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Verification of the Effectiveness of ActivePure® Technology in Decontamination of SARS-CoV-2

Final Report

FOR

Aerus, LLC

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Dallas, Texas 75254

MRIGlobal Project No. 311624.01.001

July 15, 2020

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.

Section 5.
Quality Assurance

5.1 Type of Study

This study was non-GLP, however all work was executed using established SOPs, at MRIGlobal in Kansas City, MO and all procedures utilized were technically valid in accordance with 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.

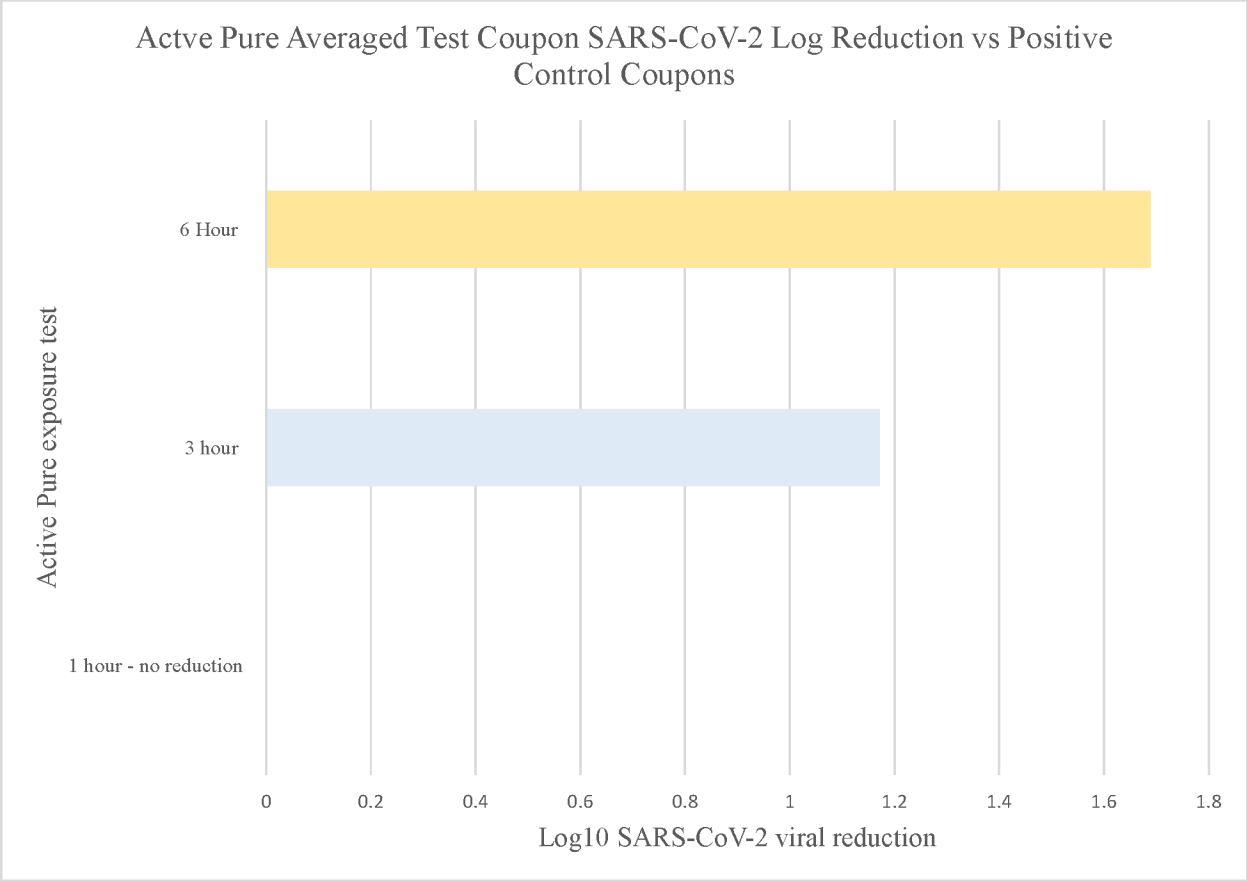

Figure 2. Test Results for Active Pure SARS-CoV-2 log reduction Efficacy

Laboratory environmental conditions in MRIGlobals BSL3 laboratories are monitored continually and data logged using an Amegaview data capture system. The laboratory temperature and humidity conditions for each test, as well as the log and percent reduction efficacy of the Active Pure unit are shown in Table 2.

Table 2. Tabulated Test Results, Test Parameters and Environmental Conditions

Test Date	Active Pure exposure time (hr)	Lab Temperature (ºF)	Lab Humidity (%RH)	Averaged SARS-CoV-2 Log viable reduction	Averaged SARS-CoV-2 viable reduction (%)
5/18/2020	1	72	47	0	0
6/15/2020	3	71.5	55	1.17	93.27
6/15/2020	6	71.5	55	1.69	97.95

Testing of the Active Pure unit showed substantial viral reduction of SARS-CoV-2 on test coupons for the 3 and 6 hour tests with results of 93.27%, and 97.95% respectively. The 1 hour exposure test showed no viral deactivation efficacy of the Active Pure system in reducing test coupon viral concentrations in relation to positive control coupons.

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 concentration and quantity of virus were available for testing. 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 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. Cells were infected with viral coupon sample extractions at 70% confluence and observed for the presence of cytopathic effect (CPE) for four (4) to five (5) days post-infection. A 10X serial dilution of coupon sample viral extractions 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. 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 dose of a virus).

Test Results:

Coupon preparation including SARS-CoV-2 inoculation, drying, exposure testing, extractions, and cell assay plating were conducted in a sterile class 2 biological safety cabinet. Following a 4 day plate assay viral incubation period, plates were read for viral infectivity and data recorded on $TCID_{50}$ test logs. Results were entered into a Reed Muench data analysis program for results and comparison of positive test control sample viral titer coupon concentrations to Active Pure exposed test coupon results. A plot showing the averaged log reduction efficacy of the ActivePure® Technology in deactivating a set of three (3) SARS-CoV-2 infected stainless steel coupons. The log reduction data shows the averaged test coupon viral deactivation in relation to the averaged positive control coupons (non-Active Pure exposured) over each exposure period, and is shown in Figure 2.

Table 1. Active Pure Test Matrix

System operating condition	System Flow Volume (cfm)	Test virus	Exposure time (hours)	Number of test coupons	Coupon viral inoculation volume (µl)	Coupon DMEM Extraction volume (ml)	Total Coupon samples/ Test	Number of Tests	Total Sample Assays
Without ActivePure	300	SARS-CoV-2	1	3	200	2	3	1	3
With ActivePure	300	SARS-CoV-2	1	3	200	2	3	1	3
Without ActivePure	300	SARS-CoV-2	3	3	200	2	3	1	3
With ActivePure	300	SARS-CoV-2	3	3	200	2	3	1	3
With ActivePure	300	SARS-CoV-2	6	3	200	2	3	1	3
Without ActivePure	300	SARS-CoV-2	6	3	200	2	3	1	3

sterile petri dishes and inoculated from a standard stock viral suspension with 200 mL of SARS-CoV-2 virus using a calibrated micropipette. The viral suspension was then evenly coated over the test coupons surface using sterile cell spreaders. Coated test coupons were air dried at standard laboratory conditions in the biological level 2 safety cabinet prior to exposure tests. Additional positive control coupons were similarly prepared and were subjected to the same environmental conditions and time course as test coupons without being subjected to Active Pure Technology exposure. The positive control coupons served as viral concentration standards to define the efficacy of the system in deactivating the SARS-CoV-2 virus from test coupons.

For each test, a set of three SARS-CoV-2 inoculated test coupons were placed on the floor of the biosafety cabinet at the exhaust outlet of the Active Pure unit, and at a 45° offset angle from the outer housing of the blower to avoid direct air flow turbulence. A diagram of the test setup is shown in Figure 1.

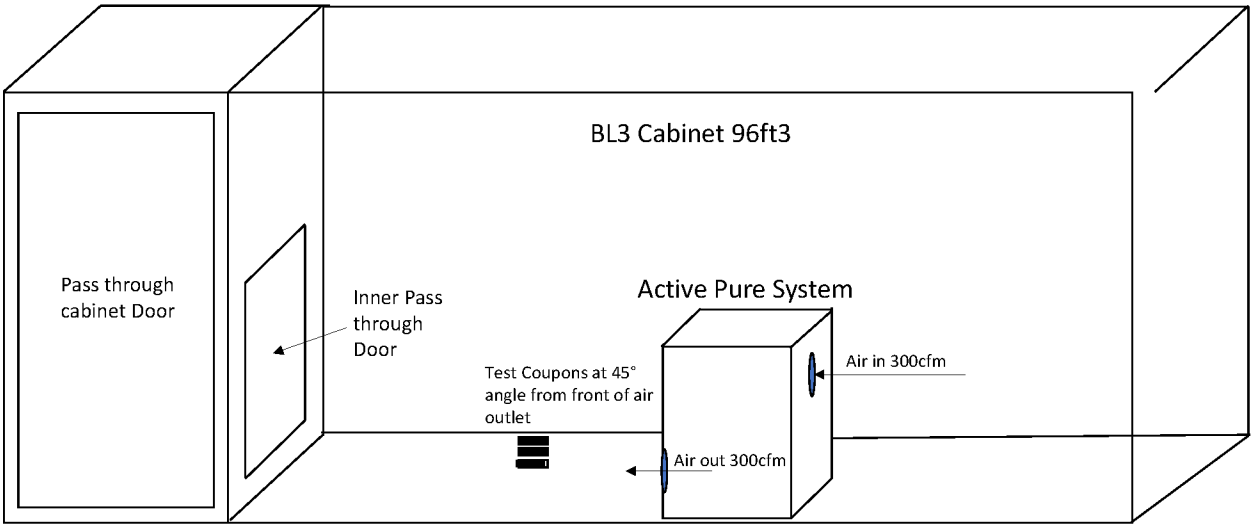

Figure 1. Diagram of Active Pure Test System

Tests were conducted over three (3) exposure times of 1, 3, and 6 hours. Test coupons were subjected to the Active Pure technology operation during the exposure process. Positive control coupons were subjected to the same exposure time course with only the unit blower flow operational without Active Pure technology operational. This provided a common and standardized test control for all system tests over each timecourse, and accurate assessment of the test units ActivePure® technology viral deactivation efficacy. A test matrix is shown in Table 1.

Section 3.
Test Systems and Methods

3.1 Equipment

Test Equipment

The ActivePure whole room disinfection system uses ActivePure® Technology and is a portable air purification system with dimensions of approximately 13" D × 13" W × 22" H. The system generates powerful oxidants that destroy bacteria, virus and odors without the use of chemicals. The system recirculates air in room environments at desired volumetric flow settings of 300 cfm using fan forced air flow. The system is designed to purify air and surfaces in rooms up to 30000 ft^3. As air enters the system, oxygen and water molecules in the air enter the unit through a honeycomb matrix which converts the molecules to powerful oxidizers that are released back into the room which destroy biological bacterial and viral contaminants. The Active Pure unit was provided to MRIGlobal by Aerus, LLC and was set for single speed air recirculation flow operation (300 cfm). The unit was also equipped with an on/off power switch and an Active Pure on/off switch for selection between blower only operation, or combined blower and Active Pure operation for testing.

SARS-CoV-2 (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.

3.2 Methods

Testing Description

MRIGlobal conducted testing characterization of a single ActivePure® portable air purification system in surface decontamination trials to evaluate the log reduction destructive kill effectiveness against an envelope virus (SARS-CoV-2) strain USA-WA1/2020. All tests were conducted in a biological class 3 facility at MRIGlobal, Kansas City, MO. The biological safety cabinet has internal dimensions of 6'W × 4'D × 4'H, with a displacement volume of approximately 96 ft^3. The cabinet is annually pressure decay tested for leak free integrity, and certified for safety. For testing of the Active Pure unit, the cabinet was sealed with the exhaust, and filter air inlet vents capped with gasketed steel plates for isolation and testing under static conditions without cabinet flow. The Active Pure unit was positioned in the center of the biosafety cabinet with position marks drawn on the bottom of the cabinet for proper placement and alignment preceding each test. The unit was tested for viral surface destruction efficacy using sterile 1" × 3" × 1 mm stainless steel test coupons inoculated with SARS-CoV-2. Test coupons were each inoculated with a 200 µL of SARS-CoV-2 stock suspension in a sterile class 2 biological safety cabinet. Individual test coupons were placed in test identification labeled

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor

Aerus, LLC
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2.2 Sponsor's Representative

Andrew Eide
Vice President, Product Development and Manufacturing

2.3 Testing Laboratories

MRIGlobal
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2.4 Personnel Responsibilities

Study Director—MRIGlobal

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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 Aerus, LLC, MRIGlobal conducted testing and evaluation of Aerus, LLC's whole room air disinfection system with ActivePure® Technology. The Active Pure room disinfection system uses free oxygen and water molecules in the air that are pulled through a honeycomb matrix. The technology creates powerful oxidizers that are released back into the room which destroy biological bacterial and viral contaminants. The Active Pure whole room disinfection system was evaluated in independent surface destruction tests of SARS-CoV-2 (Washington Isolate Strain) in laboratory trials at MRIGlobal.

Contents

Figures

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Preface

This final report was prepared at MRIGlobal (MRIGlobal) for the work performed under MRIGlobal Task No. 311624.01.001, "Verification of the Effectiveness of ActivePure® Technology in Decontamination of SARS-CoV-2"

Test devices were supplied to MRIGlobal by Aerus, LLC for the conduct of the program. The experimental phase of this task was initiated by MRIGlobal on May 18, 2020 and ended on June 19, 2020.

The Study Director of the program was Rick Tuttle. Execution of the study was assisted by Carl Gelhaus, Ph.D., Luca Popescu, Ph.D., Kristen Solocinski, Ph.D., Sam Humphries, 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.

MRIGLOBAL

Rick Tuttle
Study Director

Approved by:

Ed Sistrunk
Division Director
Medical Countermeasures

July 15, 2020