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EVERGREEN SINGLE AIR PASS EFFICACY TESTING AGAINST HCoV-229E

PROJECT: EVERGREEN BIOAEROSOL 229E

PRODUCT: EVERGREEN PROTOTYPE UNIT

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM(S):

COVID SURROGATE 229E

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Medical Director

Study Completion Date

7/19/2021

Testing Facility

Innovative Bioanalysis, Inc.

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Laboratory Project Number

1092

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Efficacy Study Summary

Study Title	EVERGREEN SINGLE AIR PASS EFFICACY TESTING AGAINST HCoV-229E
Laboratory Project #	1092
Guideline:	Modified ISO standards and as no international standards exist.
Testing Facility	Innovative Bioanalysis, Inc.
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	Human coronavirus surrogate 229E
Description	Evergreen Safe Air Solutions provided a pre-assembled device for aerosol testing. The in vitro study is a single air pass test to determine the device's efficacy in reducing an aerosolized pathogen, HCoV-229E when operating at three flow rate settings.
Test Conditions	The test was conducted using a modified ASHRAE test model within a sealed environment that complied with BSL-3 standards. The temperature during testing was approximately 75 ±2°F, with a relative humidity of 33%. The nebulizer was filled with the 6.32 x 10^6 TCID50 per mL in FBS-based viral media and nebulized at a constant rate into the device.
Test Results	A single air pass challenge was conducted for each air velocity setting: 50LPM, 100LPM, and 1000LPM. At 50LPM, viral concentration was reduced by 99.990%, 87.89% at 100LPM, and 90.47% at 1000LPM.
Control Results	A single air pass control test was conducted without the device at each of the three flow settings and served as a comparative baseline to calculate the viral reduction. The control results revealed a natural viral concentration reduction by 23.77% @ 50LPM, 36.66% @ 100 LPM, and 75.46% @ 1000LPM.
Conclusion	The Evergreen device effectively reduced aerosolized Human Coronavirus surrogate 229E concentrations during each air pass challenge.

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Study Report

Study Title: EVERGREEN SINGLE AIR PASS EFFICACY TESTING AGAINST HCoV-229E

Sponsor: Evergreen Safe Air Solutions

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

Device Testing: Evergreen prototype device

Study Report Date: 07/20/2021
Experimental Start Date: 06/28/2021
Experimental End Date: 06/28/2021
Study Completion Date: 07/19/2021

Study Objective:

Evergreen Safe Air Solutions supplied a device for single air pass testing purposes to determine efficacy against viral pathogens. This study evaluated the device's effectiveness in its ability to reduce concentrations of the SARS-CoV-2 surrogate, HCoV-229E, in the air.

Test Method:

Bioaerosol Generation:

For the control and the viral challenges, the nebulizer was filled with the same amount of viral stock (4.02×10^6 TCID50 per mL) in viral media. The solution was nebulized at a flow rate of 1mL/min. The nebulizer was driven by untreated local atmospheric air. After each completion, the nebulizer's remaining viral stock volume was weighed to confirm that roughly the same amount was nebulized.

Bioaerosol Sampling:

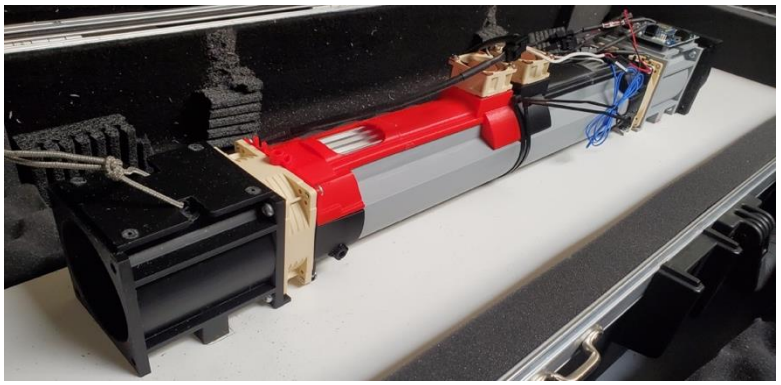
For air sampling, two Gilian 10i programmable vacuum devices were used. The air samplers were operated in conjunction with removable sealed cassettes, which were manually removed after each sampling run and pooled. The cassettes had a delicate internal filtration disc coated with a viral suspension media to aid in the collection of viral samples.

Test System Strains: Human coronavirus 229E

The following reagent was deposited by the ATCC and obtained through BEI Resources, NIAID, NIH: Human Coronavirus, 229E, NR-52726.

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 prior to arrival at the laboratory. The device was powered on to confirm functionality prior to testing.



MANUFACTURER: Evergreen Safe Air Solutions

MODEL: Prototype

SIZE: N/A

MAKE: N/A

SERIAL #: N/A

Testing Layout:

Testing was conducted inside a sealed and controlled BSL3 chamber pod measuring 74" x32" x32". The test was performed in compliance with a modified version of ANSI/ASHRAE standards. Before testing, the modified HVAC system was pressure tested using an air compressor and analog PSI meter to confirm no leaks were present. The airflow was cyclical, starting upstream with a variable fan positioned near the device's intake and moving downstream through the device. Sample collection occurred downstream with two sampling probes, each connected to a vacuum device. The chambers were visually inspected, pressure tested, and all internal lab systems and equipment were reviewed before testing.

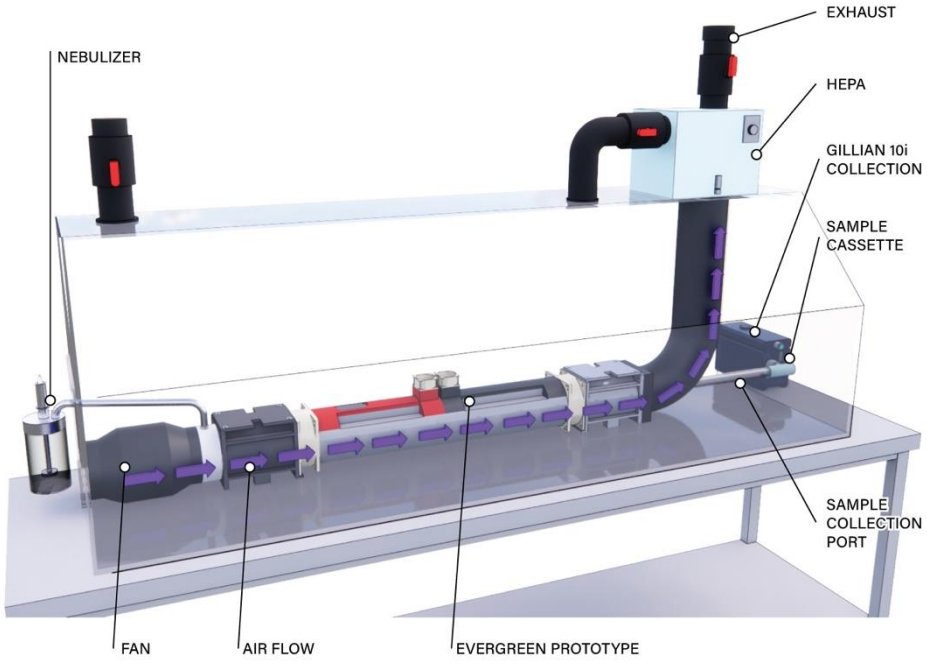


Figure 1. Testing layout for control and experimental trials.

Test Method:

Exposure Conditions:

1. The temperature during all test runs was approximately 75°F ±2°F with a relative humidity of 33%.
2. One of three air velocities (50LPM, 100LPM, and 1000LPM) were tested during each trial.

Nebulization:

1. Before the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. 4.02 x 10^6 TCID50/mL HCoV-229E viral media was nebulized into the air stream on the upstream side of the device while sample collection was done on the downstream side of the device.
3. Airflow was created using a variable speed fan on the upstream side of the device and speeds were confirmed using a digital anemometer.
4. Air samples were collected on the downstream side of the device for 10 minutes during the nebulization process and 5 minutes post nebulization.
5. After each run, the sample cassettes were manually removed from the collection system and taken to an adjacent biosafety cabinet to be pooled.

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: Human coronavirus 229E (HCoV-229E) (BEI NR-52726)

Test	Specifications	Results
Identification by Infectivity in MRC-5 Cells	Cell Rounding and Clumping	Cell Rounding and Clumping
Whole Genome Sequencing (~27310 nucleotides)	≥ 98% identity with HCoV, 229E GenBank: AF304460.1	99.9% identity with HCoV, 229E GenBank: AF304460.1
Sequencing of Species-Specific Region (~1000 Nucleotides)	≥ 98% identity with HCoV, 229E GenBank: AF304460.1	99.9% identity with HCoV, 229E GenBank: AF304460.1
Titer by TCID50 in MRC-5 Cells by Cytopathic Effect	Report Results	1.6 X 10^6 TCID50 per mL in 6 days at 35°C and 5% CO_2
Sterility (22-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 MRC-5 cells either in-house or at a partner lab to the concentrations listed within the experiment design.

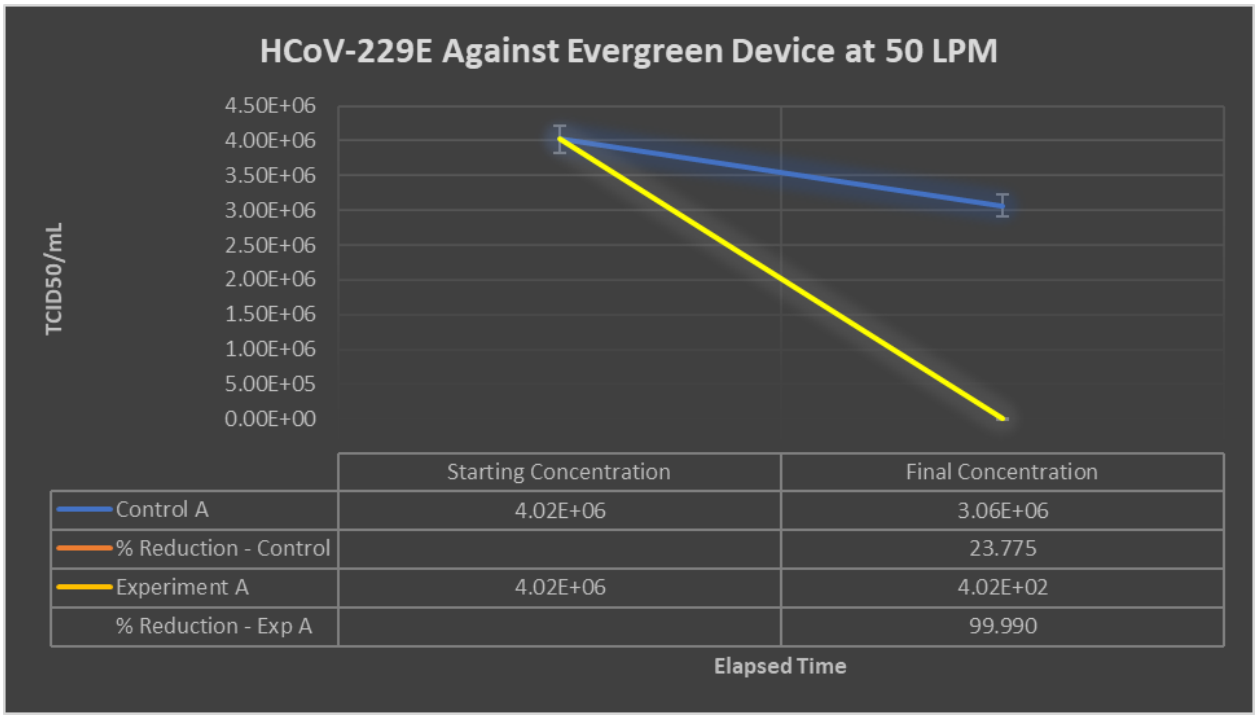
Control Protocol

Control was conducted without the device operating at each air velocity setting to accurately assess the Evergreen unit. Experimental procedures were performed in the same manner to serve as a comparative baseline to consider aerosolized viral reduction when the device was operating.

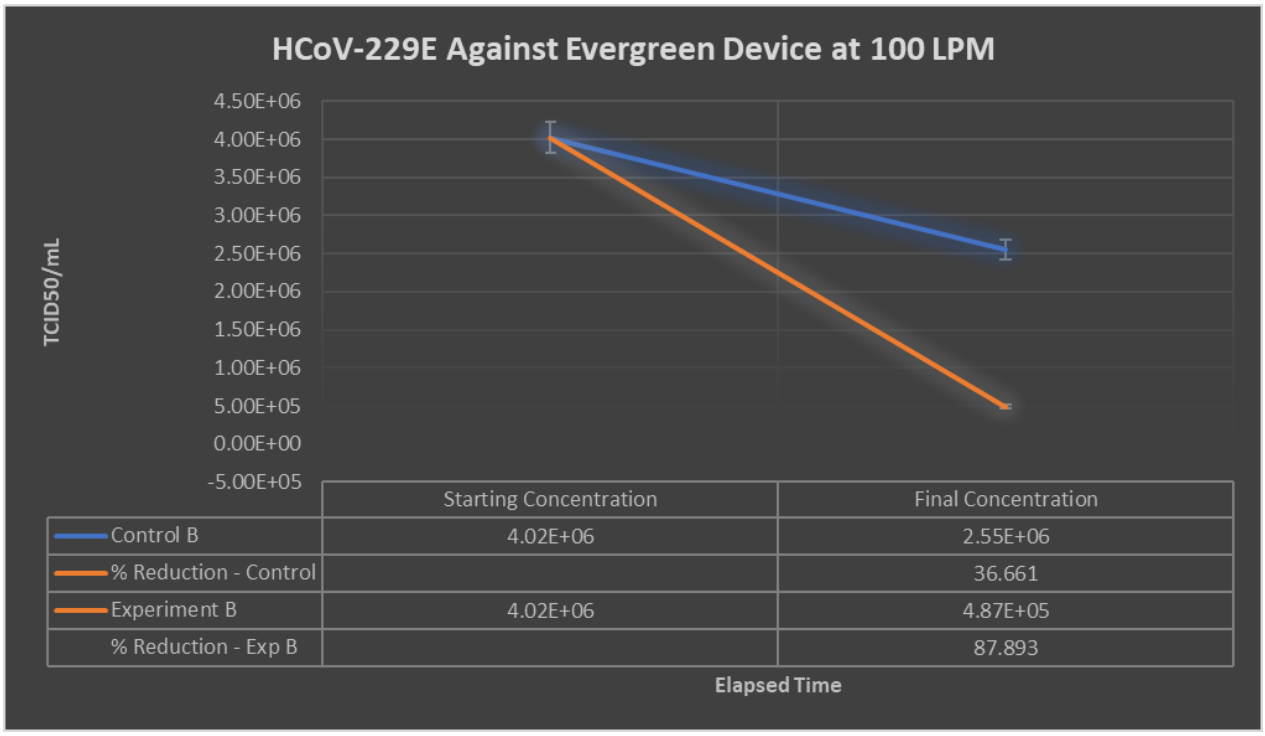
Study Results

Results from the control runs were graphed and plotted to show natural viability loss over time in the chamber. The control data served as a basis to determine the reduction achieved after a single air pass with the Evergreen device at 50 liters per minute (LPM), 100LPM, and 1000LPM. All air velocity settings observed a faster reduction in collectible active HCoV-229E than natural viability loss rates. At an air velocity of 50 L/min, the concentration decreased from 4.02 X 10^6 TCID50/mL to 4.02 X 10^2 TCID50/mL, indicative of a 99.990% reduction after a single air pass in comparison to 23.78% for the control. Increasing the velocity to 100LPM resulted in a drop from 4.02 X 10^6 TCID50/mL to 4.87 X 10^5 TCID50/mL, indicative of an 87.89% reduction in comparison to 36.66% for the control. Setting the speed to 1000LPM led to a 90.42% reduction in active collectible HCoV-229E after a single air pass, indicative of a 90.43% reduction in comparison to 75.46% for the control.

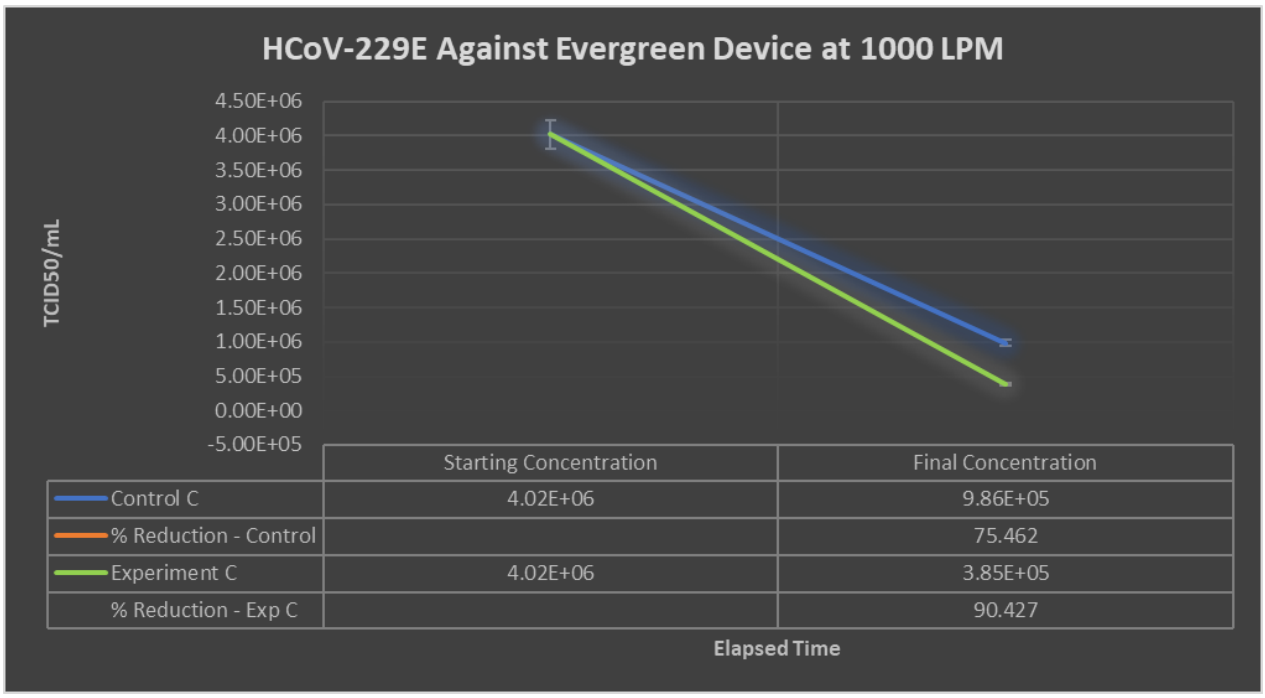
RESULTS:



	Starting Concentration	Final Concentration
Control A	4.02E+06	3.06E+06
% Reduction - Control		23.775
Experiment A	4.02E+06	4.02E+02
% Reduction - Exp A		99.990



	Starting Concentration	Final Concentration
Control B	4.02E+06	2.55E+06
% Reduction - Control		36.661
Experiment B	4.02E+06	4.87E+05
% Reduction - Exp B		87.893



	Starting Concentration	Final Concentration
Control C	4.02E+06	9.86E+05
% Reduction - Control		75.462
Experiment C	4.02E+06	3.85E+05
% Reduction - Exp C		90.427

**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 Evergreen Safe Air Solutions device consistently reduced aerosolized viral concentrations of human coronavirus 229E (HCoV-229E) at varying velocities. For example, after a single air pass with a 50LPM air velocity, the amount of quantifiable active HCoV-229E was 4.02 X 10^2 TCID50/mL. Increasing the airflow to 1000LPM resulted in a 3.85 X 10^5 TCID50/mL viral concentration, approximately a 90.427% net reduction after a single air pass. At 100LPM, there was an 87.89% reduction in active collectible HCoV-229E. As the test was designed to observe aerosol functions, it is unknown if any active pathogen remained on the surface areas inside the unit or the chamber walls.

An effort was made to simulate a real-life environment in the chamber while considering the special precautions needed when working with a Biosafety Level 3 Pathogen. When aerosolizing pathogens and collecting said pathogens, variables cannot be fully accounted for, namely, placement of pathogen, collection volume, collection points, drop rate, surface saturation, viral destruction on the collection or nebulization, 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 the virus in the control test.

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