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EFFICACY OF THE FIELD CONTROLS SKYE UNIT AGAINST AEROSOLIZED SARS-CoV-2 USA-CA1/2020

PROJECT: FIELD CONTROLS EFFICACY ON AEROLIZED SARS-COV-2

PRODUCT: AIR+HEALTH SKYE

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

1082

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Efficacy Study Summary

Study Title	EFFICACY OF FIELD CONTROLS SKYE AGAINST AEROSOLIZED SARS-COV-2 USA-CA1/2020
Laboratory Project #	1082
Guideline:	Modified ISO standards as no international standards exist.
Testing Facility	Innovative Bioanalysis, Inc.
Study Initiation Date:	05/11/2021
Study Completion Date:	05/31/2021
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	SARS-CoV-2 USA-CA1/2020
Description	The Field Controls SKYE system is a portable air purifier designed to be placed in a free-standing room to decrease the concentration of pathogens in the air when operating. This in vitro study is being conducted to determine the effectivity of the SKYE unit in reducing an aerosolized pathogen, SARS-CoV-2 when operating.
Test Conditions	The test was conducted in a large, sealed environment that complied to BSL-3 standards and was inspected for any leaks prior to usage. The temperature during all test runs was approximately 75°F ±2°F, with a relative humidity of 36%. Air samplers were calibrated by the manufacturer on September 3, 2020 and set at a standard flow of 5.02L/min. Calibration records indicate a 0.20% tolerance. The nebulizer was filled with the same amount of viral stock (6.32 x 10^6 TCID50 per mL) in FBS-based viral media and nebulized at a constant rate while four mixing fans were running simultaneously to ensure homogenous air.
Test Results	When tested against SARS-CoV-2 USA-CA1/2020 virus, the SKYE unit showed a reduction of active pathogens after 30 minutes of continued operation resulting in a loss greater than 1.20 x 10^2 TCDI50/mL.
Control Results	A control test was conducted without the air purifying unit engaged and samples were taken at the corresponding timepoints used for the challenge trial to serve as a comparative baseline in order to calculate viral reduction.
Conclusion	The SKYE unit demonstrated a 99.99% net reduction of recoverable active SARS-CoV-2 virus in the air of after 30 minutes of operation.

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Study Report

Study Title: FIELD CONTROLS SKYE AGAINST AEROSOLIZED SARS-COV-2-USA-CA1/2020

Sponsor: Field Controls, LLC

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

Device Testing: Testing the efficacy of the SKYE unit against an aerosolized known pathogen, SARS-CoV-2

Study Report Date: 6/21/2021
Experimental Start Date: 05/11/2021
Experimental End Date: 05/31/2021
Study Completion Date: 06/21/2021

Study Objective:

This in vitro study was designed to determine the efficacy of the SKYE unit against the airborne transmission of the known pathogen, SARS-CoV-2.

Test Method:

Bioaerosol Generation:

For the control and the viral challenges, the nebulizer was filled with the same amount of viral stock (6.32 x 10^6 TCID50 per mL) and 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 the same amount of viral stock was nebulized.

Bioaerosol Sampling:

For air sampling, 2 different Gilian 10i programmable vacuum devices were used. Air samplers were calibrated by the manufacturer in September 2020 and certificates were inspected prior to use. Air sample volume collections were confirmed prior to use with a Gilian Gilibrator 2, SN- 200700-12 and a high flow bubble generator SN-2009012-H. Air samplers were operated in conjunction with removable sealed cassettes, which were manually removed after each sampling time point. Cassettes had a delicate internal filtration disc to collect viral samples which were coated with a viral suspension media to aid collections.

Aerosolization of Viral Media:

Control samples were performed in the same manner as the viral test at the timepoints and rate of collection. A viral stock of SAS-CoV-2 USA-CA1/2020 with a concentration of 6.32 x 10^6 TCDI50/mL was used for this experiment.

Test System Strains: SARS-CoV-2

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 filtration systems were installed prior to arrival at the laboratory. The device was powered on to confirm functionality prior to testing.

MANUFACTURER: Air Health

MODEL: SKYE

SIZE: 26” X 11.4” X 11.3”

MAKE: Air+Health

SERIAL #: N/A

Equipment Specifics: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. The system has an internal fan system along with a multistage mechanical filtrations system.

Testing Chamber: The test was conducted inside a 10’ X 8’ X 8’, sealed chamber with active monitoring of testing conditions via calibrated wireless devices and air sampling sensor. At each corner of the chamber, low volume mixing fans were positioned to ensure homogenous, bioaerosol concentrations. Furthermore, the use of the mixing fans encouraged bioaerosol suspension and reduction in natural particle descent rates. The testing chamber was set up to allow all exhausted air after the test samples had been taken to be exhausted through a dual HEPA filtration system.

Design Layout:

The chamber used for testing was a 10' X 8' X 8', sealed air volume chamber with metal walls and epoxy floor which complied with BSL3 standards. The chamber was designed to be completely sealed from the outside environment to prevent any potential release of testing media into the atmosphere. The testing chamber was equipped with 2 sealed viewing windows and a lockable antechamber for entry and exit. Calibrated wireless devices and air sampling sensors are strategically placed to provide active monitoring of testing conditions – temperature and humidity—throughout the testing process.

At each corner of the chamber, low volume mixing fans were positioned at 45-degree angles to ensure homogenous mixing of bioaerosol concentrations when nebulized into the chamber. Furthermore, the use of the mixing fans encouraged bioaerosol suspension and reduction in natural particle descent rates. The fans help circulate the air around the room, allowing a mixing of the nebulized viral media and air within the testing chamber. The testing chamber was set up to allow all exhausted air after the test samples had been taken to be passed through a dual HEPA filtration system. The testing chamber had HEPA filtered inlets and exhaust, coupled with an active UV-C system in all ducting lines.

For air sample testing, the chamber was equipped with 2 probes that were along the centerline of the room and protruded down from the ceiling 24". Each probe was connected to a Gilian 10i programmable system with sampling cassettes from lot #24320 made by Zefon International. A single bioaerosol nebulizing port was in the center of the 10' wall opposite of the entry doors. The dissemination port protruded from the wall 24" and was connected to a programmable compressor nebulizer system.

An active air sampling sensor was used to confirm operations of the equipment and O_3 measurements were taken only for verification the system was operating. Test scenario captures O_3 data, but the conditions are not designed to be compared to EPA requirements and cannot be used for O_3 claims as the sensors and test parameters are not designed to meet O_3 certification requirements. The bioaerosol testing system was constructed to meet internal SOP requirements and all seams were sealed. During testing, the temperature was 75°F with a relative humidity of 36%. All sample collection pumps were set to a 10-minute air draw at the point of sampling.

Prior to testing, the chamber was pressure tested for leaks and visual inspections were made using a colored smoking device. All seals for the chamber were confirmed and all equipment used had a function tests to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

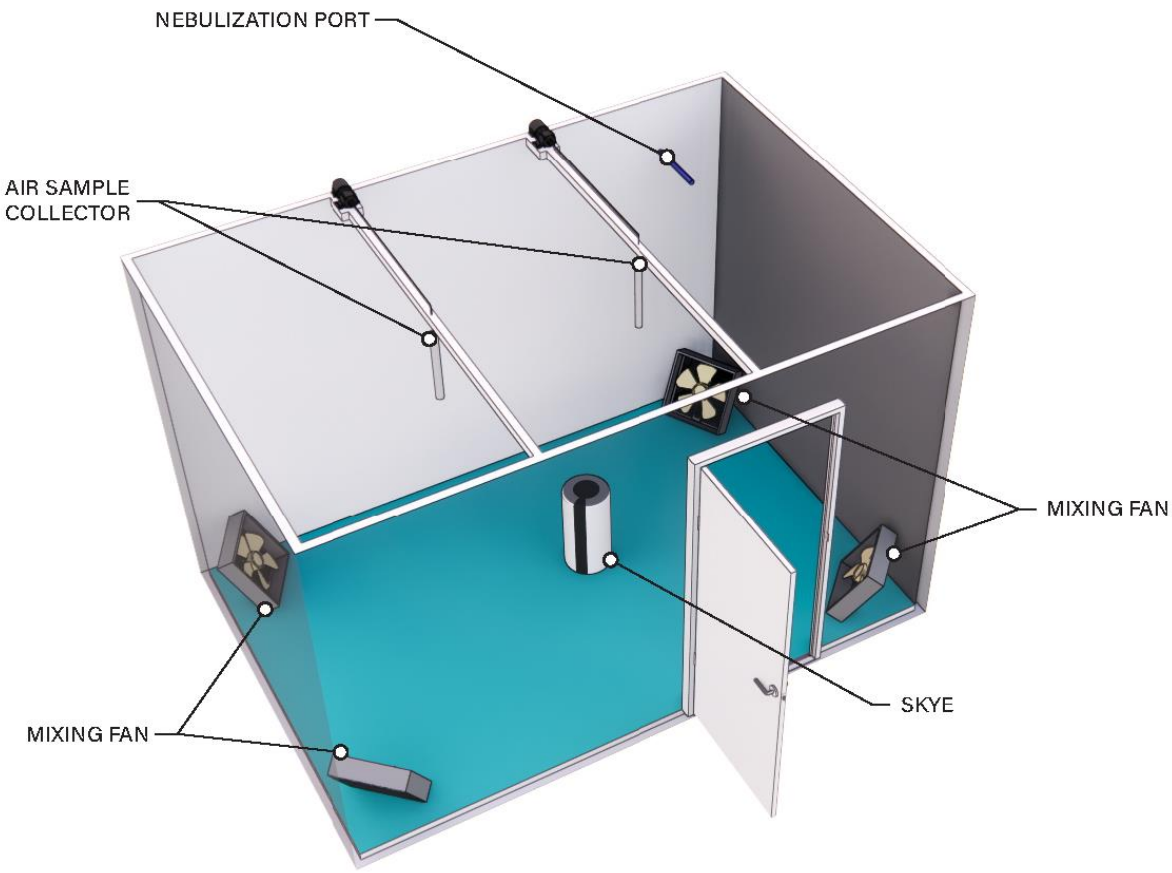


Figure 1. Room layout for control and experimental trial.

Test Method:

Field Controls supplied the SKYE air purifier system for testing purposes to determine the efficacy against aerosolized viral pathogens. More specifically, this study evaluated the device's ability to inactivate the viral strain referred to as SARS-CoV-2 in the air in a defined enclosed space.

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 75°F with a relative humidity of 36%.
3. Air samplers were calibrated by the manufacturer on September 3, 2020 and set at a standard flow of 5.02L/min. Calibration records indicate a 0.20% tolerance.
4. The air sample collection volumes were set to 10-minute continual draws at the point of sampling.
5. Low volume mixing fans were placed at each corner of the chamber at a 45-degree angle and turned on prior to nebulization.
6. Each timepoint was treated as an individual test and the chamber was reset after sample collection.
7. Testing timepoints were as follows with T equal to minutes: T-0, T-15, T-30

Nebulization:

1. Nebulization for control and viral test challenges were performed in the same manner.
2. After nebulization of the pathogen, the SKYE unit was turned on with a remote power switch.
3. Fan speed on the SKYE was set on high for the test conditions and was turned off at the defined timepoint for sample collection.
4. For the viral challenge, a known quantity of viral media was nebulized into the sealed environment from a dissemination port.
5. Viral media was nebulized at a constant rate for 12.5 minutes.
6. Air sampling collection occurred for both the challenge and control tests at the defined sample timepoints after nebulization ceased for a total of 10 minutes.
7. Sample cassettes were manually removed from the collection system after each control run and each air pass challenge.
8. Upon cassette removal after each challenge, cassette sets were taken to an adjacent biosafety cabinet for extraction and placement into viral suspension media.
9. One control and viral challenge was conducted using the same methodology.

Post Decontamination:

At the conclusion of each viral challenge test the UV-C system inside the testing chamber was activated for 30 minutes. After 30 minutes of UV-C exposure the chamber was fogged with a Hydrogen Peroxide gas mixture followed by a 30-minute air purge. 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. 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.

TCID50 Procedure:

Materials and Equipment:

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips—20uL, 200 uL, 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 (4) 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

One control test was conducted without the SKYE unit in the testing chamber. Control samples were taken at the corresponding sample time used for the challenge trial. Nebulization of viral media and collection methods were the same for the control as the viral challenge. Control testing was used for the comparative baseline to assess the viral reduction when the SKYE device was operated in the challenge trial to enable net reduction calculations to be made. During the control test, four low volume fans were operated in each corner of the testing chamber to ensure homogenous mixing of the air. During the control, temperature and relative humidity were monitored. Prior to running the viral challenges temperature and humidity were confirmed to be in relative range to the control ±5%.

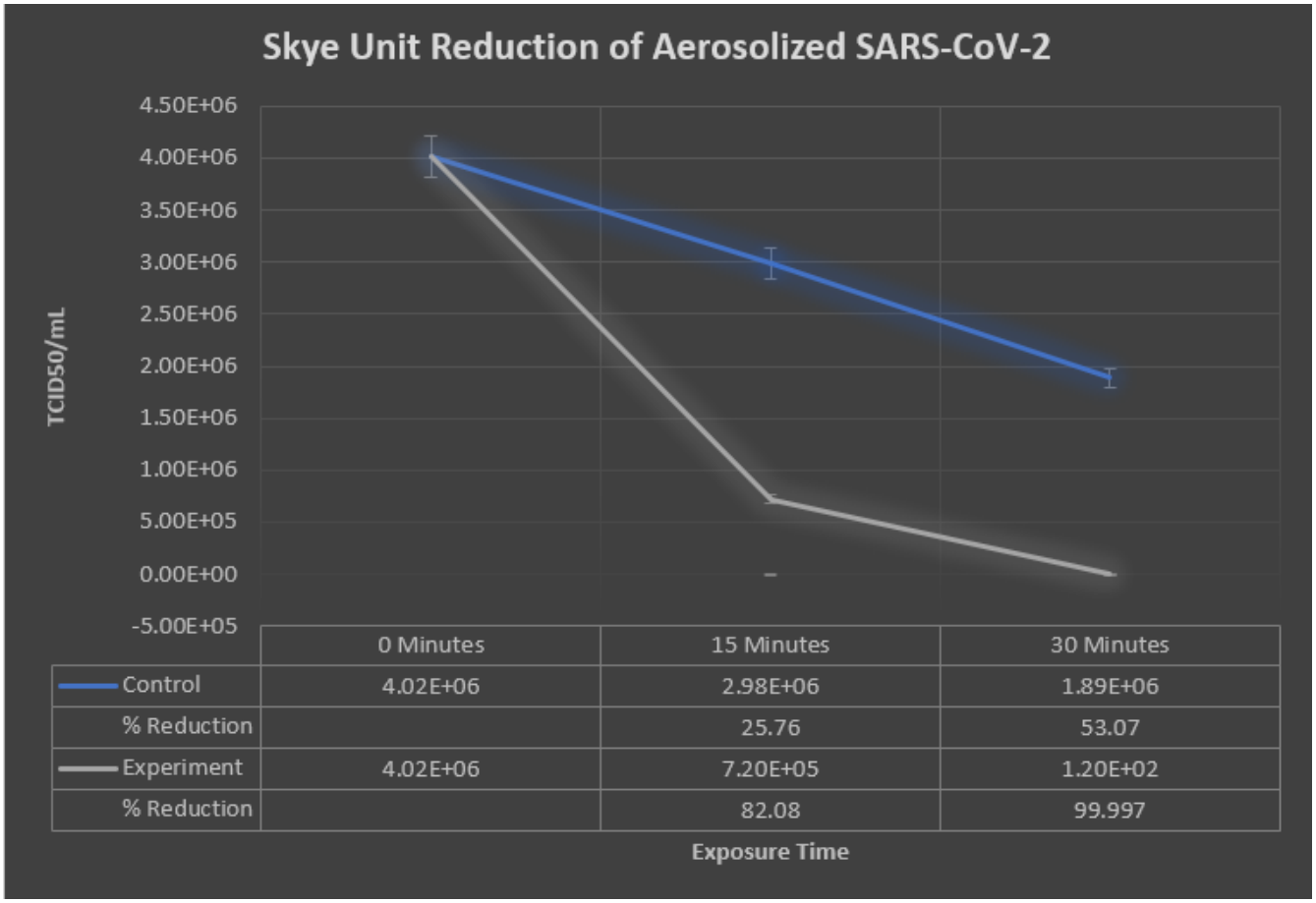
Aerosolization of Viral Media:

The control samples were performed in the same manner as the viral test regarding the time points and collection rate. 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.

Study Results

RESULTS:

When tested against SARS-CoV-2 USA-CA1/2020 virus, the SKYE unit showed a reduction of active pathogens after 30 minutes of continued operation resulting in a loss greater than 1.20 x 10^2 TCDI50/mL. This is indicative of a 99.99% net reduction of collectable virus in the air after 30 minutes of operation.



	0 Minutes	15 Minutes	30 Minutes
Control	4.02E+06	2.98E+06	1.89E+06
% Reduction		25.76	53.07
Experiment	4.02E+06	7.20E+05	1.20E+02
% Reduction		82.08	99.997

**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 Air+Health SKYE air purifier demonstrated the ability to reduce the concentration of the active pathogen, SARS-CoV-2 USA-CA1/2020 from the air. During the test scenario, collectable active SARS-CoV-2 in the air was reduced by 99.99% after 30 minutes of operation in the sealed testing environment. After 30 minutes of operation, there was a loss greater than 1.20×10^2 TCDI50/mL indicating a titer that is lower than the specified limit of quantification. The SKYE unit showed the ability to reduce collectable pathogen in the air below the lower limits of detection faster than natural loss rates. As the test was designed to observe aerosol functions, it is unknown if any active pathogen remained on the surface areas inside the SKYE unit or on the testing chamber walls.

Effort was made to simulate a real-life environment in the chamber while taking into consideration the special precautions needed when working with a Biosafety Level 3 Pathogen. Taking into consideration the starting concentration of active SARS-CoV-2 virus, the volume aerosolized and the volume inoculated, one could assume that the likelihood of entering an environment with this quantity of pathogen in a real-life circumstance to be unlikely. Furthermore, when aerosolizing and collecting said pathogens, there are variables that cannot be fully accounted for, namely, placement of pathogen, collection volume, collection points, drop rate, surface saturation, viral destruction on 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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