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Characterization of a Flow–Through Air Purification Device In Deactivation Of SARS-CoV-2

Final Report

FOR

Aurabeat Technology Limited

Unit 651, 6/F., Building 19W, No.19 Science Park West Avenue, Hong Kong Science Park, Pak Shek Kok, N.T., Hong Kong

MRIGlobal Project No. 311717.01.001

January 8, 2021

Preface

This final report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311717.01.001, "Characterization of a Flow-Through Air Purification Device in Deactivation of Aerosolized SARS-CoV-2."

Test devices were supplied to MRIGlobal by Aurabeat Technologies Limited for the conduct of the program. The Flow-Through Air Purification Device is marketed under the product names: Aurabeat AG+ Sanitizing Air Purifier (Model NSP-X1), Brondell Pro Sanitizing Air Purifier with AG+ Technology by Aurabeat (Models P700BB-W & P7004C-W), and AG+ Pro Air Purifier (Model NSP-X2). The experimental phase of this task was initiated by MRIGlobal on May 7, 2020 and ended on December 4, 2020.

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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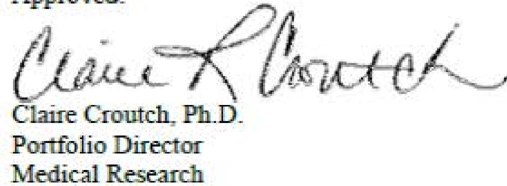
Rick Tuttle
Study Director

Approved:



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January 8, 2021

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Section 1. Objective

The emergent threat of 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 Aurabeat Technologies Limited, MRIGlobal conducted testing and evaluation of an Air Purifier product. The purifier is marketed under the following product names: Aurabeat AG+ Sanitizing Air Purifier (Model NSP-X1), Brondell Pro Sanitizing Air Purifier with AG+ Technology by Aurabeat (Models P700BB-W & P7004C-W), and AG+ Pro Air Purifier (Model NSP-X2). The air purifier will be refered to as ("Test Device") in this report. Aurabeat Technologies Limited 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 destruction of SARS-CoV-2 aerosol challenges in laboratory trials at MRIGlobal.

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Section 2. Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor

Aurabeat Technologies Limited, Unit 651, 6/F., Building 19W, No.19 Science Park West Avenue, Hong Kong Science Park, Pak Shek Kok, N.T., Hong Kong

2.2 Sponsor's Representative

Dr. Roger Sze To

2.3 Testing Laboratories

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2.4 Personnel Responsibilities

Section 3. Test Systems and Methods

3.1 Equipment

Test Equipment

Aurabeat air purifier (Test Device) with dimensions of 390 × 211 × 628 mm. The unit was delivered with 110V power supply and voltage regulator for fan speed control.

3.2 Methods

Testing Description

MRIGlobal conducted testing characterization of the Aurabeat Air Purifier in viral aerosol decontamination trials to evaluate the log reduction destructive kill 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) in Washington, USA in January 2020. 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 Biosafety Level 3(BSL-3) facility at MRIGlobal, Kansas City, MO. Due to the impracticality and potential hazards associated with conducting large area aerosol dissemination studies with safety level 3 human pathogens, MRIGlobal designed a scaled down aerosol containment cabinet to simulate a large room environment. The client provided an air purification unit (Aurabeat AG+ Sanitizing Air Purifier Model NSP-X1) with a voltage regulator to scale down the units large room flow recirculation rate for aerosol test chamber challenges. The Test Device under normal operating conditions with full power would produce a room air flow recirculation rate of approximately 7000 L/minute. A supplied voltage regulator was utilized to provide a continuous and regulated supply of 50V to regulate the Test Device flowrate to 100L/minute to accommodate the smaller size of the aerosol containment system. This provided approximately sixteen (16) test chamber air exchanges every hour to provide test results that can be extrapolated into a larger real life room size. This 100L/minute flow rate setting represents the unit running at 1.428% of capacity as compared to the standard 7000 L/min large room operation condition. For this testing, MRIGlobal fabricated a primary aerosol containment system (cabinet) to conduct evaluation of the Test Device. Tests were conducted at MRIGlobal inside a Biological Class III Safety Cabinet in a high containment BSL-3 laboratory using a common SARS-CoV-2 stock with known viral concentration. The aerosol containment cabinet was fabricated out of Plexiglas with internal dimensions of 30 inches tall × 3.5ft long × 1.5ft wide with a displacement volume of approximately 370 liters or 13 cubic feet. The Test Device fan speed was regulated for all tests at 50 volts with the power regulator plugged into a standard 110V receptacle. A diagram of the test system is shown in Figure 1.

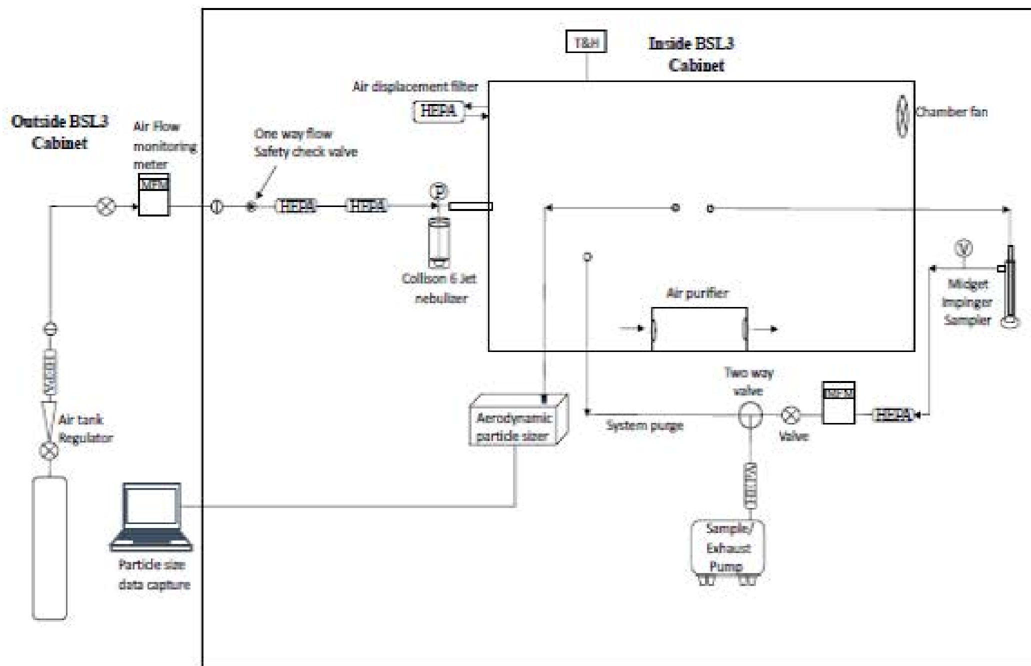

Figure 1. SARS-CoV-2 Aerosol Test System

Testing was conducted to obtain conditions that provided aerosol challenge concentrations acceptable for evaluating the Test Device in viral deactivation reduction at equal to or greater than 3 logs. 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 baseline standard and test aerosols for particle size distribution measurement and particle count concentration at time intervals corresponding to impinger samples during each test. 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. All tests were conducted using a common stock of SARS-CoV-2 prepared in DMEM suspension at a concentration of 5×10^6 plaque forming units per milliliter. Pre – device test characterization of the viral aerosol delivery efficiency and time

weighted viable aerosol concentration testing was performed to establish baseline (control) results for subsequent viral deactivation efficacy of the Test Device. A test matrix showing the baseline control and Test Device associated testing and samples 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 (min)	Midget Impinger sample flow rate (L/min)	Midget Impinger test sample times (min)	APS particle size test sample time (min)	Total number of tests	Number of Impinger samples/ test	Total TCID50 sample assays
Characterization control testing, chamber fan only operation	60	DMEM	26	15	20	t = -20-0	1.5	t = 0-15 t = 15-30 t = 30-45 t = 45-60	t = 0 t = 15 t = 30 t = 45	3	4	36
Aurabeat Air Purifier testing	60	DMEM	26	15	20	t = -20-0	1.5	t = 0-15 t = 15-30 t = 30-45 t = 45-60	t = 0 t = 15 t = 30 t = 45	3	4	36

For conducting and characterizing the viral aerosol viability and establishing natural aerosol decay results, 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 turned 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 test, the Collison nebulizer was operated over a twenty (20) minute aerosol generation period, the Nebulizer was turned off, and aerosol viral sampling from the cabinet initiated. SARS-CoV-2 aerosol sample collection and measurement of the viral deactivation efficacy were derived from impinger samples taken in sequential time order and duration from a common sample location during all conducted tests. The aerosol sample impingers (Midget, model 7531), have a high collection efficiency rating and operate at low sample flow rate requirements. Between each conducted test, resident aerosols were evacuated with a system equipped exhaust pump and verified for total particle evacuation with the APS 3321 analyzer.

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Section 4. Sample Analysis and Results

Stock virus used for test and control coupon inoculation (SARS-CoV-2, strain USA-WA1/2020) were concentration titered by serial dilution to obtain the 50% tissue culture infectious dose ($TCID_{50}$). This was conducted to ensure that sufficient quantities of virus were available for testing. Untreated virus control concentrations were assessed to ensure that titers remained consistent. 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) that were originally obtained from ATCC (CRL-1586) were used for all assays on this project. 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. Cells were infected with viral samples and observed for the presence of cytopathic effect (CPE) for four (4) to five (5) days post-infection. A 10× serial dilution of collected impinger virus samples were applied to cell assay plates at up to an 8 log dilution factor for the presence of viral growth into assay plate host cells. Plates were inoculated with 5 replicate samples at each dilution level, with each row of replicates 10 × more dilute than that used in the preceding row for viral cell infectivity detection. 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).

Test Results:

Midget impinger samples were analyzed as described above for both the in triplicate one hour characterization 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 timepoint, and transported to a dedicated Class II Biological Safety cabinet for assay and viable viral analysis. Results for the baseline control characterization testing and Test Device log reduction and percent viral deactivation efficiency were calculated by comparing the control test natural viral decay in relation to the Test Device operation results under the same conditions. Collected impinger $TCID_{50}$ concentrations at each aerosol collection time interval were calculated, and the test cabinet viral aerosol concentrations derived by defining the ratio of sampled volume in relation to the volumetric dicplacement volume of the test chamber. This ratio multiplied by the impinger sample $TCID_{50}$/mL concentration was used to extrapolate the total viable viral aerosol concentration and efficacy of the Test Device in deactivating the airborne virus. A table with results for the collected virus $TCID_{50}$ assay concentrations, and test cabinet viable aerosol concentrations for control and test device operation are shown in Table 2.

Table 2. Test Results for Aurabeat Purifier Viral Deactivation Efficacy

Characterization Control Sample Results and Aurabeat Viable Virus Deactivation Efficiency												
Sample Name	Impinger Sample Duration (mins)	Impinger Sample Flow rate (L/min)	Impinger total sample volume (L)	Test Chamber Total Displacement volume (L)	Ratio Of Impinger sample volume (L) to Test Chamber volume (L)	Plate Type	Sample concentration TCID50/mL	Ratio Of Impinger sample volume (L) to test chamber volume (L)	TCID50 viral chamber concentration	Impinger Avg TCID50/mL total chamber vol	Test Average TCID50 total chamber concentration	Averaged test sample Log10 chamber concentration TCID50
Control test 1 0-15 min							316.23	17.50	5533.99	3.74		
Control test 2 0-15 min	0-15	1.4	21	370	17.50	24-Well	100.00	17.50	1750.00	3.24	2698.75	3.30
Control test 3 0-15 min							46.42	17.50	812.28	2.91		
Control test 1 15-30 min							46.42	17.50	812.28	2.91		
Control test 2 15-30 min	15-30	1.4	21	370	17.50	24-Well	31.62	17.50	553.40	2.74	554.47	2.71
Control test 3 15-30 min							17.01	17.50	297.72	2.47		
Control test 1 30-45 min							31.62	17.50	553.40	2.74		
Control test 2 30-45 min	30-45	1.4	21	370	17.50	24-Well	3.16	17.50	55.34	1.74	221.36	2.08
Control test 3 30-45 min							3.16	17.50	55.34	1.74		
Control test 1 45-60 min							3.16	17.50	55.34	1.74		
Control test 2 45-60 min	45-60	1.4	21	370	17.50	24-Well	3.16	17.50	55.34	1.74	49.46	1.69
Control test 3 45-60 min							2.15	17.50	37.70	1.58		

Aurabeat Test Sample Results and Percent Aerosol Viral Deactivation Efficiency												
Sample Name	Impinger Sample Duration (mins)	Impinger Sample Flow rate (L/min)	Impinger total sample volume (L)	Test Chamber Total Displacement volume (L)	Ratio Of Impinger sample volume (L) to Test Chamber volume (L)	Plate Type	Sample Concentration TCID50/mL	Ratio Of Impinger sample volume (L) to Test Chamber volume (L)	TCID50 viral chamber Concentration	Impinger Avg TCID50/mL total chamber vol	Test Average TCID50 total chamber Concentration	Aurabeat Total Chamber % aerosol Log reduction in 15 minutes related to control test concentration
Test 1 15 min							2.25	17.50	39.38	1.60		
Test 2 15 min	0-15	1.4	21	370	17.50	24-Well	2.25	17.50	39.38	1.60	52.50	
Test 3 15 min							4.50	17.50	78.75	1.90		
Test 1 30 min												
Test 2 30 min	15-30	1.4	21	370	17.50	24-Well	0.00	0.00	0.00	0.00	0.00	
Test 3 30 min												99.9%
Test 1 45 min												
Test 2 45 min	30-45	1.4	21	370	17.50	24-Well	0.00	0.00	0.00	0.00	0.00	
Test 3 45 min												
Test 1 60 min												
Test 2 60 min	45-60	1.4	21	370	17.50	24-Well	0.00	0.00	0.00	0.00	0.00	
Test 3 60 min												

A plot of the averaged SARS-CoV-2 chamber aerosol concentrations for each of the in triplicate conducted tests shows the natural airborne viable viral concentration over each of the four (4) sample time intervals. The plot represents the control sample concentrations at the midpoint sample time intervals taken for each test, and shows a very linear relationship between the natural viral concentration decay in the test chamber in relation to residence time. The plot shows a near linear regession fit with an R^2 value of 0.99, and is shown in Figure 2.

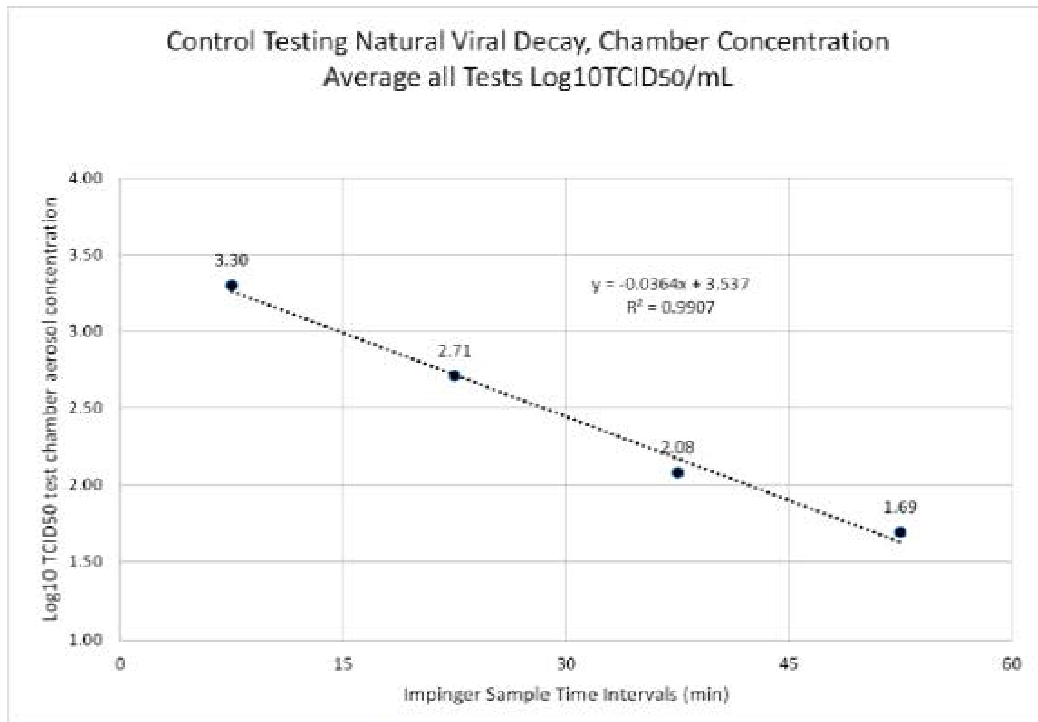

Figure 2. Control Tests SARS-CoV-2 Chamber Aerosol Concentration vs Sample Time Plot

Particle size and aerosol count analysis was conducted for each test at time intervals coinciding with the sample start time of each impinger sample. Following the initial Test Device APS sample at time = 0, it was observed that the aerosol concentration in the chamber had been totally removed within the first 15 minutes of the test with no detected aerosol 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 following the initial 0-15 minute interval impinger samples for all Test Device trials. A plot of the averaged particle counts per liter of air sampled from the aerosol cabinet with the APS at each time interval for Control characterization tests and the Test Device operation tests is shown in Figure 3.

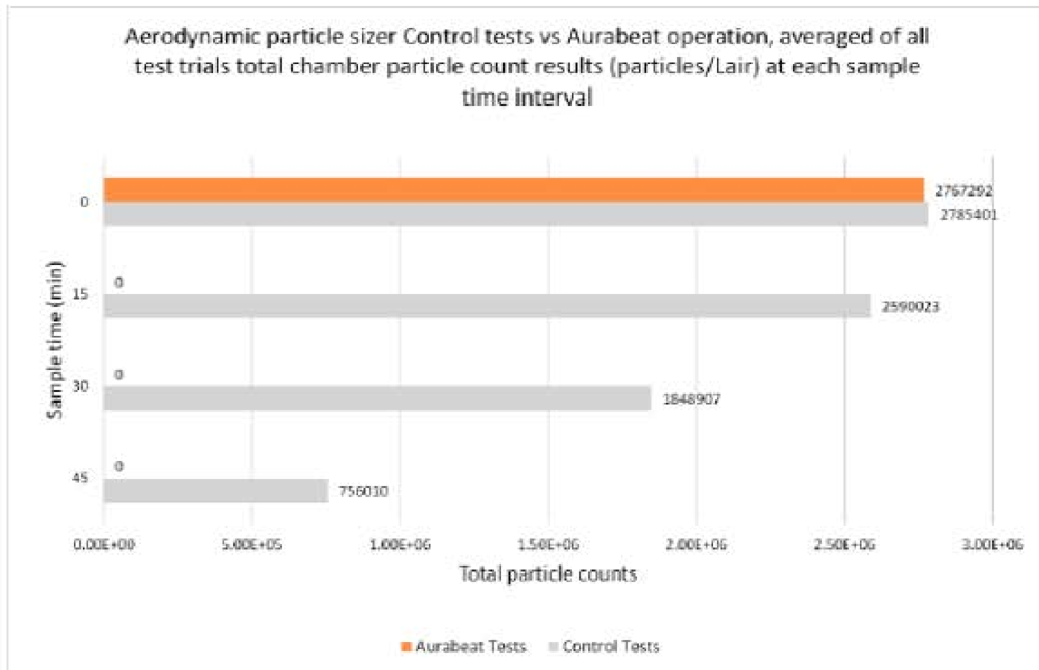

Figure 3. Aerodynamic Particle Sizer (APS) Aerosol Particle Count vs Sample Time Plot

Particle size distributions were also measured with the APS. A plot representing the particle size distribution of the resident aerosol in the test chamber following the termination of 20 minute pre-test SARS-CoV-2 aerosol generation from suspension in DMEM is shown in Figure 4. 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.

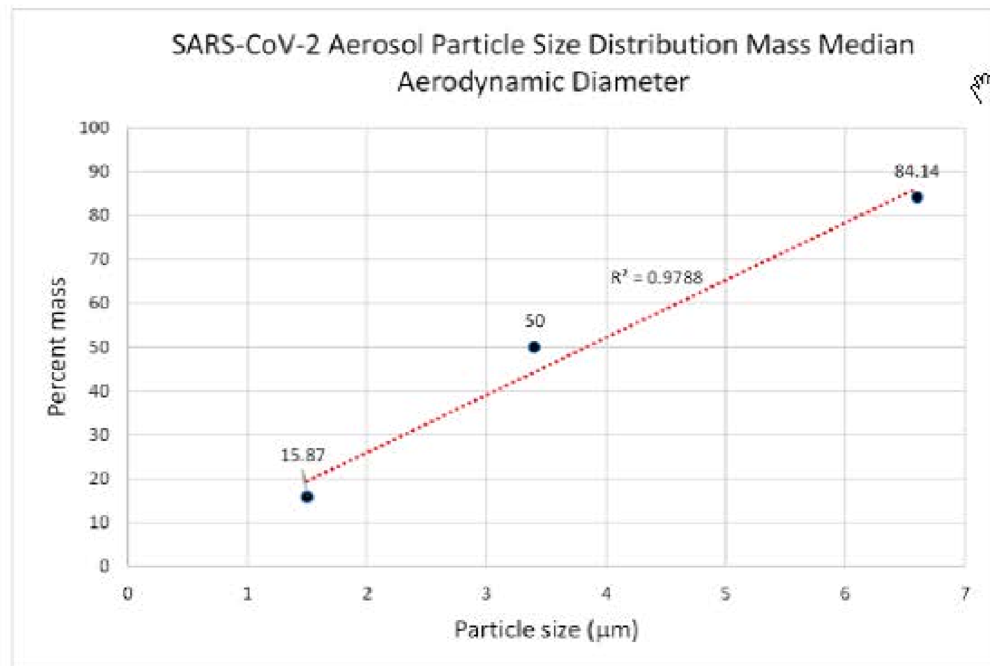

Figure 4. Aerodynamic Particle Sizer (APS) Aerosol Particle Size Plot

Conclusions:

The Test Device air purifier showed a very quick removal of resident aerosol in the test cabinet for all conducted tests. The aerosol particle concentration was reduced from an average concentration of over 2 million particles per liter of air to a zero count reading per liter of air in less than fifteen (15) minutes of operation. The viable viral concentration was also greatly reduced within the first 15 minutes of the test device operation as compared to the baseline control test data, and the control testing time dependent aerosol concentration plot shown in Figure 2. The Test Device had a calculated viable virus test cabinet concentration reduction of 99.9% within 15 minutes of Test Device operation with no detectable viral sample collection, or resident aerosol particles detected in subsequent test time sample intervals. This depletion of viable viral aerosol over the 0-15 minute operation time period equates to approximately four (4) aerosol test cabinet volume air displacement cycles through the air purifier to fully remove and deactivate the airborne virus from the test atmosphere.

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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; and all procedures utilized were technically valid. This study conducted at MRIGlobal were performed 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.