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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

Completion Date:

05/20/2022

Medical Director:

Dana Yee, M.D.

Testing Facility:

Innovative Bioanalysis, Inc.

3188 Airway Ave Suite D

Costa Mesa CA, 92626

www.InnovativeBioanalysis.com

Email: info@innovativeBioanalysis.com

Laboratory Project Number

1283

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Efficacy Study Summary

Study Title	EFFICACY OF THE GPS-FC48-AC™ AGAINST AEROSOLIZED SARS-COV-2
Laboratory Project #	1283
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 the ductwork of an HVAC system to reduce the concentration of bacteria and viruses while operational. Testing was conducted on the device to evaluate the effectiveness of the NPBI™ technology at a targeted concentration of 3,500 ions/cm^3 in reducing aerosolized SARS-CoV-2.
Test Conditions	Testing was conducted in a 22'x11'x8' chamber following BSL-3 standards. The temperature during testing was 72 ±2°F, with a relative humidity of 40 ±2%. A 10mL of 9.97 x 10^5 TCID50/mL of SARS-CoV-2 in suspension media was nebulized into the room with mixing fans before collection. Air sample collections occurred at 0, 10, 20, 25, and 30 minutes of exposure and were tested in triplicate. Ion concentrations were measured in the chamber during a dry run test each day prior to viral challenges.
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 3,490 ions/cm^3 at the testing location, the GPS-FC48-AC™ device housing NPBI™ technology decreased a starting concentration of 9.97 x 10^5 TCID50/mL SARS-CoV-2 to an average of 4.20 x 10^5 TCID50/mL after 10 minutes. Increased exposure time resulted in a higher observed reduction in recoverable active SARS-CoV-2, as shown by the 1.17 x 10^5 TCID50/mL recovered after 20 minutes, 2.34 x 10^4 TCID50/mL after 25 minutes, and 1.20 x 10^2 TCID50/mL after 30 minutes of device operation.
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 30 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 10 minutes of operation, a 57.84% gross reduction in active SARS-CoV-2 was observed and reached an 99.99% gross reduction after 30 minutes.

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Study Report

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

Sponsor: Global Plasma Solutions (dba GPS Air)

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: 05/27/2022

Experimental Start Date: 04/04/2022

Experimental End Date: 04/06/2022

Completion Date: 05/20/2022

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. The following test was to determine what reductions were possible under controlled conditions with a negative ion concentration target in the testing chamber of 3,500 ions/cm^3.

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 suspension media without the test virus present to confirm average particle size distribution. The nebulizer was filled with 9.97 x 10^5 TCID50/mL of SARS-CoV-2 in 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. 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 28875, 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 directionnel 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.

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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 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 2.0mL PBS and the subsequent tubes with 1.8mL.
4. Vortex the viral samples, then transfer 20uL of the virus to the first tube, vortex, and discard tip.
5. With a new tip, serial dilute subsequent tips transferring 200uL.

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 0.1mL of media from each well by vacuum aspiration.
4. Starting from the most dilute sample, add 0.1mL 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 0.5mL 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 GPS-FC48-AC™ device housing NPBI™ technology (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 ionizer. Before testing, the GPS-FC48-AC™ 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 each day of testing, as shown in Figure 5. Ion concentrations were measured at one point located in the center of the room across from the device's location. Average device ion concentrations were recorded as 2,650 positive ions/cm3 and 3,490 negative ions/cm3. It should be noted that due to the nature of ions, there were fluctuations in concentrations around the entire room.

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 22’x11’x8’ chamber per Biosafety Level 3 (BSL3) standards. The room had a displacement volume of 1,936 ft^3 (54,821.42 L) 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 22 ft wall protruding 24 inches from the wall opposite the door. 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. For air sample testing, the room was equipped with four probes positioned along the centerline of the room. The device was placed in the room's centerline and mounted on a movable scaffolding against the wall at an elevated position 6 ft above the ground, depicted in Figure 4. A variable-speed fan was placed behind the GPS-FC48-AC™ to create the necessary airflow to produce the required concentration of negative ions. The chamber was visually inspected, pressure tested, and all internal lab systems and equipment were reviewed before testing. Furthermore, ion measurements were measured again before testing to confirm consistent reading.

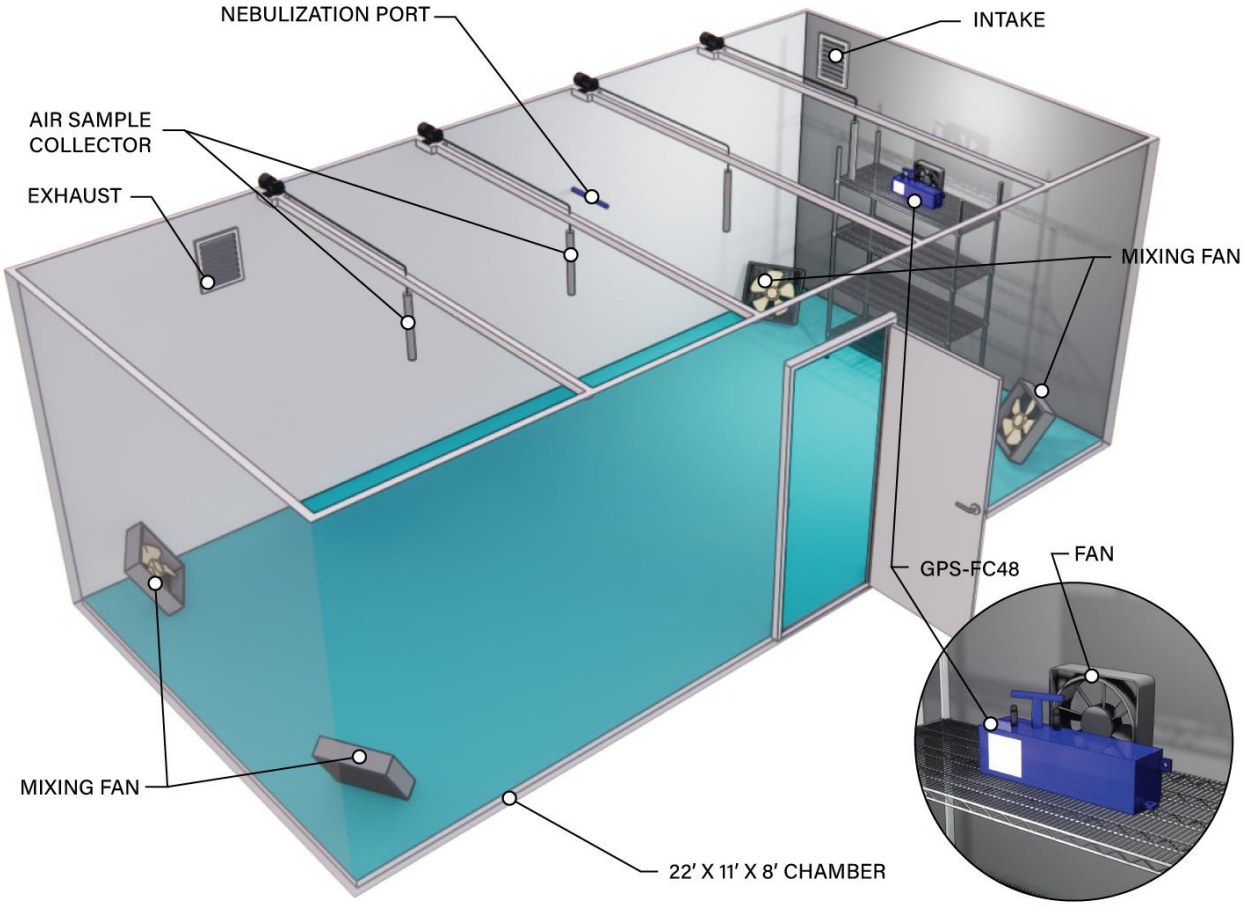

Figure 4. Room layout for control and experimental testing.

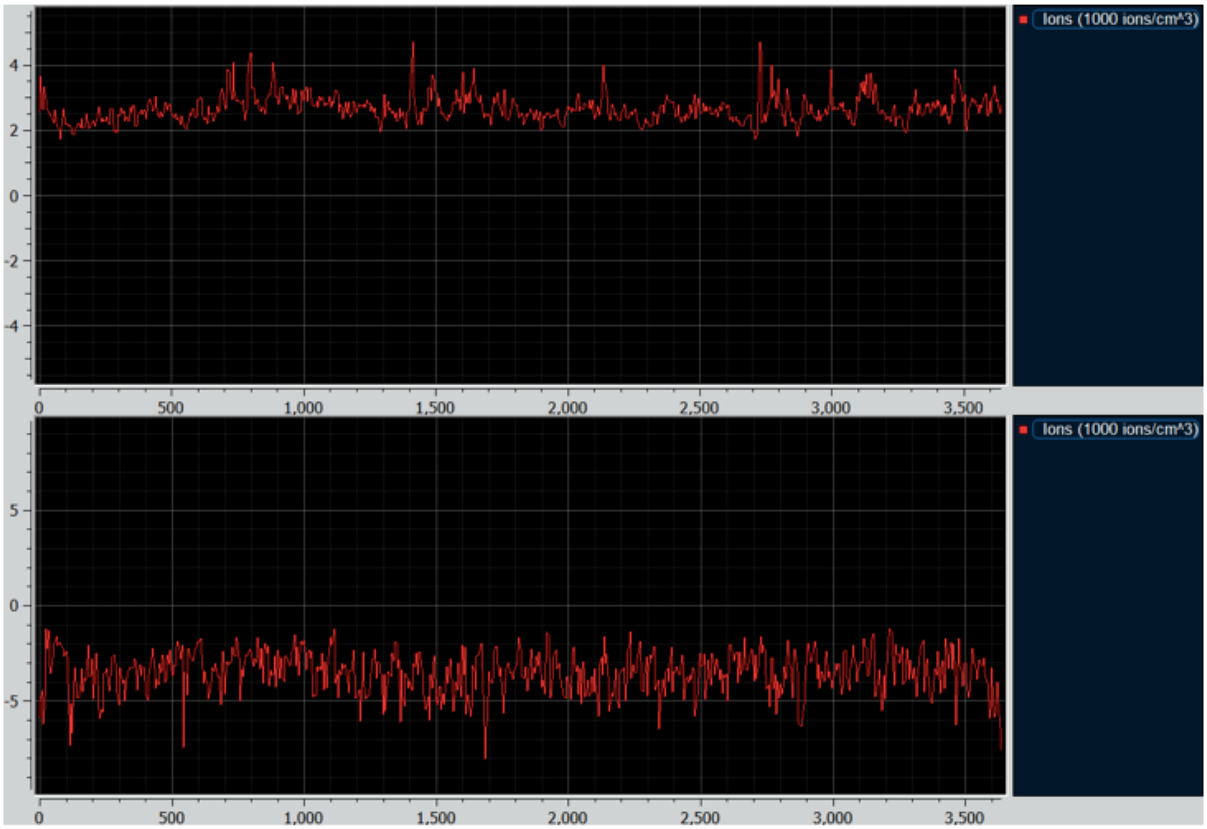

Figure 5. Positive (top) and negative (bottom) ion concentration readings from a dry run conducted prior to testing. Average ion concentration was measured at 2,650 positive ions/cm³ and 3,490 negative ions/cm³.

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 72 ±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-10, T-20, T-25, and T-30.
4. Three controls and three viral challenges were conducted using the same methodology.

Experimental Procedures:

1. Before the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. Each day prior to testing, the GPS equipment was checked during dry runs and ion concentrations were recorded to confirm similar starting environments
3. Each day of testing ion concentrations were observed to be within the acceptable test range when equipment was operated without the nebulization system running.
4. 10 mL of 9.97 x 10^5 TCID50/mL SARS-CoV-2 in suspension media was nebulized into the sealed environment via the dissemination port.
5. After nebulization, the GPS-FC48-AC™ device was turned on via remote control at T-0 after nebulization was completed.
6. The device was turned off at each predetermined time point for sample collection.
7. Air sampling collection was set to 10-minute continuous draws at the point of sampling occurring after nebulization ceased.
8. Sample cassettes were manually removed from the collection system and taken to an adjacent biosafety cabinet to be pooled.
9. 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 with a 70% isopropyl alcohol solution at the end of each day. Collection lines were soaked in a bleach bath mixture for 30 minutes and then rinsed repeatedly with DI water. The 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.

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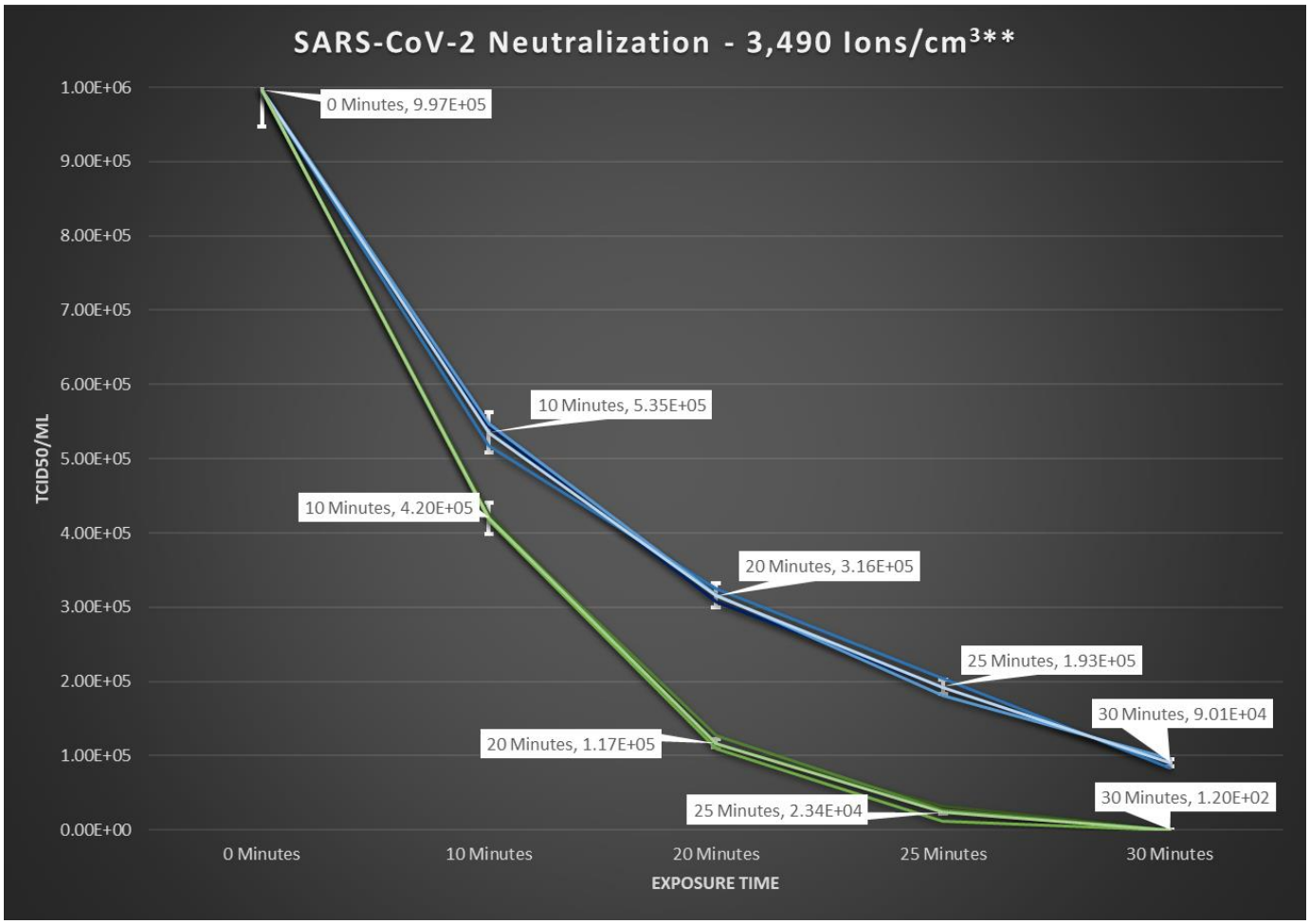
Study Results

The graph displayed recoverable active SARS-CoV-2 with and without the GPS-FC48-AC™ device housing NPBI™ technology operating over 30 minutes with a negative ion concentration of 3,490 ions/cm^3. The controls showed a natural viability loss of aerosolized SARS-CoV-2 for 30 minutes within the chamber under controlled conditions. Across three trials at an average of 3,490 negative ions/cm^3 the device reduced a starting concentration of 9.97 x 10^5 TCID50/mL to 4.25 x 10^5, 4.16 x 10^5, and 4.20 x 10^5, averaging at approximately 4.20 x 10^5 TCID50/mL after 10 minutes. After 20 minutes of operation, an average of 1.17 x 10^5 TCID50/mL was collected. Neutralization capabilities of the GPS-FC48-AC™ device housing NPBI™ technology increased with elapsed time, as observed by the decrease in recovered SARS-CoV-2 over time. After 25 minutes, 2.34 x 10^4 TCID50/mL SARS-CoV-2 was recovered and reached below the limit of quantitation represented by the value 1.20 x 10^2 TCID50/mL after 30 minutes.



**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**	**10**	**20**	**25**	**30**
Control 1	9.97E+05	5.42E+05	3.07E+05	1.92E+05	9.06E+04
Control 2	9.97E+05	5.17E+05	3.25E+05	2.05E+05	8.42E+04
Control 3	9.97E+05	5.47E+05	3.16E+05	1.81E+05	9.53E+04
3 Control Average	9.97E+05	5.35E+05	3.16E+05	1.93E+05	9.01E+04
% Reduction - Gross Control Avg.		-46.31%	-68.30%	-80.67%	-90.96%
Experiment 1	9.97E+05	4.25E+05	1.15E+05	3.24E+04	1.20E+02***
Experiment 2	9.97E+05	4.16E+05	1.09E+05	1.16E+04	1.20E+02***
Experiment 3	9.97E+05	4.20E+05	1.26E+05	2.63E+04	1.20E+02***
3 Experiment Average	9.97E+05	4.20E+05	1.17E+05	2.34E+04	1.20E+02***
% Reduction - Gross Experiment Avg.		-57.84%	-88.29%	-97.65%	-99.99%
% Net Reduction Avg.		**-21.49%**	**-63.06%**	**-87.84%**	**-99.87%**

*** 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 a controlled environment. The device achieved a 57.84% gross reduction of active viruses after 10 minutes compared to the controls, which displayed a 46.31% reduction. After 30 minutes, the device achieved a 99.99% gross reduction compared to the 90.96% 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 on 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 virus in the control test.

Considering variables, there was a measurable amount of reduction achieved by the GPS-FC48-AC™ device housing NPBI™ technology at each time point (T-10, T-20, T-25, and T-30). Overall, the device successfully reduced SARS-CoV-2 in the air by 99.99% after 30 minutes with an ion concentration of 3,490 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.

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DocuSigned by:
Dr. Dana Yee MD.
52F0012FC10C43F...

7/25/2022

Dana Yee M.D **Date**

Clinical Pathologist and Medical Director, Innovative Bioanalysis, Inc.

DocuSigned by:
Sam Kabbani
8B4B282DF4B34A3...

7/25/2022

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

Chief Scientific Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Albert Brockman
42751B72F2EF40A...

7/25/2022

Albert Brockman **Date**

Chief Biosafety Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Kevin Noble
5DF2797BAA78421...

7/25/2022

Kevin Noble **Date**

Laboratory Director, Innovative Bioanalysis, Inc.