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EFFICACY OF THE PLAY-UV AGAINST AEROSOLIZED SARS-COV-2

PROJECT: EFFECTIV HVAC™ AEROSOL SINGLE PASS TEST – SARS-COV-2

PRODUCT: PLAY-UV Diffuser

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM(S):

SARS-COV-2 USA-CA1/2020

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Laboratory Project Number

1119

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Efficacy Study Summary

Study Title	EFFICACY OF THE PLAY-UV AGAINST AEROSOLIZED SARS-COV-2
Laboratory Project #	1119
Guideline:	Modified ISO standards as no international standards exist.
Testing Facility	Innovative Bioanalysis, Inc.
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	SARS-CoV-2 USA-CA1/2020
Description	The PLAY-UV diffuser connects to an HVAC system and combines filtering, sanitizing and diffusion of the air coming from the ventilation system of a room. This in-vitro study was conducted to determine the device's efficacy against aerosolized SARS-CoV-2.
Test Conditions	The test was conducted using a modified ASHRAE 185 test model within a sealed environment. Testing was conducted in a 10'x8'x8' chamber that complied with BSL-3 standards. The measured average air velocity for all the testing was 550 fpm and the calculated air volume was approximately 458 cfm. The temperature during all testing was approximately 76 ±2°F, with a relative humidity of 32%. The nebulizer was filled with the 4.03×10^6 TCID50 per mL of SARS-CoV-2 in FBS-based viral media and nebulized at a constant rate.
Test Results	Three challenge air passes were conducted, and the amount of SARS-CoV-2 collected from each air pass challenge is as follows: 1.20×10^2, 3.86×10^3, and 2.13×10^3 TCID50/mL.
Control Results	The control was conducted in duplicate without the device, and samples were taken at the corresponding time points used for the challenge trial. The results showed a natural viability loss over time in the chamber and served as a comparative baseline to calculate viral reduction.
Conclusion	The PLAY-UV diffuser demonstrated effectiveness in a single-pass treatment test in reducing 99.949% of the aerosolized viral concentrations based on the average 2.04×10^3 TCID50/mL of SARS-CoV-2 collected.

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Study Report

Study Title: EFFICACY OF THE PLAY-UV AGAINST AEROSOLIZED SARS-COV-2

Sponsor: EffectiV HVAC™

Test Facility: Innovative Bioanalysis, Inc. 3188 Airway Ave Suite D, Costa Mesa, CA 92626

Device Testing: PLAY-UV with MERV-9 filter

Study Report Date: 09/28/2021
Experimental Start Date: 08/09/2021
Experimental End Date: 08/22/2021
Study Completion Date: 09/27/2021

Study Objective:

EffectiV HVAC™ supplied a PLAY-UV diffuser for testing purposes to determine efficacy against viral pathogens in the air. The study evaluated the unit's ability to reduce aerosolized SARS-CoV-2 when operating under a modified ASHRAE 185 test model.

Test Method:

Bioaerosol Generation:

The nebulizer was filled with a 4.03 x 10^6 TCID50/mL viral media of SARS-CoV-2 USA-CA1/2020 and nebulized at a flow rate of 1mL/min with untreated local atmospheric air. The nebulizer's remaining viral stock volume was weighed to confirm that roughly the same amount was nebulized during each run. Bioaerosol procedures for the controls and viral challenges were performed in the same manner with corresponding time points and rate of collection.

Bioaerosol Sampling:

This study used two probes for air sampling, each connected to a calibrated Gilian 10i vacuum device. Before use, the equipment was inspected for functionality. The air sampler operated in conjunction with a removable sealed cassette and manually removed after each time point. Cassettes had a delicate internal filtration disc to collect viral samples, which was moistened with a viral suspension media to aid in the collection. The filtration disc from Zefon International, Lot# 24320, was used. Air sampling was conducted for a total of 15 minutes, 10 minutes during the nebulization, and 5 minutes after nebulization.

Test System Strains: SARS-CoV-2 USA-CA1/2020

The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-CA1/2020, NR-52382.

Study Materials and Equipment:

Equipment Overview: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. All parts were assembled and installed before arrival at the laboratory, except for the UV lamp and filter. The manufacturer provided UV mercury lamps and MERV-9 pleated filters to be installed following manufacturer instructions. The device was powered on to confirm functionality. A burn-in period was performed as the UV lamp for 24 hours before starting testing.

MANUFACTURER: EffectiV HVAC™

MODEL: PLAY-UV

SIZE (W x H x D): 24" x24"

SERIAL #: PLUV-100303-M1

UV FILTER UNIT: UVFILTER-W-M9

UV LAMP UNIT: HRV-F UV

MANUFACTURER: Sanuvox

MODEL: LMPHGJ105

Design Layout:

Testing was conducted in a modified HVAC system constructed to meet internal SOP requirements and comply with modified ANSI/ASHRAE standards. The system was placed on a stainless-steel table inside a sealed 10'x8'x8' chamber following Biosafety Level 3 (BSL-3) standards. Airflow is generated upstream by a variable fan with a measured velocity of 550 ft/min with a calculated air volume of approximately 458 ft^3/min. The air travels through ducting where nebulization of the viral pathogen occurs and flows into the device where the UV lamp is mounted. The air flows through the device's MERV-9 filter and into a boxed duct, where air samples were collected using two sampling probes, each connected to a vacuum device. Any residual air was exhausted through multiple filters and into the BSL-3 lab. Before testing, the modified HVAC system was pressure tested using an air compressor and analog PSI meter to confirm no leaks were present. The BSL-3 chamber was visually inspected, pressure tested, and all internal lab systems and equipment were reviewed before testing.

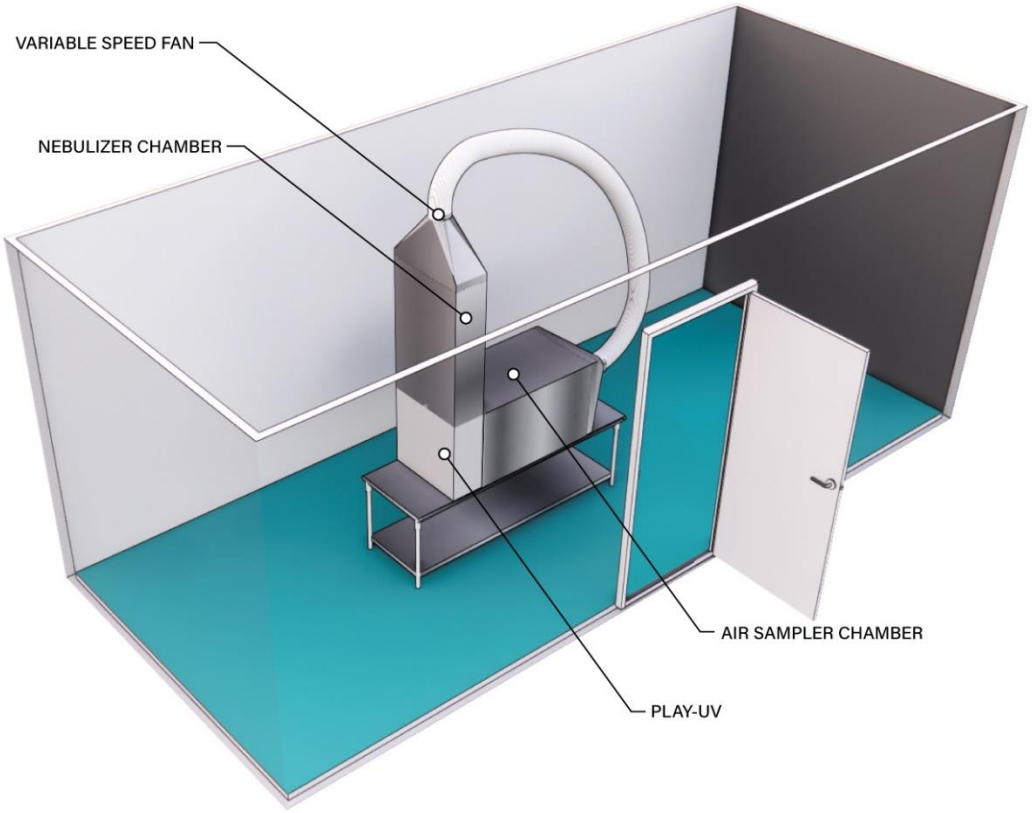


Figure 1. Room layout for control and experimental trial.

Test Method:

Exposure Conditions:

1. The temperature during all test runs was approximately 76 ±2°F with a relative humidity of 32%.
2. The air sample collection volumes were set to 15-minute continual draws at the point of sampling.

Experimental Procedures:

1. Before the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. 4.03 x 10^6 TCID50/mL SARS-CoV-2 viral media was nebulized on the upstream side for 10 minutes.
3. After nebulization, the PLAY-UV diffuser was turned on via remote control.
4. Air sampling was continuously taken from the airflow on the downstream side.
5. Air sampling collection occurred for a total of 15 minutes per challenge for each test.
6. The sample cassettes were manually removed from the collection system and taken to an adjacent biosafety cabinet for extraction and placement into viral suspension media.
7. Two controls and three viral challenges were conducted using the same methodology.

Post Decontamination:

After each viral challenge test, the UV system inside the testing chamber was activated for 30 minutes. After 30 minutes of UV exposure, there was a 30-minute air purge through the air filtration system. All test equipment was cleaned at the end of each day with a 70% alcohol solution. Collection lines were soaked in a bleach bath mixture for 30 minutes then rinsed repeatedly with DI water. The nebulizer and vacuum collection pumps were decontaminated with hydrogen peroxide mixtures.

Preparation of The Pathogen

Viral Stock: SARS-CoV-2 USA-CA1/2020 (BEI NR-52382)

Test	Specifications	Results
Identification by Infectivity in Vero 6 cells	Cell Rounding and Detachment	Cell Rounding and Detachment
Next-Generation Sequencing (NGS) of the complete genome using Illumina® iSeq™ 100 Platform	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1	99.9% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1
Approx. 940 Nucleotides	≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1	100% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1
Titer by TCID50 in Vero E6 Cells by cytopathic effect	Report Results	2.8 X 10^5 TCID50 per mL in 5 days at 37°C and 5% CO_2
Sterility (21-Day Incubation)		
Harpos HTYE Broth, aerobic	No Growth	No Growth
Trypticase Soy Broth, aerobic	No Growth	No Growth
Sabourad Broth, aerobic	No Growth	No Growth
Sheep Blood Agar, aerobic	No Growth	No Growth
Sheep Blood Agar, anaerobic	No Growth	No Growth
Thioglycollate Broth, anaerobic	No Growth	No Growth
DMEM with 10% FBS	No Growth	No Growth
Mycoplasma Contamination		
Agar and Broth Culture	None Detected	None Detected
DNA Detection by PCR of extracted test article nucleic acid	None Detected	None Detected

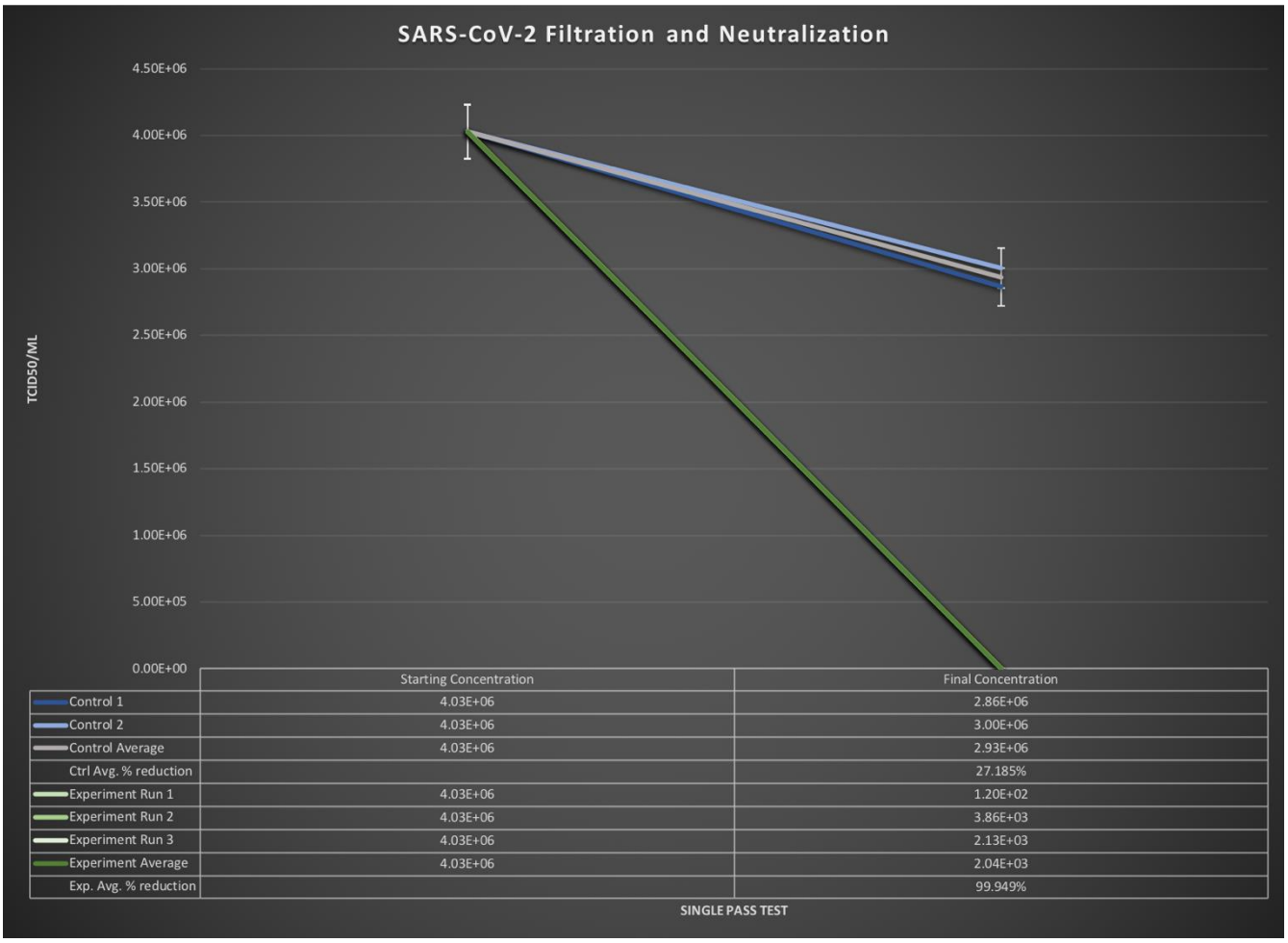
*The viral titer listed in the Certificate of Analysis is representative of the titer provided by BEI Resources. These viruses are grown on VeroE6 cells either in-house or at a partner lab to the concentrations listed within the experiment design.

Control Protocol

The control was conducted in duplicate without the device operating in the testing chamber to assess the PLAY-UV unit accurately. Control samples were taken in the same manner and at the corresponding time points used for the challenge trial to serve as a comparative baseline to assess the viral reduction when the device was operating.

Study Results

Controls were plotted to show natural viability loss after a single air pass in the chamber. After three air passes against SARS-CoV-2, the device was observed to have reduced an initial viral concentration of 4.03×10^6 TCID50/mL to a final concentration of 1.20×10^2, 3.86×10^3, and 2.13×10^3 TCID50/mL averaging approximately 2.04×10^3 TCID50/mL.



	Starting Concentration	Final Concentration
Control 1	4.03E+06	2.86E+06
Control 2	4.03E+06	3.00E+06
Control Average	4.03E+06	2.93E+06
Ctrl Avg. % reduction		27.185%
Experiment Run 1	4.03E+06	1.20E+02
Experiment Run 2	4.03E+06	3.86E+03
Experiment Run 3	4.03E+06	2.13E+03
Experiment Average	4.03E+06	2.04E+03
Exp. Avg. % reduction		99.949%

**As it pertains to data represented herein, the value of 1.2E+02 indicates a titer that is lower than the specified limit of quantitation. The limit of quantitation for this assay is 1.2E+02.

***As it pertains to data represented herein; the percentage error equates to an average of ±5% of the final concentration.

Conclusion:

The EffectiV HVAC™ Play-UV diffuser effectively reduced aerosolized SARS-CoV-2 as shown by the results collected from each air pass challenge: 1.20 x 10^2, 3.86 x 10^3, and 2.13 x 10^3 TCID50/mL. The average reduction observed after performing three air pass challenges was approximately 99.949%. One single pass challenge had a value of 1.2E+02 which indicates a titer that is lower than the specified limit of quantitation. The limit of quantitation for this assay is 1.2E+02. The measured average air velocity for all the testing was 550 fpm and the calculated air volume was approximately 458 cfm.

Therefore, with every pass-through of air, approximately 99.949% of the particulates within the air were filtered and/or neutralized. Overall, the trials demonstrated that the device with the testing combination efficiently reduces viral air concentrations that pass through the unit.

It should be noted that effort was made to simulate a real-life environment in the chamber while considering the precautions needed when working with a Biosafety Level 3 pathogen. Furthermore, when aerosolizing pathogens and collecting said pathogens, some variables cannot be fully accounted for, namely, placement of pathogen, collection volume, collection points, drop rate, surface saturation, viral destruction upon collection, viral destruction on nebulization, and possibly others. Every effort was made to address these constraints with the design and execution of the trials.

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Disclaimer

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