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Testing of 3000 Air Purification Device on Elimination of Aerosolized SARS-CoV-2

Final Report

FOR

AirDoctor, LLC
An Affiliate of Ideal Living Management, LLC

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MRIGlobal Project No. 311791.01.001

October 11, 2021

Preface

This Final Report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311791.01.001, "Testing of AirDoctor 3000 Air Purification Device on Elimination of Aerosolized SARS-CoV-2." The test was initiated by MRIGlobal on September 3, 2021 and ended on September 8, 2021.

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

All operations pertaining to this study, unless specifically defined in this protocol, were performed according to the Standard Operating Procedures of MRIGlobal or approved laboratory procedures.

All study records are stored at MRIGlobal.

Sincerely,

MRIGLOBAL

Richard Tuttle

Rick Tuttle
Principal Scientist
Life Sciences Division

Approved:

Claire R Croutch

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

October 11, 2021

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Section 1.
Objective:

The objective of this project was to measure the efficacy of the AirDoctor® 3000 air purification device ("Test Device") in elimination of aerosolized SARS-CoV-2 in controlled tests conducted at MRIGlobal. The Client provided an AirDoctor 3000 air purifier for testing. The Test Device incorporates an UltraHEPA® filter for removal of particles from the air and a high-quality carbon filter for the removal of volatile organic compounds ("VOCs"). The Test Device has four (4) different fan speed settings to choose how rapidly to clean the air, low, medium, high, and boost. For this testing, the Test Device was evaluated for aerosol removal of SARS-CoV-2 aerosols on fan speed 1 (low setting) for all conducted tests. Aerosol Test Device challenges were conducted in a primary aerosol containment system within a Class III biological safety cabinet. MRIGlobal characterized the Test Device to evaluate the log reduction effectiveness against an enveloped virus (SARS-CoV-2).

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor's Representative

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2.2 Testing Laboratories

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2.3 Personnel Responsibilities

2.3.1 Study Director—MRIGlobal

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2.3.2 Task Lead—MRIGlobal

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2.3.3 Analyst—MRIGlobal

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Section 3.
Test Materials

3.1 Test Units

AirDoctor® 3000 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.: 20210702CHB
Expiration date: 12/21

3.3 Challenge Virus

Severe Acute Respiratory Syndrome-related Coronavirus-2 (SARS-CoV-2)
Strain: USA-WA1/2020
Lot: 20210401KS-B
Passage: 12

3.4 Cell Host

Vero E6 Cells
Vendor: ATCC
Cat: CRL 1586
Passage No.: 53

3.5 Laboratory

Biosafety Level 3 (BSL-3) laboratory
MRIGlobal, Kansas City MO

Section 4.
Test System

MRIGlobal utilized the USA-WA1/2020 strain of the SARS-CoV-2 virus obtained from The University of Texas Medical Branch (UTMB) from an isolate of a patient who traveled to an infected region of China and developed the clinical disease (COVID-19) in January 2020, in Washington state, USA. The complete genome of USA –WA1/2020 has been sequenced. The Isolate-GenBank: MN985325 and after one passage in in Vero cells GenBank: MT020880. The complete genome of SARS-CoV-2 strain USA-WA1/2020 has been sequenced after four passages in collaboration with Database for Reference Grade Microbial Sequence (FDA-ARGOS; GenBank: MT246667).

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. 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.

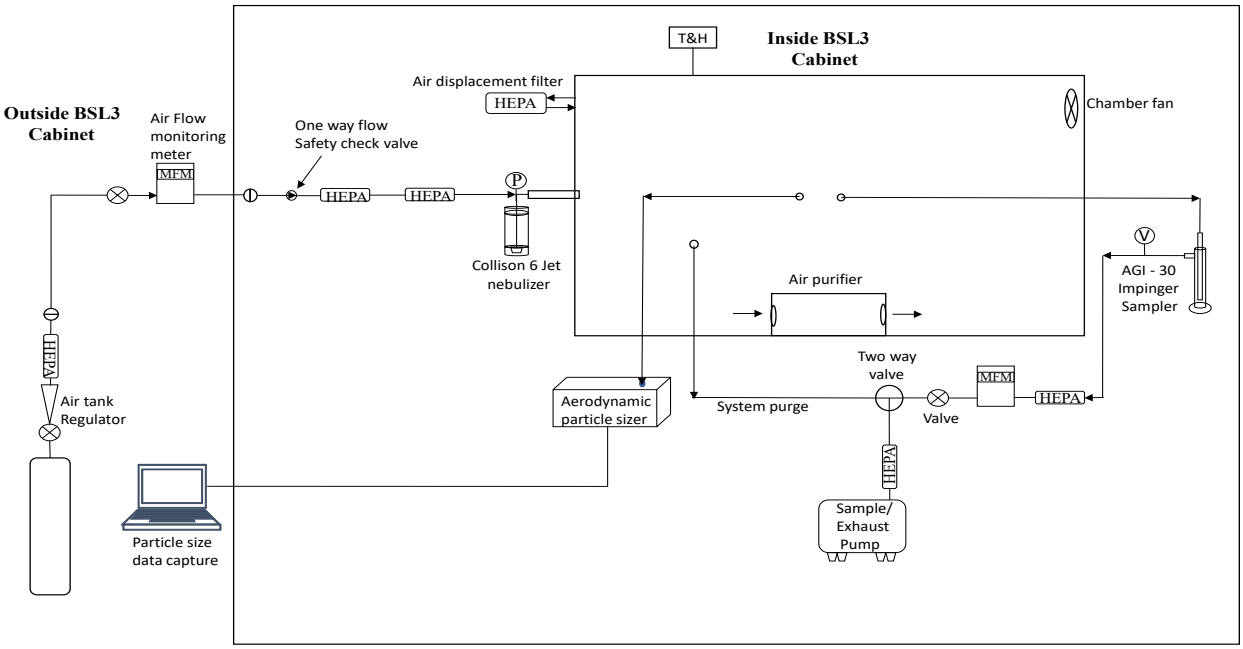

Figure 1. Aerosol System Design

Section 5.
Study Design

Testing was conducted in three (3) independent test replicates to evaluate the Test Device in removing SARS-CoV-2 aerosols from the test environment. Preceding test and evaluation of the Test Device, aerosol characterization tests were performed to establish baseline (control) standard results for subsequent evaluation of the Test Device performance. 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 the device tests. For establishing pre-test viral concentration baseline (control) standard results, the select Test 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 6.81E6 $TCID_{50}$/mL) was aerosolized with a Collison 6-jet nebulizer into a closed testing chamber for ten (10) minutes for each conducted test. The Test Device was evaluated in three independent tests. The device was tested at low fan speed setting with an air recirculation rate of approximately 90 cubic feet per minute (CFM), or 2547 liters per minute (LPM). The test matrix showing test conditions aerosol testing is shown in Table 1.

Table 1. Test Matrix

Test description	Test Time (min)	Collison 6 jet aerosol generator operation (psia)	Collison 6 jet ~ flow rate (L/min)	Collison 6 jet generation time (min)	Test Device Air displacement rate (L/min)	Test Device operation time, 10x aerosol test system diplacments (seconds)	Collison 6 jet test generation time (min)	Impinger Type	Impinger test sample times (sec:min)	APS particle size test sample times (min)	Total number of tests	Number of Impinger samples /test
Baseline characterization testing no device operation. Chamber fan only operation	30	26	15	10	NA	NA	t = -10-0	AGI-30	t = 88 seconds to t = 20 minutes	t = 0 to 20, 10 second sequential samples = 120 samples	3	1
AirDoctor 3000 test, unit operational following aerosol process	30	26	15	10	2547	t = 0 to t = 88 seconds low fan speed	t = -10-0	AGI-30	t = 88 seconds to t = 20 minutes	t = 0 to 20, 10 second sequential samples = 120 samples	3	1

For testing, an aerodynamic particle sizer (APS) was used to evaluate the count, mass, and particle size of resident aerosols during the baseline control, and Test Device trials. The APS was programmed to take sequential ten (10) second aerosol scans over the course of each test for near real time observation of the Test Device aerosol removal performance. As shown in Table 1, evaluation of the Test Device performance was established for a total air exchange rate of ten (10) times the displacement volume of the aerosol test chamber. The operation time of the Test Device following the aerosol generation process was defined to be eighty-eight (88) seconds based on a chamber displacement of 370 L as related to the device flow rate of 2547 L/min. For

aerosol collection following Test Device operation, an AGI-30 impinger model 7540 (Ace Glass, Inc.) filled with 20 mL of DMEM media was used for collection of resident aerosols. Aerosol samples were collected at the same time points from eighty-eight (88) 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 concentration characteristics in the chamber 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, the Collison 6-jet nebulizer was filled with a fresh aliquot of 8 mL of SARS-CoV-2 (6.81E6 $TCID_{50}$/mL). Aerosol samples for each test were collected from the aerosol test chamber using AGI-30 impingers (Ace Glass, Inc.) filled with 20 mL of sterile DMEM/F12 collection media. 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. For each Test Device and baseline standard (control) test, 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 $TCID_{50}$ of the samples. These dilutions were incubated forty-five (45) minutes, after which DMEM/F12 supplemented with 5% FBS was added to cells to feed them for the next four (4) to five (5) days. This incubation period allowed the virus to adsorb 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 equations below, 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.

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

Section 7.
Results

Aerosol plates were read four days after the conduct of testing. The AirDoctor 3000 air purifier reduced viral infectivity by 3.59 log (99.974%) within eighty-eight (88) seconds of operation in relation to baseline control results. Table 2 summarizes these findings and shows individual test sample results with test averaged reduction results for testing.

Table 2. $TCID_{50}$/mL Calculations for aerosol testing of AirDoctor™ 3000 Air Purifier

Sample Type	Test Duration	Test Description	Replicate #	TCID50/mL	Log10 TCID50/mL	Average TCID50/mL	Average Log10 TCID50/mL	Log Reduction	Percent Log Reduction
Air Purifier Test	20 min	Test	1	≤3.51E-01	-0.45	4.43E-01	-0.37	3.59	≥99.974%
			2	≤3.51E-01	-0.45				
			3	≤6.25E-01	-0.20				
Baseline Control	20 min	Control	1	1.76E+03	3.25	1.66E+03	3.22		
			2	1.76E+03	3.25				
			3	1.46E+03	3.16				
SARS-CoV-2 Stock		Backtiter		6.81E+06	6.83				

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.

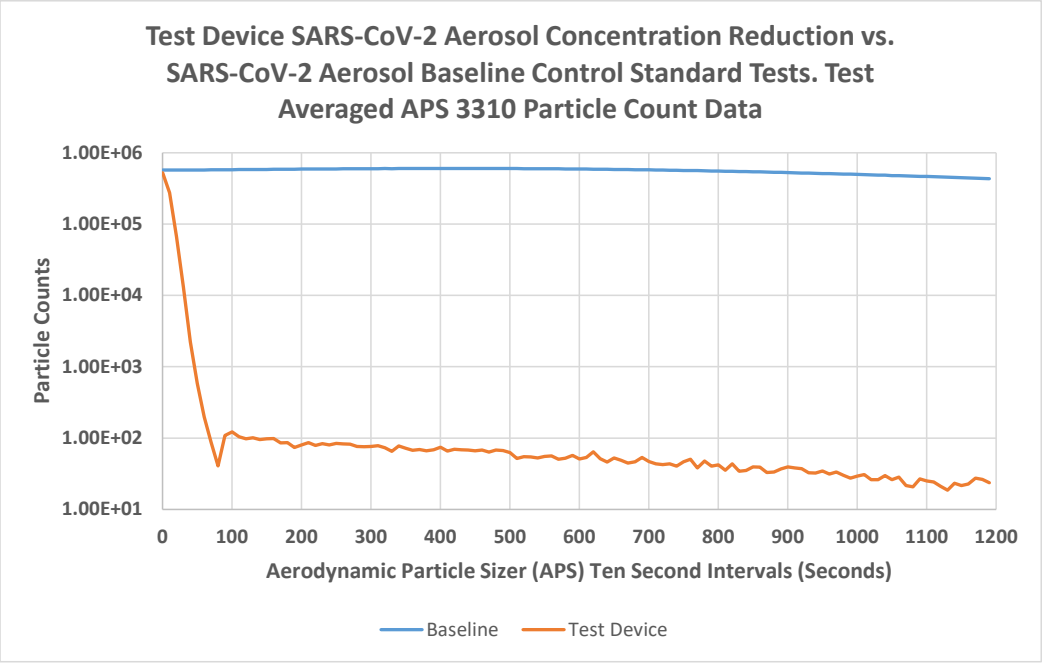

Figure 2. Aerodynamic Particle Sizer (APS) Aerosol Particle Count vs Sample Time Plot

Data showing APS scan data during representative Test Device and baseline control tests operation times representing the 10, 50, and 90 second intervals is shown in Table 3. The data shows the air purifier performance in count concentration, mass (mg/m^3), and mass median particle size reduction in relation to baseline control standard test results.

Table 3. APS Aerosol Count and Mass Test Results

Test Device	Test 1			Test 2			Test 3		
Test ID - Sample time (seconds)	T1-10	T1-50	T1-90	T2-10	T2-50	T2-90	T3-10	T3-50	T3-90
Particle counts	471671	1263	18	433968	1150	51	569749	4257	53
Conc. (mg/m^3)	6.89	9.70E-03	1.10E-04	5.68	7.80E-03	2.80E-04	5.44	8.30E-03	5.20E-04
Diameter (um)	2.77	1.72	1.56	2.71	1.65	1.72	2.67	1.76	1.68
Control	Baseline control 1			Baseline control 2			Baseline control 3		
Test ID - Sample time (seconds)	C1-10	C1-50	C1-90	C2-10	C2-50	C2-90	C3-10	C3-50	C3-90
Particle counts	565685	571393	573312	566528	569402	571937	576050	576320	578150
Conc. (mg/m^3)	12.35	12.44	12.44	12.27	12.21	12.10	12.43	12.36	12.30
Diameter (um)	3.41	3.48	3.51	3.44	3.43	3.44	3.45	3.48	3.46
	10 seconds			50 seconds			90 seconds		
	Baseline Average	Test Average	Percent Reduction	Baseline Average	Test Average	Percent Reduction	Baseline Average	Test Average	Percent Reduction
Particle counts	569421	491796	13.63	572372	2223	99.61	574466	41	99.99
Conc. (mg/m^3)	12.35	6.00	51.39	12.34	8.60E-03	99.93	12.28	3.03E-04	99.998

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 3.32µm, with 50% of the aerosol particle mass below and 50% above the median diameter. The 15.87 percent mass (1.71µm) and 84.14 percent (6.94 µm) particle mass points are also shown.

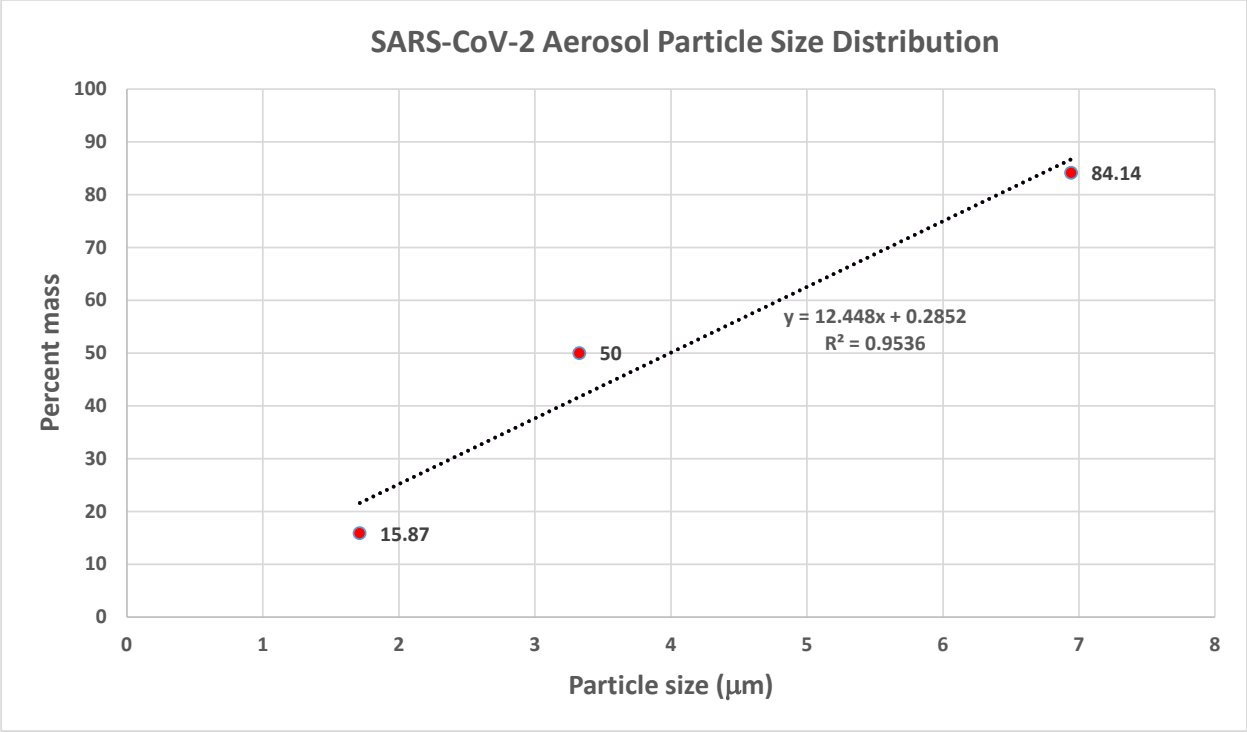

Figure 3. Aerodynamic Particle Sizer (APS) Aerosol Particle Size Distribution Plot

Section 8.
Conclusions

The AirDoctor® 3000 is effective at removing aerosolized SARS-CoV-2 virus from the air. The device showed a 3.59 log (99.97%) reduction in viable virus from the test system within eighty-eight (88) seconds of operation as compared to the natural SARS-CoV-2 aerosol viability concentrations obtained from control test results. The eighty-eight (88) second device operation represents approximately ten (10) air exchanges of the test system volume through the Test Device. The Test Device 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 purifier 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 ten (10), fifty (50), and ninety (90) second intervals. These results show a reduction in aerosol mass and median size within the first ten (10) seconds of operation, with enhanced removal (~ 3 Logs) within forty (40) to fifty (50) seconds of operation. Specifically, and as shown in Table 3, after 10 seconds, the aerosol particle count was reduced by 13.63% and the particle mass concentration was reduced by 51.39%. After 50 seconds, the aerosol particle count was reduced by 99.61% and particle mass concentration was reduced by 99.93%. After 90 seconds, the aerosol particle count was reduced by 99.99% and particle mass concentration was reduced by 99.998% . The Test Device had nearly complete removal of particles and mass within the eighty (80) to ninety (90) second operation time periods (~ 4 logs) which corresponds to the $TCID_{50}$ assay results (Table 2), and data shown in the particle reduction graph Figure 2.

Section 9.
Quality Assurance

This study was executed at MRIGlobal in Kansas City, MO that is fully qualified to conduct GLP studies. This study was not conducted under GLP, although all procedures utilized were technically valid. Testing was conducted according to MRIGlobal Standard Operating Procedures and/or approved laboratory procedures.