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

PROJECT: D6 STERIONIZER™ SURFACE 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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Laboratory Director

Study Completion Date:

6/10/2021

Testing Facility

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Laboratory Project Number

1047

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Efficacy Study Summary

Study Title	EFFICACY OF THE D6 STERIONIZER™ AGAINST 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:	4/06/2021
Study Completion Date:	6/10/2021
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	SARS-CoV-2 USA-CA1/2020
Description	An in vitro study to determine the efficacy of the Filt Air D6 Sterionizer™ against the known pathogen SARS-CoV-2.
Test Conditions	The test was conducted in a certified Bio Safety hood inside a BSL3 laboratory, which maintained negative pressure greater than 12.00 Pa. The temperature during all test runs was approximately 73°F ±2°F, with a relative humidity of 44%. Slides were inoculated with the virus by directly applying a known concentration of viral solution with a spatula and left to air dry before use.
Test Results	Active SARS-CoV-2 concentrations on the sample surfaces were reduced at both the 15-minute time point and 30-minute time point. After 15 minutes of exposure an average overall observed reduction of 84.80% was recorded, and after 30 min 99.90% was recorded which would equate to a 3-log reduction.
Control Results	Two control tests were conducted to serve as a comparative baseline for viral reduction. A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 6.32 X 10^6 TCID50/mL was used for this experiment and applied via direct pipette and evenly distributed before air drying. This value was measured and confirmed upon sample collection at T-0 minutes.
Conclusion	The Filt Air D6 Sterionizer™ demonstrated the ability to reduce the concentration of the active pathogen SARS-CoV-2 on a surface when exposed to a high negative and positive ion concentration.

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Study Report

Study Title: SARS-CoV-2 USA-CA1/2020 PATHOGEN SURFACE D6 STERIONIZER™

Sponsor: FILT AIR, Ltd.

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

Device Testing: Testing the efficacy of the Filt Air D6 Sterionizer™ system against surface, SARS-CoV-2

Study Report Date: 6/16/2021
Experimental Start Date: 5/4/2021
Experimental End Date: 5/4/2021
Study Completion Date: 6/16/2021

Study Objective:

This in vitro study was designed to determine the efficacy of the Filt Air D6 Sterionizer™ system against a surface contaminated with the pathogen SARS-CoV-2. The D6 Sterionizer™ system is designed to be placed inside an HVAC duct system or similar air movement and management system.

Test Method:

Surface Inoculation:

For the control and viral challenge, each of the testing sites were equally subjected to a 1 mL inoculation of viral media containing a known titer of 6.32 x 10^6 TCID50/mL*. The viral solution was spread with a spatula to ensure even distribution and saturation of all materials and left to air dry for 5 minutes.

Surface Sampling:

Swabs were moistened with viral media solution prior to collecting samples to maximize collection. Each slide was subjected to a 1 mL rinse in viral media and swabbed for residual pathogen material. After collection was completed, the swab and media were vortexed for 1 full minute. All samples collected were subjected to the same TCID50 assay protocol to determine viral concentration.

Test System Strains: SARS-CoV-2 USA/CA-1/2020

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.

**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 inhouse or at a partner lab to the concentrations listed within the experiment design.*

Study Materials and Equipment:

Equipment Overview: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. The device was powered on to confirm functionality prior to testing. For testing, power was supplied through a power-regulated 120v outlet with a surge protector and backup battery system. Manufacturer provided a DC power adapter for the device to be powered on.

MANUFACTURER: FILT AIR, LTD.

MODEL: IG3-025-C3. Rev B

MAKE: D6 STERIONIZER™

SERIAL #: N/A

Equipment Specifics: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. The manufacturer provided two square axial fans with pre-determined settings for directional flow and speed to generate the air flow that will blow the ions toward the samples. A sticker on the fan indicated the direction of airflow and the velocity of each fan was recorded at approximately 384 feet per minute. There were no observed filtration system or removable filters. Ion generation was confirmed prior to testing using an Alpha Lab AIC2 ion polarity meter. Airflow was checked at the sample location and was approximately 201 feet per second.

Testing Chamber: The test was conducted in a certified Bio Safety hood inside a BSL3 laboratory, which maintained negative pressure greater than 12.00 Pa. The temperature during all test runs was approximately 73°F ±2°F, with a relative humidity of 44%. Slides were inoculated with the virus by directly applying a known concentration of viral solution with a spatula and left to air dry before use.

Design Layout:

The test was conducted in a certified Bio Safety hood inside a BSL3 laboratory, which maintained negative pressure greater than 12.00 Pa. The BSL3 laboratory consisted of sealed walls, epoxy flooring, and a locking antechamber complying to BSL3 standards. The temperature during all test runs was approximately 73°F ±2°F, with a relative humidity of 44%.

The device and sample testing area were placed on opposite ends of the biosafety hood and elevated 3 inches off the surface. The sample slides were placed in a row and spread out evenly. Two AIC ion polarity meters were positioned behind the sample testing area to measure air ion concentration measurements. The average air ion concentration was 1,235k negative ion per centimeter squared and 1,804k positive ions per centimeter square. The device had a square axial fan attached to both sides of the D6 Sterionizer™ device which generated an average air flow velocity of 384 feet per minute which influenced the ion concentration in the testing environment along with sample location and air volume.

Prior to testing, all internal lab systems were reviewed and determined to be functioning. All seals for the chamber were confirmed and all equipment used had a function test to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

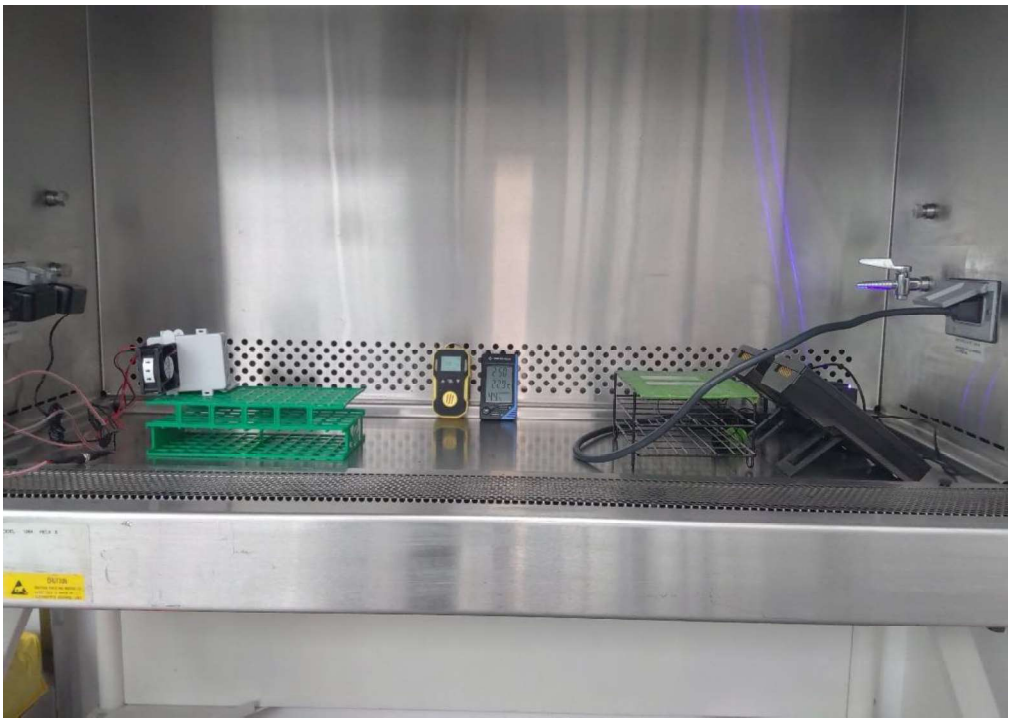

Figure 1. Room layout for control and experimental trial.

Test Method:

Filt Air, Ltd. supplied a D6 Sterionizer™ system for testing purposes to determine efficacy against viral pathogens. This study evaluated the efficacy of the D6 Sterionizer™ in its ability to inactivate the viral strain referred to as SARS-CoV-2.

Exposure Conditions:

1. Prior to the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. The temperature during all test runs was approximately 73°F ±2°F with a relative humidity of 44%.
3. The sample slides were inoculated with 1mL of a known concentration of viral media and left to air dry for 5 minutes.
4. Air ion measurements were taken directly behind the samples to confirm that the negative and positive ion concentration.

Experimental Procedure:

1. Prior to the initial control test and following the active pathogen test, the testing area was decontaminated and prepped per internal procedures.
2. The Filt Air D6 Sterionizer™ device was turned on just prior to the start of testing at the 0-minute time point.
3. Three sterile glass slides were inoculated with the viral media and labeled with a pre-determined time point.
4. Glass slides were placed in evenly spaced rows at the far end of the testing chamber in relation to the intake/upstream air side.
5. Surface samples were taken at the following pre-determined time points after ion exposure.
    - 0 minute
    - 15 minutes
    - 30 minutes
6. A swab and rinse were performed on each sample slide based on time point and cultured to determine microorganism recovery and overall efficacy.
7. All swabs were sealed after collection and provided to lab staff for analysis after study completion.
8. At the conclusion of the testing, the UV system inside the chamber was activated for 30 minutes.
9. After 30 minutes of UV exposure, all test equipment was cleaned at the end of each day with a 70% alcohol solution.

Post Decontamination:

At the conclusion of each viral challenge test, the UV system inside the biosafety chamber was activated for 30 minutes. After 30 minutes of UV exposure, all test equipment was cleaned at the end of each day with a 70% alcohol solution.

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

TCID50 Procedure:

Materials and Equipment:

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips—20uL, 200uL, 1000uL
- Inverted Microscope
- Tubes for dilution
- Hemocytometer with coverslip
- Cell media for infection
- Growth media appropriate for the cell line
- 0.4 % Trypan Blue Solution
- Lint-free wipes saturated with 70% isopropyl alcohol
- CO_2 Incubator set at 37°C or 34°C, or other temperature as indicated

Procedure:

1. One day before infection, prepare 96 well dishes by seeding each well with Vero E6 cells in DMEM plus 7.5% fetal bovine serum, 4mM Glutamine, and antibiotics.
2. On the day of infection, make dilutions of virus samples in PBS.
3. Make a series of dilutions at 1:10 of the original virus sample. Fill the first tube with 2.0 mL PBS and the subsequent tubes with 1.8mL.
4. Vortex the viral samples, then transfer 20 uL of the virus to the first tube, vortex, discard tip.
5. With a new tip, serial dilute subsequent tips transferring 200 uL.

Additions of virus dilutions to cells

1. Label the lid of a 96-well dish by drawing grid lines to delineate quadruplicates and number each grid to correspond to the virus sample and label the rows of the plate for the dilution, which will be plated.
2. Include four negative wells on each plate, which will not be infected.
3. Remove all but 0.1 mL of media from each well by vacuum aspiration.
4. Starting from the most dilute sample, add 0.1 mL of virus dilution to each of the quadruplicate wells for that dilution.
5. Infect four wells per dilution, working backward.

6. Allow the virus to absorb to the cells at 37°C for 2 hours.
7. After absorption, remove the virus inoculum. Start with the most dilute and work backward.
8. Add 0.5 mL infection medium to each well, being careful not to touch the wells with the pipette.
9. Place plates at 37°C and monitor CPE using the inverted microscope over a period of 1 to 4 weeks.
10. Record the number of positive and negative wells.

Protocol Changes:

Protocol Amendments: None

Protocol Deviations: None

Control Protocol

Two control tests were conducted with no ion exposure from the D6 Sterionizer™ in the testing chamber. Control samples were taken at the corresponding sample times used for the challenge trial to serve as a comparative baseline to assess the viral reduction when the D6 Sterionizer™ was operated. This allows for the net reduction calculations to be made for the challenge trial. Furthermore, temperature and relative humidity were monitored inside the BSL3 lab and confirmed to be in relative range, ±5% compared to control testing conditions prior to running the viral challenges. A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 6.32 X 10^6 TCID50/mL was used for this experiment and applied via direct pipette and evenly distributed before air drying. This value was measured and confirmed upon sample collection at T-0 minutes.

Inoculation of Viral Media:

A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 6.32 X 10^6 TCID50/mL was used for this experiment. Each of the testing sites were equally subjected to a 1 mL inoculation of viral media and was spread evenly on the slide using a spatula before letting it air dry for 5 minutes. The viral solution was splayed out on a sterile glass slide that was 3”x1.5” and 0.125” thick. Samples were collected using a swab moistened with viral media and rinsed to maximize collection. The control samples were prepared and collected in the same manner as the viral test regarding the time points and collection rate.

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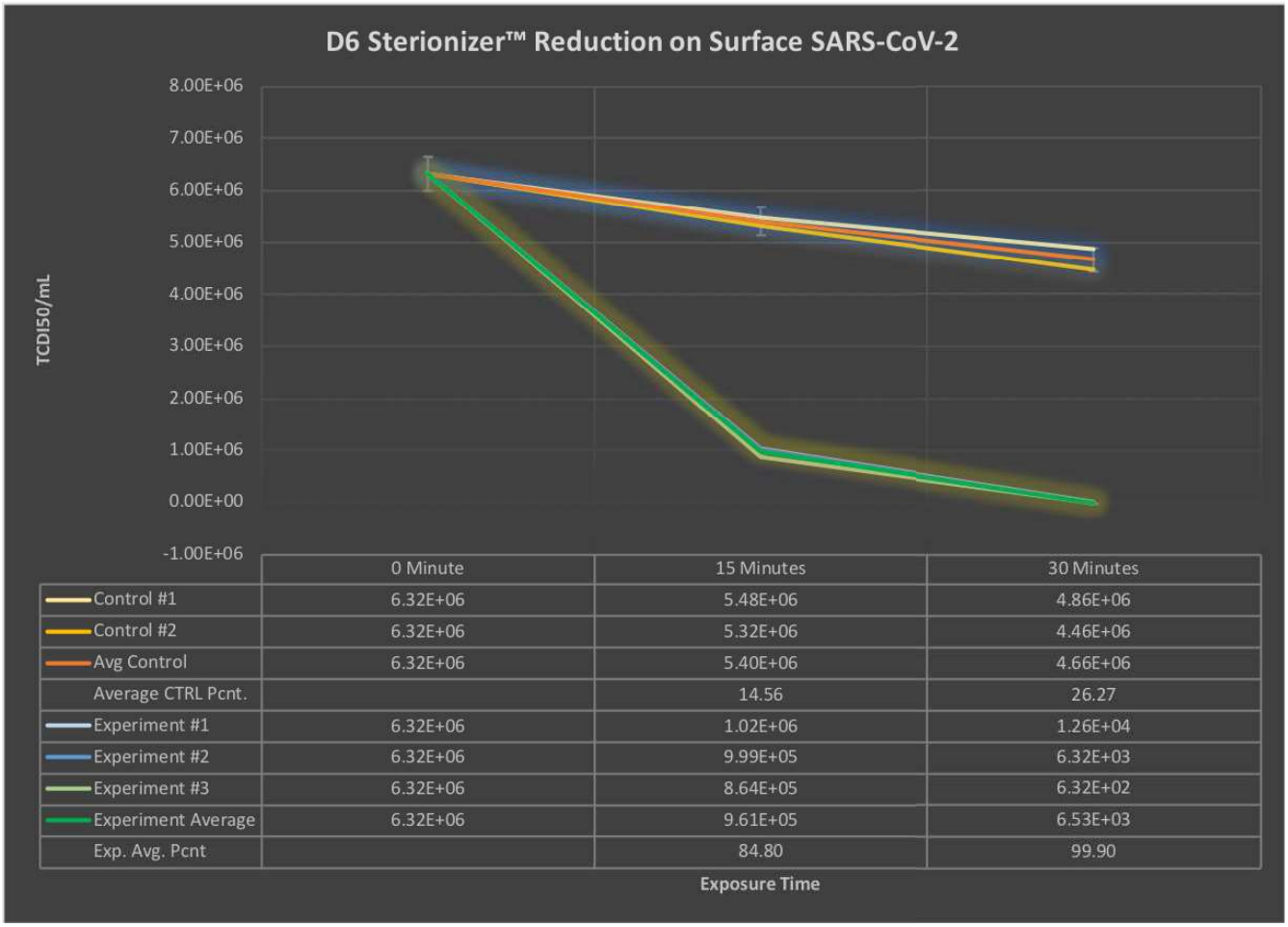
Study Results

RESULTS:



	0 Minute	15 Minutes	30 Minutes
Control #1	6.32E+06	5.48E+06	4.86E+06
Control #2	6.32E+06	5.32E+06	4.46E+06
Avg Control	6.32E+06	5.40E+06	4.66E+06
Average CTRL Pcnt.		14.56	26.27
Experiment #1	6.32E+06	1.02E+06	1.26E+04
Experiment #2	6.32E+06	9.99E+05	6.32E+03
Experiment #3	6.32E+06	8.64E+05	6.32E+02
Experiment Average	6.32E+06	9.61E+05	6.53E+03
Exp. Avg. Pcnt		84.80	99.90

**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 D6 Sterionizer™ demonstrated the ability to reduce the concentration of the active pathogen SARS-CoV-2 on a surface when exposed to a high negative and positive ion concentration. During the test scenario, collectable active SARS-CoV-2 on the surface was reduced by 99.90% after 30 minutes of ion exposure in a small, sealed testing chamber. There was a large amount of reduction achieved by the D6 Sterionizer™ device during operation in the controlled testing environment. The reduction of collectable virus off the sample surfaces was significant in the experiment design, however the effective use in a larger environment will be different depending on a multitude of factors. This design was to initiate discussion and review efficacy of what the system would be capable of doing in a small, confined area with limiting variables.

When working with and collecting microorganisms, some variables cannot be accounted for, namely, placement of microorganisms, collection volume, surface saturation, microorganism destruction upon collection, potential drying out effects, and possibly others. However, every effort was made to address these constraints with the design and execution of the trials. The efforts are reflected in the meaningful recovery of in the control test.

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Dana Yee M.D **Date**

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Laboratory Director, Innovative Bioanalysis, Inc.

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