INNOVATIVE BIOANALYSIS
creating solutions | getting results

EFFICACY OF THE GPS-FC48-AC™ AGAINST AEROSOLIZED SARS-COV-2

PROJECT: GPS – NPBI FC48 – AEROSOL SARS-COV-2

TECHNOLOGY: Needlepoint Bipolar Ionization (NPBI™)

DEVICE: GPS-FC48-AC™

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM:

SARS-COV-2 USA-CA1/2020

Medical Director:

Dana Yee, M.D.

Study Completion Date:

02/10/22

Testing Facility:

Innovative Bioanalysis, Inc.

3188 Airway Ave Suite D

Costa Mesa CA, 92626

www.InnovativeBioanalysis.com

Email: info@innovativebioanalysis.com

Laboratory Project Number:

1189S

INNOVATIVE BIOANALYSIS
creating solutions | getting results

Table of Contents

INNOVATIVE BIOANALYSIS
creating solutions | getting results

Efficacy Study Summary

Study Title	EFFICACY OF THE GPS-FC48-AC™ AGAINST AERSOLIZED SARS-COV-2
Laboratory Project #	1189S
Guideline:	GCLP, modified ISO, and BSL-3 standards were used.
Testing Facility	Innovative Bioanalysis, Inc.
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	SARS-CoV-2 USA-CA1/2020
Description	Per the manufacturer, the GPS-FC48-AC™ device housing NPBI™ technology is commercially available and designed to be installed in an HVAC system to reduce the concentration of certain bacteria and viruses while operational. Testing was conducted on the device to evaluate the effectiveness of the NPBI™ technology in reducing aerosolized SARS-CoV-2.
Test Conditions	The test was conducted in a sealed 20'x8'x8' chamber that complied with BSL-3 standards and was inspected for leaks before use. The temperature during testing was 73 ± 2°F, with a relative humidity of 40 ±2%. A 9.63×10^5 TCID50/mL of SARS-CoV-2 in viral media was nebulized into the room with mixing fans before collection. Air sample collections occurred at 0, 15, 30, 45, and 60 minutes of exposure and tested in triplicate.
Test Results	The GPS-FC48-AC™ device housing NPBI™ technology consistently reduced active SARS-CoV-2 at each time point faster than natural loss rates. With the device generating an average negative ion concentration of 4,900 ions/cm^3, the GPS-FC48-AC™ device housing NPBI™ technology decreased a starting concentration of 9.63×10^5 TCID50/mL SARS-CoV-2 to an average of 3.77×10^5 TCID50/mL after 15 minutes. Increased exposure time resulted in a higher observed reduction with an average of 1.13×10^3 TCID50/mL recovered after 30 minutes and reached below quantitation levels after 45 minutes of device operation. Ion concentrations were measured in the chamber during a dry run test prior to viral challenges.
Control Results	Control tests were conducted in triplicate without the device operational, and samples were taken at the corresponding time points used for the challenge. The results for the controls were plotted to show a natural rate of loss over 60 minutes and were used to assess the NPBI™ technology's ability to reduce SARS-CoV-2 in air.
Conclusion	The NPBI™ technology demonstrated the overall capability of reducing aerosolized SARS-CoV-2 viruses at each time point faster than the natural viability loss rates. After 15 minutes of operation, a 60.821% gross reduction in active SARS-CoV-2 was observed and reached a 99.88% gross reduction after 30 minutes.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

Study Report:

Study Title: EFFICACY OF THE GPS-FC48-AC™ AGAINST AERSOLIZED SARS-COV-2

Sponsor: Global Plasma Solutions

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

Technology Tested: NPBI™

Device Testing: GPS-FC48-AC™

Study Dates:
Study Report Date: 02/14/2022
Experimental Start Date: 12/05/2021
Experimental End Date: 12/07/2021
Study Completion Date: 02/10/2022

Study Objective:

The GPS-FC48-AC™ containing NPBI™ technology, was provided by Global Plasma Solutions for testing to evaluate the efficacy of the device against an aerosolized virus, SARS-CoV-2 Virus. The following test was to determine what reductions were possible under controlled conditions.

Test Method:

Bioaerosol Generation:

Nebulization occurred using a Blaustein Atomizing Module (BLAM), as shown in Figure 1, with a pre-set PSI and computer-controlled liquid delivery system. Before testing, the nebulizer was checked for proper functionality by nebulizing the solution without the test virus present to confirm average particle size distribution. The nebulizer was filled with 9.63 x 10^5 TCID50/mL of SARS-CoV-2 in viral suspension media and nebulized at a flow rate of 1mL/min with untreated local atmospheric air. After nebulization, the nebulizer's remaining viral stock volume was weighed to confirm roughly the same amount was nebulized during each run. Bioaerosol procedures for the controls and viral challenges were performed in the same manner with corresponding time points and collection rates.

Figure 1: BLAM Nebulizer

Bioaerosol Sampling:

This study used four probes for air sampling, each connected to a calibrated Gilian 10i vacuum device and set at a standard flow of 5.02L/min with a 0.20% tolerance. Before use, the devices were inspected for functionality, and the vacuum system calibration was confirmed using a Gilian Gilibrator-2 NIOSH Primary Standard Air Flow Calibrator. Sample collection volumes were set to 10-minute draws per time point, which allowed for approximately 50 liters of air collection per collection port. The air sampler operated with a removable sealed cassette and was manually removed after each sampling time point. Cassettes had a delicate internal filtration disc (Fig. 2) to collect virus samples, which was moistened with a virus suspension media to aid in the collection. Filtration discs from Zefon International, Lot# 26338, were used for testing. At each time point, all the sample discs were pooled into one collection tube to provide an average across the four sampling locations.

Figure 2: Sensidyne 37mm directional air flow sample cassette.

Test System Strains: SARS-CoV-2 USA-CA1/2020

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.

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 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.

Study Materials and Equipment:

Equipment Overview: The equipment (Fig. 3) arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. Due to the closed design, no assessment was conducted on the inner components of the device. Before testing, the GPS-FC48-AC™ device was powered on and operated for 1 hour in a dry run to confirm correct operations. Two Alpha Lab AIC2 ion polarity meters confirmed negative and positive ion generation. The average ion concentrations measured at a specified point in the room were 4,900 negative ions/cm^3 and 4,100 positive ions/cm^3.

MANUFACTURER: Global Plasma Solutions

MODEL: GPS-FC48-AC™

TECHNOLOGY: NPBI™

SIZE: 11.10” x 1.84” x 3.52”

SERIAL #: N/A

Figure 3. GPS-FC48-AC™ device tested.

Testing Layout:

Testing was conducted in a sealed 20’x8’x8’ chamber per Biosafety Level 3 (BSL3) standards. The overall dimensions of the test chamber provided a displacement volume of 1,280 ft^3 (36,245.56 liters) of air. The room remained closed to prevent any air from entering and leaving the room during testing. A nebulizing port connected to a programmable compressor system was located in the center of the 20-ft wall protruding 24-inches from the wall. At each chamber corner, low-volume mixing fans (approx. 30 cfm each) were positioned at 45-degree angles to ensure homogenous mixing of bioaerosol concentrations when nebulized into the chamber. The room was equipped with four probes for air sampling positioned along the room's centerline and protruded down from the ceiling 24-inches. The device was placed in the room's centerline, mounted on a movable scaffolding against the wall at an elevated position six feet above the ground, depicted in Figure 4. During testing, ion measurements were taken directly above the samples to confirm consistent readings. A variable-speed fan was placed behind the GPS-FC48-AC™ to create the necessary airflow to produce the required concentration of negative ions, 4,900 ions/cm^3. The chamber was visually inspected, pressure tested, and all internal lab systems and equipment were reviewed before testing.

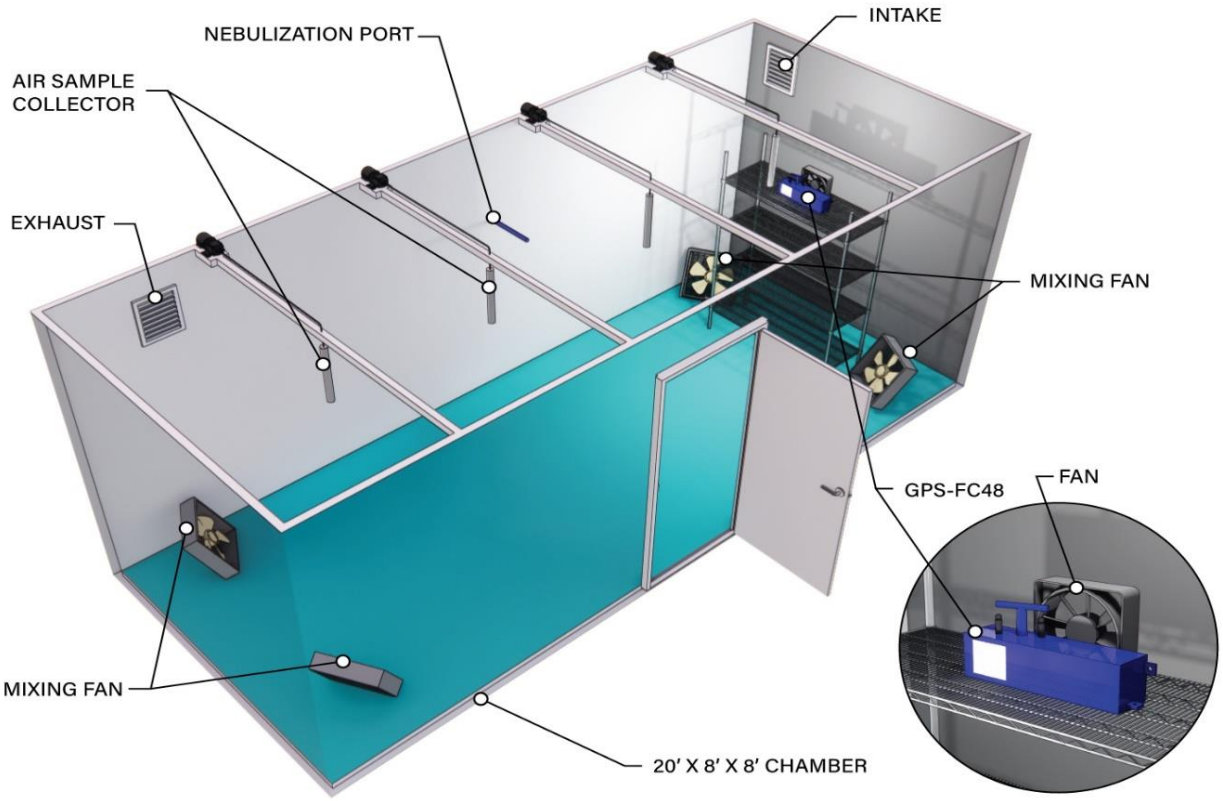


Figure 4. Room layout for control and experimental testing.

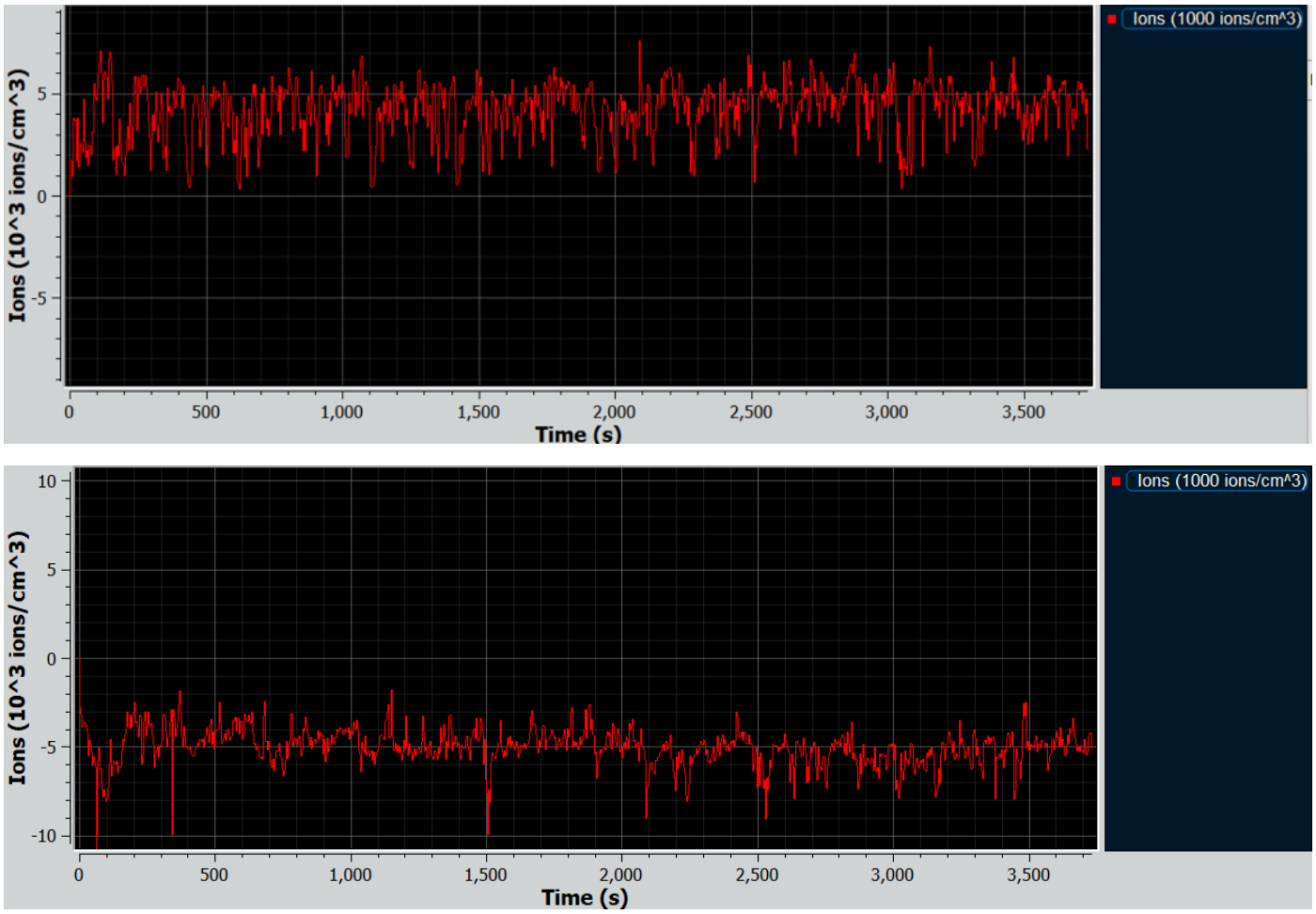


Figure 5. Positive (top) and negative (bottom) ion concentration readings during the dry run test prior to viral testing. Average ion concentration was measured at 4,100 positive ions/cm³ and 4,900 negative ions/cm³.

Control Protocol:

To accurately access the GPS-FC48-AC™ device housing NPBI™ technology a control was conducted in triplicate without the device operating in the testing chamber. The collection was taken at corresponding time points used for the challenge trial, in the same manner, to serve as a comparative baseline to assess aerosolized viral reduction when the device was operating.

Test Procedures:

Exposure Conditions:

1. The temperature during all test runs was approximately 73 ±2°F, with a relative humidity of 40 ±2%.
2. The device was off during nebulization and turned on upon completion at T-0.
3. Testing time points were as follows, with T equal to minutes: T-0, T-15, T-30, T-45, and T-60.

Experimental Procedures:

1. Before the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. 10 mL of 9.63 x 10^5 TCID50/mL SARS-CoV-2 in viral media was nebulized into the sealed environment via the dissemination port.
3. After nebulization, the GPS-FC48-AC™ device was turned on via remote control at T-0 after nebulization was completed.
4. The device was turned off at each predetermined time point for sample collection.
5. Air sampling collection was set to 10-minute continuous draws at the point of sampling occurring after nebulization ceased.
6. Sample cassettes were manually removed from the collection system and taken to an adjacent biosafety cabinet to be pooled.
7. All samples were sealed after collection and provided to lab staff for analysis after study completion.

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, the air filtration system underwent a 30-minute air purge. All test equipment was cleaned at the end of each day with a 70% isopropyl 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.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

Preparation of The Pathogen

Viral Stock: SARS-CoV-2 USA-CA1/2020 (BEI NR-52382)

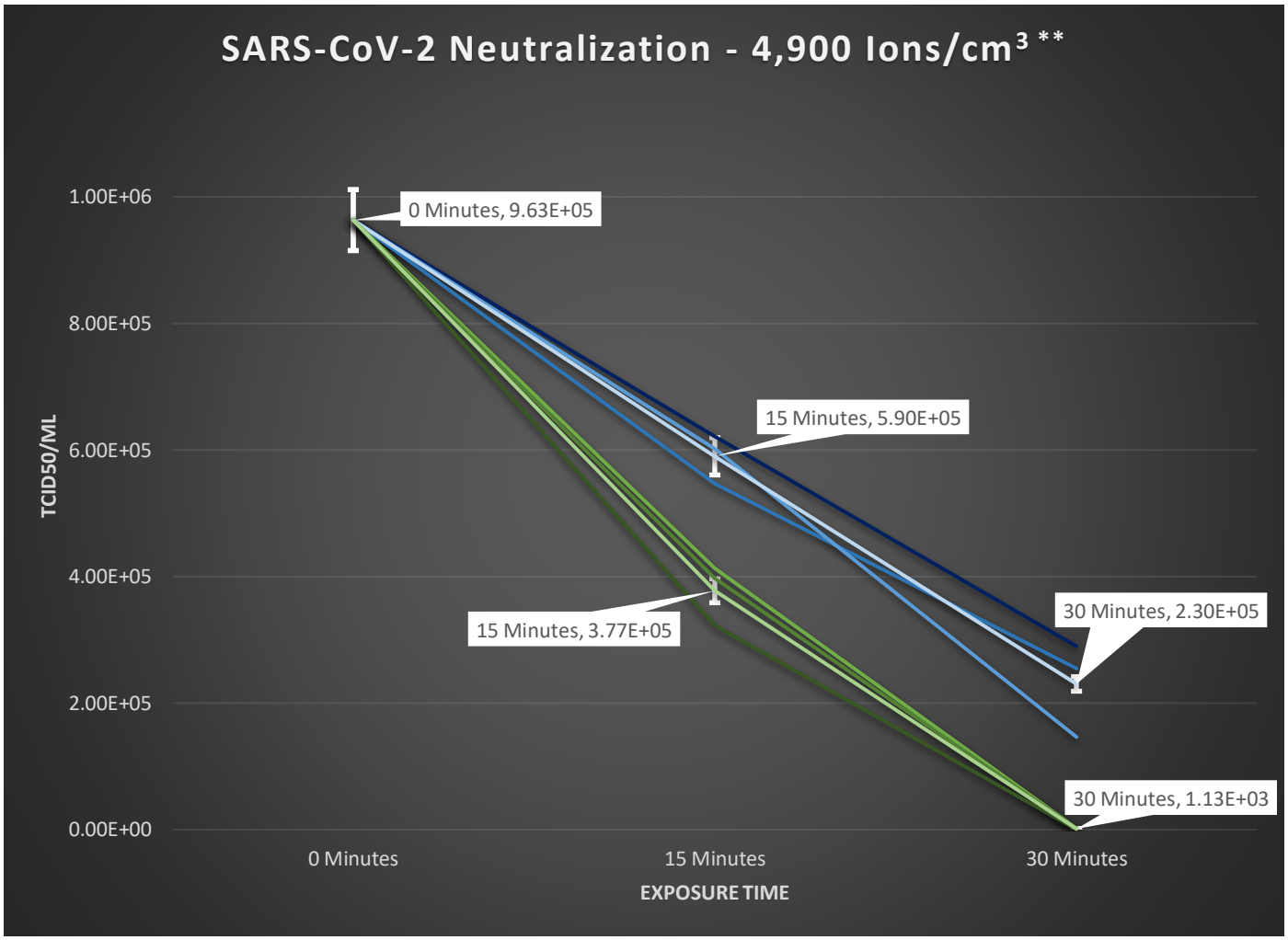
TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero 6 Cells	Cell Rounding and Detachment	Cell Rounding and Detachment
Next-Generation Sequencing (NGS) of the complete genome using Illumina® iSeq™ 100 Platform	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1	99.9% 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.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

Study Results:

The graph displayed recoverable active SARS-CoV-2 with and without the GPS-FC48-AC™ device housing NPBI™ technology operating over 60 minutes with a negative ion concentration of 4,900 ions/cm^3. The controls showed a natural viability loss of aerosolized SARS-CoV-2 for 30 minutes within the chamber under controlled conditions. At an average of 4,900 negative ions/cm^3 at each time point the average reduction against SARS-CoV-2 were as follows: 3.77 x 10^5 after 15 minutes, 1.13 x 10^3 after 30 minutes, and achieving below levels of quantitation as indicated by the value 1.20 x 10^2 TCID50/mL after 45 and 60 minutes. The 45 and 60 minutes data has been removed from the results as it all reached the limit of quantification.



***As it pertains to data represented herein, the percentage error equates to an average of ±5% of the final concentration.*

SARS-COV-2 Neutralization			
Time (min)	**0**	**15**	**30**
Control 1	9.63E+05	6.21E+05	2.90E+05
Control 2	9.63E+05	5.47E+05	2.55E+05
Control 3	9.63E+05	6.01E+05	1.46E+05
3 Control Average	9.63E+05	5.90E+05	2.30E+05
% Reduction - Gross Control Avg.		-38.76%	-76.10%
Experiment 1	9.63E+05	3.23E+05	1.20E+02***
Experiment 2	9.63E+05	4.13E+05	1.85E+03
Experiment 3	9.63E+05	3.97E+05	1.43E+03
3 Experiment Average	9.63E+05	3.77E+05	1.13E+03
% Reduction - Gross Experiment Avg.		-60.82%	-99.88%
% Net Reduction Avg.		**-36.03%**	**-99.51%**

****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.*

Conclusion:

The GPS-FC48-AC™ device housing NPBI™ technology demonstrated the ability to reduce aerosolized SARS-CoV-2 across all time points compared to the natural loss rate observed in the controlled setting. The device achieved a 60.821% gross reduction of active viruses after 15 minutes compared to the controls, which displayed a 38.755% gross reduction. After 30 minutes, the device reached a 99.882% gross reduction compared to the 76.105% gross reduction for the controls.

When aerosolizing pathogens and collecting said pathogens, some variables cannot be fully accounted for, namely, placement of pathogen, collection volume, collection points, drop rate, surface saturation, viral destruction upon collection, viral destruction on aerosolization, 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.

Considering the variables, there was a measurable amount of reduction achieved by the GPS-FC48-AC™ device housing NPBI™ technology at each time point (T-15, T-30, T-45, and T-60). Overall, the device successfully reduced SARS-CoV-2 in the air by 99.882% after 30 minutes with an ion concentration of 4,900 negative ions/cm^3 under controlled conditions. The decline of SARS-CoV-2 in the air was consistent with the manufacturer's claims that the device can decrease the concentration of active pathogens in the air. Overall, the device successfully reduced SARS-CoV-2 from the air under controlled conditions within the parameters of the test environment.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

DocuSigned by:
Dana Yee
52F0012FC10C43F...

5/27/2022

Dana Yee M.D **Date**

Clinical Pathologist and Medical Director, Innovative Bioanalysis, Inc.

DocuSigned by:
Sam Kabbani
8B4B282DF4B34A3...

5/27/2022

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

Chief Scientific Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Albert Brockman
42751B72F2FF40A...

5/27/2022

Albert Brockman **Date**

Chief Biosafety Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Kevin Noble
5DF2797BAA78421...

5/27/2022

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.