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Characterization of a Natural Convection Thermal Flow-Through (TSS Technology) Air Purification Device in Deactivation of Aerosolized SARS-CoV-2

Final Report

FOR

Airfree – Produtos Electronicos SA

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MRIGlobal Project No. 311744.01.001

May 28, 2021

Preface

This final report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311744.01.001, “Characterization of a Natural Convection Thermal Flow-Through (TSS Technology) Air Purification Device in Deactivation of Aerosolized SARS-CoV-2.”

The experimental phase of this task was initiated by MRIGlobal on April 15, 2021 and ended on April 21, 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.

The study was not performed in compliance with the FDA Good Laboratory Practice Regulations (21 *CFR* 58). All operations pertaining to this study, unless specifically defined in this protocol, were performed according to the Standard Operating Procedures or approved Laboratory Procedures of MRIGlobal. All technical procedures were overseen by a second person and data was quality checked and peer reviewed.

All study records are stored at MRIGlobal.

Sincerely,

MRIGLOBAL



Rick Tuttle
Principal Scientist

Approved:



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

May 28, 2021

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Executive Summary

Objective:

The objective of this project was to determine if the Airfree[®] Onix 3000 (sold in Asia and Europe as P150) Air Purifier (“Test Device”) can deactivate airborne SARS-CoV-2 in controlled tests conducted at MRIGlobal. The Test Device incorporates a ceramic core, which heats up to temperatures in the range of 80° to 210°C. Airflow through the unit is obtained through natural convection, which pulls in room air for recirculation and sterilization of bio-aerosols. As air exits the thermal air purification heat core, it enters a cooling chamber in the unit which cools the air to near room air temperature conditions before release back into the room. This thermal flow through air recirculation design provides maintenance free operation without the use of fans or filter units, and uses the thermal heat core for the deactivation of biological aerosols.

Study Design:

The Airfree[®] Onix 3000 (sold in Asia and Europe as P150) air purifier was evaluated at MRIGlobal under controlled conditions in a biological level 3 (“BSL-3”) laboratory. Testing was conducted using an aerosol containment system housed in a biological class 3 safety cabinet. All testing was conducted using a common stock of SARS-CoV-2 prepared in suspension at a concentration of 1.47E+07 TCID₅₀ / ml. MRIGlobal utilized the USA-WA1/2020 (Washington State 1 isolate) 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 with Nutrient Mixture F-12 (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. Pre – device test characterization of the viral aerosol delivery efficiency and time weighted viable aerosol concentration testing was performed to establish baseline (standard) results for subsequent thermal viral deactivation efficacy testing comparison. 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) for providing uniform mixing and a homogeneous concentration of generated aerosols. Aerosol samples were collected Midget Impingers (Midget, model 7531, Ace Glass Inc.) filled with 10 ml DMEM/F12 to assess the efficacy of the device. Collected Impinger samples were diluted 1:10 down a 96 deep well plate in DMEM/F12. These dilutions were transferred to a plate of Vero E6 cells with media removed. After approximately 45 minutes, DMEM/F12 supplemented with 5% Fetal Bovine Serum (FBS) was added to cells to feed them for the next four to five days. The inoculated plates were then read for cytopathic effects (CPE). Additionally, an APS 3321 aerosol particle sizer (“APS”) was used for sequential real time sampling for aerosol particle size and concentration measurements.

Section 1. Objective

The objective of this project was to determine if the Airfree[®] Onix 3000 (sold in Asia and Europe as P150) Air Purifier (Test Device) can deactivate airborne SARS-CoV-2 in controlled lab tests.

Section 2.

Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor's Representative

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Section 3. Test Materials

3.1 Test Product

3.1.1 Test Unit

Airfree[®] Onix 3000 (sold in Asia and Europe as P150) Air Purifier

3.2 Test Components

3.2.1 Cell Media

DMEM/F12 (Serum-free media)

Vendor: Gibco

Lot No.: 2239772

Expiration date: 12/21

Growth Media – 5% FBS (fetal bovine serum)

Lot No.: 202010407JW

Expiration date: 10/21

3.2.2 Challenge Virus

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

Strain: USA-WA1/2020

Vendor: BEI Resources

Virus lot: 20210401KS

3.2.3 Host

Vero E6 Cells

Vendor: ATCC

Cat: CRL 1586

Section 4.

Test Systems and Methods

4.1 Test Device

Airfree® Onix 3000 (sold in Asia and Europe as P150) Air Purifier

4.2 Methods

Testing Description

MRIGlobal conducted testing characterization of the Air Purifier (Airfree® Onix 3000 sold in Asia and Europe as P150 Air Purifier - “Test Device”) in viral aerosol trials to evaluate the log reduction deactivation effectiveness against an envelope virus (SARS-CoV-2, strain USA-WA1/2020 “Washington 1 Isolate”). The USA-WA1/2020 strain of the virus was acquired from BEI Resources (NR-52281).

All tests were conducted in a biological class 3 facility at MRIGlobal, Kansas City, MO. The client provided an air purification unit Airfree® Onix 3000 (sold in Asia and Europe as P150) Air Purifier) with an 110V power supply adapter. The Test Device is designed for room use, and is suitable for areas up to 650 ft². The Test Device incorporates a ceramic core, which heats up to temperatures in the range of 80°-210°C with optimal temperature, flow recirculation and effective air purification after approximately 30 minutes of operation. Airflow through the unit is obtained through natural convection, which pulls in room air for recirculation and sterilization/deactivation of bio-aerosols.

The aerosol containment cabinet for Test Device evaluation was fabricated out of Plexiglas with internal dimensions of 30 inches (H) × 42 inches (L) × 18 inches (W) with a displacement volume of approximately 370 liters or 13 cubic feet. A diagram of the test system is shown in Figure 1.

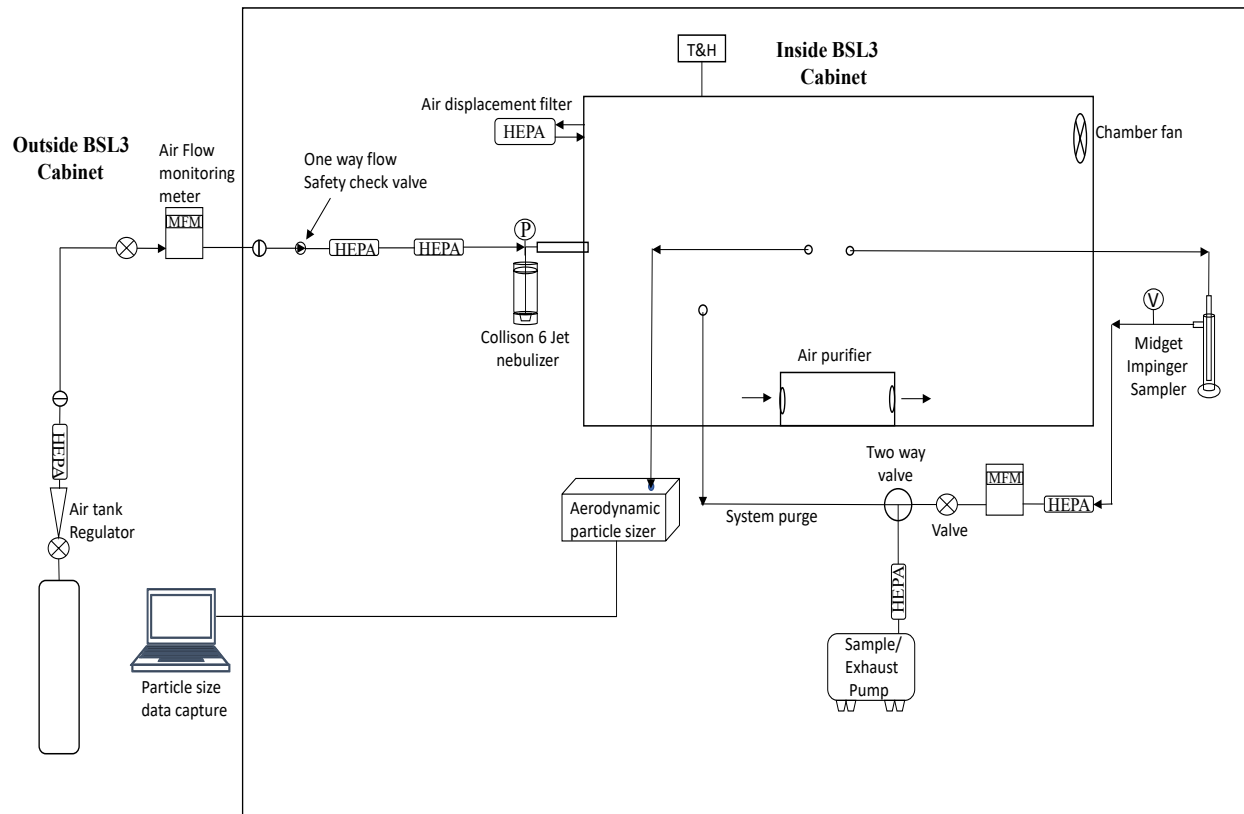


Figure 1. SARS-CoV-2 Aerosol Test System

Based on the Test Device disinfection operation and air flow being thermally regulated, a pre – study evaluation of the Airfree® Onix 3000 (sold in Asia and Europe as P150) Air Purifier internal core temperature profile and potential heat production in the aerosol test system was conducted. This testing measured the Test Device temperature range over a two (2) hour operation period followed by a 10 minute operation off period, and re - power of the unit for an additional fifty five minutes. An operation off time of ten (10) minutes to evaluate the dwell temperature decrease and temperature increase following repower was conducted to mimic the device testing operation time points as shown in the Test Matrix, Table 1 of this report. The temperature profile of the Test Device and aerosol test system were conducted in separate tests using a calibrated Fluke digital thermometer with a 1/32 inch type K thermocouple probe. The Test Device core temperature was measured by inserting the thermocouple into the ceramic heat core of the Test device for real time temperature measurements. A plot of the Test Device internal heat profile is shown Figure 2.

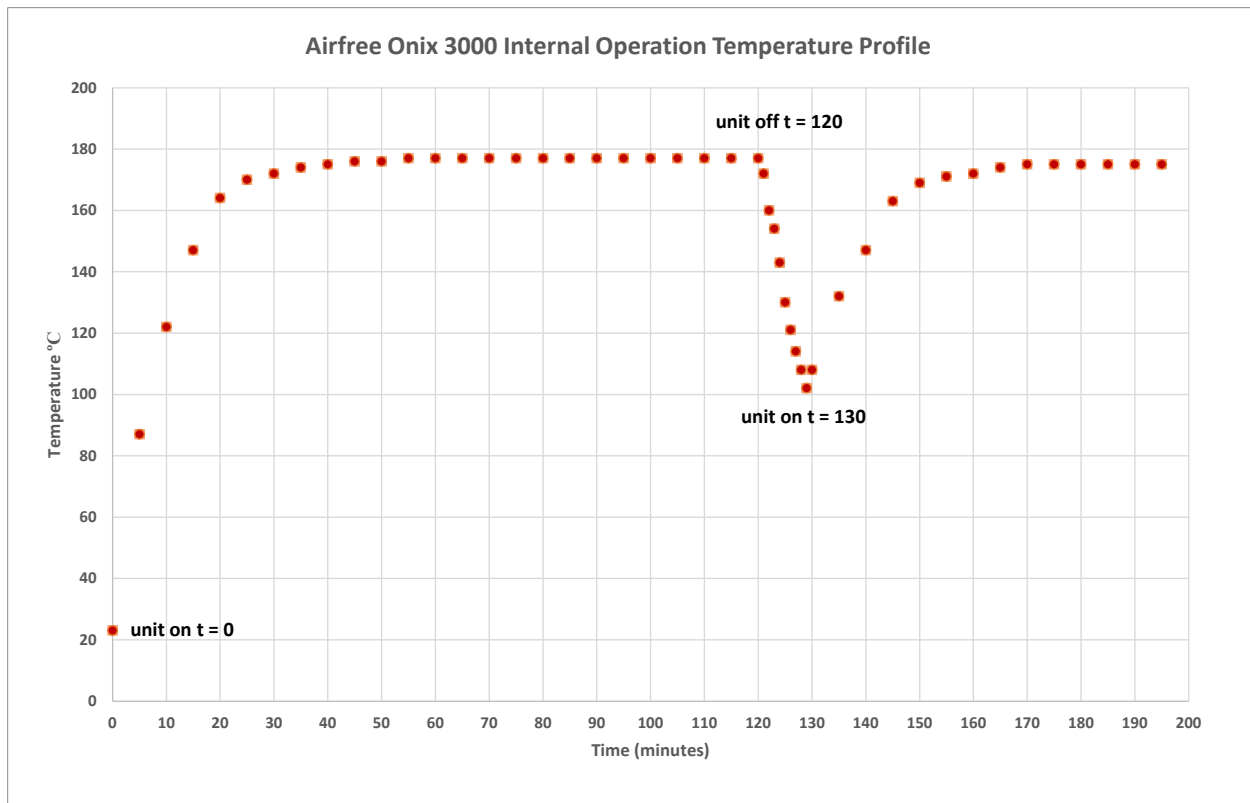


Figure 2. Test Device Temperature Profile

Results indicate a decrease of the Test Device heat core production of approximately 75°C from the Test Device off dwell time of t = 120 minutes to the re-start operation time of t = 130 minutes. An additional thirty (30) to forty (40) minutes are required for the Test device to reach optimal operation temperatures in the range of 170°-180°C. From test chamber results conducted in a separate test, there was no appreciable heat increase in the aerosol exposure system from operation of the Test Device that would have an effect on aerosol viability. The temperature increase in the aerosol test system from Test device operation over a two hour (120 minute) operation period was documented to be approximately 4°C with a temperature range of 21°-25°C.

Testing was conducted to obtain conditions that provided aerosol challenge concentrations acceptable for evaluating the Test Device for viral deactivation reduction. Preceding test and evaluation of the Test Device, aerosol characterization of the aerosol test system was 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 profile was conducted under the same generation and sampling conditions using the same SARS-CoV-2 viral working stock suspension as Test Device tests. For establishing pre-test viral concentration baseline (control) standard results, the Airfree® Onix 3000 (sold in Asia and Europe as P150) 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. For SARS-CoV-2 aerosol generation, a Collison 6 jet nebulizer (“Nebulizer”) was filled with a fresh aliquot of

10 ml of SARS-CoV-2 virus in DMEM 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 also 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 SARS-CoV-2, strain USA-WA1/2020 prepared in DMEM suspension at a concentration of $1.47\text{E}+07$ TCID₅₀ units per milliliter. Test characterization of the viral aerosol delivery efficiency and time weighted viable aerosol concentration testing was performed to establish baseline (control) results for subsequent Test Device 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 suspension media	Collison 6 jet aerosol generator operation (psia)	Collison 6 jet ~ flow rate (L/min)	Collison 6 jet generation time (min)	Airfree test operation 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 times (min)	Total number of tests	Number of Impinger samples/test
Baseline characterization testing, no device operation. Chamber fan only operation	60	DMEM	26	15	10	NA	t = -10-0	1.4	t = 0-15 t = 15-30 t = 30-45 t = 45-60	t = 0 t = 15 t = 30 t = 45	3	4
Device test, AirFree unit operational	60	DMEM	26	15	10	Warmup, t = -130 to -10 Unit off, t = -10 to 0 Unit On, t = 0 to 60	t = -10-0	1.4	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) results and for device testing, the Test Device was placed in the center of the chamber with the power supply cord routed through a sealed bulkhead adapter located on the back wall of the aerosol test system. This provided remote on/off power supply operation.

As shown in Table 1, and as directed by the client, the test device was pre – test operated for approximately one hour preceding aerosol challenge generation. This was conducted to allow the thermal core of the device to reach a stable state temperature preceding aerosol challenge generation. Following the one (1) hour warmup period, the Test Device was turned off for ten (10) minutes during aerosol generation, and re-started following the generation process.

Evaluation of the Test Device was conducted using the same operation and sample collection parameters as Control Testing with the exception of the pretest device warmup process and operation.

For each conducted test, the Collison nebulizer was operated over a ten (10) minute aerosol generation period, the Collison 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 sequential fifteen (15) minute 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.

Once impinger samples were collected, they were transferred in individually labeled sterile conical tubes 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 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 the incubation time, cells were examined for the presence of cytopathic effect (CPE) associated with viral presence and replication. Examination was done using a microscope (10× 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 5. 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 6. Results

Aerosol plates were read four days after baseline control standard and device testing. Baseline control test results and Test Device sample results were compared for viable viral concentration at each corresponding aerosol impinger sample time iteration. The Test Device deactivated viral infective viability by 0.03 log (6.4%), 0.38 log (58.5%), 0.64 log (76.9%), and 0.71 log (80.5%) over the 0 to 15, 15 to 30, 30 to 45, and 45 to 60 minute sample time periods respectively.

Table 2 summarizes these results.

Table 2. TCID₅₀/ml Calculations for Aerosol Testing of the Airfree® Onix 3000 (sold in Asia and Europe as P150)

Sample Name	Test Description	Test Replicate	Sample Interval	Samples Interval Time (min)	TCID ₅₀ /ml	Log10 TCID ₅₀ /ml	Avg TCID ₅₀ /ml	Avg Log10 TCID ₅₀ /ml	Log10 Reduction	Percent Reduction
C1-1	Control	1	1	0-15	515.73	2.71	1.49E+03	3.07	N/A	
C2-1		2			1111.11	3.05				
C3-1		3			2854.92	3.46				
C1-2		1	2	15-30	432.44	2.64	6.86E+02	2.80		
C2-2		2			515.73	2.71				
C3-2		3			1111.11	3.05				
C1-3		1	3	30-45	624.82	2.80	4.07E+02	2.58		
C2-3		2			298.08	2.47				
C3-3		3			298.08	2.47				
C1-4		1	4	45-60	29.81	1.47	8.40E+01	1.86		
C2-4		2			111.11	2.05				
C3-4		3			111.11	2.05				
T1-1	Test	1	1	0-15	432.44	2.64	1.32E+03	3.04	0.03	6.4%
T2-1		2			1760.99	3.25				
T3-1		3			1760.99	3.25				
T1-2		1	2	15-30	176.10	2.25	2.71E+02	2.42	0.38	58.5%
T2-2		2			351.36	2.55				
T3-2		3			285.49	2.46				
T1-3		1	3	30-45	29.81	1.47	2.09E+02	1.95	0.64	76.9%
T2-3		2			556.87	2.75				
T3-3		3			41.42	1.62				
T1-4		1	4	45-60	17.61	1.25	1.44E+01	1.15	0.71	80.5%
T2-4		2			n/a	na				
T3-4		3			11.11	1.05				

The device efficacy based on time of operation and temperature is further shown in Figure 3 below.

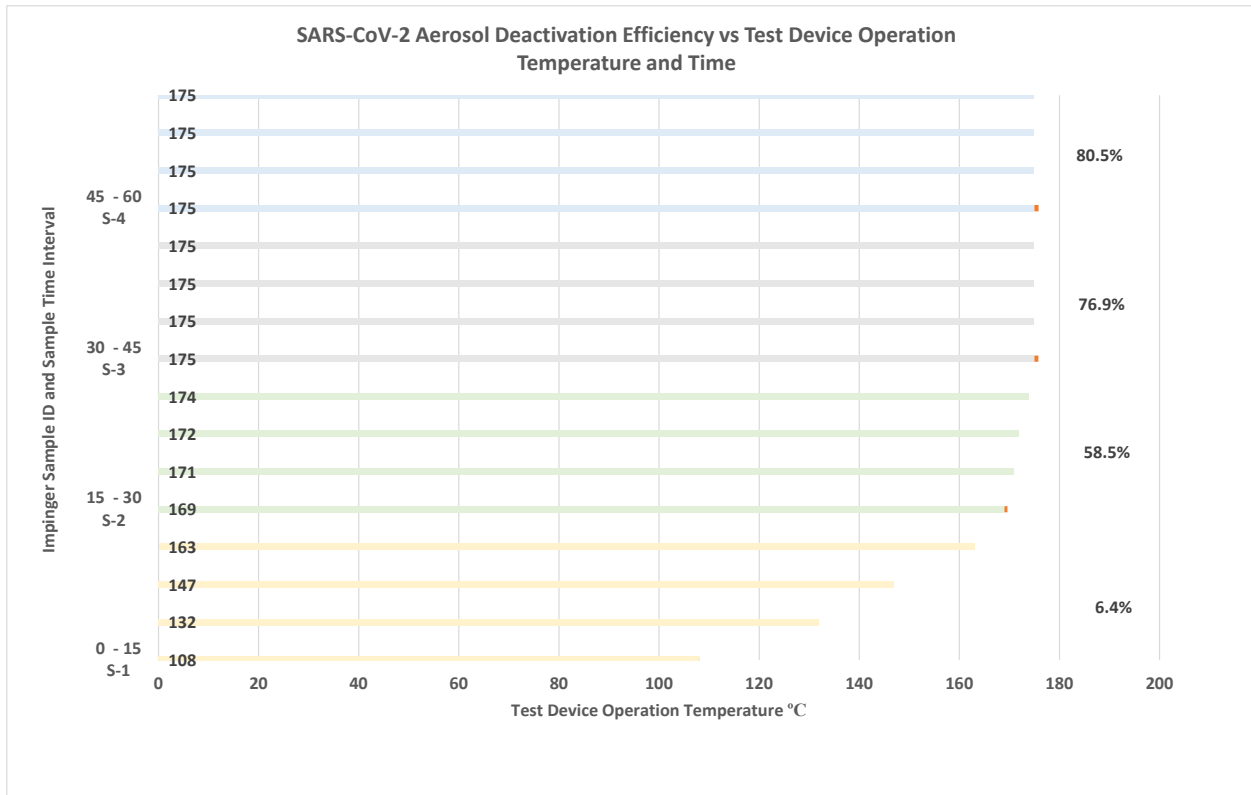


Figure 3. SARS-CoV-2 Aerosol Deactivation Efficiency of Test Device Operation Temperature vs Time

APS particle counts were taken for 30 seconds at the initiation of each impinger sample time interval following the aerosol generation process. The APS 3321 pulls aerosol measurement samples at a controlled flow rate of one (1) liter per minute. A plot of the averaged control and Device Test APS particle count samples (0.5 liters/sample) is shown in Figure 4.

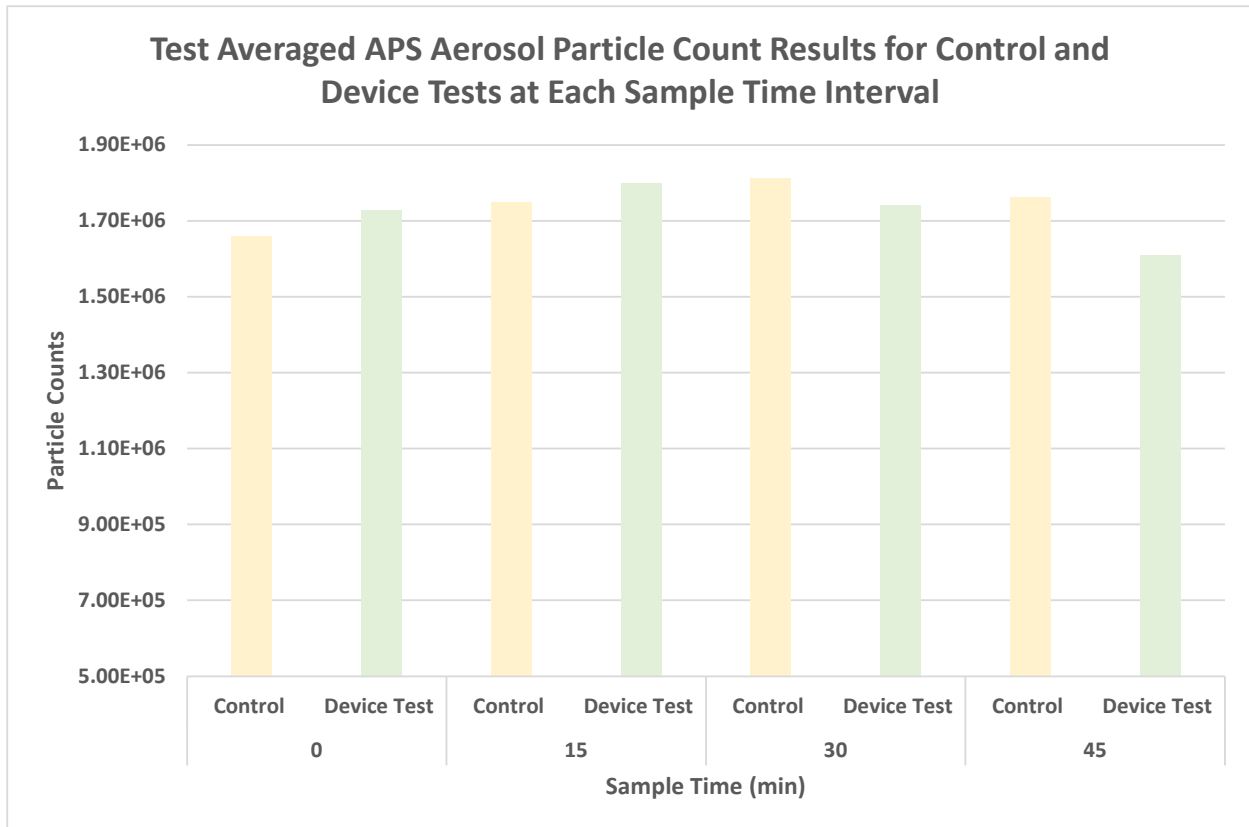


Figure 4. Aerodynamic Particle Sizer (APS) Aerosol Particle Count vs Sample Time Plot

Particle size distributions were also measured with the APS. A plot showing a representative SARS-CoV-2 aerosol particle size distribution derived from control testing data is shown in Figure 5. The plot shows the percent mass of the aerosol distribution in relation to particle size. The Mass Median Aerodynamic Diameter (MMAD) shown in the graph reflects a median diameter of approximately 3.51 μm , with 50% of the aerosol particle mass below and 50% above the median diameter. The 15.87 percent mass (1.71 μm) and 84.14 percent mass (7.03 μm) particle size points are also shown.

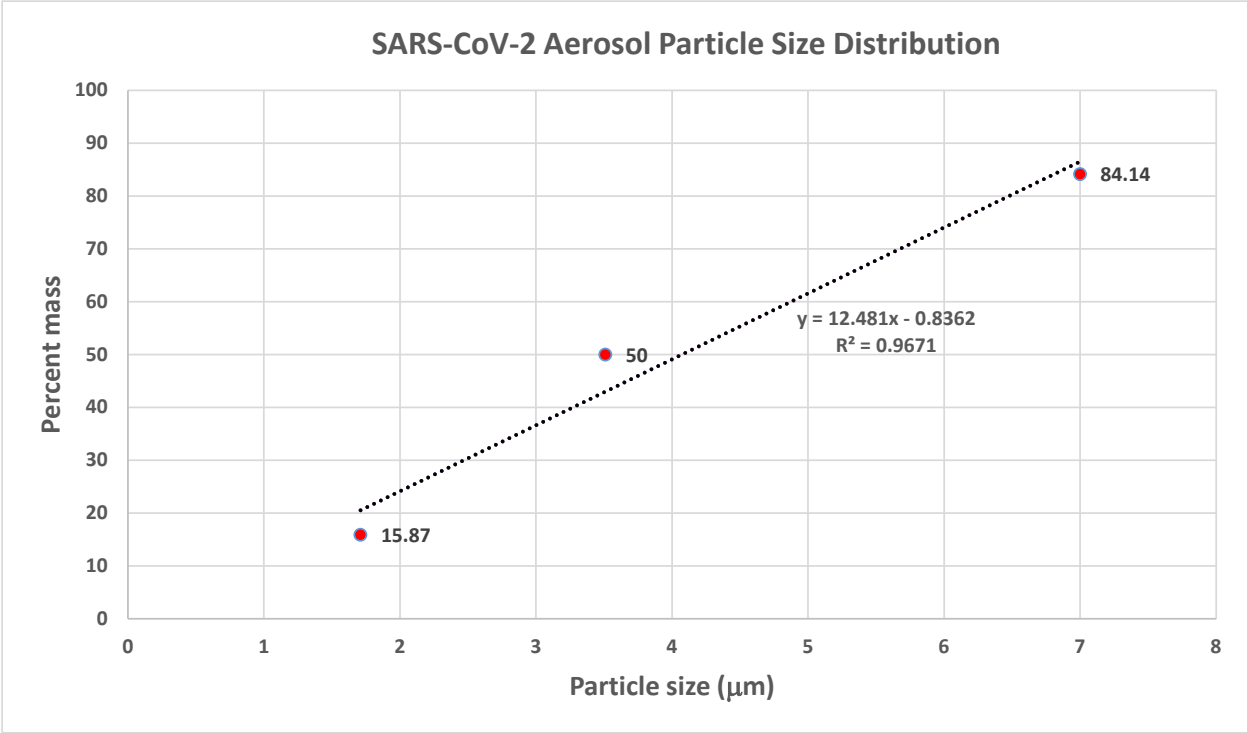


Figure 5. Aerodynamic Particle Sizer (APS) Aerosol Particle Size Distribution Plot

Section 7. Conclusions

The Airfree[®] Onix 3000 (sold in Asia and Europe as P150) Air Purifier used in this experiment is designed to be run continuously “On” with its commercial use. In this approved experiment the Air Purifier was purposely powered “Off” for the first 10 min of the test during the SARS CoV-2 aerosol nebulization. The unit was powered off to provide an aerosol concentration level without interference from test unit operation. This off period was conducted for comparative deactivation efficacy for comparative analysis to baseline control standard operation test results. Preliminary temperature profile tests, conducted before the study, revealed that the device core ducts operating temperature would drop from approximately 175° to 100°C while the device was powered off during the 10 (ten) minute period consistent with the timed nebulization process. Subsequently, the 10 (ten) minutes powered off required an additional 30 (thirty) to 40 (forty) minutes to reach the prior stabilized operation range of 170°-180°C. According to the Client, this temperature operation range is approximately 15% to 20% below the manufacturer’s 200°C peak operating temperature. Nevertheless, based on the results of our testing, the device was able to deactivate 80.5% of the virus. Further testing is proposed against SARS-CoV-2 aerosols with the Test Device continuously powered on, throughout the test, and at its full temperature core capacity.