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EFFICACY OF THE D6 STERIONIZER™ AGAINST AEROSOLIZED SARS-CoV-2

PROJECT: FILT AIR LTD. D6 STERIONIZER™ AEROSOL SARS-COV-2

PRODUCT: D6 STERIONIZER™ BIPOLAR NEEDLEPOINT IONIZER

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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Medical Director

Study Completion Date

8/3/2021

Testing Facility

Innovative Bioanalysis, Inc.

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Laboratory Project Number

1047

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Efficacy Study Summary

Study Title	EFFICACY OF THE D6 STERIONIZER™ AGAINST AEROSOLIZED SARS-CoV-2
Laboratory Project #	1047
Guideline:	Modified ISO standards as no international standards exist.
Testing Facility	Innovative Bioanalysis, Inc.
Study Dates:	
Study Initiation Date:	04/12/2021
Study Completion Date:	08/03/2021
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	SARS-CoV-2 USA-CA1/2020
Description	Filt Air Ltd. provided a D6 Sterionizer™, a compact ionizing device designed to be integrated into an air movement and management system such as an HVAC duct system, air conditioner or humidifier. The in vitro study evaluates the efficacy of the D6 Sterionizer™ against aerosolized SARS-CoV-2.
Test Conditions	The study conducted two control tests and 3 viral challenges in a certified Biosafety hood inside a BSL-3 laboratory. The temperature during testing was approximately 73 ±2°F, with a relative humidity of 44%. Bioaerosol was generated using a nebulizer filled with 6.32×10^6 TCID50/mL SARS-CoV-2 in FBS based media. Air samples were collected after 0, 15, and 30-minute exposure to the operating device.
Test Results	Active SARS-CoV-2 concentrations were observed to have been significantly reduced at the 15-minute and 30-minute time point. After 30 minutes of operation, the trial observed a decrease in the initial viral concentration of 7.02×10^6 to an average of 8.86×10^4 TCID50/mL.
Control Results	The results plotted on the graph showed a natural viability loss over a 30-minute period and served as a comparative baseline in order to calculate viral reduction.
Conclusion	The Filt Air Ltd. D6 Sterionizer™ demonstrated the ability to reduce the concentration of aerosolized SARS-CoV-2 when exposed to a high negative and positive ion concentration.

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Study Report

Study Title: EFFICACY OF THE D6 STERIONIZER™ AGAINST AEROSOLIZED SARS-CoV-2

Sponsor: Filt Air Ltd.

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

Device Testing: D6 Sterionizer™

Study Report Date: 08/03/2021
Experimental Start Date: 05/04/2021
Experimental End Date: 05/04/2021
Study Completion Date: 08/03/2021

Study Objective:

The D6 Sterionizer™ device, was provided by Filt Air Ltd. for testing to determine its effectiveness against an aerosolized virus, SARS-CoV-2, under controlled conditions.

Test Method:

Bioaerosol Generation:

The nebulizer was filled with 6.32 x 10^6 TCID50 per mL of SARS-CoV-2 in FBS-based viral media and nebulized at a flow rate of 1mL/min with untreated local atmospheric air. Upon each completion, 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:

A calibrated Gilian 10i vacuum device was used for air sampling in this study. Prior to usage, the device was inspected for functionality. The air sampler operated in conjunction with a removable sealed cassette, which was manually removed after each sampling time point. Cassettes had a delicate internal filtration disc to collect viral samples which was moistened with a viral suspension media to aid in collection. The filtration disc from Zefon International, Lot# 24320, were used for testing.

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. Due to the closed design, no assessment was conducted on the inner components of the device. The D6 Sterionizer™ came with two square axial fans that were attached to both sides of the device. A sticker on the fan indicated the directional airflow and the velocity of each fan was measured at approximately 384 feet per minute. A DC power adapter provided by the manufacturer was used to power on the device. Prior to testing, positive and negative ion generation was confirmed using two Alpha Lab AIC2 ion polarity meters.

MANUFACTURER: FILT AIR, LTD.

MODEL: IG3-025-C3. RevB

DIMENSIONS: 3.82”x3.5”x0.96”

MAKE: D6 STERIONIZER™

SERIAL #: N/A

Testing Layout:

The test was conducted in a custom 72”x32”x32” pathogen handling hood. It was designed to be fully sealed to prevent unwanted exposure of pathogens into the external surrounding. The plexiglass hood was built to be viewable from all sides of the chamber with an intake and HEPA-filtered exhaust system. The testing chamber is located inside a BSL3 laboratory that maintains a negative pressure greater than -12.00 Pa and consists of sealed walls, epoxy flooring, and a locking antechamber complying to BSL3 standards.

Inside the biosafety hood, the device was positioned at the opposite end from the site of nebulization. The device had square axial fans attached to both sides of the D6 Sterionizer™ that propelled ions toward the aerosolized pathogens at an average air velocity of 733 ft/min. For air sample collection, the chamber was equipped with a single probe which was connected to a Gilian 10i programmable system with a sampling cassette. Power was supplied to the device through a power-regulated 120v outlet.

Prior to testing, the chamber was pressure tested and visually inspected for leaks. All seals for the chamber were confirmed and all equipment required for testing underwent a function test to confirm proper working conditions.

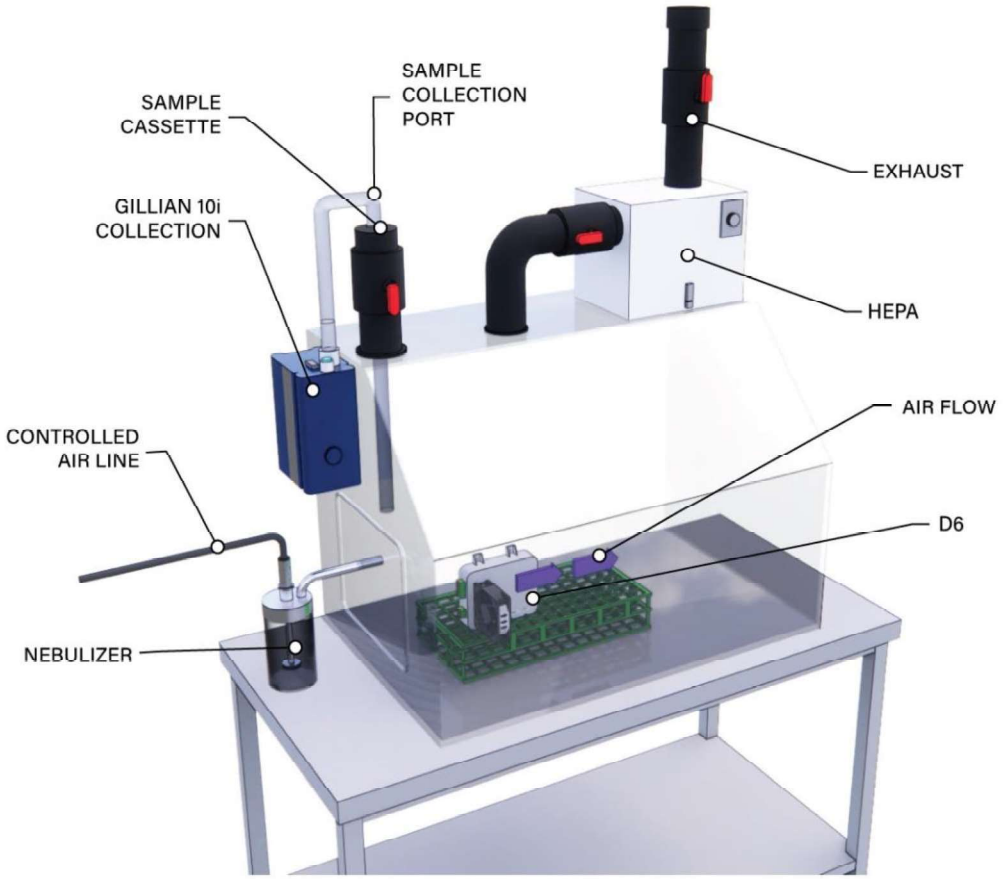

Figure 1. Test layout for control and experimental trial.

Test Method:

Exposure Conditions:

1. The testing chamber was decontaminated and prepped per internal procedures prior to start of testing.
2. The temperature during all test runs was approximately 73 ±2°F with a relative humidity of 44%.
3. The air samplers were calibrated by the manufacturer and set at a standard flow of 5.02L/min. Calibration records indicate a 0.20% tolerance.
4. Each time point was treated as an individual test and the chamber was reset after sample collections.
5. Testing time points were as follows with T equal to minutes: T-0, T-15, and T-30.
6. Two controls and three viral challenges were conducted using the same methodology.

Nebulization:

1. Nebulization for control and viral test challenges were performed in the same manner.
2. After nebulization of the pathogen, the D6 Sterionizer™ was turned on via remote control.
3. A viral stock of 6.32 x 10^6 TCID50/mL in FBS media was nebulized into the sealed environment via a dissemination port at a constant rate for 10 minutes.
4. During the pathogen challenge, the D6 Sterionizer™ was turned off at the defined time points for sample collection to start.
5. Air sample collection was set to a 10-minute continuous draw at the point of sampling.
6. Sample cassettes were manually removed from the collection system after each control and experimental trial.
7. The cassette was taken to an adjacent biosafety cabinet for extraction and placement into a viral suspension media.

Post Decontamination:

At the conclusion of 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

To accurately assess the Filt Air Ltd. D6 Sterionizer™, two control trials were conducted without the device operating in the testing chamber. Control samples were taken 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. This allows for the percent reduction calculations to be made for the viral challenges.

Study Results

When tested against aerosolized SARS-CoV-2, the D6 Sterionizer™ showed overall higher percent reductions for each time point when compared to the control trials. The device reduced SARS-CoV-2 from a starting concentration of 7.02×10^6 to 2.97×10^6, 3.09×10^6, 2.85×10^6 TCID50/mL after 15 minutes of operation. After 30 minutes, a higher reduction of approximately 98.73% was achieved with an average concentration of 8.86×10^4 TCID50/mL.

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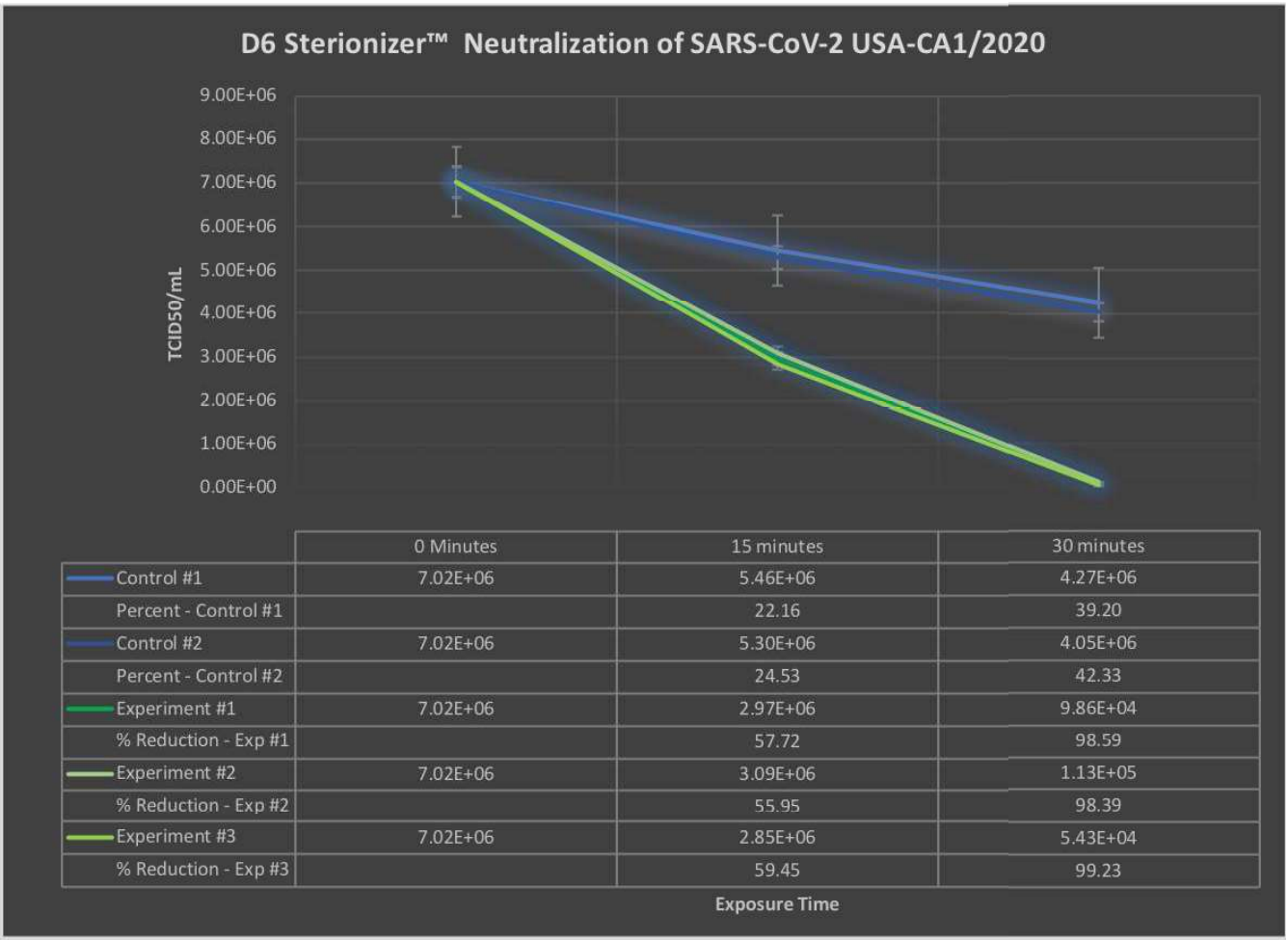
RESULTS:



	0 Minutes	15 minutes	30 minutes
Control #1	7.02E+06	5.46E+06	4.27E+06
Percent - Control #1		22.16	39.20
Control #2	7.02E+06	5.30E+06	4.05E+06
Percent - Control #2		24.53	42.33
Experiment #1	7.02E+06	2.97E+06	9.86E+04
% Reduction - Exp #1		57.72	98.59
Experiment #2	7.02E+06	3.09E+06	1.13E+05
% Reduction - Exp #2		55.95	98.39
Experiment #3	7.02E+06	2.85E+06	5.43E+04
% Reduction - Exp #3		59.45	99.23

**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 Filt Air Ltd. D6 Sterionizer™ demonstrated the ability to reduce the amount of active aerosolized SARS-CoV-2 when exposed to a high negative and positive ion concentration. During the test trials, collectable active SARS-CoV-2 in the air was reduced to 9.86 x 10^4, 1.13 x 10^5, and 5.34 x 10^4 TCID50/mL which is indicative of an overall net reduction of approximately a 98.73% after 30 minutes of ion exposure in a sealed testing chamber.

These test scenarios were designed to observe the ability of negatively and positively charged ions to have a quantifiable effect on a pathogen in the air. The study was in a smaller sized environment to control as many variables as possible. When the system is applied to different sized room environments the results will scale with variables present and the ion concentrations present. Room size, occupancy rating, how many units are being used, air velocity, furniture, and amount of pathogen in the air will all play a significant factor in the efficacy of the system which cannot be determined based on the outcome of this report. Air moves differently in all spaces and as humans interact with the environment, they can change subtle movements of airflow. This study is to open the discussion on the efficacy of ionization and the ability to reduce a pathogens activity in the air.

When aerosolizing pathogens and collecting said pathogens, some variables cannot be accounted for, namely, placement of pathogen, collection volume, collection points, drop rate, surface saturation, viral destruction upon collection, viral destruction on aerosolization, and possibly others. Every effort was made to address these constraints with the design and execution of the trials. And these efforts are reflected in the meaningful recovery of virus in the control test.

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