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EFFICACY OF THE PLASMA AIR PA662 AGAINST AEROSOLIZED SARS-COV-2 OMICRON

PROJECT: WELLAIR – PA662 – SARS-COV-2 – 18K ION CC

PRODUCT: PLASMA AIR PA662

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM (S):

SARS-CoV-2 OMICRON VARIANT

Medical Director

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Laboratory Project Number

1226

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Efficacy Study Summary

Study Title	EFFICACY OF THE PLASMA AIR PA662 AGAINST AEROSOLIZED SARS-COV-2 OMICRON
Laboratory Project #	1226
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	SARS-CoV-2 Omicron Variant
Description	Per the manufacturer, the Plasma Air PA662 is an ionizer installed in an air handling unit or an air management system to reduce the presence of pathogens. WellAir provided the device for an in-vitro study to determine the efficacy of the Plasma Air PA662 at reducing aerosolized SARS-CoV-2 Omicron variant.
Test Conditions	Testing was conducted in a sealed 20'x8'x8' chamber following BSL-3 standards. The temperature during testing was approximately 74 ±2°F (23.3 ±1.1°C), with a relative humidity of 41%. A 1.08×10^7 TCID50/mL of SARS-CoV-2 Omicron variant in viral media was nebulized into the chamber with mixing fans before collection. Air samples were collected at 30, 60, and 90 minutes after exposure.
Test Results	At 18,000 negative ions/cm^3, the device decreased a starting concentration of SARS-CoV-2 Omicron from 1.08×10^7 TCID50/mL to an average 4.08×10^6 TCID50/mL after 30 minutes. At 60 minutes, the Plasma Air PA662 reduced collectible SARS-CoV-2 Omicron to an average of 1.73×10^5 TCID50/mL and neutralized active pathogen to 5.01×10^2 TCID50/mL after 90 minutes.
Control Results	Control testing was conducted without the device operating in duplicate, and samples were taken at the corresponding time points used for the challenge. The results displayed a natural viability loss over time in the chamber and were used as a comparative baseline to calculate viral reduction.
Conclusion	The Plasma Air PA662 demonstrated an overall capability in reducing aerosolized SARS-CoV-2 Omicron viruses at each time point faster than the natural viability loss rates. After 30 minutes of operation, a 62.401% gross reduction was observed and increased with longer exposure time, as shown by the 99.995% reduction achieved after 90 minutes.

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Study Report

Study Title: EFFICACY OF THE PLASMA AIR PA662 AGAINST AEROSOLIZED SARS-COV-2 OMICRON

Sponsor: WellAir

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

Device Testing: Plasma Air PA662

Study Report Date: 02/10/2022

Experimental State Date: 12/20/2021

Experimental End Date: 01/15/2022

Study Completion Date: 02/09/2022

Study Objective:

The Plasma Air 660 series (PA662) device is a compact ionizing module designed to be integrated into an air movement and management system. WellAir provided the device for testing to evaluate the efficacy against aerosolized SARS-CoV-2 Omicron 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 1.08 x 10^7 TCID50/mL of SARS-CoV-2 Omicron variant 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

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. Before use, the devices were inspected for functionality, and the vacuum system calibration was confirmed using a Gilian Gilibrator-2 NIOSH Primary Standard Air Flow Calibrator. 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# 26338, 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 sample cassette.

Test System Strains: SARS-CoV-2 B.1.1.529, Omicron Variant

The following reagent was obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP20874/2021 (Lineage B.1.1.529; Omicron Variant), NR-56461, contributed by Andrew S. Pekosz.

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

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 0.1 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 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 0.5 mL 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 equipment 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 device. The device was powered on to confirm functionality before testing. Before testing, the Plasma Air PA662 device was powered on and operated for 1 hour in a dry run to verify correct operations. One Holbach IM806v3 meter was used at the center of the room to confirm positive and negative ion generation, as shown in Figure 4. The average ion concentrations measured in the center of the room were 19.36 x 10^3 negative ions/cm^3 and 57.92 x 10^3 positive ions/cm^3. It should be noted that the specified target average ion concentration was 18,000 negative ions/cm^3 for testing purposes.

MANUFACTURER: Plasma Air

MODEL: 660 Series (PA662)

DIMENSIONS: 3.875" x1.5" x1.5"

MAKE: Plasma Air

SERIAL #: N/A

Figure 3. Plasma Air 660 Series (PA662) tested.

Testing Layout:

Testing was conducted in a sealed 20'x8'x8' chamber per Biosafety Level 3 (BSL3) standards. The overall dimensions of the test chamber provided a displacement volume of 1,280 ft^3 (approximately 36,245.56 liters) of air. The chamber remained closed during testing, with no air entering or leaving the room. A nebulizing port connected to a programmable compressor system was in the center of the 20 ft wall protruding 24-inches from the wall. 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. The room was equipped with four probes for air sampling positioned along the room's centerline and located 6 feet off the chamber floor (Figure 7). The device was placed on one side of the test chamber with a small variable-speed fan positioned behind the device to create the necessary airflow to produce the required concentration of 18.0 x 10^3 negative ions/cm^3. A ductwork system with an 8.5-inch round flex duct ran from the variable speed fan from the floor to the ceiling and across to the center of the chamber, as shown in Figure 6. The chamber was visually inspected, pressure tested, and all internal lab systems and equipment were reviewed before testing.

Figure 4. Holbach ionmeter IM806v3 provided by WellAir for ion measurements.

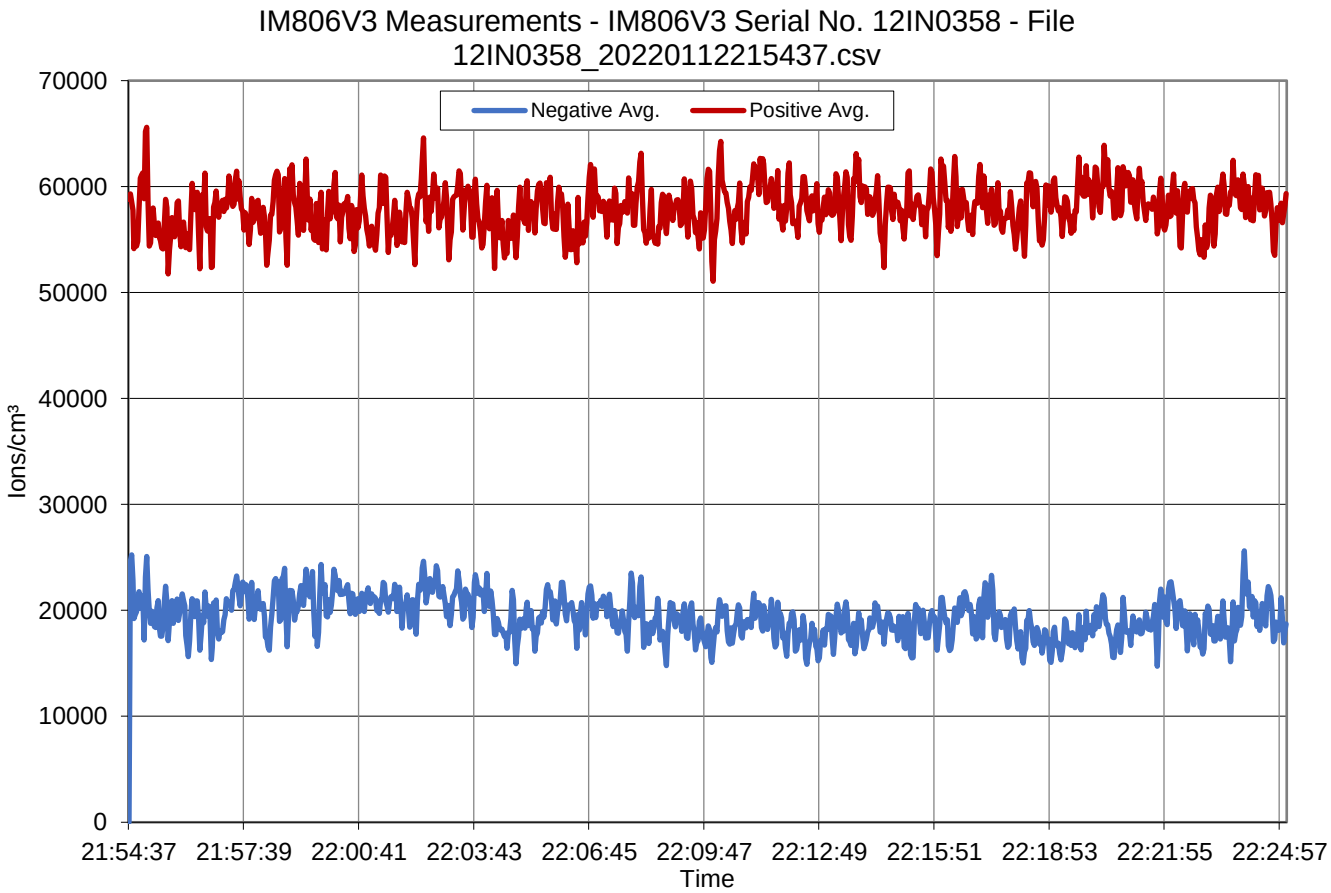

Figure 5. Ion measurements from the Holbach IM806V3 ionometer provided by WellAir. Average ion concentrations were measured at 57.92 x 10^3 positive ions/cm^3 and 19.36 x 10^3 negative ions/cm^3.

Figure 6. Images of the ductwork system set up within the chamber. The fan (in black) connected on the upstream provides air flow that moves the ions through the ducting and up through the diffuser.

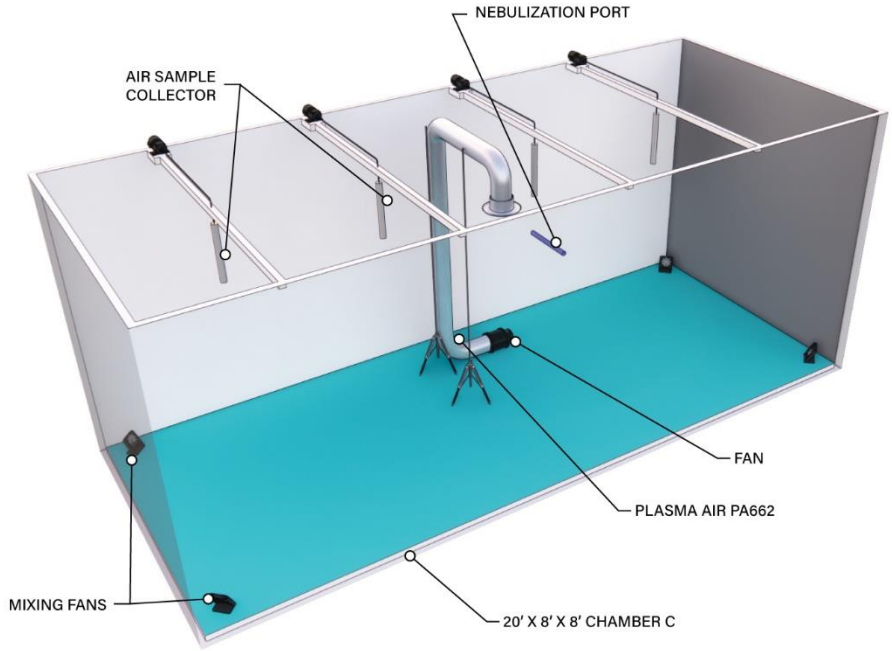

Figure 7. A 3D visual model of the testing chamber for the control and experimental trials.

Control Protocol:

Controls were conducted in duplicate without the device operating in the testing chamber to assess the Plasma Air PA662 accurately. Control samples were collected in the same manner and at the corresponding time points used for the challenge trial to serve as a comparative baseline to assess the viral reduction when the device was operating.

Test Procedures:

Exposure Conditions:

1. The temperature during all test runs was approximately 74 ±2°F (23.3 ±1.1°C) with a relative humidity of 41 ±2%. Before running the viral challenges temperature and humidity were confirmed to be in relative range to the control ±5%
2. Samples were collected after nebulization stopped (T-0) at the following time points with T equal to minutes: T-30, T-60, and T-90.
3. Two 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. 10 mL of 1.08 x 10^7 TCID50/mL SARS-CoV-2 Omicron variant in viral media was nebulized via the dissemination port into the room.
3. After nebulization, the Plasma Air PA662 was turned on via remote.
4. The device was turned off at the pre-determined time points for sample collection.
5. Air sample collections were set to 10-minute continuous draws at the point of sampling, which allowed for approximately 50 liters of air collection per collection port.
6. Sample cassettes were manually removed from the collection system and brought to an adjacent biosafety cabinet for extraction and placement into a viral suspension media.
7. After collection, all samples were sealed 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 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.

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Preparation of The Pathogen

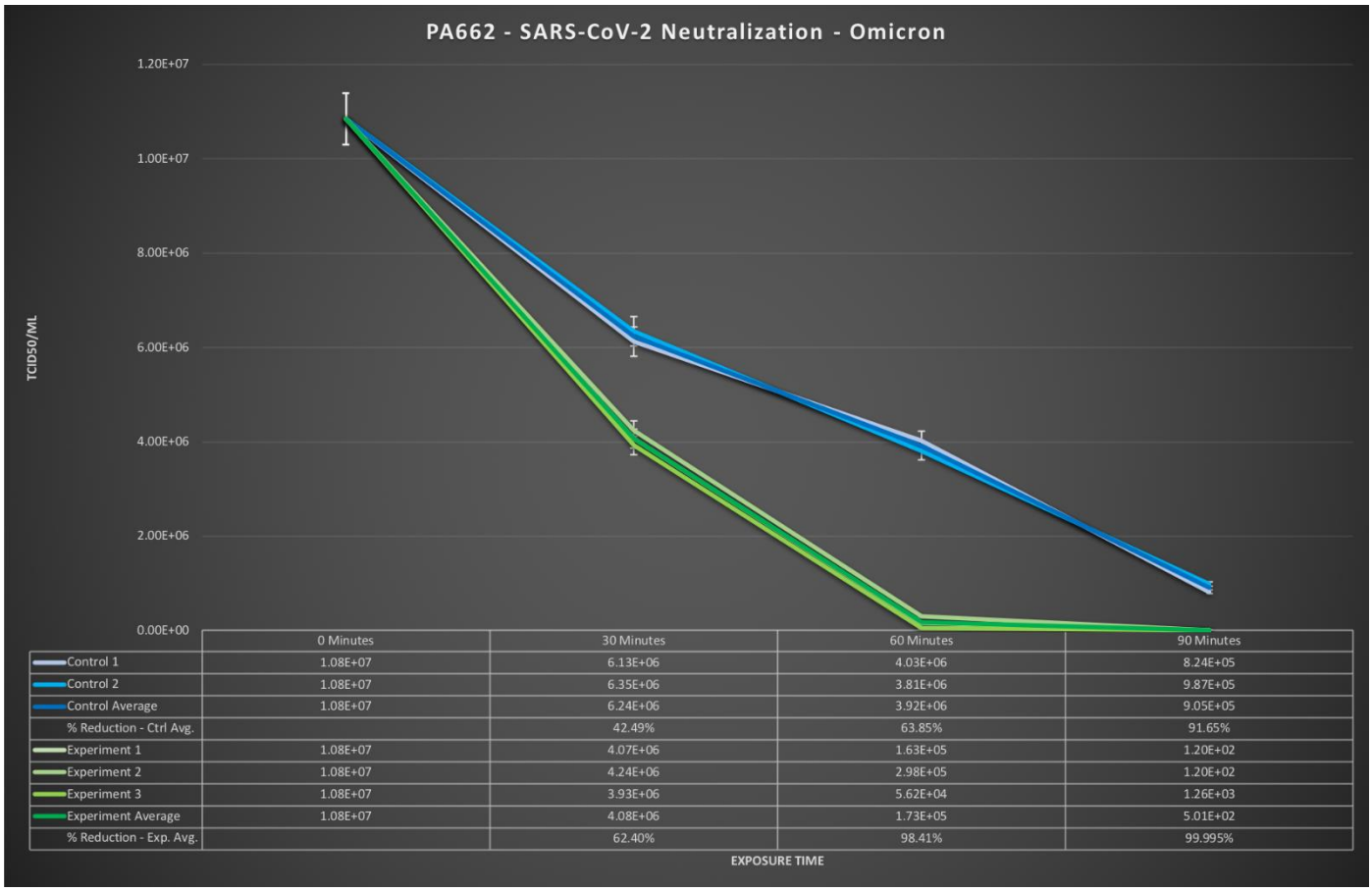
Viral Stock: SARS-CoV-2, Lineage B.1.1.529; Omicron Variant (BEI NR-56461)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Calu-3 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, hCoV-19/USA/MD-HP20874/2021 (GISAID: EPI_ISL_7160424)	99.99% identity with SARS-CoV-2, hCoV-19/USA/MD-HP20874/2021 (GISAID: EPI_ISL_7160424)
Titer by $TCID_{50}$ in Calu-3 Cells by Cytopathic Effect	Report Results	4.4 X 10^5 $TCID_{50}$ per mL^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 represents the titer provided by BEI Resources.

Study Results

The graph below displayed recoverable active SARS-CoV-2 Omicron with and without the Plasma Air PA662 operating. The control showed a natural viability loss of aerosolized SARS-CoV-2 Omicron for 90 minutes within the chamber under controlled conditions. For three trials against SARS-CoV-2 Omicron, an initial concentration of 1.08 x 10^7 TCID50/mL was reduced to 4.07 x 10^6, 4.24 x 10^6, and 3.93 x 10^6 TCID50/mL averaging to 4.08 x 10^6 TCID50/mL at 30 minutes. After 60 minutes of device operation, recoverable SARS-CoV-2 Omicron was reduced to 1.63 x 10^5, 2.98 x 10^5, and 5.62 x 10^4 TCID50/mL averaging to 1.73 x 10^5 TCID50/mL. Aerosolized SARS-CoV-2 Omicron was reduced to 1.20 x 10^2, 1.20 x 10^2, and 1.26 x 10^3 TCID50/mL after 90 minutes of operation, averaging 5.01 x 10^2 TCID50/mL.



	0 Minutes	30 Minutes	60 Minutes	90 Minutes
Control 1	1.08E+07	6.13E+06	4.03E+06	8.24E+05
Control 2	1.08E+07	6.35E+06	3.81E+06	9.87E+05
Control Average	1.08E+07	6.24E+06	3.92E+06	9.05E+05
% Reduction - Ctrl Avg.		42.49%	63.85%	91.65%
Experiment 1	1.08E+07	4.07E+06	1.63E+05	1.20E+02
Experiment 2	1.08E+07	4.24E+06	2.98E+05	1.20E+02
Experiment 3	1.08E+07	3.93E+06	5.62E+04	1.26E+03
Experiment Average	1.08E+07	4.08E+06	1.73E+05	5.01E+02
% Reduction - Exp. Avg.		62.40%	98.41%	99.995%

**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.

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

SARS-COV-2 Omicron Variant Neutralization 18,000 ion/cm3 (TCID50/mL)				
Time (min)	**0**	**30**	**60**	**90**
Control 1	1.08E+07	6.13E+06	4.03E+06	8.24E+05
Control 2	1.08E+07	6.35E+06	3.81E+06	9.87E+05
Test 1	1.08E+07	4.07E+06	1.63E+05	1.20E+02
Test 2	1.08E+07	4.24E+06	2.98E+05	1.20E+02
Test 3	1.08E+07	3.93E+06	5.62E+04	1.26E+03
Control (average)	**1.080E+07**	**6.240E+06**	**3.920E+06**	**9.055E+05**
Test (average)	**1.080E+07**	**4.080E+06**	**1.724E+05**	**5.000E+02**
Net Reduction (average)	**0.000%**	**-34.615%**	**-95.602%**	**-99.945%**

Conclusion

The Plasma Air PA662 demonstrated the ability to reduce aerosolized SARS-CoV-2 Omicron across all time points compared to the natural loss rate observed in the controlled setting. The device achieved a 78.77% overall reduction of active viruses after 30 minutes and reached a 99.995% reduction after 90 minutes of exposure.

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.

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