displacement volume of approximately 370 liters or 13.1 cubic feet. The bio-aerosol test system is fabricated for nebulizer adaptation, aerosol and sample dilution air displacement filtration, air supply regulation and control, exhaust flow regulation, aerosol sampling, particle size measurement, and temperature and humidity monitoring. Aerosol generation and sampling system pressures and flow rates were monitored and controlled for maintaining reproducible test conditions using calibrated digital mass flow meters and controllers. SARS-CoV-2 aerosol nebulizer generation was provided with flow and pressure regulated tank supplied breathing grade air. A diagram of the aerosol test system is shown in Figure 1.

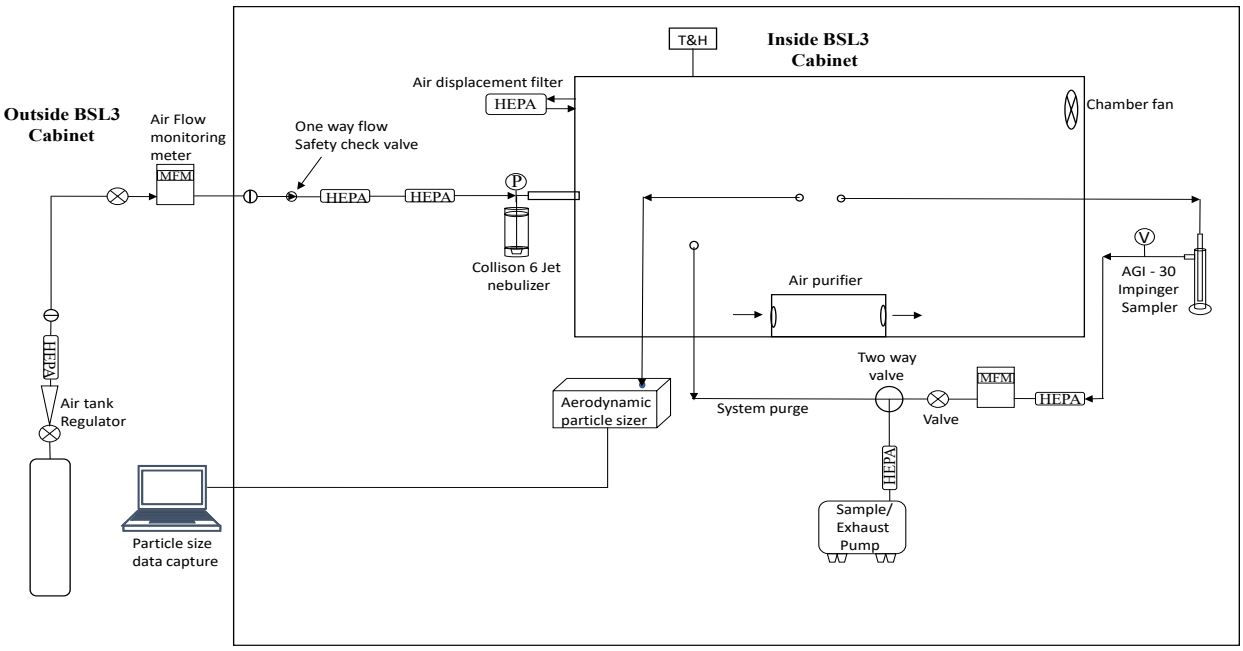

Figure 1. Aerosol System Design

Testing was conducted in three (3) independent test replicates to evaluate each of the two (2) Test Devices. The Test Devices evaluated were the pureAir 3000+ air purifier, and the HEPA+ air purifier. The Test Devices were independently tested in removal of viable SARS-CoV-2 aerosols from the test environment. Preceding test and evaluation of the Test Devices, aerosol characterization tests were performed to establish baseline (control) standard results for subsequent evaluation of the performance of each Test Device. Characterization testing to establish the viral aerosol baseline (control) standard concentration profiles was conducted under the same operating conditions and using the same SARS-CoV-2 viral working stock suspension as device tests. For establishing pre-test viral concentration baseline (control) standard results, the select device was placed in the center bottom of the test system with only an air recirculation mixing fan operational, and the Test Device off. The chamber mixing fan (low flow) provided

uniform mixing and a homogeneous concentration of generated aerosols within the test system during virus aerosol generation and the aerosol sampling period.

SARS-CoV-2 virus (titer of 1.47E7 $TCID_{50}$/ml) was aerosolized with a Collison 6-jet nebulizer into a closed testing chamber for ten (10) minutes for each conducted test. The Test Devices were evaluated in two (2) separate aerosol trials; Task 1 evaluated the aerosol elimination efficacy of Test Device 1 (pureAir 3000+), and Task 2 evaluating the aerosol elimination efficacy of Test Device 2 (HEPA+ small unit) in a biological level 3 facility at MRIGlobal. Tests evaluated the Test Devices operation flows of 23.43 cubic feet per minute (cfm) for Test Device 1, and 39.27 cfm for Test Device 2. The test device volumetric air exchanges, and operation times were based on 10 complete air exchanges of the 370 L aerosol test system for each test device. Therefore, Test Device 1 and Test Device 2 test procedures utilized different operation times. Aerosol sampling and particle size analysis times were maintained at the same times respectively for baseline control and each device test for accurate comparative performance evaluation. The test matrix with test conditions for Task 1 and Task 2 aerosol testing is shown in Table 1.

Table 1. Test Matrix for SARS-CoV-2 Deactivation Testing

Task Description	SARS-Cov-2 stock supension media	Collison 6 jet nebulizer operation (psia)	Collison 6 jet flow rate (L/min)	Collison 6 jet generation time (min)	Device Flow rate (cfm)	Device Operation time (min:sec)	Estimated aerosol system Test device air exchanges per test	Collison 6 jet test generation time (min)	Test device operation times (min)	AGI-30 Impinger test sample times (min)	APS particle size test sample time (min)	Total number of tests
Characterization baseline control, Test system mixing fan only	DMEM	26	15	10	NA	NA	NA	t = -10-0	NA	t = 10 to 20	t = 0 to 20, (30 second sequential samples) 40 total samples	3
Task 1. pureAir 3000+ test with filtration, fan speed 5	DMEM	26	15	10	23.43	5:35	10	t = -10-0	t = 0 to 5:35	t = 5:35 to 20	t = 0 to 20, (30 second sequential samples) 40 total samples	3
Task 2. Device HEPA+ with filtration ,fan speed 1	DMEM	26	15	10	39.27	3:20	10	t = -10-0	t = 0 to 3:20	t = 5:35 to 20	t = 0 to 20, (30 second sequential samples) 40 total samples	3

For the pureAir 3000+ this 10 volumetric air exchanges in the test system is defined as 5 min 35 sec and 3 min 20 sec for the HEPA + unit based on the CFM flow speeds of the units. Each device was activated for its respective operation time following ten (10) minutes of aerosol challenge generation into the test chamber. For all trials, baseline and test, aerosol collection was conducted with an AGI-30 impinger model 7540 (Ace Glass, Inc.) filled with 20 ml of DMEM media. Aerosol samples were collected at the same time points from five (5) minutes thirty-five (35) seconds to the twenty (20) minute time for both Test Devices and baseline characterization control tests. The set of three baseline characterization tests were conducted to measure the natural aerosol test chamber concentration characteristics without the Test Device operational. This characterization testing was conducted under the same aerosol generation, system operation conditions, and sampling intervals as device tests. The resultant baseline control results provided

a standard with which to compare the Test Device results and calculation of the device efficacy in eliminating airborne SARS-CoV-2.

For each baseline (control) standard, and device test conducted in Task 1 and Task 2, the Collison 6-jet nebulizer was filled with a fresh aliquot of 8 ml of SARS-CoV-2 (1.47E7 $TCID_{50}$/ml). Aerosol samples for each test were collected from the aerosol test chamber using impingers (Ace Glass, Inc.) filled with sterile DMEM/F12 collection media for each sample collection iteration. Additional aerosol characteristic analysis was conducted for each baseline (control) standard and Test Device test using the TSI Aerodynamic Particle Sizer® 3321 (APS™) spectrometer. The APS is an aerodynamic time of flight particle measurement instrument that provides accurate particle size analysis and has a dynamic particle size measurement range of 0.3 to 20 µm. The APS provides mass median aerodynamic diameter ("MMAD"), Geometric Standard Deviation ("GSD"), total sample aerosol mass (mg/cc), and aerosol particle counts (#/cc) in real time.

For each test, the Collison 6-jet nebulizer was operated with tank supplied breathing grade air at a pressure of 26 psi to generate viral aerosol into the test cabinet at a flow rate of approximately 15 L/min. Following a ten (10) minute aerosol generation period, the nebulizer was turned off, and testing initiated. The aerosol test system has a HEPA capsule filter adapted to allow for the introduction of generated air supply flows, and air displacement introduction for aerosol sampling which was uniform and consistent for all respective testing. This provides near ambient pressure conditions in the test system during each test trial and provides natural test environmental conditions for Test Device evaluation. Test sampling and Test Device operation parameters were followed as shown in Table 1, and as described above. For Task 1 and Task 2 testing and baseline standard (control) characterization tests, impinger samples were collected, and placed in sample identification labeled sterile conical tubes. Samples were transferred in a secondary container to another BSL-3 laboratory where the samples were then diluted 1:10 down a 24-well plate in DMEM/F12 to assess the TCID50 of the samples. These dilutions were incubated approximately thirty (30) minutes, after which DMEM/F12 supplemented with 5% FBS was added to cells to feed them for the next four to five days. This incubation period allowed the virus to adsorb to cells without interference from FBS.

After a four (4) to five (5) day incubation time, cells were examined under magnification for the presence of cytopathic effect (CPE) associated with viral presence and replication. Examination was done using a microscope (10x objective to view the entire well at once) and observing the morphology of the cells. Healthy Vero E6 cells are semitransparent with a fusiform appearance (pinched or narrowing ends and more round in the middle) in a monolayer of cells with little to no space between cells. Dead cells displaying CPE are often detached from the plate, round, less transparent, and much smaller than living cells. Furthermore, the healthy Vero E6 cells cover much of the surface of the well but wells containing cells with CPE have areas of the well where no cells are adherent, described as empty space. Any well displaying CPE is marked as positive whether the whole well is affected or only a small patch as both are indicative of the presence of viable virus.

Section 6.
Statistical Analysis of Data

The number of positive and negative wells were entered into a modified Excel spreadsheet that was published as part of Lindenbach BD. Measuring HCV infectivity produced in cell culture and *in vivo*. Methods Mol Biol. 2009; 510:329-336. doi:10.1007/978-1-59745-394-3_24. The $TCID_{50}$/ml is calculated using the below equations, all using Microsoft Excel.

$$\text{Proportionate Distance (PD)} = \frac{\%\text{CPE at dilution above 50\%} - 50\%}{\%\text{ CPE at next dilution above 50} - \%\text{ CPE at next dilution below 50}}$$

$$\text{TCID50} = 10^{\text{log of dilution above 50\% CPE}} - \text{PD}$$

$$\text{TCID50/ml} = \frac{1}{\text{volume used per well}} x \frac{1}{\text{TCID50}}$$

The log10 of the three technical replicates was averaged for control and treatment samples. This number for the treatment is subtracted from the number for the control and is reported as "log reduction." This log reduction is converted into a percent log reduction via the following equation.

$$\%\text{ Log Reduction} = \left(1 - 10^{-\log \text{reduction}}\right) x\ 100$$

Section 7.
Results

Aerosol plates were read four days after the conduct of each test trial. The pureAir 3000+ reduced viral infectivity by 3.71 log (99.98%) within five (5) minutes and thirty-five (35) seconds of operation in relation to baseline control results. The HEPA+ reduced viral infectivity by 3.63 log (99.98%) within three (3) minutes and twenty (20) seconds of operation in relation to baseline control results. Table 2 summarizes these findings and shows individual test sample results with test averaged reduction/deactivation results for Task 1 and Task 2 testing.

Table 2. $TCID_{50}$/ml Calculations for aerosol testing of pureAir 3000+ and HEPA+ devices

Sample Name	Sample Type	Replicate #	$TCID_{50}$/mL	Log10 $TCID_{50}$/mL	Average $TCID_{50}$/mL	Average Log10 $TCID_{50}$/mL	Log Reduction	Percent Log Reduction
T3000-1	pureAir 3000+	1	≤ 3.51E-01	-0.45	3.72E-01	-0.43	3.71	99.98%
T3000-2		2	≤ 3.51E-01	-0.45				
T3000-3		3	≤ 4.14E-01	-0.38				
TW-1	HEPA+	1	≤ 5.16E-01	-0.29	4.48E-01	-0.35	3.63	99.98%
TW-2		2	≤ 4.14E-01	-0.38				
TW-3		3	≤ 4.14E-01	-0.38				
C1	Control	1	7.01E+02	2.85	2.41E+03	3.28		
C2		2	2.39E+03	3.38				
C3		3	4.14E+03	3.62				

APS 3321particle counts were taken sequentially over the same time periods for baseline control standard tests and Test Device tests. A plot of the test averaged control and Device Test APS particle count profiles vs time is shown in Figure 2.

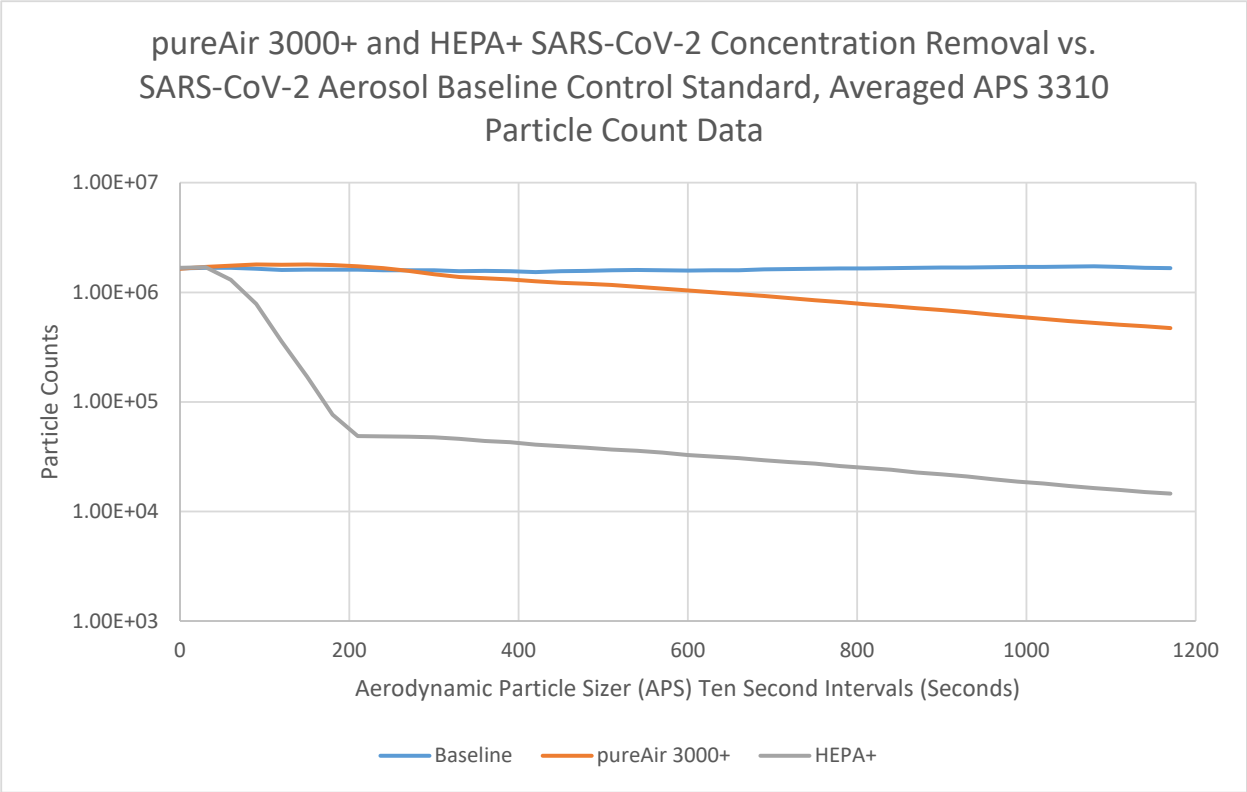

Figure 2. Aerodynamic Particle Sizer (APS) Aerosol Particle Count vs Sample Time Plot

The averaged values from APS scans with aerosol count concentration, mass (mg/m^3), and mass median particle size diameter for baseline control standard tests, and Task 1 and Task 2 testing sample time periods is shown in Table 3.

Table 3. APS Aerosol Count and Mass Test Results

pureAir 3000+ and HEPA+ APS Data									
	pureAir 3000+ Test 1			pureAir 3000+ Test 2			pureAir 3000+ Test 3		
Test ID-(seconds)	T1-0	T1-600	T1-1200	T2-0	T2-600	T2-1200	T3-0	T3-600	T3-1200
Particle counts	1674792	589442	179910	1678727	1305236	666236	1617393	1234573	567155
Conc. (mg/m^3)	9.30	1.55	0.314	9.33	3.22	1.13	8.68	3.20	8.82E-01
Diameter (um)	3.27	2.90	1.70	3.25	2.95	2.25	3.41	3.20	2.31
	HEPA+ Test 1			HEPA+ Test 2			HEPA+ Test 3		
Test ID-(seconds)	T1-0	T1-600	T1-1200	T2-0	T2-600	T2-1200	T3-0	T3-600	T3-1200
Particle counts	1605276	39988	14670	1743511	17790	7703	1749327	40715	21430
Conc. (mg/m^3)	6.27	2.56E-02	9.42E-03	7.59	1.48E-02	5.97E-03	7.43	3.01E-02	1.42E-02
Diameter (um)	3.78	1.14	1.08	3.68	1.14	1.06	3.63	1.17	1.08
	Baseline control 1			Baseline control 2			Baseline control 3		
Test ID-(seconds)	C1-0	C1-600	C1-1200	C2-0	C2-600	C2-1200	C3-0	C3-600	C3-1200
Particle counts	1740274	1614877	1640893	1740274	1614877	1640893	1490082	1507736	1713187
Conc. (mg/m^3)	12.3	9.15	8.58	9.82	8.53	8.85	7.56	7.12	7.46
Diameter (um)	3.35	4.06	4.24	3.57	4.04	4.61	3.46	4.03	4.44

Particle size distributions were also measured with the APS. A plot with representative SARS-CoV-2 aerosol particle size distribution derived from control testing data is shown in Figure 3.

The plot shows the percent mass of the particle size distribution in relation to particle size. The Mass Median Aerodynamic Diameter (MMAD) shown in the graph reflects a median diameter of approximately 3.46 µm, with 50% of the aerosol particle mass below and 50% above the median diameter. The 15.87 percent mass (1.62µm) and 84.14 percent (7.44 µm) particle mass points are also shown.

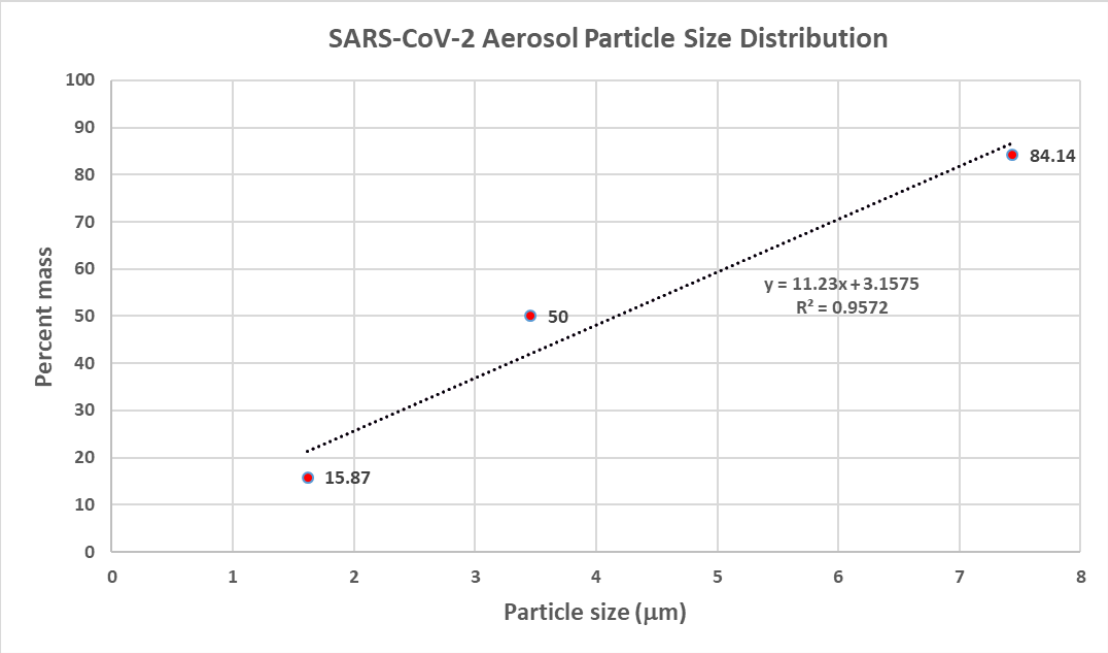

Figure 3. Aerodynamic Particle Sizer (APS) Aerosol Particle Size Distribution Plot

Section 8.
Conclusions

Based on these experiments, we conclude that both the pureAir 3000+ and HEPA+ devices are highly effective at reducing the infectivity of aerosolized SARS-CoV-2 virus with a 3.71 log (99.98%) reduction within five (5) minutes and thirty (30) seconds of operation, and a 3.63 log (99.98%) reduction within three (3) minutes and twenty (20) seconds of operation, respectively, in relation to baseline control results.

Each device operation time represents approximately ten (10) air exchanges of the test system volume through the Test Devices. The Test Devices' reduction of particle counts is depicted in Figure 2, which shows the device particle removal results in relation to the non - operation control test aerosol concentration profile. Data in Table 3 shows the air purifiers' performance in count concentration, mass (mg/m^3), and mass median particle size reduction in relation to baseline control standard test results. The data shows the APS measured data for each baseline control and Test Device test corresponding to times zero (0), six hundred (600), and twelve hundred (1200) seconds. These results show that HEPA+ had a reduction in aerosol mass and median size within the (5) minutes and thirty (30) seconds of operation of operation, with a mass removal of approximately 2 logs. Whereas the pureAir 3000+ had a smaller reduction in aerosol mass and median size within the three (3) minutes and twenty (20) seconds of operation, with a mass removal of approximately 1 log. However, while particle mass reductions of only 1 to 2 logs were observed, both devices demonstrated viral infectivity reductions greater than 3.5 logs.