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Characterization of the Model AH01 Air Purification Device in Deactivation of Aerosolized SARS-CoV-2

Final Report

FOR

Kave Industries, LLC

8605 Santa Monica Boulevard #53476
Los Angeles, California 90069

MRIGlobal Project No. 311728.01.001

March 30, 2021

Preface

This Final Report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311728.01.001, "Characterization of the Model AH01 Air Purification Device in Deactivation of Aerosolized SARS-CoV-2."

The test device was designed and supplied to MRIGlobal by Kave Industries, LLC for the conduct of the program. The device is marketed under the product name AirKAVE Portable (Model #AH01) and is manufactured by A-One Tech Limited ("A-One"). The experimental phase of this task was initiated by MRIGlobal on February 20, 2020 and ended on February 26, 2020.

The Study Director of the program was Rick Tuttle. Execution of the study was assisted by Kristen Solocinski, Ph.D., Sam Humphrey, 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

March 30, 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 with severe illness and fatalities, has created a needed response for rapid development and evaluation of effective countermeasures. In response to testing for Kave Industries, LLC, MRIGlobal conducted testing and evaluation of a plasma generation Air Purifier product. The test device was designed and supplied to MRIGlobal by Kave Industries, LLC for the conduct of the program. The device is marketed under the product name AirKAVE Portable (Model #AH01) and is manufactured by A-One Tech Limited ("A-One"). The device incorporates a flow-through air recirculation design that utilizes airKAVE's Plascide plasma technolology platform without the use of air filters. The Test Device was evaluated in independent tests for efficacy in deactivation of SARS-CoV-2 aerosol challenges in laboratory trials at MRIGlobal.

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor

Kave Industries, LLC.
8605 Santa Monica Boulevard #53476
Los Angeles, CA 90069

2.2 Sponsor's Representative

Brett Lieberman
Kave Industries, LLC

2.3 Testing Laboratories

MRIGlobal
425 Volker Boulevard
Kansas City, MO 64110
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2.4 Personnel Responsibilities

2.4.1 Study Director—MRIGlobal

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Phone: (816) 753-7600, Ext. 5752
Email: rtuttle@mriglobal.org

Section 3.
Test Systems and Methods

3.1 Equipment

Test Equipment

AirKAVE Portable (Model #AH01) with USB power adapter. Unit dimensions of 83mm (W) × 45mm (L) × 628 mm (H).

3.2 Methods

Testing Description

MRIGlobal conducted testing characterization of the AirKAVE Portable Model #AH01 ("Test Device") in viral aerosol decontamination trials to evaluate the log reduction deactivation 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).

All tests were conducted in a biological class 3 facility at MRIGlobal, Kansas City, MO. Due to the impracticality and potential hazards associated with conducting large area aerosol dissemination studies with class III human pathogens, MRIGlobal designed a scaled down aerosol containment cabinet to simulate a large room environment. The client provided an air purification unit (AirKAVE Portable Model #AH01) with a USB to 110V power supply adapter. The Test Device is designed for small personal areas, and operates with an internal fan at a flowrate in the range of 80 to 100 L/minute. The Test Device utilizes plasma generation for air purification . Tests were conducted at MRIGlobal in 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 × 42 inches long × 18 inches wide with a displacement volume of approximately 370 liters or 13 cubic feet. A diagram of the test system is shown in Figure 1.

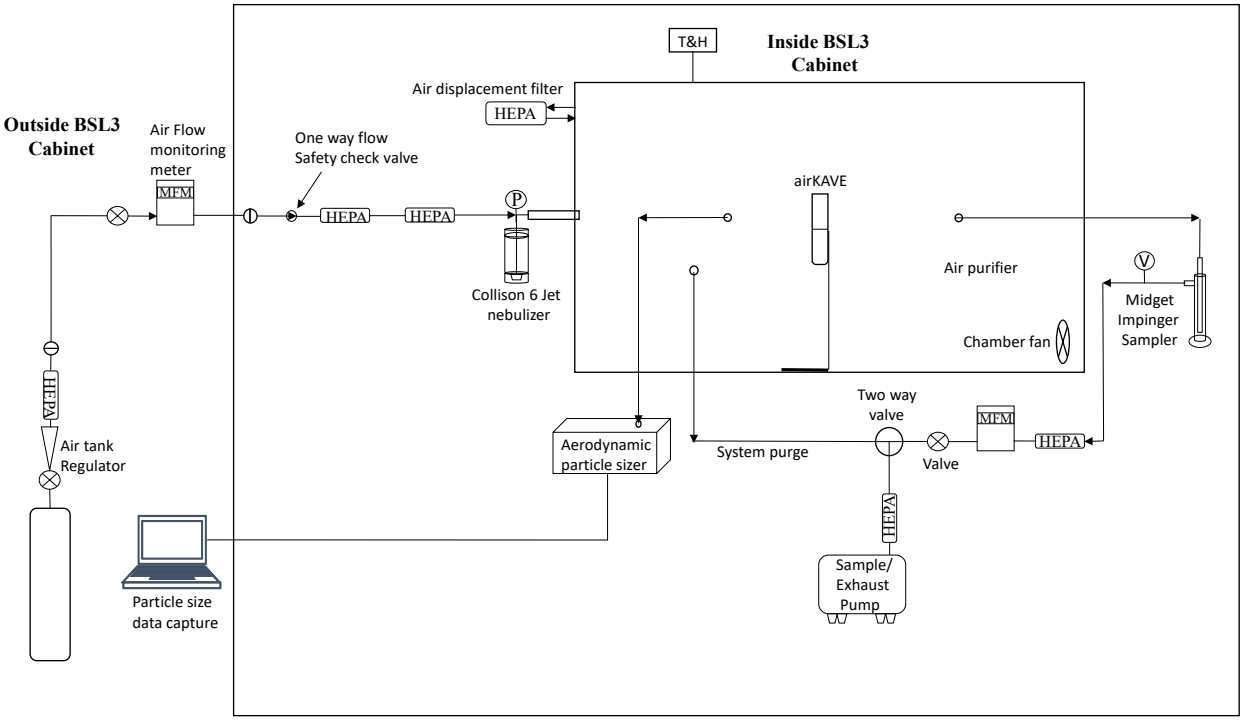

Figure 1. SARS-CoV-2 Aerosol Test System

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 2 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 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 3×10^7 $TCID_{50}$ 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 plasma viral deactivation efficacy. 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 nebulizer operation (psia)	Collison 6 jet flow rate (L/min)	Collison 6 jet generation time (min)	Collison 6 jet test generation time (min)	airKAVE Operation 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
Characterization testing, no plasma, test chamber fan only operation	60	DMEM	24 - 28	15	15	t = -15-0	NA	1.5	t = 0-15 t = 15-30 t = 30-45 t = 45-60	t = 0 t = 15 t = 30 t = 45	3	4
Device test, plasma with fan operation	60	DMEM	24 - 28	15	15	t = -15-0	0 - 60	1.5	t = 0-15 t = 15-30 t = 30-45 t = 45-60	t = 0 t = 15 t = 30 t = 45	3	4

For establishing aerosol concentration baseline control tests and for device testing, the Test Device was placed in the center of the chamber and supported using a ring stand at a mid level location between the top and bottom of the test chamber. For aerosol characterization of viral aerosol viability and establishing natural aerosol decay results (control testing), testing was conducted with only a low flow test chamber recirculation fan operational (Test Device plasma off). This provided uniform mixing and a homogeneous concentration of generated aerosols within the aerosol test chamber for accurate sample collection of viable aerosols. Evaluation of the Test Device was conducted using the same operation parameters as control testing with the units plasma operational following the nebulization process.

For each conducted test, the Collison nebulizer was operated over a Fifteen (15) 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 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, Ace Glass, inc.), were filled with 10 ml of sterile DMEM collection media for each sample iteration. The Midget impingers have a high collection efficiency rating and operate at a low sample flow rate requirement. For all tests, impinger sample flows were controlled with a calibrated critical flow orifice with flows monitored using a calibrated mass flow meter. Sample flow was supplied with a valve equipped rotary vane vacuum pump (Gast Manufacturing, Benton Harbor, MA). 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.

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 (Gibco) supplemented with 5% fetal bovine serum (Avantor), 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 these assays. 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 three (3) days post-infection. A dilution of collected impinger virus samples was diluted and 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 one hour 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. A table with results for the collected virus $TCID_{50}$ assay concentrations, and test chamber viable aerosol concentrations for control and Test Device operation are shown in Table 2.

Table 2. Test Results for airKave Portable Model #AH01 Purifier Viral Deactivation Efficacy

Midget Impinger Sample ID	Testing Description	Estimated Test Device Aerosol Chamber volume cycle time range (min)	Post Aerosol Generation Impinger Sample Time Interval (min)	Impinger Sample TCID50 Assay Replicate	Sample Results TCID50/mL	Sample Log10 TCID50/mL	Averaged Sample TCID50/mL	Average Log10 TCID50/mL	Log Reduction	Percent Log Reduction
T1-1	Device Operation	3.2 to 4.1	0-15	1	5.62E+02	2.75	8.64E+02	2.8	0.7	80.05%
T2-1				2	2.51E+02	2.4				
T3-1				3	1.78E+03	3.25				
C1-1	Control	NA	0-15	1	3.16E+03	3.5	3.52E+03	3.5	N/A	N/A
C2-1				2	1.78E+03	3.25				
C3-1				3	5.62E+03	3.75				
T1-2	Device Operation	6.5 to 8.1	15-30	1	3.98E+01	1.6	5.99E+01	1.73	1.37	95.72%
T2-2				2	3.98E+01	1.6				
T3-2				3	1.00E+02	2				
C1-2	Control	NA	15-30	1	1.43E+03	3.16	1.50E+03	3.1	N/A	N/A
C2-2				2	5.62E+02	2.75				
C3-2				3	2.51E+03	3.4				
T1-3	Device Operation	9.7 to 12.2	30-45	1	1.00E+01	1	7.08E+00	0.83	1.75	98.22%
T2-3				2	5.62E+00	0.75				
T3-3				3	5.62E+00	0.75				
C1-3	Control	NA	30-45	1	3.16E+02	2.5	3.98E+02	2.58	N/A	N/A
C2-3				2	3.16E+02	2.5				
C3-3				3	5.62E+02	2.75				
T1-4	Device Operation	13.0 to 16.2	45-60	1	3.98E+00	0.6	2.03E+00	0.12	1.97	98.92%
T2-4				2	3.16E-01	-0.5				
T3-4				3	1.78E+00	0.25				
C1-4	Control	NA	45-60	1	1.43E+02	2.16	1.66E+02	2.09	N/A	N/A
C2-4				2	3.98E+01	1.6				
C3-4				3	3.16E+02	2.5				

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 in relation to the Test Device viable deactivation reduction. The plot represents the control and Test Device sample concentrations at the midpoint sample time intervals taken for each test, and shows a very linear relationship between the natural viral concentration decay samples and Test Device deactivation sample concentrations. The plot shows a linear regession fit with an R^2 value of 0.997 for the control samples, and a value of 0.992 for Test Device viral deactivation, and is shown in Figure 2.

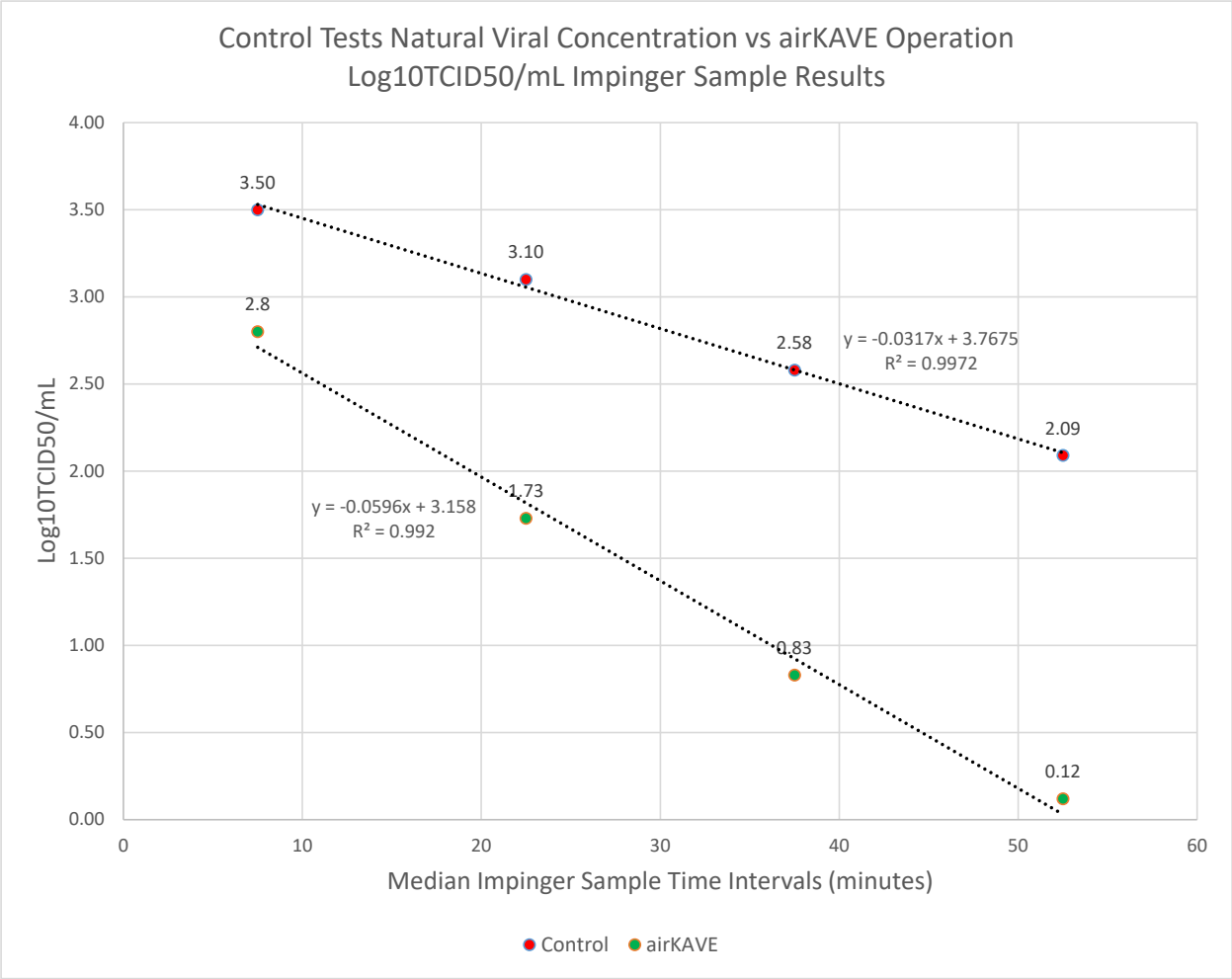

Figure 2. Test Device and Control Test SARS-CoV-2 Chamber Aerosol Concentration vs Sample Time Plot

Particle size distributions of the SARS-CoV-2 aerosol challenge were measured with the APS. Aerosol challenges of SARS-CoV-2 were generated from suspension in DMEM. A plot showing a representative particle size distribution of the resident aerosol in the test chamber following the termination of 15 minute pre-test SARS-CoV-2 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 3.8 µm, with 50% of the aerosol particle mass below and 50% above the median diameter. It was also observed that the resident aerosol particle concentration in the test chamber was not affected by operation of the Test device.

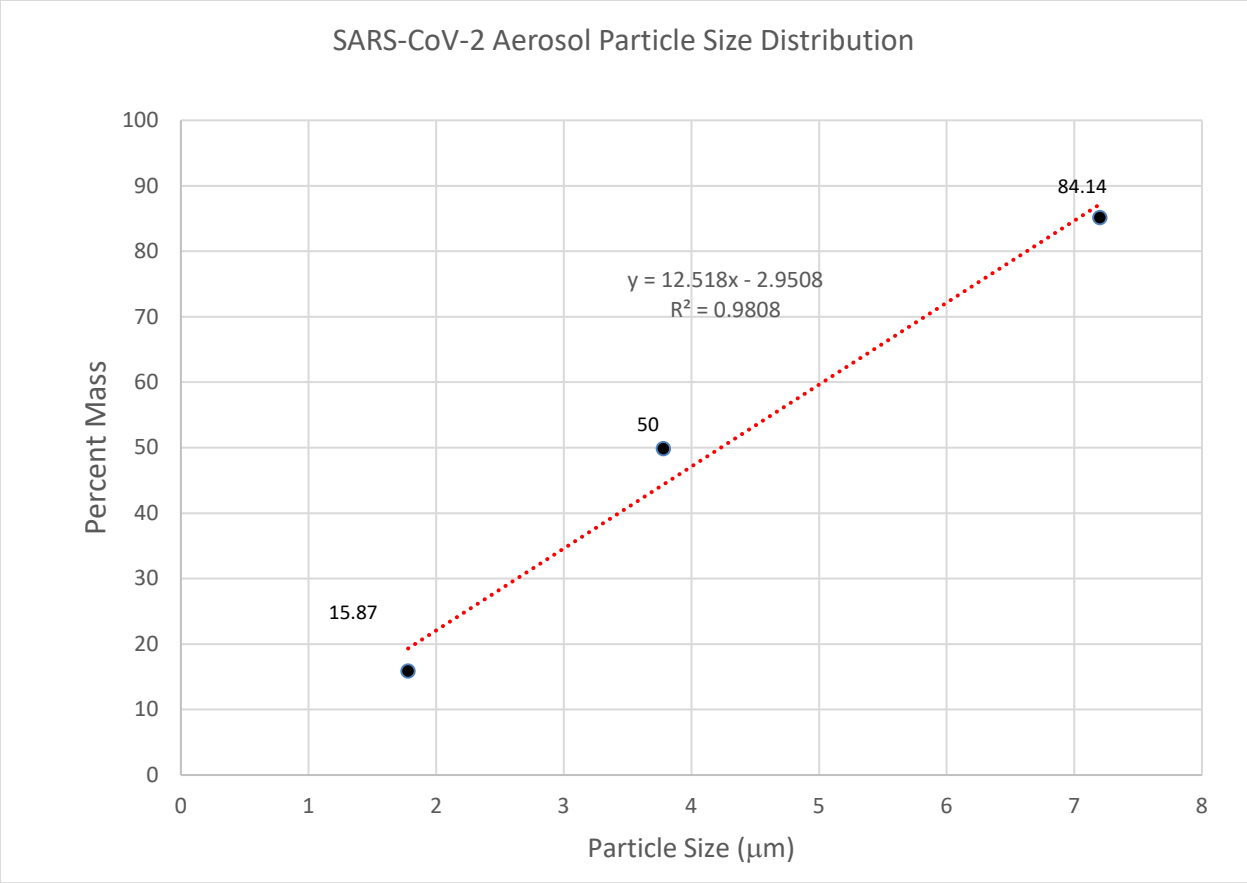

Figure 3. Aerodynamic Particle Sizer (APS) Aerosol Particle Size Plot

Conclusions:

The Test Device (airKAVE portable) with Plaside plasma technology, showed a progresive viral deactivation trend over the one (1) hour testing trials. Comparative SARS-CoV-2 aerosol percent viability (Table 2) with the Test Device operational in relation to baseline (Control) test results, showed viral deactivation rates of 80.05%, 95.72%, 98.22%, and 98.92% for sample time intervals of 7.5, 22.5, 37.5, and 52.5 minutes respectively. It was observed that the aerosol concentration in the test chamber was not affected by the filter-less Test Device. The data reflects that the Air Halo portable model AH01 is efficacious at deactivating high airborne challenge concentrations of SARS-CoV-2 without the use of filters or particle collection mechanisms, and could be beneficial in deterring bioaerosol transmission and infectivity.

Section 5.
Quality Assurance

5.1 Type of Study

This study was executed using established SOPs, at MRIGlobal in Kansas City, MO. 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.