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Testing of Airocide Air Purification System against Aerosolized SRS-CoV-2

Final Report

FOR

SteriLumen, Inc.

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Attn: Keyoumars Saeed
CEO

MRIGlobal Project No. 311788.01.001

September 17, 2021

Preface

This Final Report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311788.01.001, “Testing of Airocide Air Purification System against Aerosolized SARS-CoV-2.” The experimental phase of this task was initiated by MRIGlobal on July 27, 2021 and ended on August 1, 2021.

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

The study was not performed in compliance with the FDA Good Laboratory Practice Regulations (21 *CFR* 58) or EPA Good Laboratory Practice Regulations (40 *CFR* 160). All operations pertaining to this study, unless specifically defined in this protocol, were performed according to the Standard Operating Procedures of MRIGlobal.

All study records are stored at MRIGlobal.

Sincerely,

MRIGLOBAL



Rick Tuttle
Principal Scientist
Life Sciences Division

Approved:



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September 17, 2021

Contents

Preface.....	i
Section 1. Objective:.....	1
Section 2. Sponsor, Testing Laboratory, and Personnel Responsibilities.....	2
2.1 Sponsor’s Representative	2
2.2 Testing Laboratories.....	2
2.3 Personnel Responsibilities.....	2
Section 3. Test Materials.....	3
3.1 Test Units	3
3.2 Cell and Viral Growth Media.....	3
3.3 Challenge Virus.....	3
3.4 Cell Host.....	3
Section 4. Test System.....	4
Section 5. Study Design.....	5
Section 6. Statistical Analysis of Data.....	9
Section 7. Results.....	10
Section 8. Conclusions.....	14

Figures

Figure 1. Aerosol System Design.....	5
Figure 2. Aerodynamic Particle Sizer (APS) Aerosol Particle Count vs Sample Time Plot	11
Figure 3. Aerodynamic Particle Sizer (APS) Aerosol Particle Size Distribution Plot.....	13

Tables

Table 1. Phase 1 and Phase 2 Test Matrix	6
Table 2. TCID ₅₀ /ml Calculations for aerosol testing of AP100 and AP300 devices.....	10
Table 3. APS Aerosol Count and Mass Test Results.....	12

Section 1. Objective:

The objective of this project was to measure the efficacy of the Client's Airocide HD Air Purification System ("Test Device") in elimination and neutralization of aerosolized SARS-CoV-2 in controlled tests conducted at MRIGlobal. The Client provided a set of two (2) Airocide HD Air Purifiers. One Test Device was provided with a filter unit and Photocatalytic Oxidation System (PCO) unit for testing the complete air purification system in viral reduction/elimination. The other Test Device was delivered with the filter removed for evaluation of the Photocatalytic Oxidation System (PCO) only for testing the PCO deactivation efficacy of viable virus. Each Test Device was tested independently for efficacy in aerosol removal/deactivation of SARS-CoV-2 aerosols. Aerosol Test Device challenges were conducted 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

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Section 3. Test Materials

3.1 Test Units

Phase 1. Airocide HD Air Purification System with filtration + Photocatalytic Oxidation (PCO)
Phase 2. Airocide HD Air Purification System with Photocatalytic Oxidation (PCO) only

3.2 Cell and Viral Growth Media

DMEM/F12 (Serum-free media)

Vendor: Gibco

Lot No.: 2239746

Expiration date: 4/30/22

Growth Media – 5% FBS (fetal bovine serum)

Lot No.: 20210723BC

Expiration date: 01/23/22

3.3 Challenge Virus

Severe Acute Respiratory Syndrome-related Coronavirus-2 (SARS-CoV-2)

Strain: USA-WA1/2020

Vendor: BEI Resources

Lot: 202010401KS-A

Passage: 10

3.4 Cell Host

Vero E6 Cells

Vendor: ATCC

Cat: CRL 1586

Passage No.: 53

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.

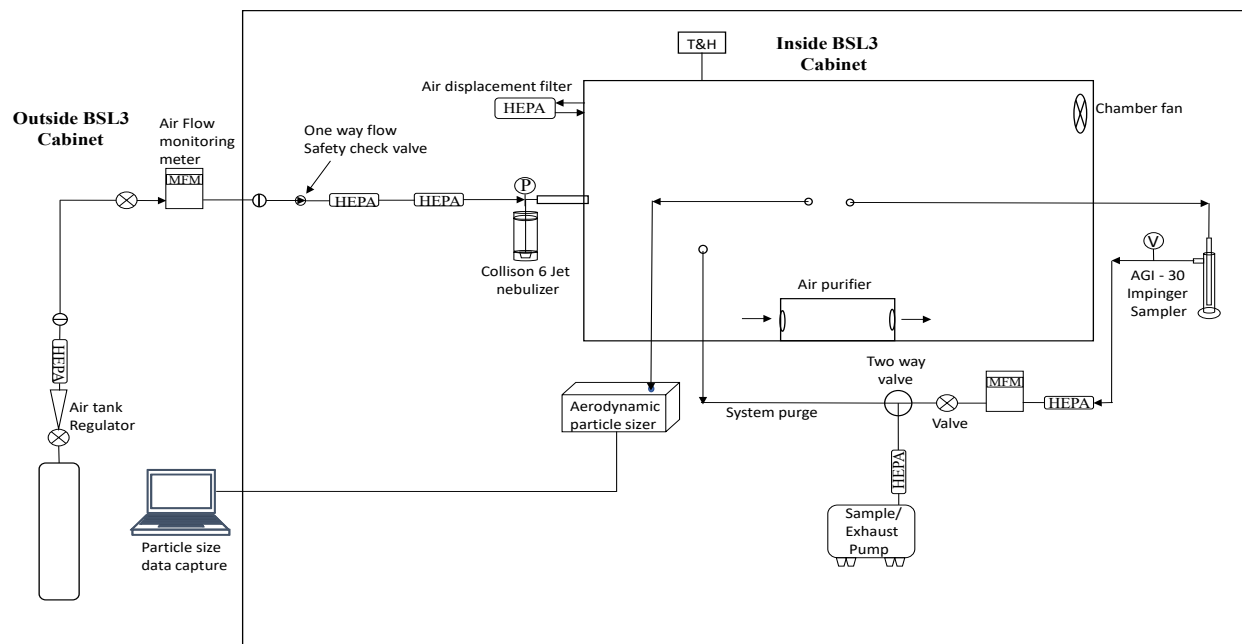


Figure 1. Aerosol System Design

Testing was conducted in three (3) independent test replicates to evaluate each of the two (2) Test Devices. The Test Devices evaluated were the Airocide HD Air Purification System with filtration + PCO in Phase 1, and the Airocide HD Air Purification System with PCO only in Phase 2. The Test Devices were independently tested in removing/deactivating 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 TCID₅₀/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 consisting of Phase 1 (filtration + PCO) and Phase 2 (PCO only) operation in a biological level 3 facility at MRIGlobal. Tests were conducted using both devices with the same set flow of approximately 30 cubic feet per minute (cfm). It was determined that Phase 1 (filtration + PCO) tests would provide a quicker aerosol reduction/elimination than Phase 2 (PCO only) tests based on the Test Device aerosol filter capture process. Therefore, Phase 1 and Phase 2 test procedures utilized different aerosol collection, and sample time methods for evaluation. The test matrix showing test conditions for Phase 1 and Phase 2 aerosol testing is shown in Table 1.

Table 1. Phase 1 and Phase 2 Test Matrix

Phase 1 Airocide Filter and PCO Operation Testing											
Test description	Test Time (min)	Collison 6 jet aerosol generator operation (psia)	Collison 6 jet ~ flow rate (L/min)	Collison 6 jet test generation time (min)	Impinger Type	Airocide test operation time (min)	Estimated Test Device, aerosol test system air displacements	Impinger test sample times (min)	APS particle size test sample times (min)	Total number of tests	Number of Impinger samples /test
Baseline characterization testing for filter and PCO, no device operation. Chamber fan only operation	30	26	15	t = -10-0	AGI-30	NA	NA	t = 5:30 to t = 20:00	t = 0 to 20, 30 second sequential samples = 40 samples	3	1
Device test, filter and PCO, no warmup, unit operational following aerosol process	30	26	15	t = -10-0	AGI-30	5 min: 30 sec	12.6	t = 5:30 to t = 20:00	t = 0 to 20, 10 second sequential samples = 120 samples	3	1
Phase 2 Airocide PCO Only Operation Testing											
Baseline characterization testing for PCO, no device operation. Chamber fan only operation	40	26	15	t = -10-0	Midget	NA	NA	t = -10 to 0 t = 0 to 10 t = 10 to 20 t = 20 to 30	t = 0 t = 10 t = 20	3	4
Device test, PCO only with 20 min warmup - no filter, unit operational during aerosol process and full test	40	26	15	t = -10-0	Midget	t = - 30 continuous operation throughout testing	NA	t = -10 to 0 t = 0 to 10 t = 10 to 20 t = 20 to 30	t = 0 t = 10 t = 20	3	4

Phase 1 Testing: Airocide HD (filtration + PCO)

For Phase 1 testing, it was agreed upon by the client to establish the Test Device (filtration + PCO) operation time for aerosol challenge removal based on aerodynamic particle sizer (APS) count data. To establish this operation time, the APS was programmed to take sequential ten (10) second aerosol scans over the course of each Phase 1 Test Device test for near real time observation of aerosol removal performance. It was determined that the Airocide HD (filtration + PCO) had an effective removal of a majority of the aerosol from the test system following a five (5) minute thirty (30) second operation time based on the APS data. With this aerosol removal time established, subsequent testing was based on a five (5) minute thirty (30) second operation time following ten (10) minutes of aerosol challenge generation into the test chamber. This equated to approximately 12.6 aerosol test system air exchanges based on a Test Device air flow rate of 30 cfm. For aerosol collection following Test Device operation in Phase 1, an AGI-30 impinger model 7540 (Ace Glass, Inc.) filled with 20ml of DMEM media was used for collection of resident aerosols. Aerosol samples were collected at the same time points from five (5) minutes thirty (30) seconds to the twenty (20) minute time for both Test Device 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.

Phase 2 Testing: Airocide HD (PCO only)

Testing in Phase 2 evaluated the Airocide HD Test Device (PCO only) source independently for deactivation of SARS-CoV-2 aerosols with the filter element removed from the unit. Phase 2 tests utilize low flow Midget impingers model 7531(Ace Glass, Inc.) filled with 10ml of sterile DMEM collection media. The midget impingers are widely used for biological aerosol collection, have a high collection efficiency rating, and operate at low sample flow rate conditions. These low flow samplers are more suitable for evaluating non - filtration open source disinfection systems which may require increased exposure times for efficacy. Due to the PCO source requiring an initial warm up time to reach maximum operation intensity, we evaluated the PCO following twenty (20) minutes of operation preceding the viral aerosol generation. The PCO source was continually operated during the aerosol generation and for the subsequent 30 minute sampling interval processes to measure the PCO efficacy in deactivating viral viability. Impinger samples were also taken starting at the initiation of the aerosol generation period with three sequential ten (10) minute impinger samples taken following aerosol generation. This sampling interval process was replicated for both Test Device and baseline (standard) characterization tests.

As shown in Table 1, evaluation of the Airocide HD Test Devices for Phase 1 (filtration + PCO) and Phase 2 (PCO only) included conducting baseline control aerosol test to establish baseline (standard) results for the subsequent Test Device viral deactivation efficacy calculation for both test phases. For each baseline (control) standard, and device test conducted in Phase 1 and Phase 2, the Collison 6-jet nebulizer was filled with a fresh aliquot of 8 ml of SARS-CoV-2 (1.47E7 TCID₅₀/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 in Phase 1 and Phase 2 discussion sections above. For Phase 1 and Phase 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 forty five (45) 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 TCID₅₀/ml is calculated using the below equations, all using Microsoft Excel.

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

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

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

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.

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

Section 7. Results

Aerosol plates were read four days after the conduct of each Phase 1 and Phase 2 test trial. The Airocide HD Test Device (filtration + PCO) reduced viral infectivity by 3.25 log (99.94%) within five (5) minutes and thirty (30) seconds of operation in relation to baseline control results. The Airocide HD Test Device (PCO only) had a progressive deactivation of viable virus from 0.61 log (75.52%) during the aerosol generation process to 2.02 log (99.05%) during the twenty (20) to thirty (30) minute test sample time iteration in relation to baseline control results. Table 2 summarizes these findings and shows individual test sample results with test averaged reduction/deactivation results for Phase 1 and Phase 2 testing.

Table 2. TCID₅₀/ml Calculations for aerosol testing of AP100 and AP300 devices.

Sample Name	Sample Type	Test Description	Sample Time Interval (min)	Test Sample Replicate	TCID ₅₀ /mL	Log ₁₀ TCID ₅₀ /mL	Average TCID ₅₀ /mL	Average Log ₁₀ TCID ₅₀ /mL	Log Reduction	Percent Log Reduction
T1-1	Airocide HD with filter and PCO	Test	5 min 30 sec to 20 min	1	3.51E-01	-0.45	3.51E-01	-0.45	3.25	99.943%
T1-2				2	3.51E-01	-0.45				
T1-3				3	3.51E-01	-0.45				
C1-1	Airocide HD with filter and PCO off	Control	5 min 30 sec to 20 min	1	4.14E+02	2.62	6.80E+02	2.79	control (NA)	
C1-2				2	1.11E+03	3.05				
C1-3				3	5.16E+02	2.71				
T2-1.1	Airocide HD with PCO only	Test	-10-0	1	3.51E+01	1.55	6.05E+01	1.71	0.61	75.516%
T2-2.1				3	3.51E+01	1.55				
T2-3.1				2	1.11E+02	2.05				
T2-1.2	Airocide HD with PCO only	Test	0-10	1	5.16E+00	0.71	2.89E+00	0.38	2.06	99.120%
T2-2.2				3	1.11E+00	0.05				
T2-3.2				2	2.39E+00	0.38				
T2-1.3	Airocide HD with PCO only	Test	10-20	1	3.51E+00	0.55	2.71E+00	0.38	1.81	98.458%
T2-2.3				3	3.51E+00	0.55				
T2-3.3				2	1.11E+00	0.05				
T2-1.4	Airocide HD with PCO only	Test	20-30	1	2.39E+00	0.38	2.09E+00	0.16	2.02	99.048%
T2-2.4				3	3.51E-01	-0.45				
T2-3.4				2	3.51E+00	0.55				
C2-1.1	Airocide HD with PCO off	Control	-10-0	1	3.51E+02	2.55	2.34E+02	2.32	control (NA)	
C2-2.1				2	2.39E+02	2.38				
C2-3.1				2	1.11E+02	2.05				
C2-1.2	Airocide HD with PCO off	Control	0-10	1	1.11E+03	3.05	5.05E+02	2.43	control (NA)	
C2-2.2				2	5.16E+01	1.71				
C2-3.2				2	3.51E+02	2.55				
C2-1.3	Airocide HD with PCO off	Control	10-20	1	6.53E+02	2.81	2.72E+02	2.19	control (NA)	
C2-2.3				2	1.11E+02	2.05				
C2-3.3				2	5.16E+01	1.71				
C2-1.4	Airocide HD with PCO off	Control	20-30	1	3.51E+02	2.55	1.97E+02	2.18	control (NA)	
C2-2.4				2	1.89E+02	2.28				
C2-3.4				2	5.16E+01	1.71				

APS 3321 particle counts were taken sequentially for Phase 1 Airocide HD Test Device (filtration + PCO) testing, and at the initiation of each impinger sample iteration for Phase 2 Test Device (PCO only) testing. This sampling was also conducted at the same time periods for baseline control standard tests. A plot of the averaged control and test APS particle count profiles based

on 0.5 liters/sample for the baseline control and Airocide HD Test Device (filtration + PCO) testing, and Airocide HD Test Device (PCO only) Test Devices is shown in Figure 2.

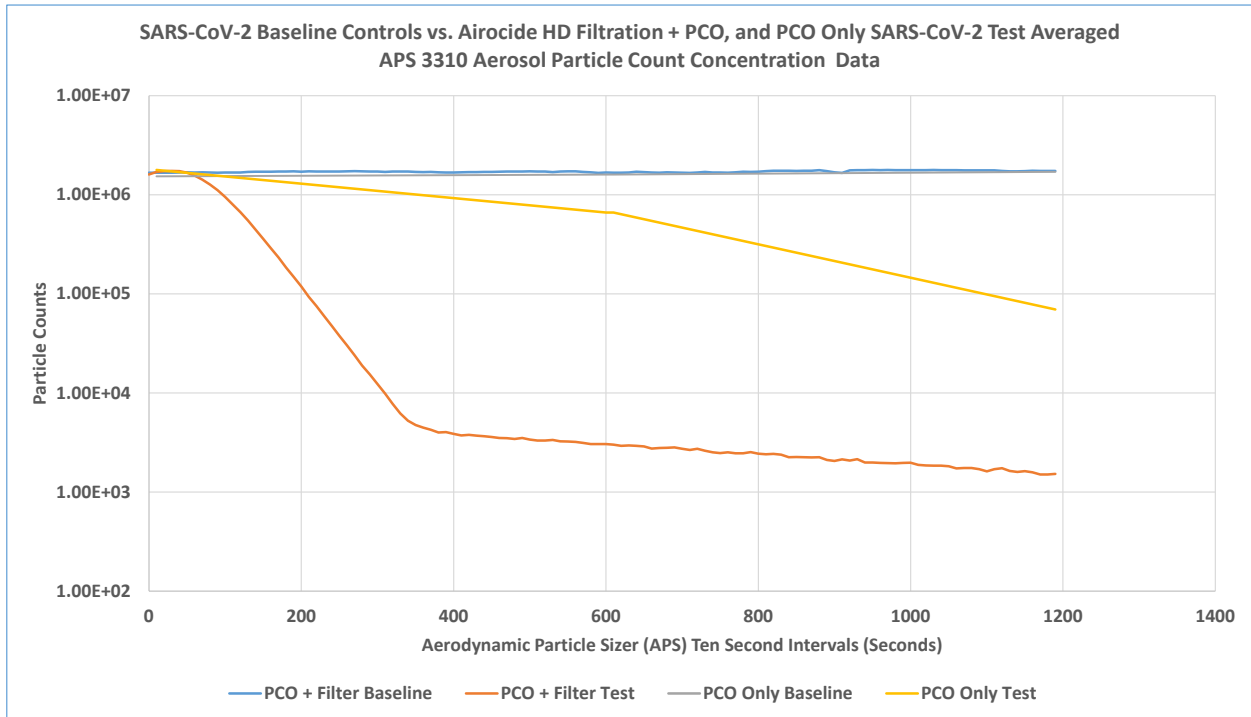


Figure 2. Aerodynamic Particle Sizer (APS) Aerosol Particle Count vs Sample Time Plot

Data showing 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 Phase 1 and Phase 2 testing sample time periods is shown in Table 3.

Table 3. APS Aerosol Count and Mass Test Results

Phase 1. Airocide HD Test Device (PCO and Filter) APS (0.5L sample)									
	Test 1			Test 2			Test 3		
Test ID - Sample time	T1-0	T1-10	T1-20	T2-0	T2-10	T2-20	T3-0	T3-10	T3-20
Particle counts	1606776	3657	1851	1766370	3498	1815	1775955	1968	909
Conc. (mg/m ³)	9.58	5.97E-02	2.34E-03	8.48	5.12E-03	2.43E-03	9.95	2.94E-03	1.14E-03
Diameter (um)	3.25	1.57	1.29	3.15	1.5	1.42	3.31	1.47	1.23
	Baseline control 1			Baseline control 2			Baseline control 3		
Test ID - Sample time	C1-0	C1-10	C1-20	C2-0	C2-10	C2-20	C3-0	C3-10	C3-20
Particle counts	1614219	1723023	1808763	1643337	1771818	1753686	1755189	1510587	1656726
Conc. (mg/m ³)	9.18	10.00	9.12	8.98	12.00	11.00	12.00	8.42	9.19
Diameter (um)	3.22	3.79	4.23	3.36	4.19	4.71	3.7	4.55	4.65
Phase 2. Airocide HD Test Device (PCO only) APS (0.5L sample)									
	Test 1			Test 2			Test 3		
Test ID - Sample time	T1-0	T1-10	T1-20	T2-0	T2-10	T2-20	T3-0	T3-10	T3-20
Particle counts	1761114	1076734	139151	1807031	551365	45295	1775004	359750	24909
Conc. (mg/m ³)	12.8	6.34	0.421	12.6	2.72	0.109	12	1.42	4.58E-02
Diameter (um)	4.45	4.97	3.74	4.66	4.93	3.32	4.74	4.43	2.99
	Baseline control 1			Baseline control 2			Baseline control 3		
Test ID - Sample time	C1-0	C1-10	C1-20	C2-0	C2-10	C2-20	C3-0	C3-10	C3-20
Particle counts	1499471	1509495	1660007	1552659	1659681	1755062	1554703	1628002	1696045
Conc. (mg/m ³)	9.85	9.42	10.9	9.78	10.9	11.7	9.82	10.4	11
Diameter (um)	3.37	3.56	3.95	3.51	3.9	4.21	3.44	3.8	4.13

Particle size distributions were also measured with the APS. A plot showing a 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 3.74 μm , with 50% of the aerosol particle mass below and 50% above the median diameter. The 15.87 percent mass (1.76 μm) and 84.14 percent (7.39 μm) particle mass points are also shown.

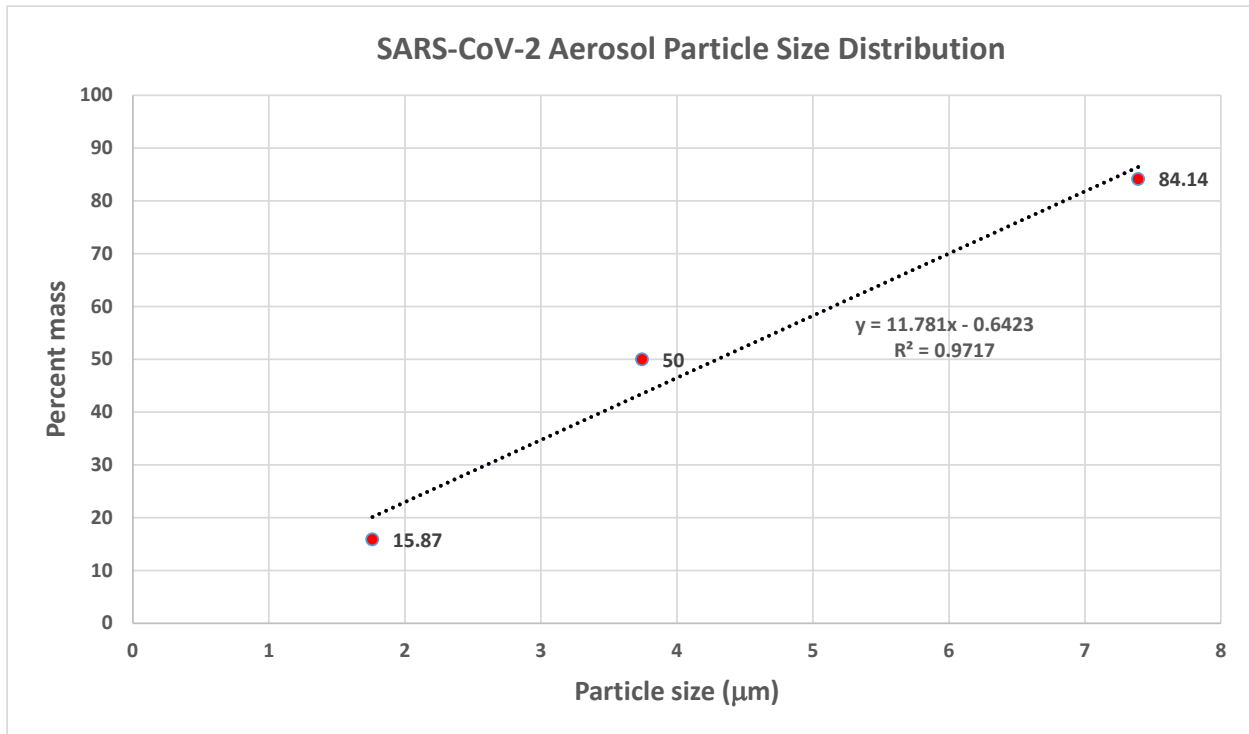


Figure 3. Aerodynamic Particle Sizer (APS) Aerosol Particle Size Distribution Plot

Section 8. Conclusions

Based on these experiments, we conclude that the Airocide HD (filtration + PCO) is very effective at removing aerosolized SARS-CoV-2 virus from the air with a 3.25 log (99.94%) within five (5) minutes and thirty (30) seconds of operation. The Airocide HD (PCO only) Test Device showed a high level of progressive viable virus deactivation from 0.61 log (75.52%) during the aerosol generation process to 2.02 log (99.05%) during the twenty (20) to thirty (30) minute test sample time iteration in relation to baseline control results.