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

Final Report

FOR

Aerus, LLC

14841 Dallas Parkway
Aberdeen Bldg., Suite 500
Dallas, Texas 75254

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Preface

This final report was prepared at MRIGlobal (MRIGlobal) for the work performed under MRIGlobal Task "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 August 2, 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

Richard Tuttle

Rick Tuttle
Study Director

Approved by:

Claire L Cantrell

for Ed Sistrunk
Division Director
Medical Countermeasures

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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 ActivePure® 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 ActivePure® whole room disinfection system was evaluated in independent surface destruction tests of SARS-CoV-2 (Washington Isolate Strain) in laboratory trials at MRIGlobal.

Test Systems and Methods

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 3000 ft . 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 ActivePure® 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 ActivePure® on/off switch for selection between blower only operation, or combined blower and ActivePure® 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.

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 ActivePure® 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 ActivePure® 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

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 ActivePure® 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.

Tests were conducted over four (4) exposure times of 1, 3, 6 and 7 hours. Test coupons were subjected to the ActivePure® 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 ActivePure® 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.

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. Testing for 1, 3, and 6 hour tests with the ActivePure® technology were conducted in the BL3 cabinet without the addition of humidity. An additional test over a 7 hour ActivePure® technology exposure period was conducted on 8/2/2020, with the humidity maintained in the test cabinet using a Honeywell digital humidity monitor with humidity level setpoint control that provided a continuous and regulated humidity level of 63% RH (relative humidity) during the test. 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 ActivePure®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- ActivePure® exposured) over each exposure period, and is shown in Figure 2.

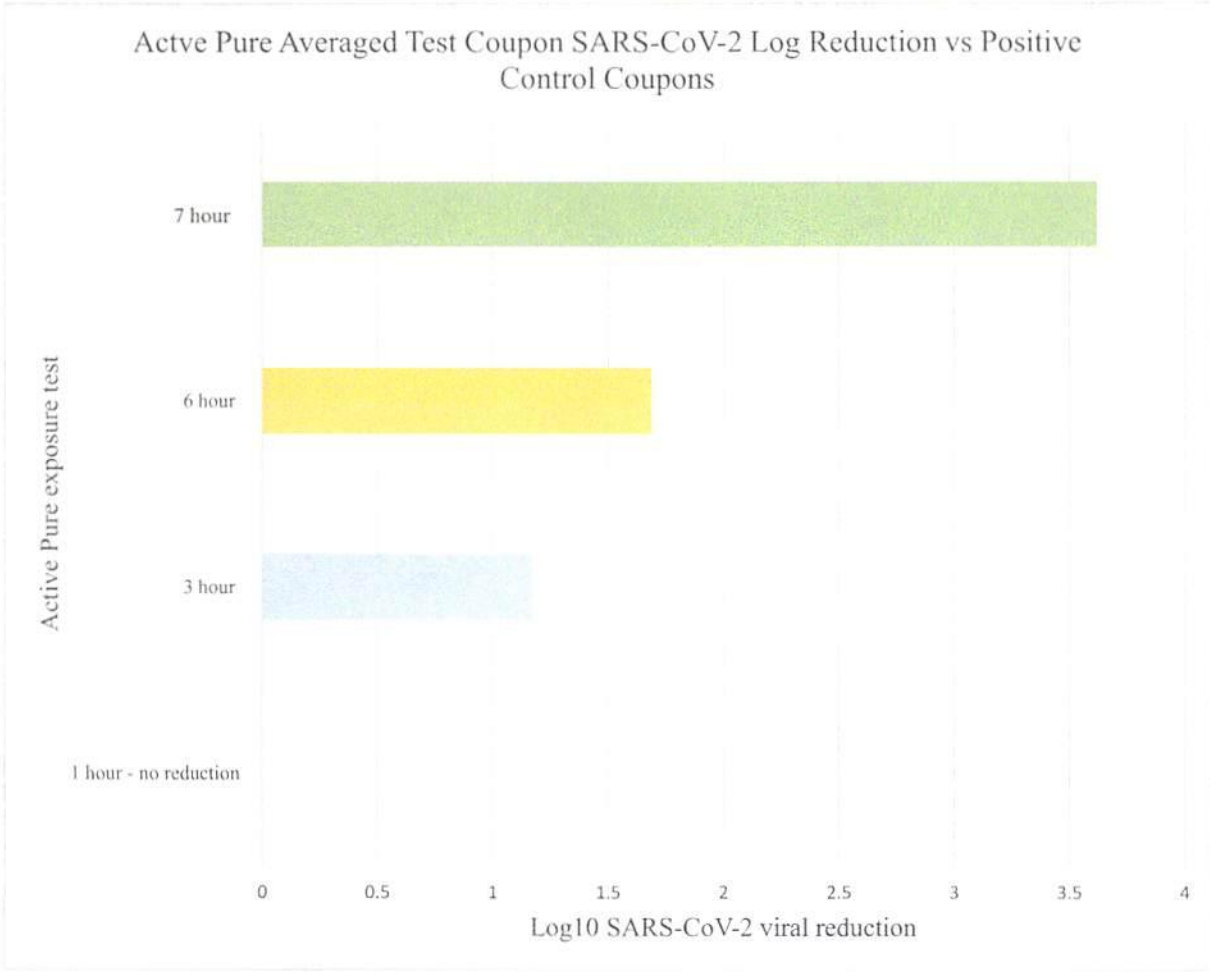

Figure 2. Test Results for ActivePure® SARS-CoV-2 log reduction Efficacy

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, and test cabinet %RH (if applicable) as well as the log and percent reduction efficacy of the ActivePure® 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 (°F)	Lab Humidity (%RH)	BL3 Test Cabinet Humidity (%RH)	Averaged SARS-CoV-2 Log viable reduction	Averaged SARS-CoV-2 viable reduction (%)
5/17/2020	1	72	47	NA	0	0
6/15/2020	3	71.5	55	NA	1.17	93.27
6/15/2020	6	71.5	55	NA	1.69	97.95
8/2/2020	7	72	52	63	3.62	99.98

Testing of the ActivePure® 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 7 hour test included controlled humidification of the test cabinet at 63% RH throughout the test with a viable reduction of 99.98% of SARS-CoV-2 on test coupons. It is theorized that the humidity levels in the test cabinet (air tight seal), may have reduced over the ActivePure® operation and the ActivePure® production of powerful oxidizers may have reduced or depleted during 1, 3, and 6 hour testing, thus reducing effectiveness against SARS-CoV-2. The 7 hour test, with the addition of humidity control regulated at 63% showed almost a 2 log viral kill increase over the 6 hour test, and approximately a 2.5 log increased virus kill in relation to the 3 hour test.