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

AEROSOL SARS-CoV-2 USA-CA1/2020 NEUTRALIZATION BY GPS FC48-AC (FC48)

CLIENT: GLOBAL PLASMA SOLUTIONS

PROJECT: FC48 AEROSOL V-1.0

PRODUCT: GPS-FC48-AC
CAP LIC NO: 886029801
CLIA LIC NO: O5D0955926
STATE ID: CLF 00324630

STUDY REPORT DATE: 3/18/2021
STUDY REPORT REVISION DATE: 8/19/2021

CHALLENGE ORGANISIM(S):

- **SARS-CoV-2 USA-CA1/2020**

INNOVATIVE BIOANALYSIS
creating solutions | getting results

ABSTRACT: EFFICACY OF THE GPS FC48-AC NPBI™ IONIZATION UNIT AGAINST AIRBORN SARS-CoV-2

Background: This in vitro study was designed to determine the efficacy of GPS FC48 NPBI™ ionization unit on an aerosol pathogen. The following test was to determine what log reductions were possible under controlled conditions after a pathogen was aerosolized. The FC48 is commercially available and designed for installation in an HVAC system. The FC48 is engineered as a disinfection device manufactured by GPS. The system is designed to emit ions at different polarities to decrease the concentration of bacteria and pathogens in the air and on surfaces while operational. Coronavirus can be spread through the air and by touching contaminated surfaces. There is a demand for disinfectant devices that have a proven ability to reduce infectious pathogens on surfaces and in air, thereby reducing the risk of human infection and transmission. GPS supplied a pre-packaged FC48 unit for testing purposes. For the testing, power was supplied through a power regulated 120v outlet with surge protector and backup battery system. Test procedures were followed using internal SOPs for viral pathogen challenges and subsequent decontamination. All internal SOPs and processes follow GCLP guidelines and recommendations.

For this challenge, the following viral strain was used.

- **SARS-CoV-2 USA-CA1/2020**

EQUIPMENT PROVIEDED:

MANUFACTURER: Global Plasma Solutions

MODEL: GPS FC48-AC

SERIAL#NONE

FC-48 EQUIPMENT:

The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. Prior to starting the challenge, the FC48 was operated for 1 hour in a dry run in a sealed bioaerosol to confirm correct operations. Chamber was the same BSL3 chamber used for the viral challenge testing.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

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 4 sealed viewing windows and a lockable chamber door for entry and exit. The overall dimensions of the test chamber were approximately 8'x8'x20.

For this test, no outside air was brought in during testing and the intake remained sealed. Humidity and temperature were monitored inside the chamber using a calibrated wireless device. For air sample testing, the chamber was equipped with 4 probes that were along the centerline of the room and protruded down from the ceiling 48". Each probe tube was connected to a Gilian 10i programmable system with sampling cassettes from lot #23166 made by Zefon International. A single bioaerosol nebulizing port was in the center of the 10' wall. The dissemination port protruded from the wall 24" and was connected to a programmable compressor nebulizer system.

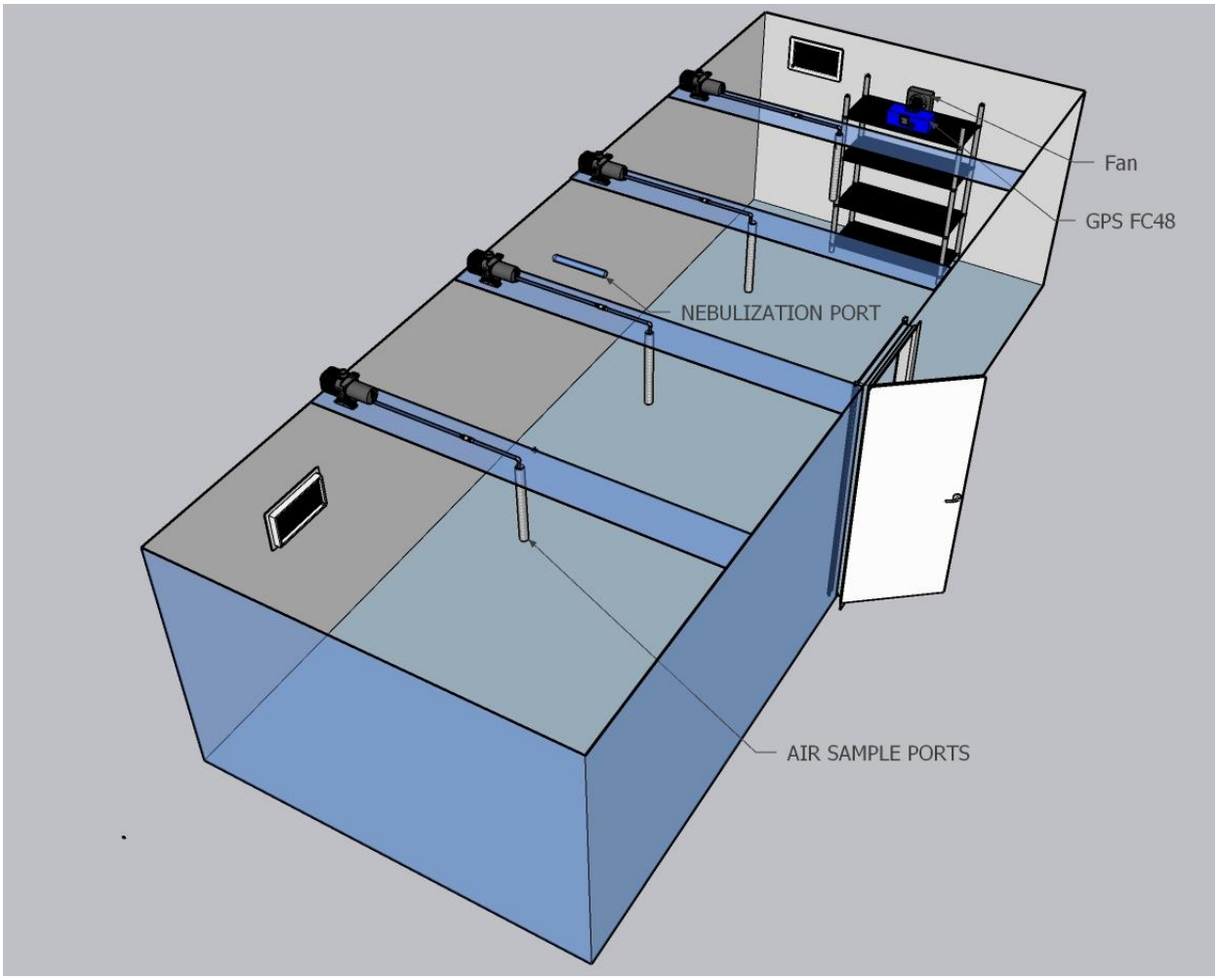
Four low volume mixing fans were placed on the floor in each corner of the room to assist with homogeneous mixing of the nebulized virus. Mixing fans were moving at approximately 120 CFM and were angled up at 45 degrees. Mixing fan speed were confirmed prior to testing with a vane anemometer.

One FC48 unit was used in the testing environment. The FC48 unit was placed in the center line of the room on the far wall depicted in Figure 1 below. The FC48 was placed on an adjustable plane six feet above the floor. A variable speed fan was placed behind the unit to create the necessary airflow for ion generation. Due to the nature of ions, there were fluctuations of concentrations around the entire room. Ion readings were taken from multiple points in the room prior to aerosol testing to adjust test environment and get close to a room average.

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 test to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

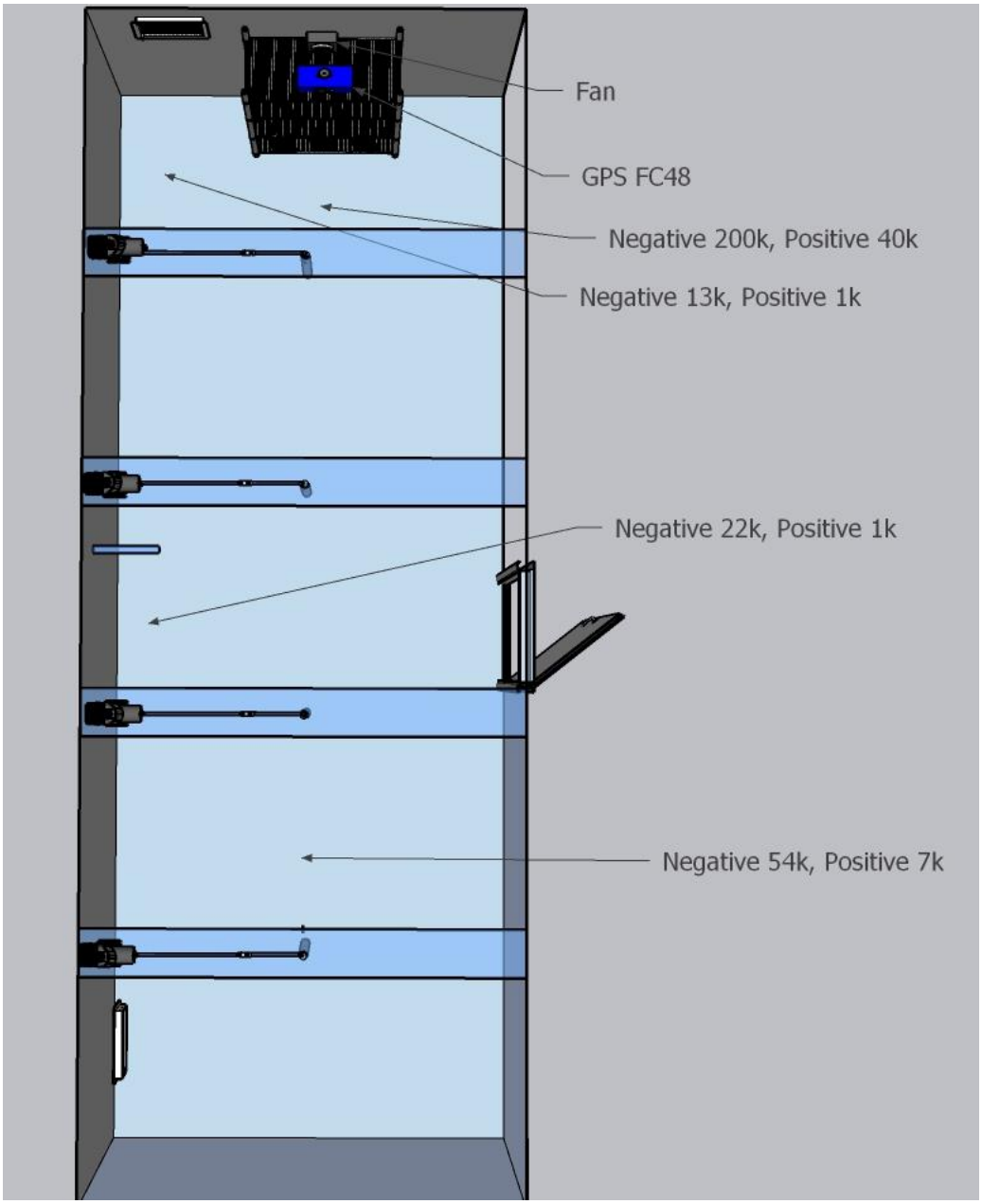
INNOVATIVE BIOANALYSIS
creating solutions | getting results

TESTING CHAMBERS:



INNOVATIVE BIOANALYSIS
creating solutions | getting results

DRY RUN ION CONCENTRATION OBSERVATIONS:



INNOVATIVE BIOANALYSIS
creating solutions | getting results

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 72 +/- 2f with a relative humidity of 47%.
- 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 volumes were set to 10-minute draws per time point.
- Sampling time points were T-0, T-15, T-30, T-45, T-60 measured in minutes from completed nebulization.
- 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.
- Ion concentration were checked in various locations prior to nebulization to confirm system functionality.
- FC48 and the airflow to create the ionization was turned on at the 0 time point after the nebulization process.
- Nebulization for control and viral test challenges were performed in the same manner.
- After nebulization was completed the 0-time point was set, samples were timed for exposure after the completion of nebulization.
- After each time point collection was completed the chamber was decontaminated and the air was completely evacuated.
- 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.
- 1 control was completed, and 1 viral challenge was completed. No more than one time point was taken per nebulization, and the chamber was prepped and decontaminated the same way each time.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

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 in FBS based viral media. Solution was 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. Nebulizer was calibrated prior to testing and a particle counter confirmed average size disbursement to be .8 microns.

BIOAEROSOL SAMPLING:

For air sampling, 4 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. Bioaerosol sample cassettes were prepped and handled per internal procedures to confirm uniform collections. The low volume mixing fans stayed active throughout all testing scenarios and conditions.

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.

POST DECONTAMINATION:

At the conclusion of each viral challenge timepoint/ 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.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

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.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

CONTROL:

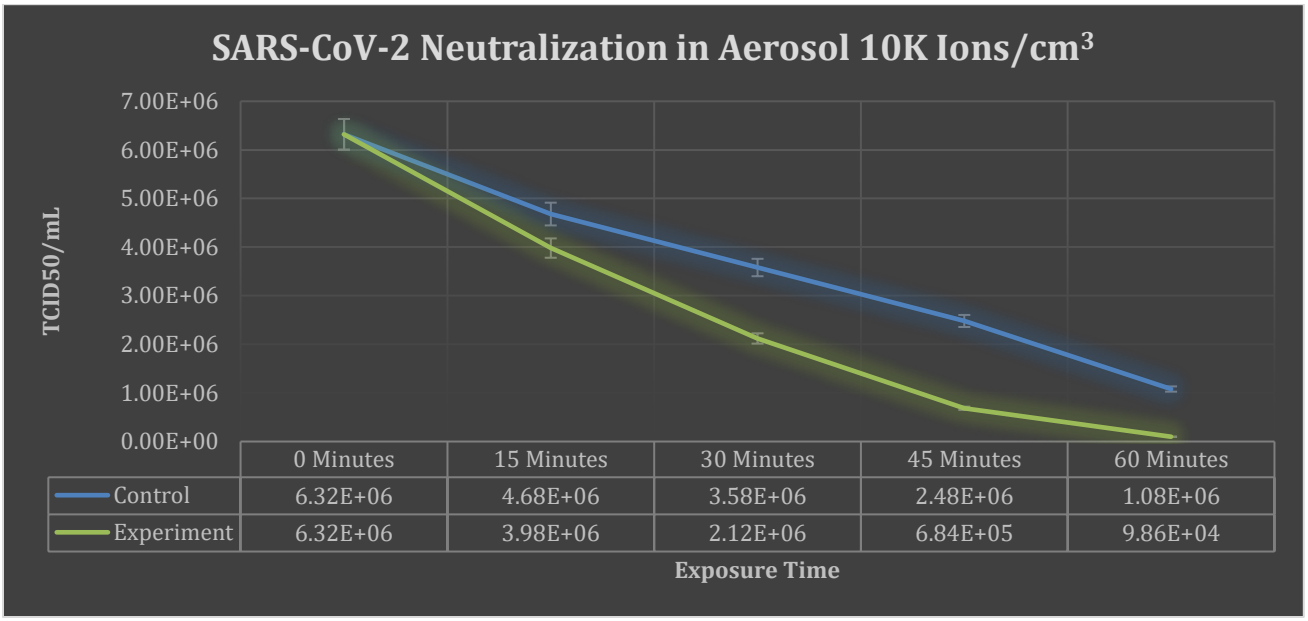
One Control test was conducted without the ion devices activated in the testing chamber. Control samples were taken at each of the corresponding sample times for the viral challenges. Nebulization of viral media was the same for the control as the viral challenge. Control testing was used for the comparative baseline to assess the viral reduction when the GPS FC48 NPBI™ unit was operated in the challenge trials, 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. 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:

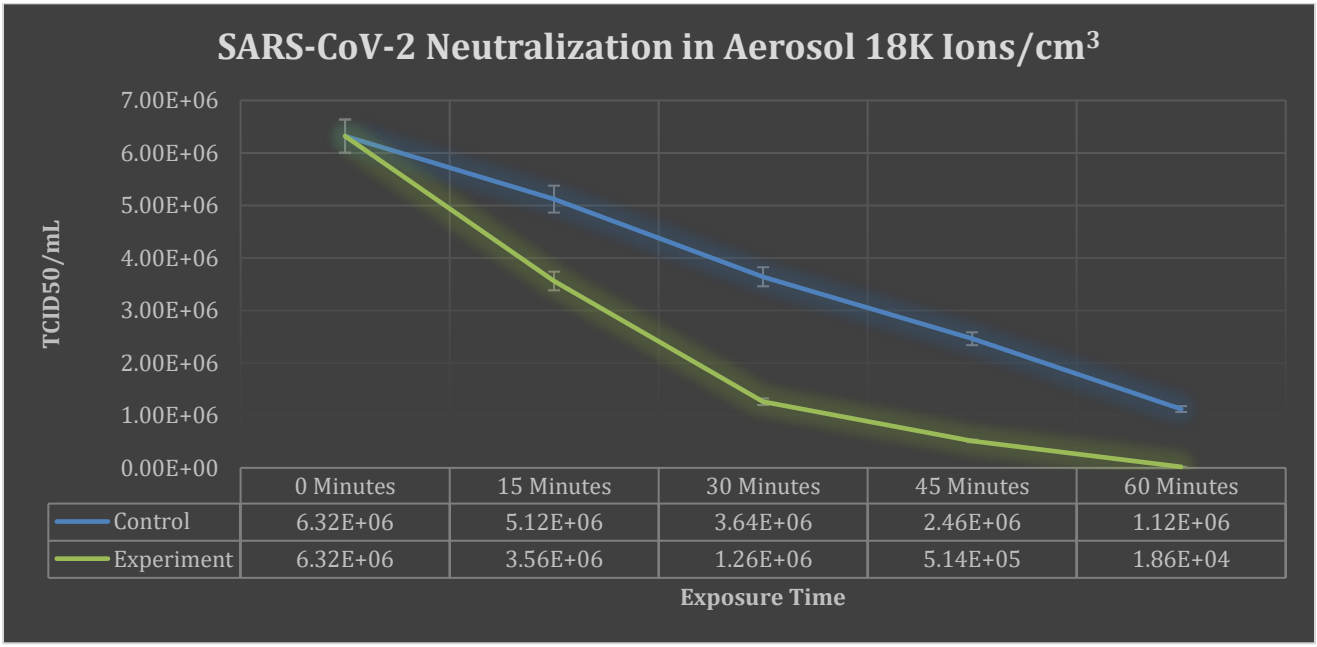
The challenge pathogen, SARS-CoV-2-USA-CA1/2020, was used for testing the efficacy of the GPS FC48 NPBI™. During the challenge tests the pressure in the challenge chamber was monitored to confirm no portion of the chamber was leaking. The bioaerosol efficacy challenge was completed in two distinct trials with the live pathogen to create a baseline of data. Prior to nebulizing the viral pathogen, the GPS FC48 NPBI™ was turned on and allowed to run for 1 hour than turned off for 10 minutes prior to nebulization. At the 0-time point when the nebulization system was turned off and the GPS FC48 NPBI™ was activated. Four low volume mixing fans were used throughout the entire control test and viral pathogen test. Sample times were as follows with T equal to minutes, T-0, T-15, T-30, T-45, T-60. Sampling occurred using 4 automatic air volume samplers that operated simultaneously for each collection. Samplers were pre-set to automatically shut off after 10 minutes of collection. Collections were made via the equipment 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.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

RESULTS:



	0 Minutes	15 Minutes	30 Minutes	45 Minutes	60 Minutes
Control	6.32E+06	4.68E+06	3.58E+06	2.48E+06	1.08E+06
Experiment	6.32E+06	3.98E+06	2.12E+06	6.84E+05	9.86E+04



	0 Minutes	15 Minutes	30 Minutes	45 Minutes	60 Minutes
Control	6.32E+06	5.12E+06	3.64E+06	2.46E+06	1.12E+06
Experiment	6.32E+06	3.56E+06	1.26E+06	5.14E+05	1.86E+04

INNOVATIVE BIOANALYSIS
creating solutions | getting results

CONCLUSIONS:

The GPS FC48 NPBI™ unit performed to manufacturer specifications and demonstrated a progressive reduction of active virus in the air after 15 minutes of exposure in aerosol form. The live SARS-CoV-2 virus was reduced. Taking into consideration the starting concentration of active SARS-CoV-2 virus, the volume aerosolized, 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. The reduction of collectable virus in the air was significant over the course of 60 minutes. Overall, the GPS FC48 NPBI™ unit showed efficacy in the reduction of SARS-CoV-2USA_CA1/2020 in air. Based on the understood method by which ion exposure deactivates pathogens it would be expected similar results on the various genetic mutations of SARS-CoV-2 would occur.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

DocuSigned by:
Dana Yee
7D5A69A0907947B...

8/24/2021

Dana Yee M.D **Date**

Clinical Pathologist and Medical Director, Innovative Bioanalysis, Inc.

DocuSigned by:
Sam Kabbani
8B4B282DF4B34A3...

8/24/2021

Sam Kabbani, MS, BS, MT(ASCP), CLS **Date**

Chief Scientific Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Albert Brockman
06DF5C77A0D2400...

8/24/2021

Albert Brockman **Date**

Chief Biosafety Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Kevin Noble
5DF2797BAA78421...

8/24/2021

Kevin Noble **Date**

Laboratory Director, Innovative Bioanalysis, Inc.

Disclaimer:

The Innovative Bioanalysis, Inc., LLC. ("Innovative Bioanalysis") laboratory is not certified or licensed by the United States Environmental Protection Agency and makes no equipment emissions claims pertaining to ozone, hydrogen peroxide gas, reactive oxygen species, volatile organic compounds, or byproduct of any device. Innovative Bioanalysis, Inc. makes no claims to the overall efficacy of any device. 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. focuses heavily of variable mitigation as it pertains to our experiments and uses several proprietary methods of culturing pathogens and recovering experiment samples. Tests conducted in the same manner with attention to all experimental variables should come within a margin of error to the results stated within this report. 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