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Characterization of purAir HVAC ARC + BPI Technology in Surface Deactivation of SARS-CoV-2

Final Report

FOR:

GreenTech Environmental, LLC

6118 Kingsport Highway
Johnson City, Tennessee 37615

Attn: Kim Wilson, Director of Communications

MRIGlobal Project No. 311757.01.001

July 9, 2021

Preface

This report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311757.01.001, "Characterization of purAir HVAC ARC + BPI Technology in Surface Deactivation of SARS-CoV-2."

The experimental phase of this task was initiated by MRIGlobal on May 14, 2021 and ended on May 18, 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 of MRIGlobal, and any deviations were documented.

All study records are stored at MRIGlobal.

Sincerely,

MRIGLOBAL

Rick Tuttle
Principal Scientist
Life Sciences Division

Approved:

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

July 9, 2021

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Executive Summary

Objective:

The objective of this project was to measure purAir HVAC ARC + BPI Technology in surface deactivation of SARS-CoV-2 *in vitro*. The device was tested on SARS-CoV-2 virus inoculated on stainless steel coupons.

Study Design:

Stainless steel coupons were inoculated with 200 µL virus stock (1.47E+07 $TCID_{50}$/ml). Virus was evenly spread over the coupons and allowed to dry. Test coupons were transferred to into an aerosol test room and exposed to the test device for four hours. After the exposure time, any remaining virus was resuspended with a cell scraper and 2 ml DMEM/F12. Samples were diluted 1:10 down a 96 deep well plate in DMEM/F12. These dilutions were transferred to a plate of Vero cells with media removed. After approximately 20 minutes, DMEM/F12 supplemented with 5% FBS was added to cells to feed them for the next four days. The inoculated plates were then read for cytopathic effects (CPE).

Results and Conclusions:

Based on this experiment, we conclude that the purAir HVAC ARC + BPI technology is minimally effective at reducing surface SARS-CoV-2 infectivity *in vitro* after 4h of exposure. Test samples had 0.89 log (86.98%) lower infectivity of SARS-CoV-2 as compared to control samples.

Section 1.
Objective

The objective of this project was to determine if the purAir HVAC ARC + BPI Technology device has the ability to perform surface deactivation of SARS-CoV-2 *in vitro*. The device was tested on SARS-CoV-2 virus inoculated on stainless steel coupons.

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor's Representative

Kim Wilson
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2.2 Testing Laboratories

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2.3 Personnel Responsibilities

2.3.1 Study Director—MRIGlobal

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2.3.2 Task Lead– MRIGlobal

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2.3.3 Analyst – MRIGlobal

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Section 3.
Test Conditions

3.1 Test Product

3.1.1 Test Unit

purAir HVAC ARC + BPI technology

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.: 202010505JW
Expiration date: 11/21

3.2.2 Challenge Virus

Severe Acute Respiratory Syndrome-related Coronavirus-2 (SARS-CoV-2)
Strain: USA-WA1/2020
Vendor: BEI Resources
Lot: 202010401KS-A
Passage: 10

3.2.3 Host

Vero E6 Cells
Vendor: ATCC
Cat: CRL 1586
Passage No.: +32

Section 4.
Test System

MRIGlobal utilized the USA-WA1/2020 strain of the virus, acquired from BEI Resources (NR-52281). This was propagated in Vero E6 cells (ATCC CRL-1586); these cells were also used for the neutralization assay. Vero E6 cells were cultured in growth media consisting of Dulbecco's Modified Eagle Medium/F12 (DMEM/F12) supplemented with 5% FBS (Fetal Bovine Serum), and PSN (penicillin, streptomycin, and neomycin).

Section 5.
Study Design

The Vero E6 cells were plated on 96-well plates the day before the assay and were allowed to grow to ~ 60%-70% confluence. Stainless steel coupons (approximately 1" × 3") were inoculated with 200 µL virus stock (1.47E+07 $TCID_{50}$/ml). Coupons were allowed to dry in the biosafety cabinet for approximately 40 minutes.

Following the drying process of viral inoculated coupons, the coupons were placed in labeled petri dishes, transferred to the test room, and placed on a wire rack shelving unit at the designated locations within the test room. The Test Device was placed on a shelving unit in the center of the test room. The Test Device was remotely powered to initiate testing and remotely powered off at the conclusion of the specified test period with a remote power switch. Additionally, the room humidity level was pre-test conditioned and maintained in the range of approximately 65% RH during the test using a Dayton® Model 1UHGB Humidifier control sensor that regulated operation of a mist humidifier. Ion levels were read prior to testing to ensure device operation using the Alpha Labs Inc. Air Ion Counter (Settings: 1999, Polarity +, Offset Medium, Measure). An initial reading of 0.00 was taken prior to the device being activated, followed by a reading ranging between 408 to 435 once the device was turned on. Ion level readings were measured at approximately one (1) foot in front of the device.

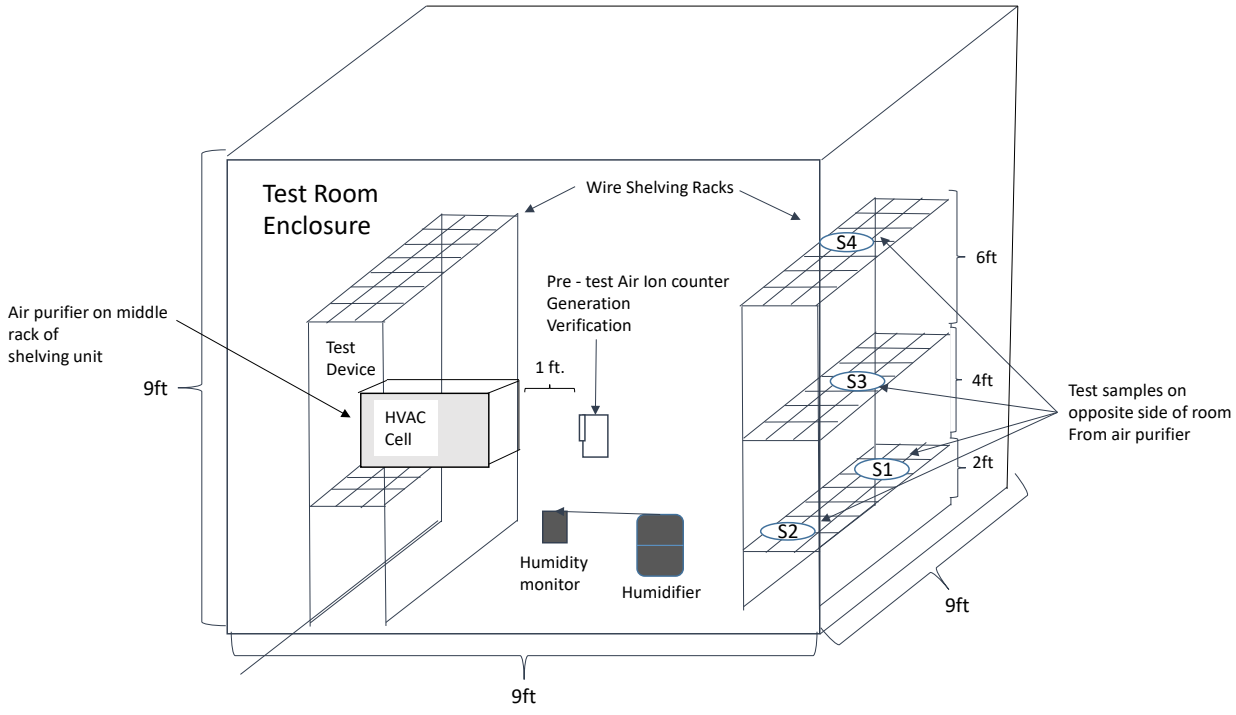

Figure 1. Room Set Up and Coupon Placement for Surface Testing

Coupons were exposed to test device operation for 4 hours. After the exposure time was complete, a visual inspection assuring operation of the test device was conducted, and the device

turned off. Test coupons were removed from the test room and placed in the biosafety cabinet. Two (2) ml of DMEM/F12 was added to each coupon and any remaining viral film was resuspended with a cell scraper. Samples were diluted 1:10 down a 96 deep well plate in DMEM/F12. These dilutions were transferred to a plate of Vero cells with media removed. After 20 minutes, DMEM/F12 supplemented with 5% FBS was added to cells to feed them for the next four days. This incubation period allowed the virus to adsorb to cells without interference from FBS.

Section 6.
Statistical 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 $TCID_{50}$/ml is calculated using the below equations, all using Microsoft Excel.

$$\text{Proportionate Distance (PD)} = \frac{\%\text{CPE at dilution above 50\%} - 50\%}{\%\text{ CPE at next dilution above 50} - \%\text{ CPE at next dilution below 50}}$$

$$\text{TCID50} = 10^{\text{log of dilution above 50\% CPE}} - \text{PD}$$

$$\text{TCID50/ml} = \frac{1}{\text{volume used per well}} x \frac{1}{\text{TCID50}}$$

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.

$$\%\text{ Log Reduction} = (1 - 10^{-\text{log reduction}}) \times 100$$

Section 7.
Results

Plates were read 4 days after the initiation of the assay. Test samples had 0.89 log (86.98%) lower infectivity of SARS-CoV-2 as compared to control samples. However, this is a moderate reduction and does not indicate great efficacy at reducing surface SARS-CoV-2 infectivity *in vitro*.

Table 1. Surface testing $TCID_{50}$/mL table

Sample Name	Test Description	Replicate No.	$TCID_{50}$/mL	Log10 $TCID_{50}$/mL	Average $TCID_{50}$/mL	Average Log10 $TCID_{50}$/mL	Log Reduction	Percent Log Reduction
T1	4h Test	1	4.81E+05	5.68	2.00E+05	5.11	0.89	86.981%
T2		2	4.22E+04	4.63				
T3		3	2.08E+05	5.32				
T4		4	6.81E+04	4.83				
C1	Control	1	3.16E+06	6.50	1.41E+06	6.00		
C2		2	3.16E+05	5.50				
C3		3	1.47E+06	6.17				
C4		4	6.81E+05	5.83				
20210401KS-A	Back titer		1.47E+07	7.17				

Section 8.
Conclusions

Based on this experiment, we conclude that the purAir HVAC ARC + BPI technology is minimally effective at reducing surface SARS-CoV-2 infectivity *in vitro* after 4h of exposure. Test samples had 0.89 log (86.98%) lower infectivity of SARS-CoV-2 as compared to control samples.