Innovative Bioanalysis, Inc.
3188 Airway Ave Suite D
Costa Mesa, CA 92626
www.InnovativeBioanalysis.com
Email: Albert.Brockman@innovativebioanalysis.com

SARS-CoV-2 USA-CA1/2020

CLIENT: FIELD CONTROLS

PROJECT: BIO AEROSOL EFFICACY

PRODUCT: TRIO PLUS™

CAP LIC NO: 886029801

CLIA LIC NO: O5D0955926

STATE ID: CLF 00324630

CHALLENGE VIRUS: SARS-CoV-2 USA-CA1/2020

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ABSTRACT: EFFICACY OF THE FIELD CONTROLS TRIO PLUS™ AGAINST AEROSOLIZED SARS-CoV-2

Background: This in vitro study was designed to determine the efficacy of the Field Controls TRIO PLUS™. The product is a commercially available mobile air purification device manufactured by Field Controls. The TRIO Plus™ unit is designed to be placed free standing in a room and decrease the concentration of pathogens in the air when it is operating, to reduce the spread of pathogens. For this challenge, the SARS-CoV-2 USA-CA1/2020 pathogen was used. There is a demand for air purifications devices that have a proven ability to reduce infectious pathogens in the air thereby reducing the risk of human infection and transmission. Field Controls supplied a pre-packaged TRIO PLUS™ free standing unit for testing purposes. For the testing, power was supplied through a Power Bright step-up voltage transformer set to 220W. Test procedures were followed using internal SOPs for aerosolized viral pathogen challenges and subsequent decontamination. All internal SOPs and processes follow GCLP guidelines and recommendations.

EQUIPMENT PROVIDED:

MANUFACTURER: FIELD CONTROLS

MODEL: TRIO PLUS™

SERIAL #: N/A

TRIO PLUS™ EQUIPMENT:

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 check for normal operation upon arrival and the UV-C lamps were inspected to confirm working conditions. The system was operated continuously for 100 hours prior to testing to simulate normal working conditions.

VIRAL CHALLENGE TESTING CHAMBER:

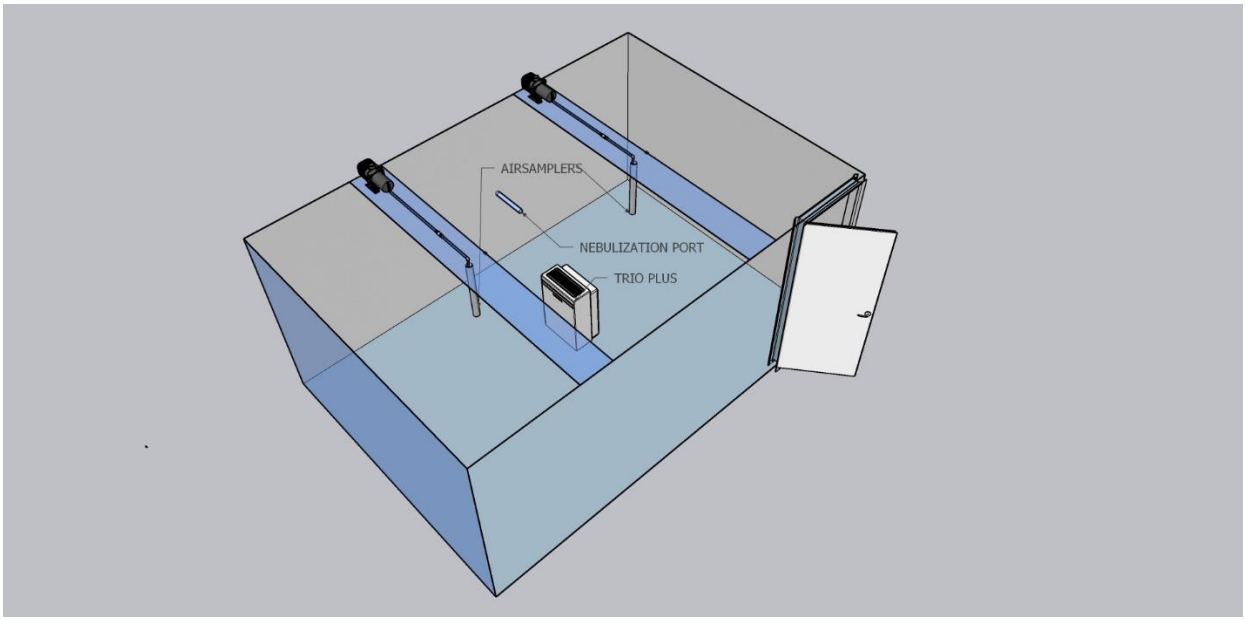
The testing chamber was a large, sealed air volume testing chamber consisting of metal walls and epoxy floor which complied with BSL3 standards. The chamber was designed to be completely sealed from the outside environment to prevent any potential release of testing media into the atmosphere. The testing chamber was equipped with 2 sealed viewing windows and a lockable chamber door for entry and exit. The overall dimensions of the test chamber were approximately 10'x8'x8'.

The testing chamber had HEPA filtered inlets and exhaust, coupled with an active UV-C system in all ducting lines. Humidity and temperature were monitored inside the chamber using a calibrated wireless device. 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 tube was connected to a Gilian 10i programmable system with sampling cassettes from lot # 23166 made by Zefron 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 O3 measurements were taken only for verification the system was operating. Test scenario captures O3 data, but the conditions are not designed to be compared to EPA requirements and cannot be used for O3 claims as the sensors and test parameters are not designed to meet 03 certification requirements.

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.

DESIGN LAYOUT:



EXPERIMENTAL SUMMARY:

- Prior to the initial control test and following each trial run the testing area was decontaminated and prepped per internal procedures.
- Temperature during all test runs was approximately 74F +/- 2F with a relative humidity of 43%.
- Relative humidity and temperature were taken in two sections of the chamber during all tests to confirm there was no more than a 3% deviation from each side.
- 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.
- All sample collection pumps were set to a 10-minute air draw at the point of sampling.
- Low volume mixing fans were turned on prior to nebulization to confirm homogenous concentrations in the test chamber.
- Mixing fans remained on and positioned at a 45-degree angle to encourage bioaerosol suspension and reduce natural particle descent rates.
- Test condition had the TRIO PLUS™ operating on the highest setting and air samples were taken after 30 minutes.
- O3 samples taken during dry runs of system with RKI air sampling array to confirm there was no safety hazard to staff.
- Nebulization for control and viral test challenges were performed in the same manner.

- Sample cassettes were manually removed from the collection system and stored after each time point and replaced with new cassettes.
- Upon cassette removal at each time point, cassette sets were taken to an adjacent bio safety cabinet and pooled.
- Two total sample times were collected per bioaerosol challenge.
- 1 control was completed, and 1 viral challenge was completed using the same methodology.

BIOAEROSOL GENERATION:

For the control and viral challenges, the nebulizer was filled with the same amount of viral stock (4.50 x 10^6 TCID50 per mL* and nebulized at a flow rate of 1ml/min). Nebulizer was driven by untreated local atmospheric air. The nebulizer's remaining viral stock volume was weighed after each completion to confirm the same amount of viral stock that was nebulized.

BIOAEROSOL SAMPLING:

For air sampling, 2 different Gillian 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:

Controls samples were performed in the same manner as the viral test at the time-points and rate of collection. A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 4.50 X 10^6 TCID50/mL* was used for this experiment.

VIRUS STRAIN BACKGROUND:

The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through the BEI Resources, BIAID, 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.*

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 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.

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 cover slip
- Cell Media for infection
- Growth Media appropriate for 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 indicated.

Procedure:

1. One day prior to 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 sample in PBS.
3. Make a series of dilutions at 1:10 of the original virus sample. First tube with 2.0 mL PBS and subsequent tubes with 1.8mL.
4. Vortex Viral samples, transfer 20 uL of virus to first tube, vortex, discard tip.
5. With new tip, serial dilute subsequent tips transferring 200 uL.

Additions of virus dilutions to cells:

1. Label lid of 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 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 4 wells per dilution, working backward.
6. Allow the virus to absorb to cells at 37°C for 2 hours.
7. After absorption, remove virus inoculum. Start with the most dilute and work backwards.
8. Add 0.5 mL infection medium to each well being careful to not 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.

CONTROL:

One Control test was conducted without the TRIO PLUS™ unit in the testing chamber. Control samples were taken at 30 minutes for 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 TRIO PLUS™ 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%.

VIRAL CHALLENGE:

The challenge pathogen, SARS-CoV-2 USA-CA1/2020, was used for testing the efficacy of the TRIO PLUS™ air purifier. During the challenge test the pressure in the challenge chamber was monitored to confirm no portion of the chamber was leaking. The bioaerosol efficacy challenge was completed in one trial with the live pathogen to create a baseline of data. For the test scenario the fan speed was set to the maximum air speed setting.

Test condition the system ran for 30 minutes post nebulization. Sampling occurred using 2 automatic air volume samplers that operated simultaneously for each collection. Samplers were pre-set to automatically shut off after 10 minutes of air sampling collection. Collections were made via the Gillian 10i vacuum pumps utilizing viral media coated filters for maximum pathogen trapping and stability. Collection samples were provided to lab staff for pooling after each collection time point.

After the control run and each aerosol test run the chamber was decontaminated per internal SOPs and prepped for the subsequent test run. After testing was completed the TRIO PLUS™ was decontaminated and placed in mandatory quarantine to confirm no active pathogens were left untreated.

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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 complete genome using Illumina® iSeq™ 100 Platform (Approx. 940 Nucleotides)	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1 ≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1	99.9% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1 100% 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% CO2
Sterility (21-Day Incubation) Harpos HTYE Broth, aerobic Trypticase Soy Broth, aerobic Sabourad Broth, aerobic Sheep Blood Agar, aerobic Sheep Blood Agar, anaerobic Thioglycollate Broth, anaerobic DMEM with 10% FBS	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth
Sterility (21-Day Incubation) Harpos HTYE Broth, aerobic Trypticase Soy Broth, aerobic Sabourad Broth, aerobic Sheep Blood Agar, aerobic Sheep Blood Agar, anaerobic Thioglycollate Broth, anaerobic DMEM with 10% FBS	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth
Mycoplasma Contamination Agar and Broth Culture DNA Detection by PCR of extracted Test Article nucleic acid.	 None Detected None Detected	 None Detected None Detected

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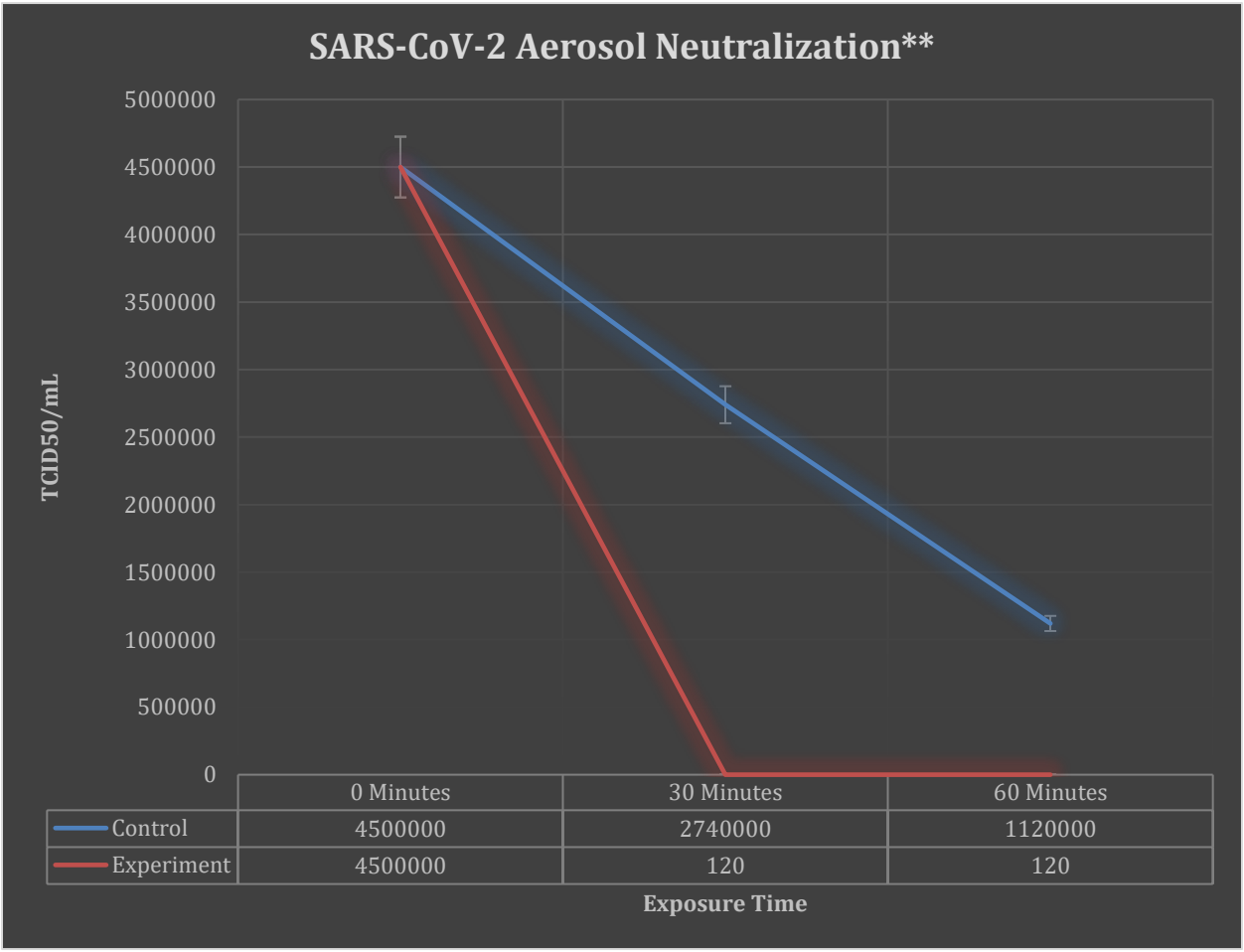
RESULTS:

When tested against SARS-CoV-2 USA-CA1/2020 virus, the TRIO PLUS™ unit showed a reduction during the time it was operated resulting in a loss greater than 1.20 x 10^2. The TRIO PLUS™ showed the ability to reduce collectable pathogen in the air below the lower limits of detection. This would result in a 99.99% reduction of collectable virus in the air after one hour of operation.



***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.*

CONCLUSIONS:

The TRIO PLUS™ air purification device performed to manufacturer specifications and demonstrated an overall reduction of recoverable active virus in the air after 30 minutes. The active SARS-CoV-2 virus was not detectable after the 30-minute timepoint, (levels were below the 120 TCID50/ml limit of quantification).

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.

When aerosolizing pathogens 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. And these efforts are reflected in the meaningful recovery of virus in the control test.

Testing confirmed TRIO PLUS™ unit showed a high-level reduction of pathogens in the air during the time it was operated resulting in a loss greater than 1.20 x 10^2 after 30 minutes. When operated in the test environment the TRIO PLUS™ air purification device demonstrated a 99.99% reduction of recoverable active SARS-CoV-2 virus in the air after 30 minutes.

DISCLAIMER:

The Innovative Bioanalysis, Inc. (“Innovative Bioanalysis”) laboratory is not certified or licensed by the United States Environmental Protection Agency and makes no equipment emissions claims pertaining to ozone, volatile organic compounds, or byproduct of any Field Controls TRIO PLUS™ device. Innovative Bioanalysis, Inc. makes no claims to the overall efficacy of any TRIO PLUS™. The experiment results are solely applicable to the device used in the trial. The results are only representative of the experiment design described in this report. Innovative Bioanalysis, Inc. makes no claims as to the reproducibility of the experiment results given the possible variation of experiment results even with an identical test environment, viral strain, collection method, inoculation, nebulization, viral media, cell type, and culture procedure. Innovative Bioanalysis, Inc. makes no claims to third parties and takes no responsibility for any consequences arising out of the use of, or reliance on, the experiment results by third parties. Ozone production was not monitored for verification levels and test was not an EPA ozone test. Ozone was monitored only as a function to confirm the system was turned on and operating.

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DocuSigned by:
Dana Yee
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5/3/2021

Dr. Dana Yee M.D
Clinical Pathologist and Medical Director

Date

DocuSigned by:
Sam Kabbani
8B4B282DF4B34A3...

5/2/2021

Sam Kabbani, MS, BS, MT(ASCP), CLS
Chief Scientific Officer, Innovative Bioanalysis

Date

DocuSigned by:
Albert Brockman
06DE5C77A0D2400...

5/2/2021

Albert Brockman
Chief Biosafety Officer, Innovative Bioanalysis

Date

DocuSigned by:
Kevin Noble
5DF2797BAA78421...

5/2/2021

Kevin Noble
Chief Operating Officer, Innovative Bioanalysis

Date