INFLUENZA B VIRUS AEROSOL EFFICACY TESTING

PROJECT: GPS AEROSOL INFLUENZA B

TECHNOLOGY: Needle Point Bipolar Ionization

DEVICE: GPS-FC48-AC™

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM (S):

INFLUENZA B VIRUS

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Medical Director

Study Completion Date

7/26/2021

Testing Facility

Innovative Bioanalysis, Inc.

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Laboratory Project Number

1034-B

INNOVATIVE BIOANALYSIS
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Efficacy Study Summary

Study Title	INFLUENZA B VIRUS AEROSOL EFFICACY TESTING
Laboratory Project #	1034-B
Guideline:	No standard exists; GLP and modified ISO standards were used.
Testing Facility	Innovative Bioanalysis, Inc.
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	Influenza B Virus
Description	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 pathogens while operational. Testing was conducted on the device to evaluate the effectiveness of the NPBI™ technology in reducing aerosolized Type B Influenza Virus.
Test Conditions	The test was conducted in an airtight 20'x8'x8' chamber with a redundant negative pressure system connected to HEPA filters and an in-duct UV-C system. The temperature during testing was 72 ± 2°F, with a relative humidity of 43%. Aerosolization was generated by filling a nebulizer with an Influenza B concentration of 3.86 x 10^6 TCID50/mL in FBS-based media. Air samples were collected after 0, 15, 30, 45, and 60 minutes of exposure to the operating device.
Test Results	The GPS-FC48-AC™ device housing NPBI™ technology decreased Influenza B Virus concentrations to 4.16 x 10^5 TCID50/mL after 60 minutes. The device showed a consistent reduction of collectible Influenza B at each time point faster than natural loss rates. Ion concentrations were measured in the chamber during a dry run test prior to viral challenges with an average of 22,000 negative ions per cm^3.
Control Results	For the duration of testing, Influenza B decreased from 3.86 x 10^6 TCID50/mL to 2.59 x 10^6 TCID50/mL. 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 Influenza B.
Conclusion	The NPBI™ technology exhibited the overall capability to reduce aerosolized Influenza B viruses at each time point faster, with an 89.23% reduction observed after 60 minutes of operation.

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Study Report

Study Title: INFLUENZA B VIRUS AEROSOL EFFICACY TESTING

Sponsor: Global Plasma Solutions

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

Technology Tested: NPBI™

Device Tested: GPS-FC48-AC™

Study Report Date: 07/28/2021
Experimental State Date: 04/12/2021
Experimental End Date: 04/16/2021
Study Completion Date: 07/26/2021

Study Objective:

An ionization unit, 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, Influenza Type B Virus.

Test Method:

Bioaerosol Generation:

The nebulizer was filled with a 3.86 x 10^6 TCID50 per mL FBS-based viral media of Influenza B and nebulized at a flow rate of 1mL/min with untreated local atmospheric air. Upon each completion, the nebulizer's remaining viral stock volume was weighed to confirm that 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.

Bioaerosol Sampling:

Four probes connected to calibrated Gilian 10i vacuum devices set at a standard flow of 5.02L/min with a 0.20% tolerance were inspected for functionality before being used. Sample collection volumes were set to 10-minute draws per time point. The air sampler operated with a removable sealed cassette and manually removed after each sampling time point. Cassettes had a delicate internal filtration disc to collect viral samples, which was moistened with a viral suspension media to aid in the collection. Filtration discs from Zefon International, Lot# 24320, were used for testing.

Test System Strains: Influenza B Virus (NR-48660)

Study Materials and Equipment:

Equipment Overview: The GPS-FC48-AC™ device housing NPBI™ technology arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. Before starting the challenge, the GPS-FC48-AC™ was operated for 1 hour in a dry run to confirm correct operations.

MANUFACTURER: Global Plasma Solutions

MODEL: GPS-FC48-AC™

SERIAL #: N/A

Testing Layout:

Testing was conducted in a 20'x8'x8' sealed chamber per Biosafety Level 3 (BSL3) standards. The overall dimensions of the test chamber provided a displacement volume of 1,280 cubic feet and approximately 36,245.56 liters of air. 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 1. A variable-speed fan was placed behind the GPS-FC48-AC™ to create the necessary airflow to produce the required concentration of negative and positive ions. During testing, ion measurements were taken to confirm consistent readings, as shown in Figure 2 & 3.

At each chamber corner, low-volume mixing fans moving at approximately 120 CFM 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 that were positioned along the centerline of the room and protruded down from the ceiling 24". A nebulizing port connected to a programmable compressor system was located in the center of the 20' wall protruding 24" from the wall. 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 before aerosol testing, as shown in Figure 2. The chamber was visually inspected, pressure tested, and all internal lab systems and equipment were reviewed before testing.

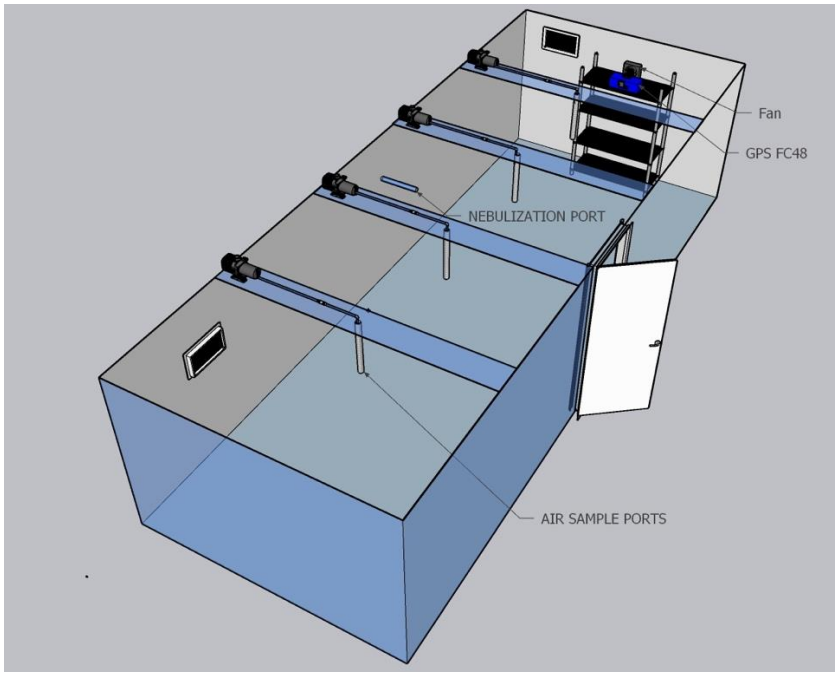


Figure 1. Room layout for the control and experimental trials.

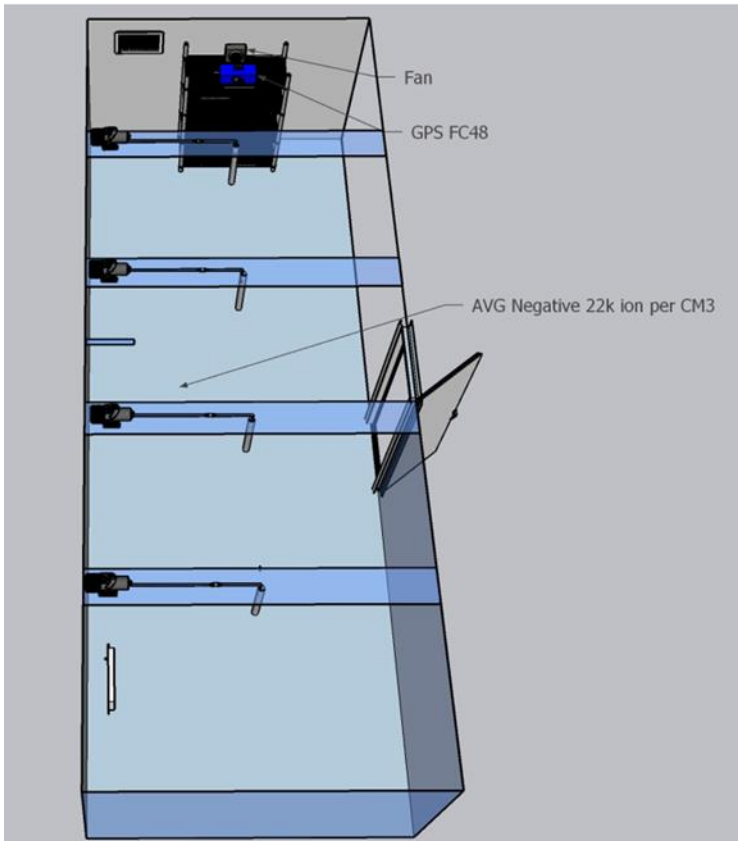


Figure 2. Overhead view of the dry run ion concentration observations.

Test Method:

Exposure Conditions:

1. The temperature during all test runs was approximately 72°F ±2°F with a relative humidity of 43%.
2. Testing time points were as follows, with T equal to minutes: T-0, T-15, T-30, T-45, and T-60.

Nebulization:

1. The testing area was decontaminated and prepped per internal procedures before the initial control test and following each trial run.
2. An Influenza B stock of 3.86 x 10^6 TCID50/mL in suspension media was nebulized into the sealed environment via the dissemination port.
3. After nebulization, the GPS-FC48-AC™ device housing NPBI™ technology was turned on via remote control.
4. Air sampling collection occurred after nebulization ceased for the challenges and control test.
5. After each run, sample cassettes were manually removed from the collection system and taken to an adjacent biosafety cabinet to be pooled.

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, there was a 30-minute air purge through the air filtration system. 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. The nebulizer and vacuum collection pumps were decontaminated with hydrogen peroxide mixtures.

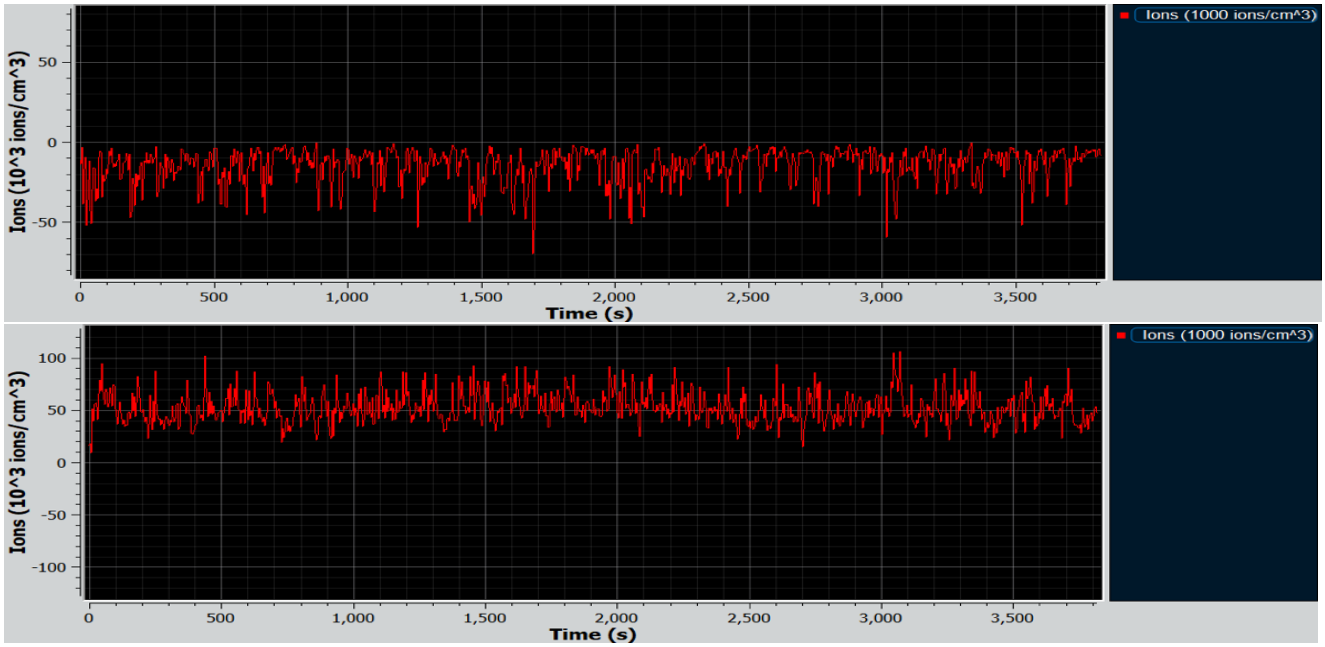


Figure 3. Device ion concentration recordings while in operation during testing.

Preparation of The Pathogen

Viral Stock: Influenza B Virus (BEI NR-48660)

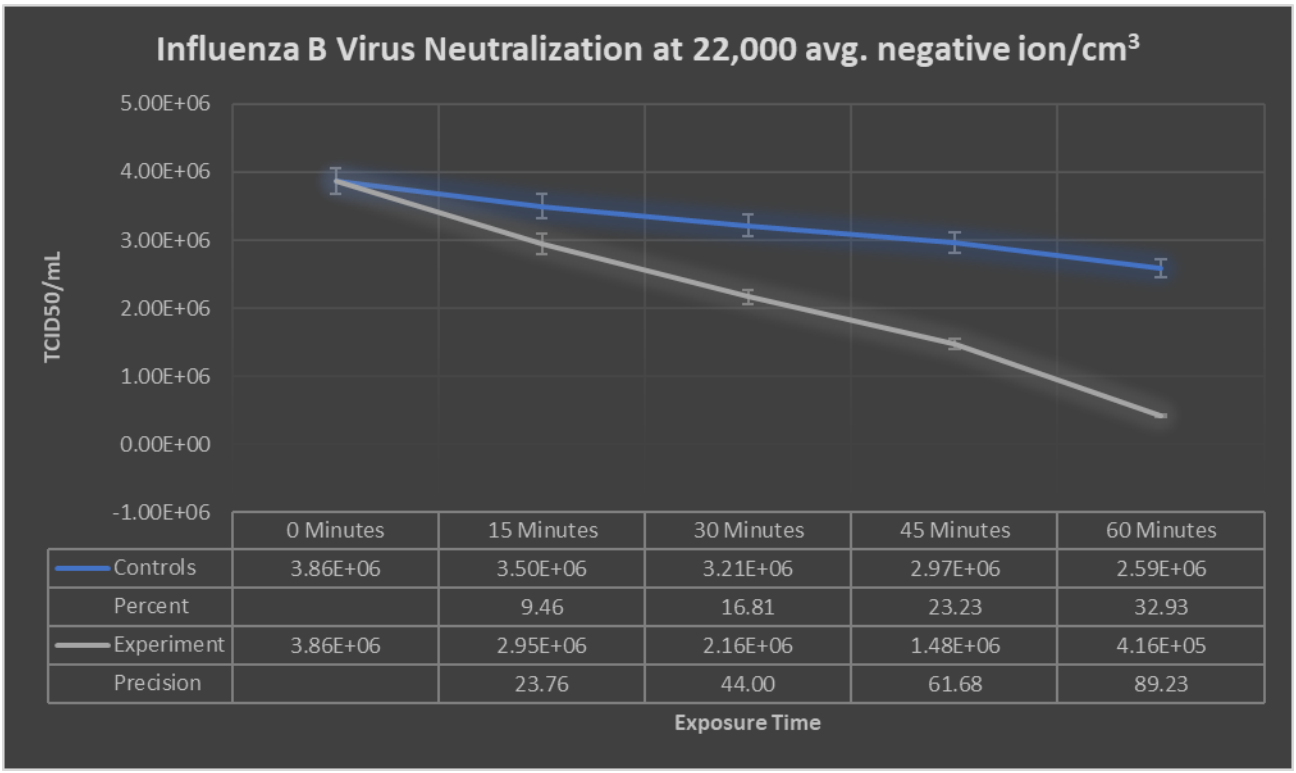
Test	Specifications	Results
Identification by Infectivity in MDCK Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Neuramindase Coding Regions (~900 nucleotides)	≥ 98% identity with B/New York/1055/2003 GenBank: CY174331.1	100% identity with B/New York/1055/2003 GenBank: CY174331.1
Titer by TCID50 Assay in MDCK Cells by Cytopathic Effect	Report Results	5 X 10^6 TCID50 per mL
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 represents the titer provided by BEI Resources.

Control Protocol

To accurately assess the GPS-FC48-AC™ device housing NPBI™ technology a control was conducted 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.

Study Results



	0 Minutes	15 Minutes	30 Minutes	45 Minutes	60 Minutes
Controls	3.86E+06	3.50E+06	3.21E+06	2.97E+06	2.59E+06
Percent		9.46	16.81	23.23	32.93
Experiment	3.86E+06	2.95E+06	2.16E+06	1.48E+06	4.16E+05
Precision		23.76	44.00	61.68	89.23

Conclusion:

Against Influenza B, the GPS-FC48-AC™ device housing NPBI™ technology was able to reduce the concentration of active pathogen quicker than the natural viability loss rate. The device reduced the initial concentration of 3.86×10^6 TCID50/mL to 4.16×10^5 TCID50/mL after 60 minutes of exposure, indicating an 89.23% reduction. Ion concentrations were measured in the chamber during a dry run test prior to viral challenges with an average of 22,000 negative ions per cm^3.

Considerations:

When working with microorganisms and collecting said microorganisms, some variables cannot be fully accounted for, namely, placement of microorganisms, collection volume, collection points, surface saturation, microorganism destruction on collection, 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 microorganisms in the control test.

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