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SARS-CoV-2 Neutralization Through Bi-Polar Ionization

CLIENT: BIG ASS FANS

PROJECT: BAF-ION-FAN-1

PRODUCT: PLASMA AIR PA-603
CAP LIC NO: 886029801
CLIA LIC NO: O5D0955926
STATE ID: CLF 00324630

SAMPLE RECEIVED: 07/22/2020
START DATE: 07/29/2020
REPORT DATE: 08/24/2020
CHALLENGE VIRUS: SARS-CoV-2 USA-WA1/2020

ABSTRACT:

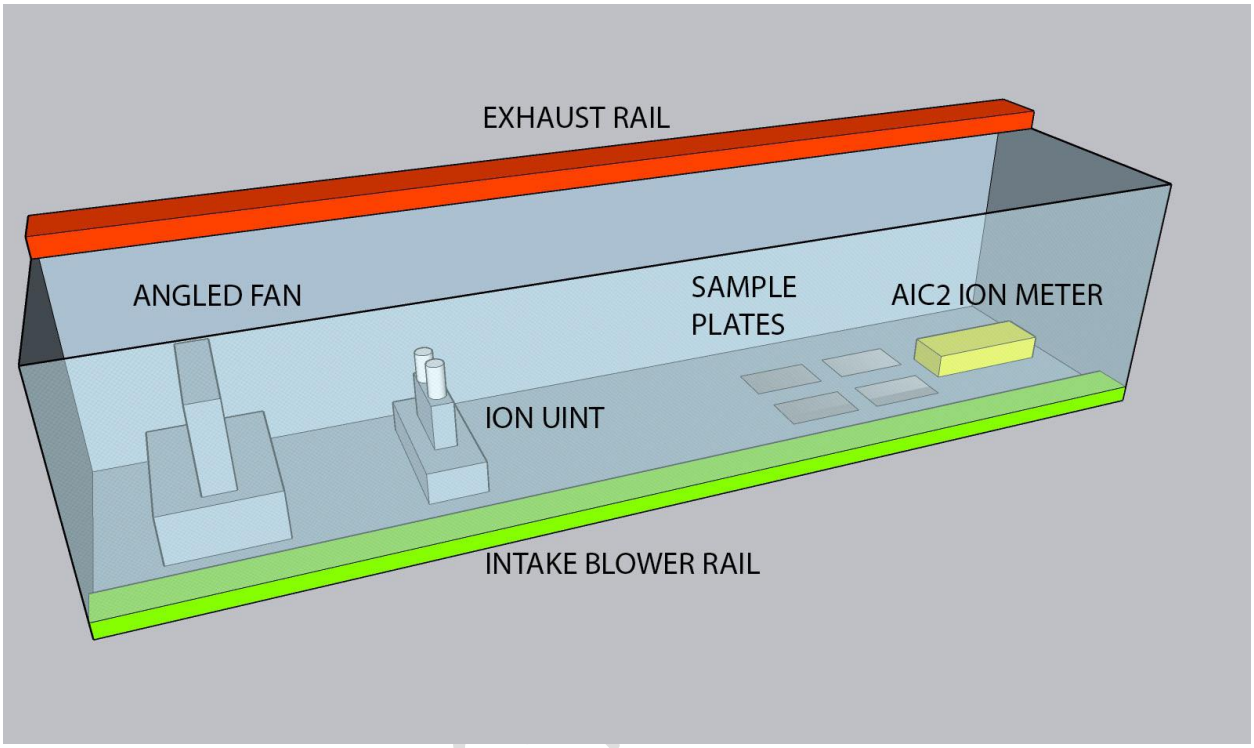
This in vitro study was to characterize the **PLASMA AIR PA-603** system and determine efficacy against the SARS COVID-19 virus. The Plasma Air PA-603 is designed to be attached to the winglet of a fan blade. When operational the unit is intended to deactivate viral pathogens on surfaces and in the air to sanitize enclosed areas. This study was to evaluate the efficacy of one viral strain referred to as SARS COVID-19 in a large setting.

EXPERIMENTAL SUMMARY: T0, T10, T20, T30, T60

A metal and glass bio safety chamber, 72" W x 30" H x 30" D with sealed seams was used for a direct inoculation testing site. An intake vent with filter was affixed to the front of the chamber and the exhaust was routed through the top of the unit terminating at a bleach soaked HEPA filter. The air temperature fluctuated slightly through the test and ranged from 69.36F to 71.28F. During the control testing and the viral load tests the temperature fluctuation was consistent. The ambient humidity inside the test chamber was 61.7%.

- At each testing site there was an AIC2 Air Ion Counter continually logging the negative ion count. Each test site contained four 3" x 1.5" piece of sterile glass, 0.125" thick. Test pieces were inoculated with the virus by spraying them with an aerosolized viral solution from 6 inches away to get even coverage of the testing area. 1 sample swab was taken from each test piece at a 10-minute time point, 20-minute time point, 30-minute time point, 60-minute time point 120 - minute time point, and 180-minute timepoint, respectively.
- Test A consisted of obtaining an average ion count between 40k and 50k per cubic centimeter over the test samples for a total elapsed time of one hour.
- Test B consisted of obtaining an average ion count between 140k and 150k per cubic centimeter for a total elapsed time of one hour.
- Test C consisted of obtaining an average ion count between 10k and 20k per cubic centimeter for a total elapsed time of 3 hours two additional time points were added to this test at 120-minute time and 180-minute time.
- To adjust over ion concentration in each set of tests a variable speed fan was used, and the pitch angle of the fan was altered as needed. Further manipulation of distance from the fan the ion distribution was placed effected overall ion distribution per cubic centimeter. Test sites and distance from the AIC2 ion counter were consistent in all tests and marked for accurate placement. Swabs were moistened with the viral suspension liquid and rubbed across the testing surfaces to pull as much standing viral media as possible. Swabs were sealed in individual tubular containers and stored in a sealed box for the duration of the test so no further ions could interact with them.
- Upon testing completion, samples were provided to lab staff for viral count via TCID50 assay.

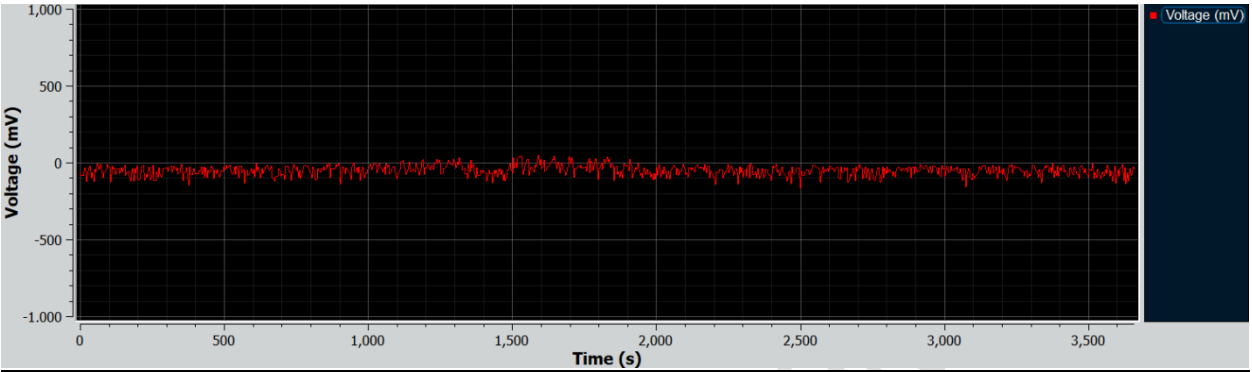
DESIGN LAYOUT:



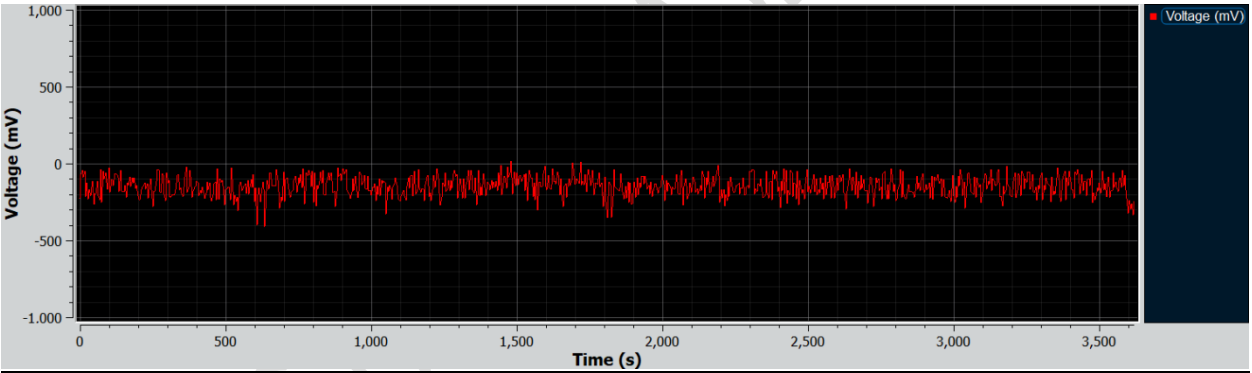
CONTROL SUMMARY:

For the control section one AIC2 Air Ion counter was placed in the center of the of the testing chamber. The natural state of ions was counted, and little fluctuations were observed until the door to the chamber was opened and equipment was moved at the end of the test. Ion counts were recorded every 0.5 seconds and the average for the duration of the test was 80 ions per cm3 without the ionization unit running.

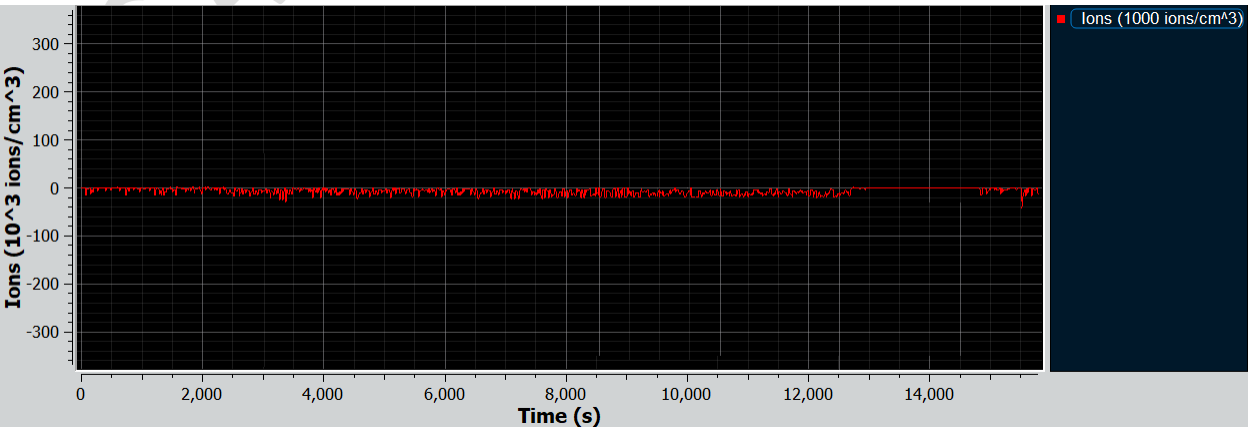
TEST {A} 1 HOUR 40K – 50k



TEST {B} 1 HOUR 140K – 150k



TEST {C} 3 HOUR 10k – 20k



TESTING PROCEDURE:

VIRAL STOCK: SARS-CoV-2 USA_WA1/2020 (BEI NR-52281)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero 6 cells	**Cell Rounding and Detachment**	**Cell Rounding and Detachment**
Sequencing of Species-Specific Region (Approx. 940 Nucleotides) **(Approx. 940 Nucleotides**	**≥ 98% identity with SARS-CoV 2, isolate USA-WA1/2020 GenBank: MN985325.1** **≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-WA1/2020 GenBank: MT246667.1**	**100% identity with SARS-CoV 2, isolate USA-WA1/2020 GenBank: MN985325.1** **100% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-WA1/2020 GenBank: MT246667.1**
Genome Copy Number using Biorad QX200 Droplet Digital PCR.	**Report Results**	**2.07 X 10^9 genome equivalents per mL**
Titer by TCID50 in Vero 6 Cells by Cytopathic effect	**Report Results**	**2.8 X 10^5 TCID50 per mL in 6 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**
Mycoplasma Contamination **Agar and Broth Culture** **DNA Detection by PCR of extracted Test Article nucleic acid.**	 **None Detected** **None Detected**	 **None Detected** **None Detected**

- **Viral sub culturing was performed in order to increase the overall concentration of the virus to the final value on 6.32 X 10^6 TCID50/mL**

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 before infection, prepare 48 well dishes by seeding each well with cells 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 viral samples and 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 48-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

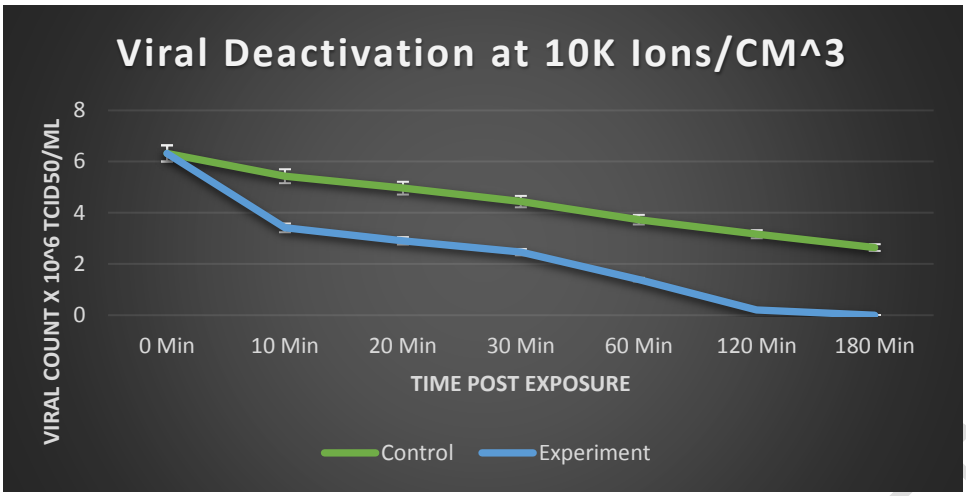
Each of the 18 samples collected were subject to the same TCID50 assay protocol to determine viral concentration. Each collected swab was vortexed for 1 full minute in 1ml viral preservation media prior to serial dilution.

INNOCULATION OF THE TEST CARRIERS:

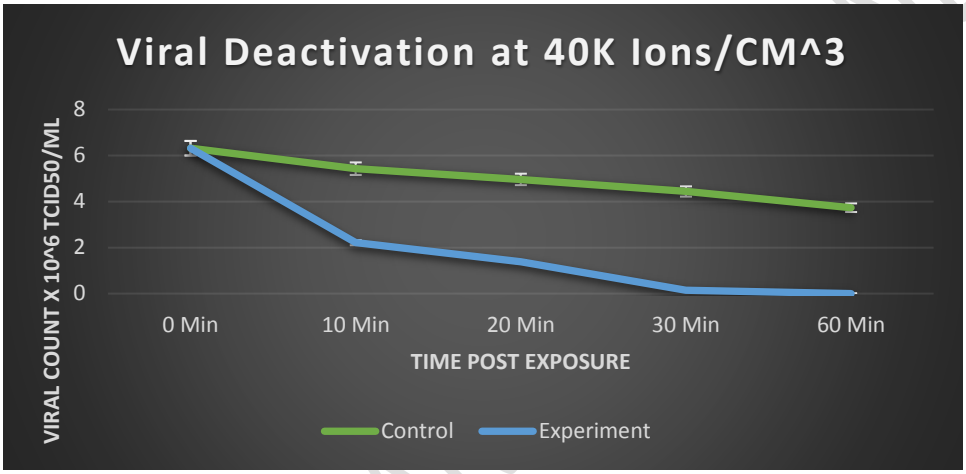
Each of the testing sites were simultaneously and equally subjected to a 1ml inoculation of viral media containing a known titer of 6.32 X 10^6 TCID50 per mL to ensure saturation of all materials. The material inoculated was stainless steel.

EFFICACY TESTING:

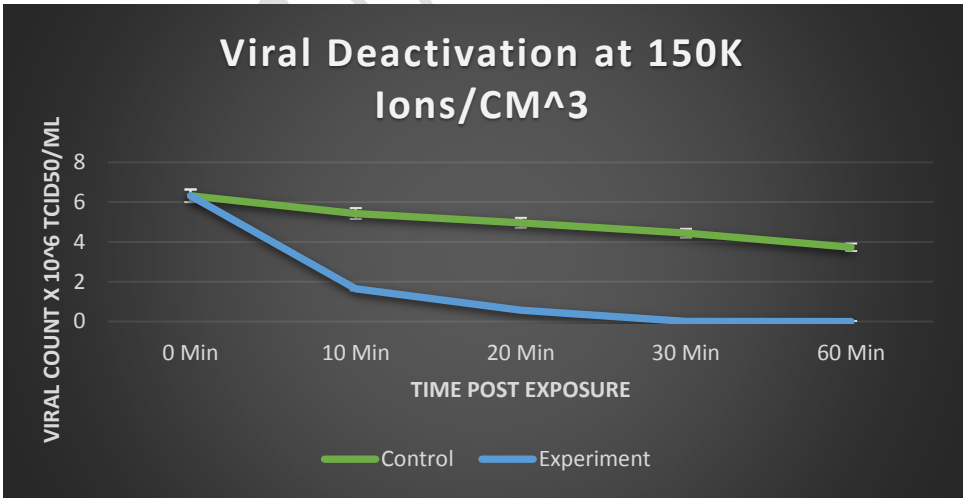
Viral media with a known concentration was applied via direct inoculation to the materials in locations throughout the containment unit and exposed to bipolar ionization for a period of 10, 20, 30, 60, 120, and 180 minutes, respectively. Swabs were taken of all material and cultured by the same means as the original viral titration performed on the BEI Resources provided SARS-CoV-2 USA-WA1/2020 viral culture. Results are as follows



Log 10 Reduction 10 Min: 0.27, 20 Min:0.34, 30 Min: 0.41, 60 Min: 0.66, 120 Min: 1.49, 180 Min: 3.94



Log 10 Reduction 10 Min: 0.46, 20 Min:0.66, 30 Min: 1.62, 60 Min: >5.00



Log 10 Reduction 10 Min: 0.59, 20 Min:1.05 Min, 30 Min: >5.00, 60 Min: >5.00

CONCLUSIONS:

With regards to Test A, the results we are as expected based on previous experiments involving the bi-polar ionization systems and SARS-CoV-2 USA_WA1/2020. At an average count of 40,000 ions per cm^3, a substantial log reduction was seen at 30-min post inoculation followed by exponential degradation at the 60-minute post inoculation time point. This ion count is by far the most manageable and easy to achieve given the provided equipment and setup. It is concluded that the bi-polar ionization system can effectively deactivate SARS-CoV-2 USA_WA1/2020 at a rate significant enough to implement in any facility and provide a valuable level of sterilization and decontamination to the environment in which it is implemented.

With regards to Test B, the results were greater than expected based on previous experiments involving the bi-polar ionization systems and SARS-CoV-2 USA_WA1/2020. At an average count of 150,000 ions per cm^3, a substantial log reduction was seen at 10 min post inoculation followed by exponential degradation at the 20-minute, 30-minute, and 60-minute post inoculation time. This ion count is manageable given the provided equipment and setup with an increased airflow and direct exposure to high speed circulating air. It is concluded that the bi-polar ionization system can effectively deactivate SARS-CoV-2 USA_WA1/2020 at a rate significant enough to implement in any facility and provide a valuable level of sterilization and decontamination to the environment in which it is implemented.

With regards to Test C, the results were as expected given the lower ion count and based on previous experiments involving the bi-polar ionization systems and SARS-CoV-2 USA_WA1/2020. At an average count of 10,000 ions per cm^3, a substantial log reduction was seen at 60 min post inoculation followed by exponential degradation at the 120-minute and 180-minute post inoculation time. This ion count is the least manageable to simulate in the given environment given the provided equipment. It is concluded that the bi-polar ionization system can effectively deactivate SARS-CoV-2 USA_WA1/2020 at a rate significant enough to implement in any facility and provide a valuable level of sterilization and decontamination to the environment in which it is implemented. Taking into consideration the time to deactivate the virus given the lower ion level, additional testing may be required given the environment in which the technology will be implemented.

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