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

CLIENT: GLOBAL PLASMA SOLUTIONS

PROJECT: RC48 10-20

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

STUDY REPORT DATE: 3/13/2021
STUDY REPORT REVISION DATE: 8/19/2021

CHALLENGE ORGANISIM(S):

- **SARS-CoV-2 USA-CA2/2020**

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

Background: This in vitro study was designed to determine the efficacy of the GPS FC48 against a known pathogen. The following tests were to determine what log reductions were possible under controlled conditions. 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 and in the air 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 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 surface 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**

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. RKI air monitoring systems continuously sampled air for O3, H202, N20 production which could put staff members at risk. Air monitoring was in place as a safety mechanism for staff and no alarms for unsafe elevated O3 were activated during testing.

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 outside variables from entering the test chamber. 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'.

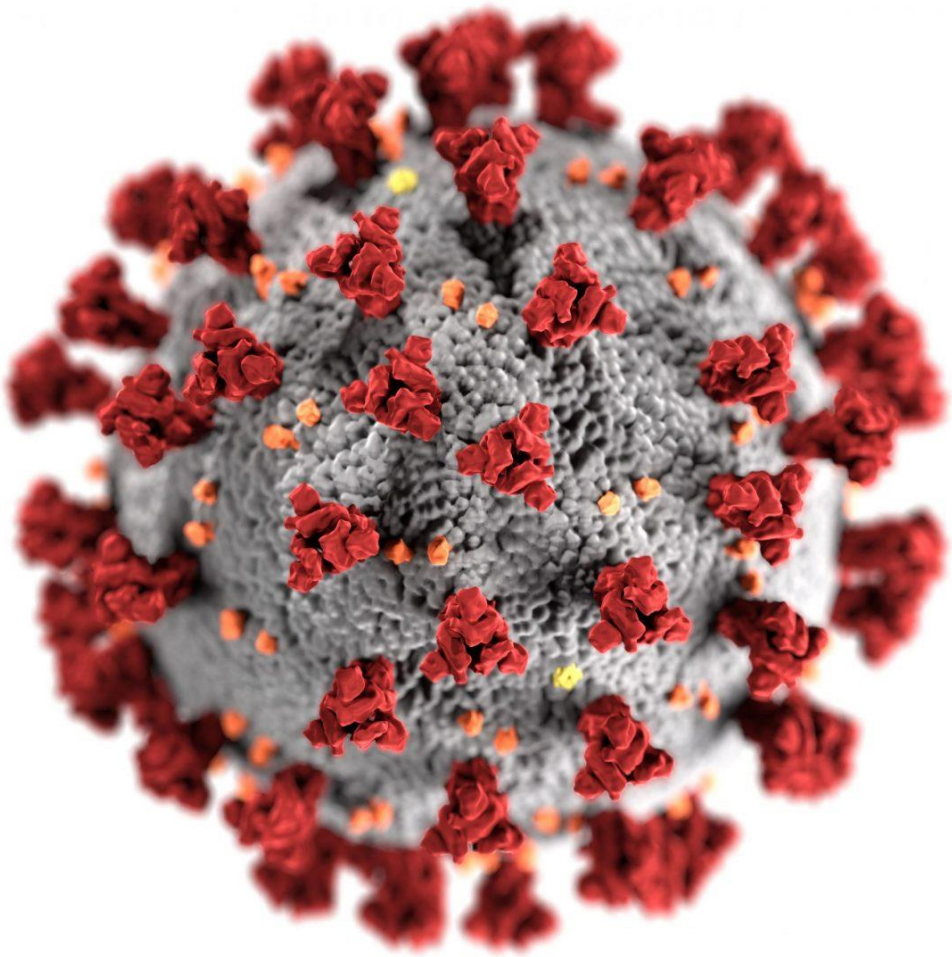
The testing chamber had HEPA filtered inlets and exhaust, coupled with an active UV-C system in all ducting lines. Humidity and temperature were monitored inside the chamber using a calibrated wireless device. Prior to testing, the chamber was pressure tested for leaks and visual inspections were made 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.

CONTROL SUMMARY:

One control was conducted for each of the time points used for a comparative analysis. For the control, two Air Ion counters were placed in the center of the of the testing chamber for 5 minutes prior to the control test. The natural state of positive and negatively charged ions were counted, and little fluctuations were observed. Ion counts were recorded every 0.5 seconds and the average for the duration of the test was 66 negative ions per cm3 53 positive ions per cm3 without the ionization unit running.

Five glass slides previously inoculated with SARS-CoV2 were placed on a table inside the room and the door closed to prevent outside environmental contaminants. Swabs were taken at the pre-defined time points of 0 Minutes, 10 minutes, 15 minutes, 30 minutes and 60 minutes for the viral pathogen test and all swabs were sealed after collection and provided to lab staff. The door to the chambers remained closed the entirety of the test and all air entering the test chamber was filtered through a HEPA filter. The intake with the HEPA filter was a fixed housing and air entering or exiting the room would be at a natural rate which was not at a measurable flow due to the low volume of exchange.

SARS-CoV-2



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EXPERIMENT SUMMARY:

GPS supplied an **FC48** system for testing purposes to determine efficacy against viral pathogens. The purpose of this study was to evaluate the efficacy of the FC48 to inactivate the viral strain referred to as SARS-CoV-2. Two negative ion concentrations were evaluated (figures 2 & 3) for viral inactivation efficacy (figures 4 & 5)

- Temperature during all test runs was approximately 71F +/- 2F with a relative humidity of 52%.
- Relative humidity and temperature were taken next to the sample slides.
- Swabs were taken at predefined time points of the following with T=minute
  - T-0
  - T-5
  - T-10
  - T-15
  - T-30
  - T-60
- Testing chamber interior seal was not breached during the test. All air entering passed through a HEPA filter at a natural rate the same as the control run.
- Air intake with HEPA filter consisted of a fixed opening which allowed for the natural passage of air into and out of the chamber at a low volume.
- Test condition (A) consisted of an average ion concentration of 9.7k negative ions per cm^3.
- Test condition (B) consisted of an average ion concentration of 23.6k negative ions per cm^3.
- No drop in humidity was observed and based on previous studies a fluctuation of a few % of humidity change will not have negative impact on the challenge study. The average humidity range was 52% during testing.
- Behind the rowed test site there were two AIC2 Air Ion Counter continually logging the negative and positive ion counts.
- A single row of test slides was used, test was not combined with other pathogens.
- Test row contained 5 glass slides provided by the lab inoculated with 1mL of viral media.
- All sample slides were labeled with their virus strain and the time point they were to be used with. One sample swab was taken from each slide, as well as a swab collected for residual viral media at the 0 minute, 5-minute time point, 10-minute time point, 15-minute time point, 30-minute time point, and 60-minute time point.
- The FC48 system was placed in front of a variable speed fan on an adjustable platform and angled up at a 45-degree angle to allow the ions to cascade down throughout the room.
- Fan speeds were adjusted until the desired concentration was reached prior to exposing test samples. All samples were brought into the testing chamber sealed and removed from the testing chamber sealed.
- Upon testing completion, samples were provided to lab staff for further review.

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. This was the chosen pathogen strain because it was taken from a 38-year-old subject with severe acute respiratory syndrome in California as was part of the A lineage. This was a non-fatal case which represents most of the cases in the United State. The age group the patient belonged to was not elderly or juvenile, which was determined to be a good medium average.

DESIGN LAYOUT:

The same room layout (figure 1) was used to test condition (A) and test condition (B). Air speed across the FC48 was varied between test (A) and test (B) to achieve the desired negative ion concentrations in the testing environment. Controls were conducted in the same testing chamber and under the same conditions as the live viral challenge. Test condition (A) target ion concentration was approximately 10,000 negative ions per cubic centimeter. Test condition (B) target ion concentration was approximately 20,000 negative ions per cubic centimeter. The actual ion concentrations for test conditions (A) and (B) are reported below in figure 2 & 3 respectively.

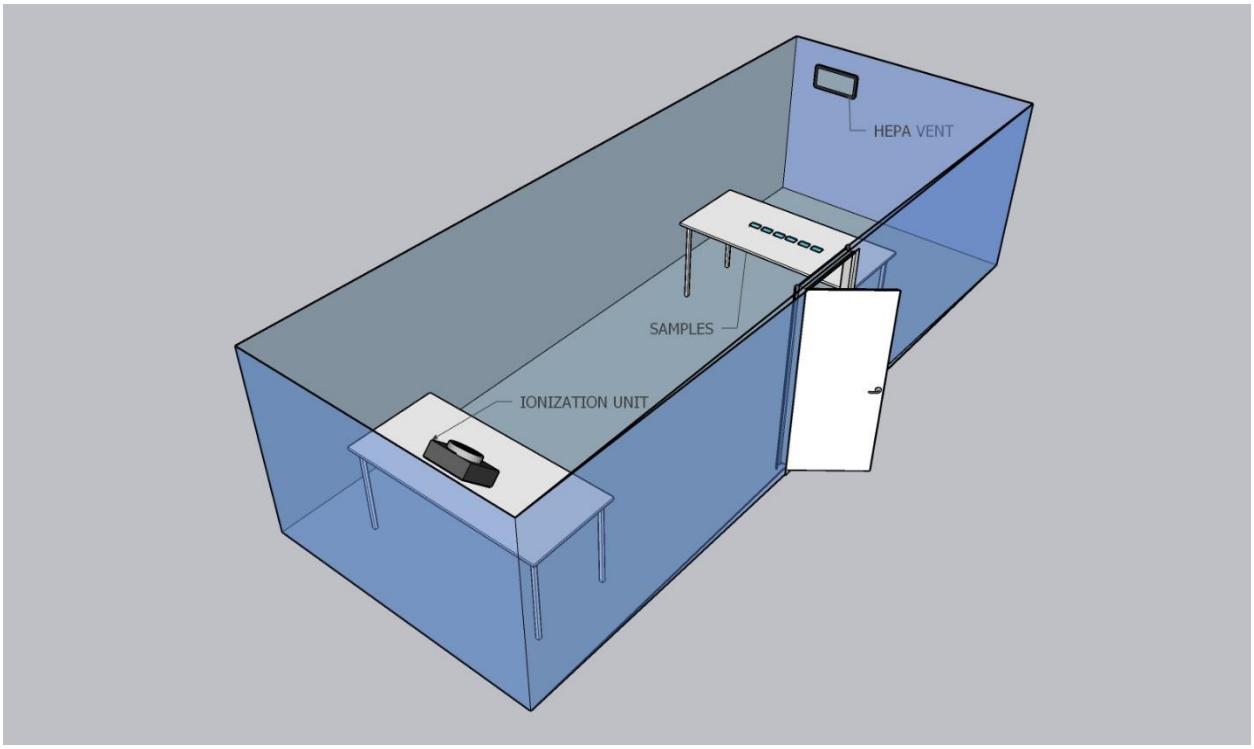
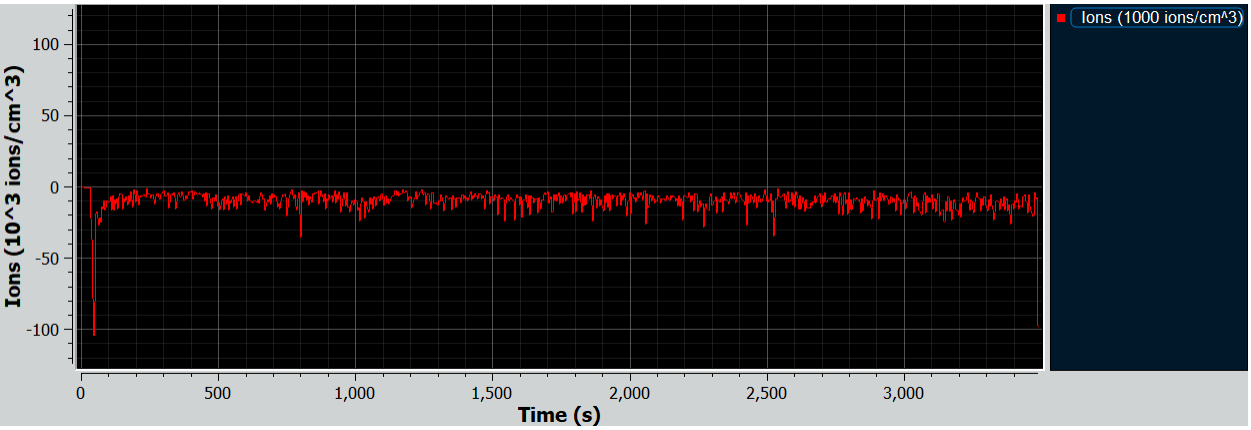


Figure 1. Room layout for control and experimental trial.

TEST (A) AVERAGE 9,000 NEGATIVE IONS PER CM3



TEST (A) AVERAGE 7,000 POSITIVE IONS PER CM3

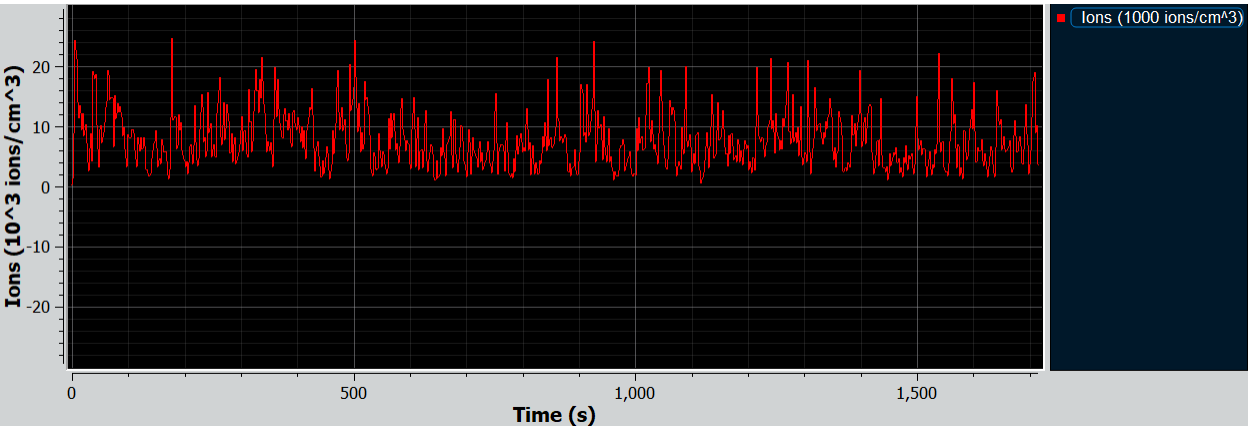
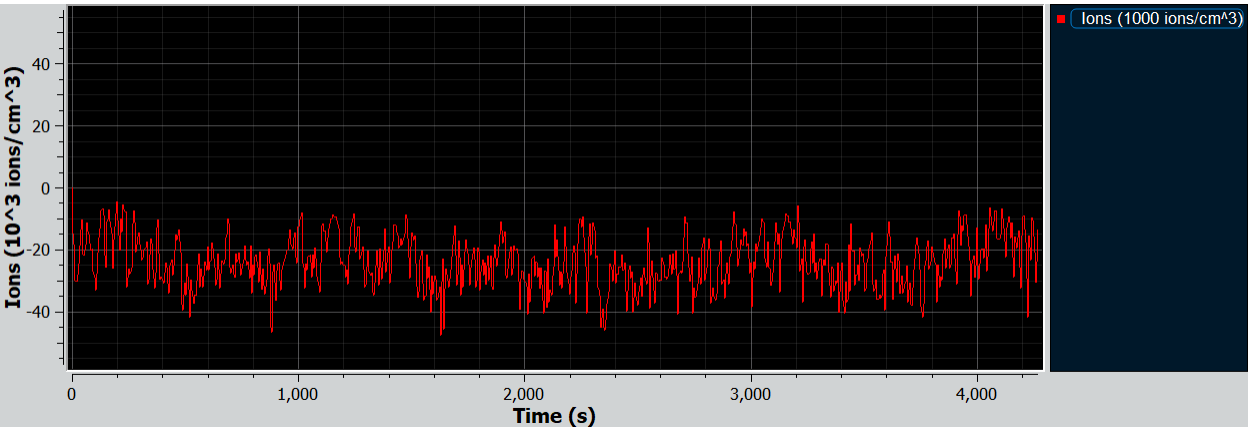


Figure 2. Test Condition (A) negative ion concentration (top) , and corresponding, positive ion concentration (bottom) reported as time versus ion concentration.

TEST(B) AVERAGE 23000 NEGATIVE IONS PER CM3



TEST(B) AVERAGE 32000 POSITIVE IONS PER CM3

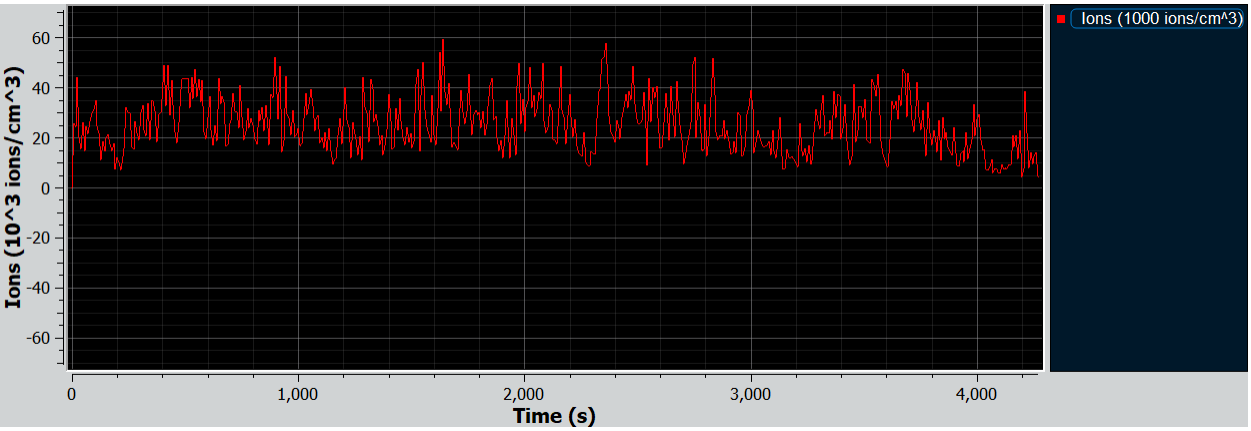


Figure 3. Test condition (B) negative ion concentration (top), and corresponding positive ion concentration (bottom) reported as time vs ion concentration.

VIRAL STOCK:

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero 6 cells	Cell Rounding and Detachment	Cell Rounding and Detachment
Next Generation Sequencing (NGS) of complete genome using Illumina® iSeq™ 100 Platform (Approx. 940 Nucleotides	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1 ≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1	99.9% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1 100% 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	1.6 X 10^6 TCID50 per mL in 5 days at 37°C and 5% CO2
Sterility (21-Day Incubation) Harpos HTYE Broth, aerobic Trypticase Soy Broth, aerobic Sabourad Broth, aerobic Sheep Blood Agar, aerobic Sheep Blood Agar, anaerobic Thioglycollate Broth, anaerobic DMEM with 10% FBS	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth

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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 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**
- **CO2 Incubator set at 37°C or 34°C or other temperature indicated.**

Procedure:

1. **One day previous to infection, prepare 96 well dishes by seeding each well with in DMEM plus 7.5 % fetal bovine serum, 4mM Glutamine, and antibiotics.**
2. **On 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 Virus 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.**
11. **Calculate TCID50.**

VIRAL TITRATION DETERMINED BY TCID50 ASSAY PROTOCOL

Each of the samples collected were subject to the same TCID50 assay protocol to determine viral concentration. Each collected swab was vortexed for 30 seconds in 2ml viral preservation media prior to serial dilution.

INOCULATION OF SURFACE SAMPLES:

Surface inoculation consisted of applying exactly 1 ml of viral media to each coupon with a calibrated Eppendorf pipette utilizing filtered pipette tips. Coupons were standard sterile 25mm x 75mm slides. Once applied, the media was spread thin using a disposable spatula and allowed to dry for 10 minutes. The starting concentration of virus that was applied was 6.23 X 10^6 TCID50/mL. After several tests for recovery, it was determined that the most efficient method of recovering viable virus would be a 2 mL rinse in viral media followed by a swab of the inoculated area. The maximum recovery achieved was 93.2% or 5.81 X 10^6 TCID50/mL. This value was used as the “0 Minute” starting concentration for all surface testing to account for the recovery.

TEST RESULTS: SURFACE INOCULATION:

Performed in the same manner as the control testing, the following deactivation rates were observed for direct surface inoculation in the challenge trials. Collection at each time point was done by swab and rinse of the coupon. Samples were collected by a technician at specified time points inside the container and stored in viral medial until testing and recovery were complete. Technicians were wearing full hazmat coveralls an had no direct exposure to the pathogen at any time. The graphs below (figures 4 & 5) represent the date for the experiments and the controls, as it pertains to surface inoculation.

EFFICACY TESTING:

Viral media with a known concentration was applied via direct inoculation to the glass slides in single location in the containment unit and exposed to bipolar ionization for a period of 0 Min, 5 Min, 10 Min, 15 Min, 30 Minutes, 60 Minutes. Swabs were taken of all material after a rinse with viral media and cultured by the same means as the original viral titration performed on the BEI Resources provided certificates of analysis.

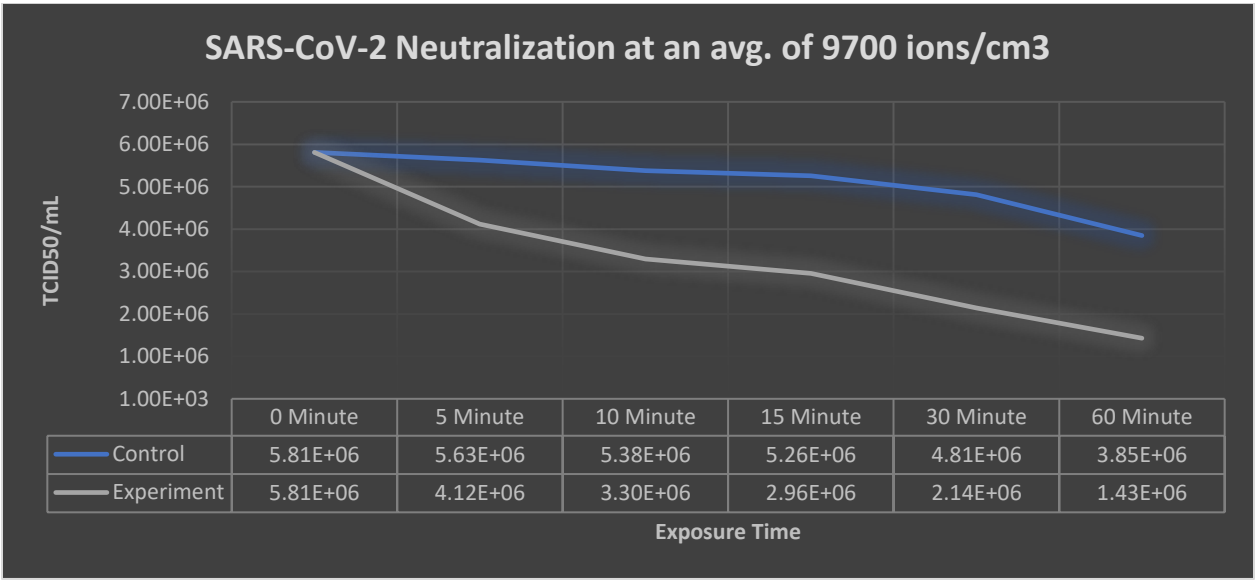

Figure 4. Condition (A) time series trials. Effect of ion exposure on infectivity of SARS-CoV-2 during FC48 ionization (gray lines) versus the control trial without FC48 ionization (blue line).

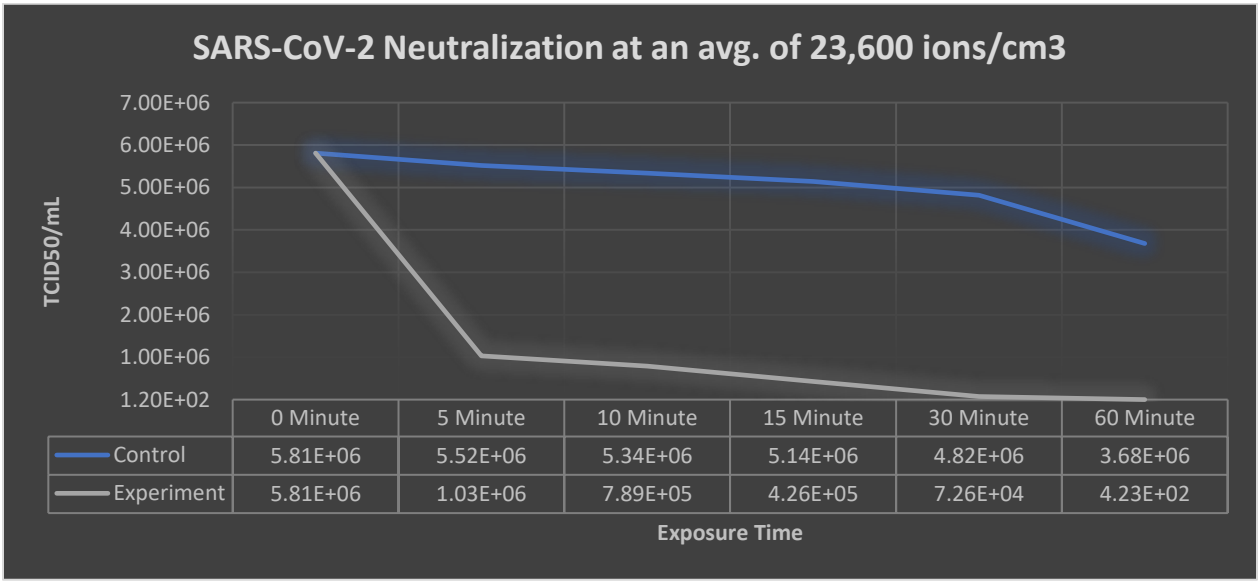

Figure 4. Condition (B) time series trials. Effect of ion exposure on infectivity of SARS-CoV-2 during FC48 ionization (gray lines) versus the control trial without FC48 ionization (blue line).

CONCLUSION:

Given the results displayed above it is understood that the pathogens tested in the testing environment ionized by GPS product FC48 were neutralized much more rapidly than the control in an environment lacking the ionization technology. The graphs above show that as ion concentration increases, pathogen neutralization increases as well. The equipment provided by Global Plasma Solutions has demonstrated a significant reduction in pathogens at both ion concentrations in relation to the controls.

CONSIDERATIONS:

- This experiment was conducted in a controlled environment in accordance with all standards set forth by the CDC for handling infectious pathogens.
- The specific viral titers utilized for this experiment are likely much higher than you would come across in a normal mode of transmission.
- The environment, although controlled, did not account for all environmental factors that could be experienced in a real-world scenario.
- All pathogens utilized in this experiment were quality tested prior to use.

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