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Characterization of a Flow-Through Air Purification Filtration Device in Removal of Aerosolized SARS-CoV-2

Final Report

FOR

Blueair, Inc.

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MRIGlobal Project No. 311727.01.001 (A)

April 13, 2021

Preface

This Final Report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311727.01.001 (A), "Characterization of a Flow-Through Air Purification Filtration Device in Removal of Aerosolized SARS-CoV-2."

Test devices were supplied to MRIGlobal by Blueair, Inc. for the conduct of the program. The experimental phase of this task was initiated by MRIGlobal on March 2, 2021 and ended on March 8, 2021.

The Study Director of the program was Rick Tuttle. Execution of the study was assisted by Kristen Solocinski, Ph.D., and Jacob Wilkinson, and managed by William Sosna.

The studies were performed in compliance with MRIGlobal QA procedures. 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, and any deviations were documented.

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Rick Tuttle
Study Director

Approved by:

Claire Croutch, Ph.D.
Portfolio Director
Medical Research

April 13, 2021

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Section 1.
Objective

COVID-19 infection originating from SARS-CoV-2 and the high rate of transmission associated severe illness and fatalities, has created a needed response for rapid development and evaluation of effective countermeasures. In response to testing for Blueair Inc., MRIGlobal conducted testing and evaluation of a Blueair Healthprotect™ Series 7400, Model 7413352000 Air Purifier product ("Test Device"). The Test Device has HEPASilent Ultra™ filtration technology which combines electrostatic and mechanical filtration to purify air. Blueair Inc., developed this technology to be effective for air disinfection in room size environments using an internal fan for multi pass large volume air recirculation and disinfection. The Test Device was evaluated in independent tests for efficacy in removal of SARS-CoV-2 aerosol challenges in laboratory trials at MRIGlobal.

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor

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2.2 Testing Laboratories

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2.3 Personnel Responsibilities

Study Director—MRIGlobal

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Section 3.
Test Systems and Methods

3.1 Equipment

Test Equipment

Blueair Healthprotect™ Series 7400, Model 7413352000 Air Purifier (Test Device).

3.2 Methods

Testing Description

MRIGlobal conducted testing characterization of the Blueair Healthprotect™ Series 7400, Model 7413352000 Air Purifier (Test Device) in viral aerosol removal to evaluate effectiveness against an envelope virus (SARS-CoV-2) strain USA-WA1/2020. USA-WA1/2020 was 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) January 2020 in Washington, 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). Each vial used on study contains approximately 0.5 mL of cell lysate and supernatant from Cercopithecus aethiops kidney cells infected with SARS-CoV-2 isolate USA-WA1/2020.

All tests were conducted in a high containment Biosafety Level 3 (BSL-3) laboratory at MRIGlobal, Kansas City, MO. Due to the impracticality and potential hazards associated with conducting large area aerosol dissemination studies with Class 3 human pathogens, MRIGlobal designed a scaled down aerosol containment cabinet to simulate a large room environment. The client provided an air purification unit (Test Device) for aerosol test chamber challenges. All tests were conducted using a common SARS-CoV-2 stock with known viral concentration. Stock virus was propagated at concentrations to obtain aerosol challenge conditions acceptable for evaluating the Test Device in viral removal at equal to or greater than 3 logs.

MRIGlobal fabricated a primary aerosol containment system (cabinet) to conduct evaluation of the Test Device within a biological Class III cabinet. The aerosol containment cabinet was fabricated out of Plexiglas with internal dimensions of 2.5ft tall × 3.5ft long × 1.5ft wide with a displacement volume of approximately 370 liters or 13.1 cubic feet. The Test Device fan speed was regulated at low fan speed (rating of ~ 60 cfm) for all tests. A diagram of the test system is shown in Figure 1.

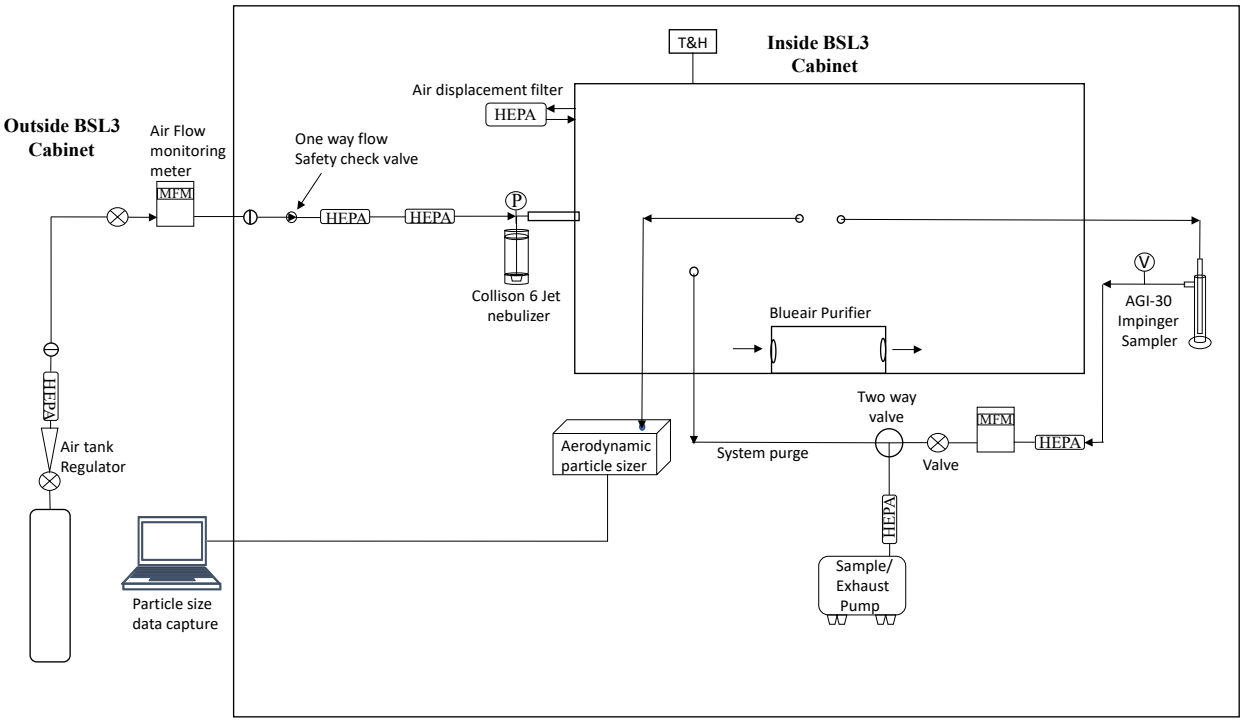

Figure 1. SARS-CoV-2 Aerosol Test System

For SARS-CoV-2 aerosol generation, a Collison 6 jet nebulizer ("Nebulizer") was filled with a fresh aliquot of 10 ml of viral DMEM stock suspension for each test. The Nebulizer was operated with tank supplied breathing grade air at a supply pressure of 26 psi to generate viral aerosol into the test cabinet at a flow rate of approximately 15 L/min. The test cabinet is adapted with a HEPA capsule filter to allow for the introduction of generated viral aerosol air supply flows, and air displacement introduction for aerosol sampling during testing. The bio-aerosol test system was fabricated for nebulizer adaptation, aerosol and sample dilution air displacement filtration, air supply regulation and control, sample flow regulation, particle size measurement, and temperature and humidity monitoring. Aerosol generation and sampling system pressures and flow rates were monitored using calibrated and regulated digital mass flow meters.

An Aerodynamic Particle Sizer (APS) was utilized to sample during baseline standard testing, and Test Device challenges for particle size distribution measurement and particle count concentration measurements. 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 20µm. 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. All tests were conducted using a common stock of SARS-CoV-2 prepared in DMEM suspension at a concentration of 1.47×10^7 $TCID_{50}$ per milliliter. Pre – device test characterization of the viral aerosol concentration was performed to establish baseline (control) results for subsequent Test Device viral removal efficacy. A test matrix showing the baseline control and Test Device associated testing parameters is shown in Table 1.

Table 1. Test Matrix

Test description	Test Time (min)	SARS-Cov-2 stock supension media	Collison 6 jet aerosol generator operation (psia)	Collison 6 jet ~ flow rate (L/min)	Collison 6 jet generation time (min)	Collison 6 jet test generation time pre - test (min)	AGI-30 Impinger sample flow rate (L/min)	AGI-30 Impinger test sample times (min)	APS particle size test sample time (min)	Total number of tests
Baseline characterization testing, no device operation. Chamber fan only operation	30	DMEM	26	15	10	t = -10-0	12.5	t=5 to t=20	t = 0 to 10 (30 second sequential) = 20 samples	3
Device test, unit operational From T=0 to T=5 minutes after aerosol generation	30	DMEM	26	15	10	t = -10-0	12.5	Device operation t=0 to t=5min, Impinger Sample t=5 to t=20min	t = 0 to 10 (30 second sequential) = 20 samples	3

For conducting and characterizing the viral aerosol viability and establishing natural aerosol decay, the Test Device was placed in the center bottom of the test system with only a low flow test chamber recirculation fan operational (Test Device off). This provided uniform mixing and a homogeneous concentration of generated aerosols. The test chamber fan was operated throughout the entirety of characterization control testing to provide aerosol mixing and recirculation conditions in the chamber similar to that produced during operation of the Test Device. For each conducted control test, the Collison nebulizer was operated over a ten (10) minute aerosol generation period, the nebulizer was turned off, and aerosol viral sampling from the chamber initiated. SARS-CoV-2 aerosol sample collection and measurement of the viral removal efficacy were derived from impinger samples (AGI-30, model 7540) taken over set time durations and sample flows from a common sample location during all conducted tests. The aerosol sample impingers have a high collection efficiency rating and are designed for biological aerosol collection. Between each conducted test, resident test system aerosols were evacuated with an equipped exhaust pump and the condition verified for total particle evacuation with the APS 3321 analyzer.

Section 4.
Sample Analysis and Results

A common stock virus suspension was used for all conducted tests (SARS-CoV-2, strain USA-WA1/2020) at a verified concentration of 1.47×10^7/ml by serial dilution to obtain the 50% tissue culture infectious dose ($TCID_{50}$). This was conducted to ensure that a sufficient concentration and quantity of virus were available for testing. The titer was confirmed prior to each test day and verified that the concentration remained at 1.47×10^7/mL for all tests. For cell and virus cultures, sterile DMEM (Mediatech) supplemented with 7% fetal bovine serum (HyClone), GlutaMax (Gibco), and penicillin-streptomycin-neomycin antibiotic mixture (Gibco) were utilized. Vero E6 cells (monkey kidney cells) originally obtained from ATCC (CRL-1586) were used for assays with ASFV. All cells were maintained at 36°-38°C and 5% CO_2 in a humidified atmosphere, and cells were seeded into flasks for propagation and expanded into 96 well plates for titration of SARS-CoV-2 virus.

Impinger collection media samples from control and Test Device testing were diluted 1:10 down a 96 deep well plate in DMEM/F12. These dilutions were transferred to a plate of Vero cells with media removed. The 10X serial dilution of Impinger sample media were applied to cell assay plates at up to an 8 log dilution factor for the presence of viral growth into the plate host cells. Plates were inoculated with 5 replicate samples at each dilution level, with each row of replicates 10X more dilute than that used in the preceding row for viral cell infectivity detection. After approximately 20 minutes, DMEM/F12 supplemented with 5% FBS was added to cells to feed them for the next 5 days. This incubation period allowed the virus to adsorb to cells without interference from FBS. Cells were infected with viral impinger sample media at 70% confluence and observed for the presence of cytopathic effect (CPE) for four (4) to five (5) days post-infection.

After the incubation time, cells were examined for the presence of cytopathic effect (CPE) associated with viral presence and replication. Examination is 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.

Viral propagation plate readings were conducted under high intensity magnification of each plate cell for viral host cell infectivity and recorded on a sample test log for positive (+) or negative (-) viral propagation. Data was entered into a Reed Muench calculation for sample concentration measurement and determination of the $TCID_{50}$ (50% tissue culture infectious concentration of virus). 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.

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

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

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

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.

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

Test Results:

AGI-30 impinger samples were analyzed as described above for both in triplicate control tests, and the in triplicate Test Device efficacy tests. Collected samples were poured into sterile 50 ml labeled sterile conical tubes following each aerosol collection time point, and transported to a dedicated Class II biological safety cabinet for assay and viable viral analysis. Log reduction with the percent viral removal efficiency results for baseline control characterization testing, and the Test Device is shown in Table 2.

Table 2. Test Results for Blueair Purifier Viral Removal Efficacy

Test Description	AGI-30 Impinger Sample ID	Viral Sample Concentration TCID50/ml	log10 TCID50	Averaged Control Testing TCID50	Averaged Control Testing log10	Test Device log 10 Reduction	Test Device Calculated Percent (%) Viral Reduction
Baseline characterization testing, no device operation. Chamber fan only operation	Control 1	7.01E+03	3.845757	1.81E+04	4.188615	n/a	n/a
	Control 2	1.76E+04	4.245757				
	Control 3	2.98E+04	4.474329				
Device test, unit operational From T=0 to T=5 minutes after aerosol generation	Test 1	3.51E-01	-0.45469	3.51E-01	-0.45469	4.643307517	99.998%
	Test 2	3.51E-01	-0.45469				
	Test 3	3.51E-01	-0.45469				

Particle size and aerosol count analysis was conducted for each of the in triplicate control, and Test Device aerosol tests with the APS programmed for sequential 30 second sampling. Particle samples were initiated following the aerosol nebulization process. Following the initial Test Device APS sample at time = 0 following aerosol generation, it was observed that the aerosol concentration in the chamber had been totally removed within the first one (1) minute of the test with little to no detected aerosol counts from APS sample analysis in subsequent test samples. This was evident for all three of the tests conducted with the Test Device operational, which also coincides with the negative $TCID_{50}$ Plate assay results shown in Table 2. A table showing APS sample concentration results is shown in Table 3.

Table 3. APS Aerosol Count and Mass Test Results

APS aerosol particle samples - control and test data results, 30 second sequential samples (0.5 L/sample)													
Sample		Control 1		Control 2		Control 3		*Test 1		Test 2		Test 3	
Time (sec)	sample #	counts	mass mg/m3	counts	mass mg/m3	counts	mass mg/m3	counts	mass mg/m3	counts	mass mg/m3	counts	mass mg/m3
0-30	1	1232069	9.75	1202113	8.42	1107829	8.16	340876	1.4	740084	5.18	700332	5.6
30-60	2	1215020	9.28	1178661	7.97	1125466	8.08	33	4.10E-05	159	5.52E-03	155	4.54E-04
60-90	3	1219999	9.02	1190075	8.16	1101134	7.95	2	3.43E-06	1	2.92E-07	3	5.71E-06
90-120	4	1260289	9.19	1183077	8.05	1111493	8.38	1	1.16E-06	1	6.67E-06	1	4.26E-03
120-150	5	1266272	9.1	1158966	7.61	1153704	8.13	3	2.63E-06	0	0	1	7.58E-04
150-180	6	1267513	8.88	1153552	7.84	1165693	7.95	2	2.62E-06	0	0	1	9.40E-07
180-210	7	1270160	8.67	1173695	8.22	1161128	7.78	1	9.41E-04	0	0	0	0
210-240	8	1283726	8.67	1195222	8.5	1162060	7.78	0	0.00E+00	2	4.50E-06	0	0
240-270	9	1313710	8.84	1194677	8.54	1185311	7.68	1	2.71E-04	2	3.89E-06	1	1.67E-04
270-300	10	1333064	8.96	1172570	8.21	1195402	7.8	0	0	2	3.02E-03	0	0
*Note operator error. Test 1, 0 - 30 second APS sample interval activated approximately 10 seconds late following Test Device operation start, resulting in low initial aerosol counts													

The tabulated data also shows the net particle mass (mg/M^3) from each APS sample with the resident mass concentration at each sample interval for control tests, as well as the test Device trials. A plot of particle counts per sample (0.5 liters/sample) derived from the APS data in Table 3 for Control and Test Device operation tests is shown in Figure 2.

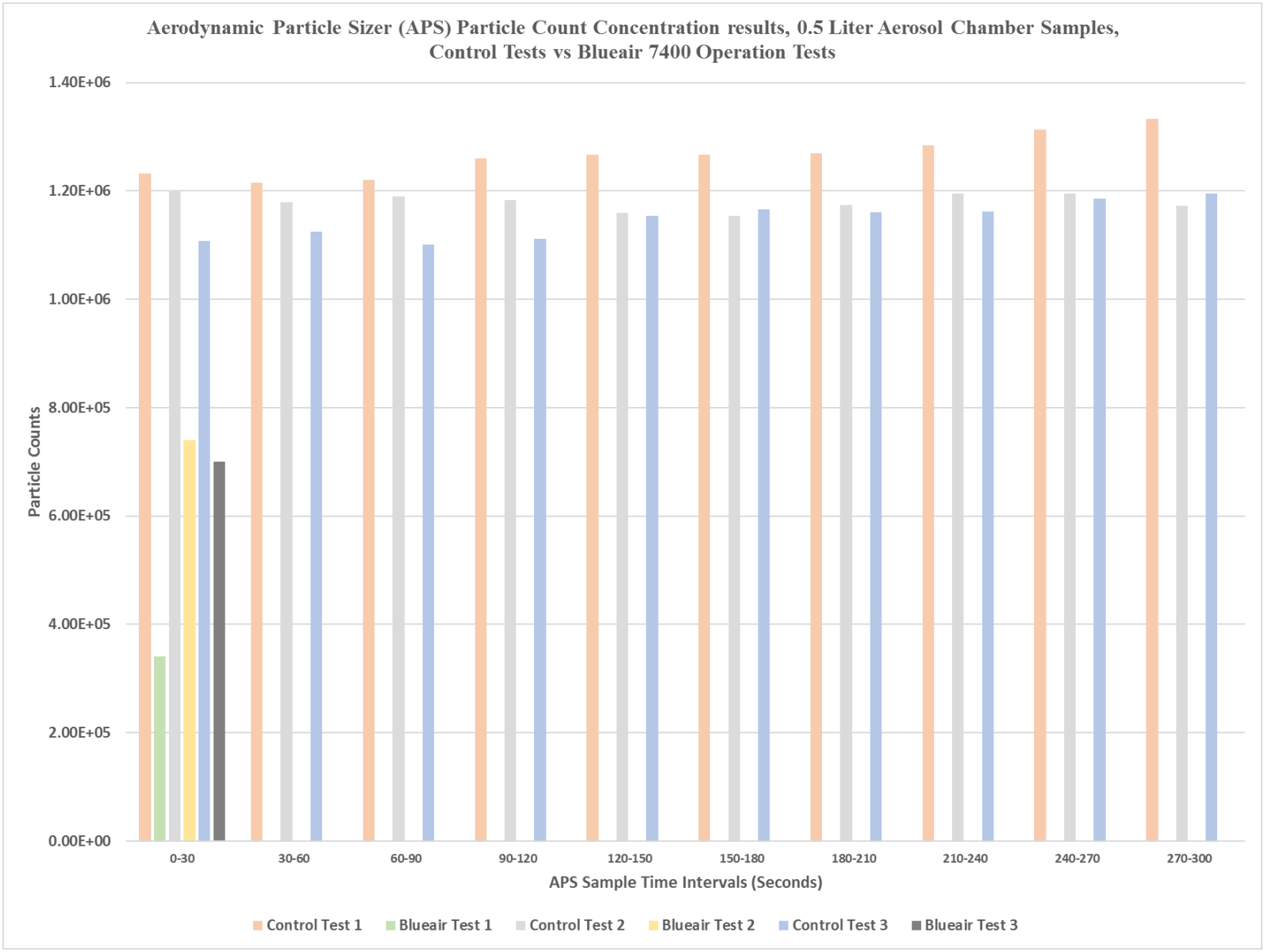

Figure 2. Aerodynamic Particle Sizer (APS) Aerosol Particle Count vs Sample Time Plot

Particle size distributions were also measured with the APS. A plot showing a representative SARS-CoV-2 aerosol particle size distribution 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 3.4 µm, with 50% of the aerosol particle mass below and 50% above the median diameter.

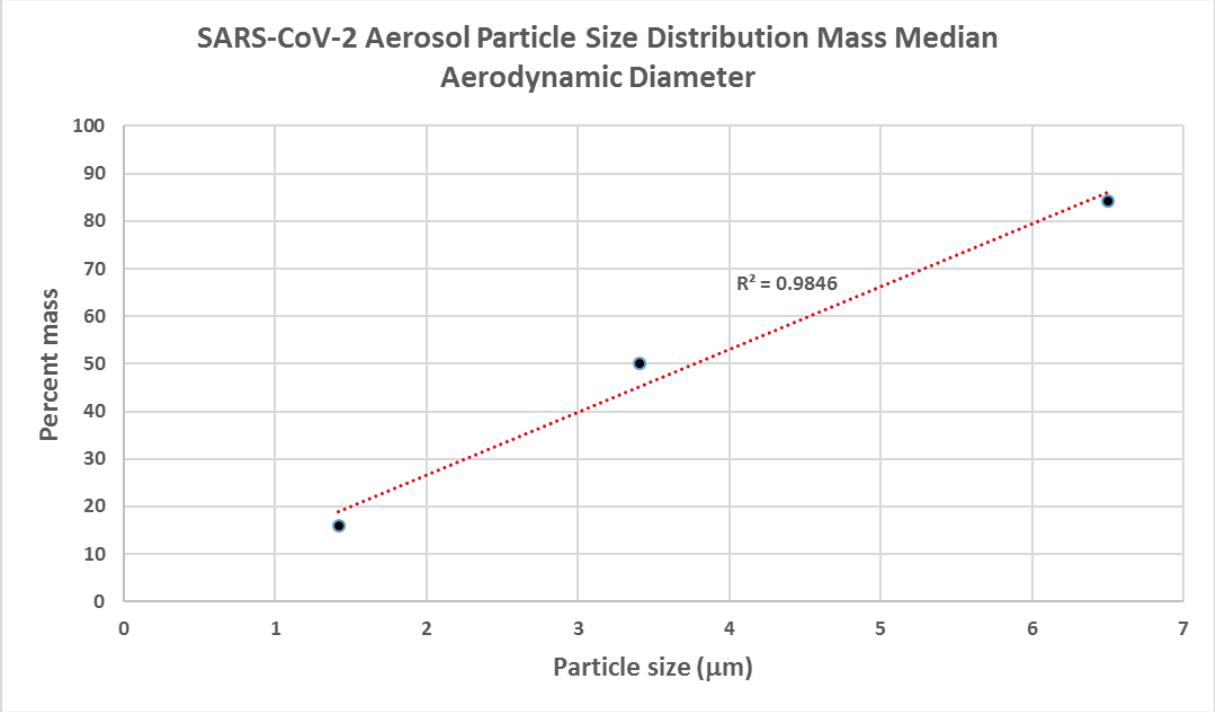

Figure 3. Aerodynamic Particle Sizer (APS) Aerosol Particle Size Plot

Conclusions:

The Blueair Series 7400 (Model 7413352000) air purification system showed very quick removal of resident SARS CoV-2 aerosol in the test chamber for all conducted tests. The aerosol particle concentration was reduced from an average concentration of over 1 million particles per half liter of air sampled to a near zero count reading per liter of air within one (1) minute of operation. The viable viral concentration was also non - detectable in any of the Test Device impinger test samples in three (3) conducted tests. The Test Device showed a greater than 3 log (99.998%) reduction of viable viral aerosol as compared to the three (3) conducted control tests conducted without the Test Device operational. The Test Device also showed a high particle removal efficiency and reduction of SARS-CoV-2 aerosols within the size distribution range shown from the particle size distribution graph in Figure 3.

Section 5.
Quality Assurance

5.1 Type of Study

This study was executed using established SOPs, 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. The study was conducted according to MRIGlobal Standard Operating Procedures and/or laboratory procedures.

5.2 Standard Operating Procedures

The study was performed according to the relevant standard operating procedures and/or laboratory procedures of MRIGlobal.

Section 6.
Location of Study Data

Exact copies of all raw data, correspondence, records, final protocol, amendments, and deviations, and any other study documentation necessary for reconstruction of the study will be archived at MRIGlobal. All raw data (including original study records, data sheets, work sheets, and computer printouts) will be archived by MRIGlobal.