



## EFFICACY OF THE GPS-FC48-AC™ AGAINST RESPIRATORY SYNCYTIAL VIRUS (RSV)

**PROJECT: GPS – NPBI FC48 – AEROSOL RSV**

TECHNOLOGY: Needlepoint Bipolar Ionization (NPBI™)

DEVICE: GPS-FC48-AC™

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

**CHALLENGE ORGANISM:**

RESPIRATORY SYNCYTIAL VIRUS (RSV)

**Medical Director:**

Dana Yee, M.D.

**Study Completion Date:**

03/07/22

**Testing Facility:**

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**Laboratory Project Number:**

1189R



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## Efficacy Study Summary

<b>Study Title</b>	EFFICACY OF THE GPS-FC48-AC™ AGAINST RESPIRATORY SYNCYTIAL VIRUS (RSV)
<b>Laboratory Project #</b>	1189R
<b>Guideline:</b>	GCLP, modified ISO, and BSL-3 standards were used.
<b>Testing Facility</b>	Innovative Bioanalysis, Inc.
<b>GLP Compliance</b>	All internal SOPs and processes follow GCLP guidelines and recommendations.
<b>Test Substance</b>	Respiratory Syncytial Virus (RSV)
<b>Description</b>	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 Respiratory Syncytial Virus (RSV).
<b>Test Conditions</b>	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 72°F, with a relative humidity of 41 ±1%. A $4.15 \times 10^6$ TCID50/mL of RSV in suspension 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 were tested in triplicate. Ion concentrations were measured in the chamber during a dry run test prior to viral challenges.
<b>Test Results</b>	The GPS-FC48-AC™ device housing NPBI™ technology consistently reduced active Respiratory Syncytial Virus (RSV) at each time point faster than natural loss rates. With an average concentration of 23,600 negative ions/cm <sup>3</sup> , the GPS-FC48-AC™ decreased a starting concentration of $4.15 \times 10^6$ TCID50/mL RSV to an average $3.06 \times 10^6$ TCID50/mL after 15 minutes. Increased exposure time resulted in a higher observed reduction in recoverable active RSV, with an average of $5.96 \times 10^4$ TCID50/mL recovered after 60 minutes.
<b>Control Results</b>	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 RSV in air.
<b>Conclusion</b>	The NPBI™ technology demonstrated the overall capability to reduce aerosolized Respiratory Syncytial Virus (RSV) at each time point faster than the natural viability loss rates. After 60 minutes of operation, a 97.00% gross reduction in active RSV in the air was achieved with the device producing an average of 23,600 negative ions/cm <sup>3</sup> compared to a 51.15% loss without the device.



## Study Report

Study Title: EFFICACY OF THE GPS-FC48-AC™ AGAINST RESPIRATORY SYNCYTIAL VIRUS (RSV)

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: 03/10/2022

Experimental Start Date: 12/19/2021

Experimental End Date: 12/22/2021

Study Completion Date: 03/07/2022

### 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, Respiratory Syncytial Virus (RSV). 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 the average particle size distribution. The nebulizer was filled with  $4.15 \times 10^6$  TCID50/mL of Respiratory Syncytial Virus (RSV) 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

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## 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 and viability. Filtration discs from Zefon International, Lot# 28144, 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 sampler cassette.*

## Test System Strains: Respiratory Syncytial Virus (RSV)

The following reagent was obtained through BEI Resources, NIAID, NIH: Human Respiratory Syncytial Virus, A2001/2-20, NR-28525.



### 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<sub>2</sub> 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.

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## 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 ion generation, 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 concentration was recorded at 9,600 positive ions/cm<sup>3</sup> and 23,600 negative ions/cm<sup>3</sup> with the manufacturer's pre-set airflow. 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: 11.1" x 1.84" x 3.52"

SERIAL #: N/A



Figure 3. The GPS-FC48-AC™ as tested.

## Testing Layout:

Testing was conducted in a sealed 20'x8'x8' chamber per Biosafety Level 3 (BSL3) standards. The room had a displacement volume of 1,280 ft<sup>3</sup> (36,245.56 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 20-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 and protruded down from the ceiling 24-inches. The device was placed in the room's centerline and mounted on a movable scaffolding against the wall at an elevated position six feet above the ground, as 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, and 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.

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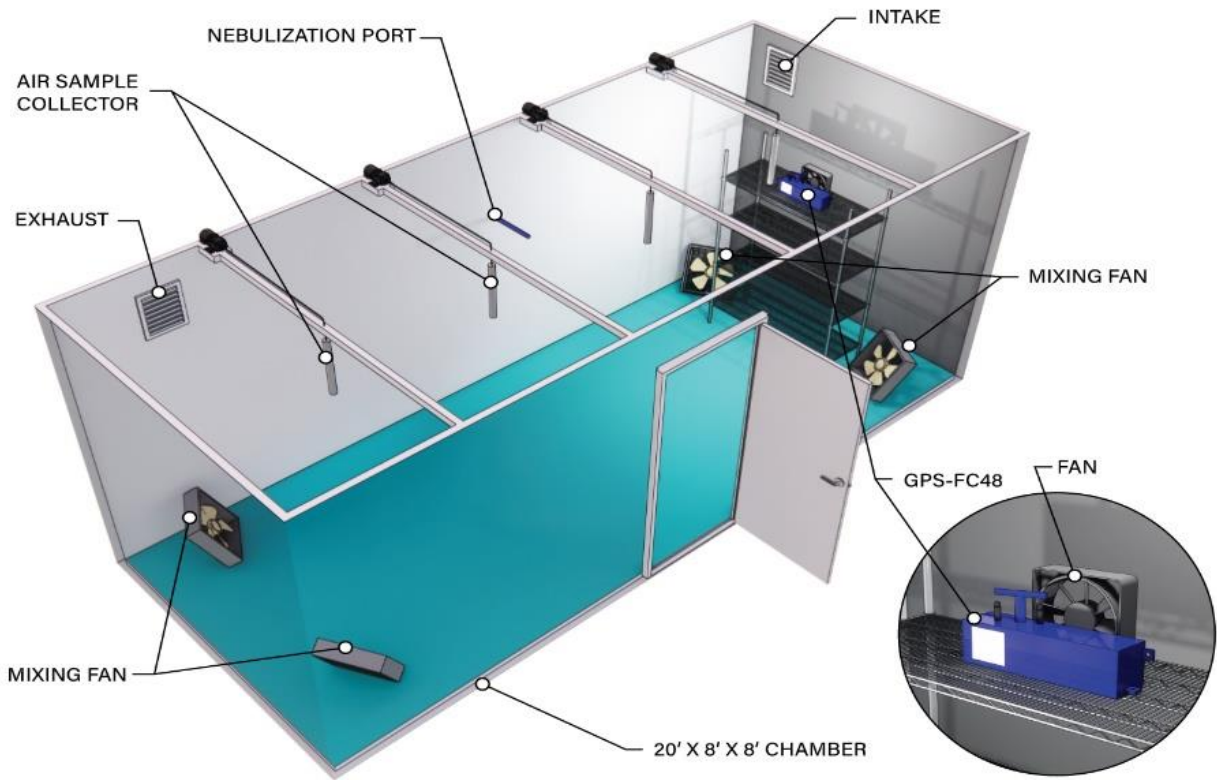


Figure 4. Room layout for control and experimental testing.



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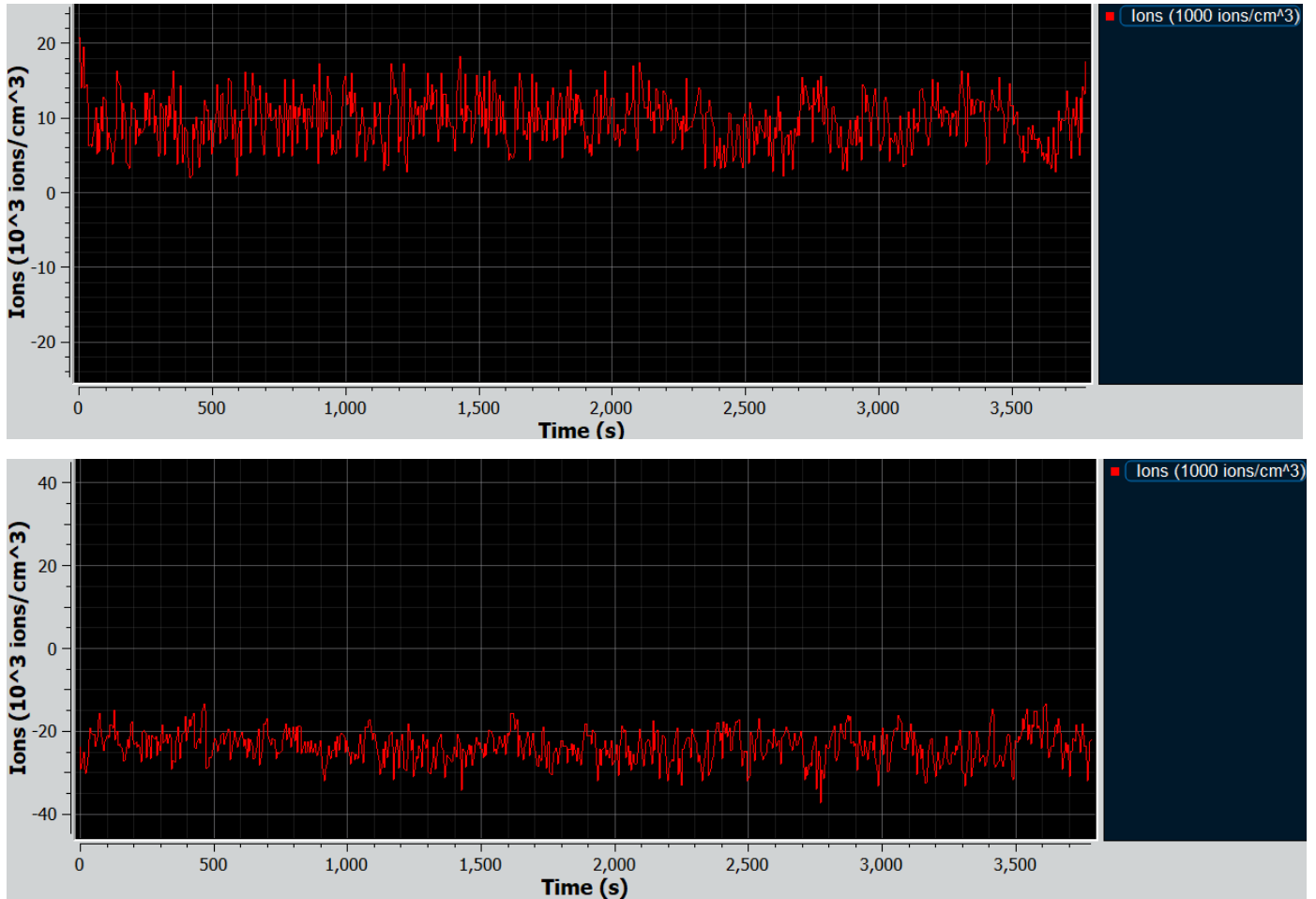


Figure 4. Positive (top) and negative (bottom) ion concentration readings during the 1-hour dry run. Average ion concentration was measured at 9,600 positive ions/cm<sup>3</sup> and 23,600 negative ions/cm<sup>3</sup>.

## Control Protocol:

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



## Test Procedures:

### Exposure Conditions:

1. The temperature during all test runs was approximately 72°F, with a relative humidity of 41 ±1%.
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  $4.15 \times 10^6$  TCID50/mL RSV viral suspension 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.
4. The device was turned off at the pre-determined time points 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 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: Human Respiratory Syncytial Virus (NR-28525, Lot #: 60109225)

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in HE-p2 Cells</b>		
	Cell rounding and sloughing	Cell rounding and sloughing
<b>Sequencing of Species-Specific Region (822 nucleotides)</b>		
	Consistent with human respiratory syncytial virus, A2001/2-20	Consistent with human respiratory syncytial virus, A2001/2-20 GenBank: JX069798
<b>Titer by TCID50 in Hep-2 Cells</b>		
	Report Results	2.8 X 10 <sup>8</sup> TCID50 per mL
<b>Sterility (21-Day Incubation)</b>		
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
<b>Mycoplasma Contamination</b>		
Agar and Broth Culture	None Detected	None Detected
DNA Detection by PCR of extracted test article nucleic acid	None Detected	None Detected

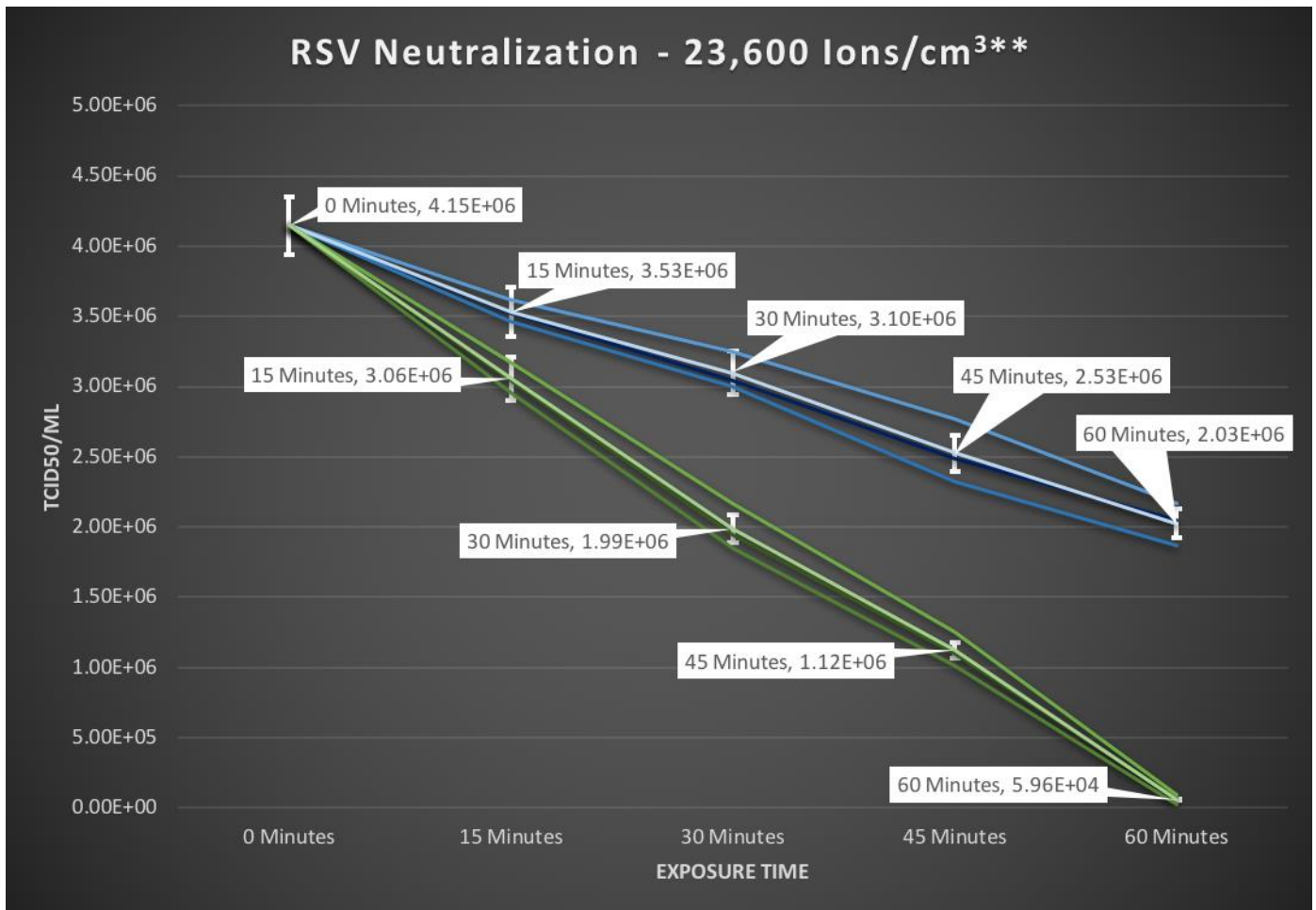
\*The viral titer listed in the Certificate of Analysis represents the titer provided by BEI Resources.

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## Study Results

The graph displayed the recoverable Respiratory Syncytial Virus (RSV) with and without the GPS-FC48-AC™ device housing NPBI™ technology operating over 60 minutes. The control shows a natural viability loss over time within the chamber under controlled conditions. At an average concentration of 23,600 negative ions/cm<sup>3</sup> against RSV, a starting concentration of 4.15 x 10<sup>6</sup> TCID50/mL was reduced to 3.05 x 10<sup>6</sup>, 3.17 x 10<sup>6</sup>, 2.95 x 10<sup>6</sup>, and averaging to approximately 3.06 x 10<sup>6</sup> TCID50/mL at 15 minutes. At 30 minutes, an average 1.99 x 10<sup>6</sup> TCID50/mL RSV was recovered and 1.12 x 10<sup>6</sup> TCID50/mL after 45 minutes. In 60 minutes, the GPS-FC48-AC™ reduced aerosolized RSV to 5.96 x 10<sup>4</sup> TCID50/mL.



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

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Respiratory Syncytial Virus (RSV) Neutralization					
Time (min)	0	15	30	45	60
Control 1	4.15E+06	3.53E+06	3.05E+06	2.49E+06	2.05E+06
Control 2	4.15E+06	3.46E+06	3.00E+06	2.32E+06	1.86E+06
Control 3	4.15E+06	3.62E+06	3.25E+06	2.76E+06	2.16E+06
3 Control Average	4.15E+06	3.53E+06	3.10E+06	2.53E+06	2.03E+06
% Reduction - Gross Control Avg.		-14.76%	-25.31%	-39.08%	-51.16%
Experiment 1	4.15E+06	3.05E+06	1.95E+06	1.10E+06	6.58E+04
Experiment 2	4.15E+06	3.17E+06	2.16E+06	1.25E+06	9.24E+04
Experiment 3	4.15E+06	2.95E+06	1.85E+06	1.01E+06	2.05E+04
3 Experiment Average	4.15E+06	3.06E+06	1.99E+06	1.12E+06	5.96E+04
% Reduction - Gross Experiment Avg.		-26.29%	-52.09%	-73.03%	-98.56%
<b>% Net Reduction Avg.</b>		<b>-13.53%</b>	<b>-35.85%</b>	<b>-55.72%</b>	<b>-97.06%</b>

## Conclusion

The GPS-FC48-AC™ device housing NPBI™ technology demonstrated the ability to consistently reduce aerosolized Respiratory Syncytial Virus (RSV) in a controlled environment with negative ion concentration measured at an average of 23,600 negative ions/cm<sup>3</sup> from a central point in the room. The device displayed the following gross reductions at each time point: 26.29% gross reduction at 15 minutes, 52.09% at 30 minutes, 63.40% at 45 minutes, and a 97.00% gross reduction after 60 minutes of exposure. Ion concentrations were measured in the chamber during a dry run test prior to viral challenges.

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 the virus in the control test.

Considering variables, there was a measurable amount of reduction achieved by the GPS-FC48-AC™ device at each time point (T-15, T-30, T-45, and T-60). The decline of RSV 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 RSV from the air under controlled conditions within the parameters of the test environment.

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