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EFFICACY OF THE PLASMA AIR AUTOCLEAN 1500 AGAINST AEROSOLIZED SARS-COV-2

PROJECT: PLASMA AIR – AUTOCLEAN 1500 – SARS-COV-2

PRODUCT: AUTOCLEAN 1500

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM (S):

SARS-CoV-2 USA-CA1/2020

Medical Director

Dana Yee, M.D.

Testing Facility

Innovative Bioanalysis, Inc.

3188 Airway Ave Suite D

Costa Mesa CA, 92626

www.InnovativeBioanalysis.com

Email: info@innovativeBioanalysis.com

Laboratory Project Number

1101

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Efficacy Study Summary

Study Title	EFFICACY OF THE PLASMA AIR AUTOCLEAN 1500 AGAINST AEROSOLIZED SARS-COV-2
Laboratory Project #	1101
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 USA-CA1/2020
Description	Per manufacturer, the AutoClean 1500 device, provided by Plasma Air, is a compact ionizing module designed to be integrated into an air movement and management system such as an HVAC duct system, air conditioner, and humidifier, to name a few. This study sought to determine device's efficacy against aerosolized SARS-CoV-2 USA-CA1/2020.
Test Conditions	Testing was conducted in a 20'x8'x8' chamber following BSL-3 standards. The temperature during testing was approximately 72 ±2°F (22.2 ±1.1°C), with a relative humidity of 37%. A 7.01 x 10^6 TCID50/mL of SARS-CoV-2 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	In the test setup the device decreased a starting concentration of SARS-CoV-2 from 7.01 x 10^6 TCID50/mL to an average 1.49 x 10^6 TCID50/mL after 30 minutes. At 60 minutes reduced collectible SARS-CoV-2 to an average of 2.50 x 10^5 TCID50/mL and neutralized active pathogen to below levels of assay quantification (1.20 x 10^2 TCID50/mL) after 90 minutes.
Control Results	A control test was conducted without the device, 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 gross viral reduction.
Conclusion	The AutoClean 1500 ionizing module demonstrated an overall capability in reducing aerosolized SARS-CoV-2 viruses at each time point faster than the natural viability loss rates. After 30 minutes of operation, a 78.77% gross reduction was observed and increased with longer exposure time, as shown by the 99.998% gross reduction achieved after 90 minutes.

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Study Report

Study Title: EFFICACY OF THE PLASMA AIR AUTOCLEAN 1500 AGAINST AEROSOLIZED SARS-COV-2

Sponsor: WellAir and Plasma Air

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

Device Testing: AutoClean 1500

Study Report Date: 01/05/2022
Experimental State Date: 07/02/2021
Experimental End Date: 11/31/2021
Study Completion Date: 12/27/2021

Study Objective:

WellAir/Plasma Air supplied the AutoClean 1500 for testing purposes to determine efficacy against viral pathogens. This study evaluated the effectiveness of the AutoClean 1500 in its ability to reduce the viral strain referred to as SARS-CoV-2 within the air.

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 7.01 x 10^6 TCID50/mL of SARS-CoV-2 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 USA-CA1/2020

The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-CA1/2020, NR-52382.

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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 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, ion generation from the device were taken from the opposite side of the room using the Holbach Ionometer IM806 V3 (S/N: 12IN0358, Fig. 4) provided by WellAir/Plasma Air. The PlasmaPURE AutoClean 1500 (Fig. 3) generated an average 19,000 negative ions/cm^3 and 18,000 positive ions/cm^3. To set the target ion concentration, the values of the negative ion readings from this ion meter were used.

MANUFACTURER: Plasma Air

MODEL: AutoClean 1500

DIMENSIONS: 5" x 5" x 3"

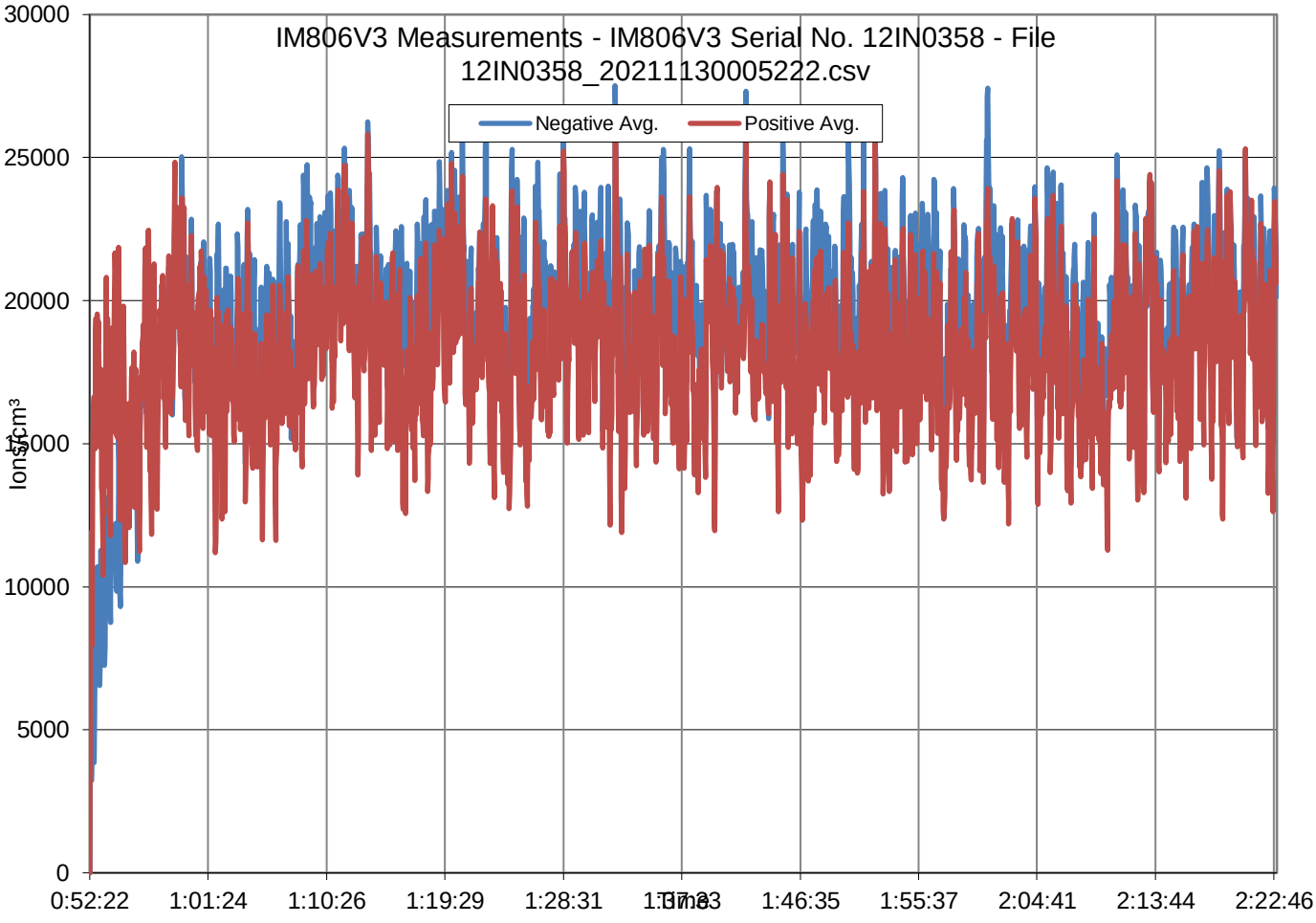
MAKE: PlasmaPURE

Figure 3. AutoClean 1500 Series (1500) as 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 located 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. 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 ions. 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. The chamber was visually inspected, pressure tested, and all internal lab systems and equipment were reviewed before testing.

Figure 4. Holbach Ionometer IM806V3 device provided by WellAir/Plasma Air for ion measurements.



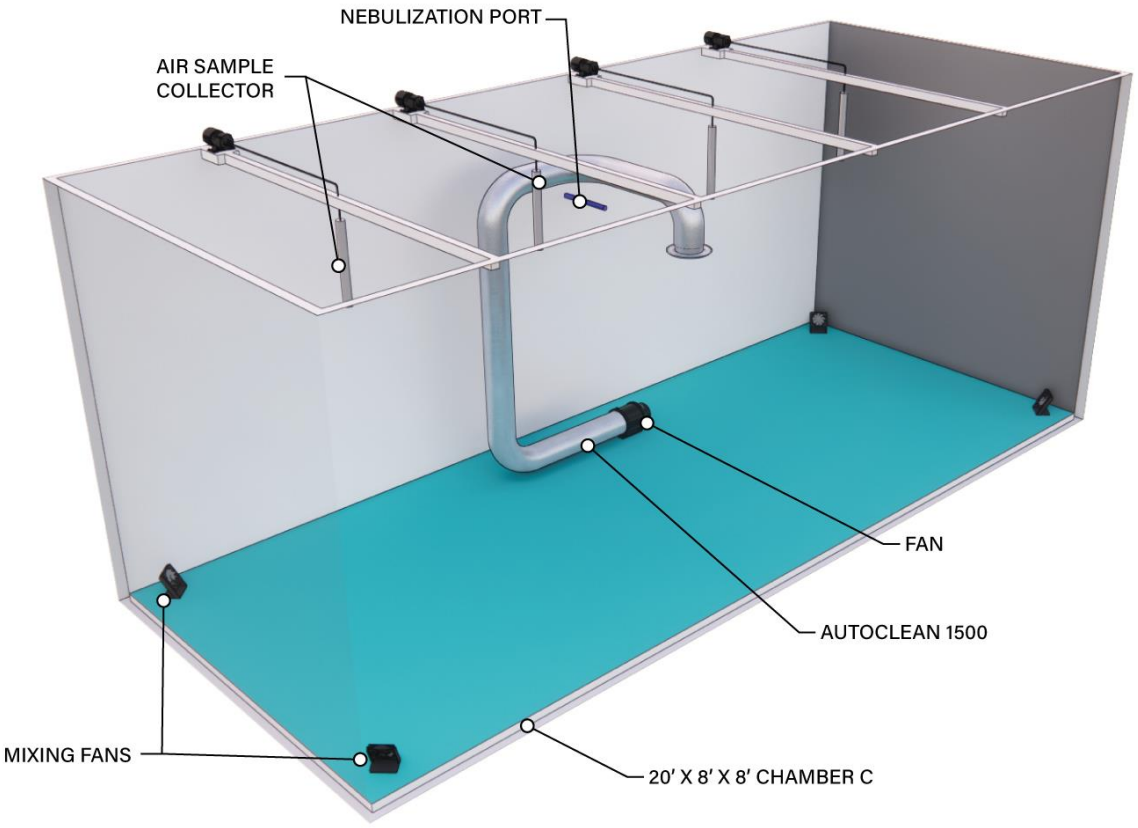


Figure 5. Testing layout for control and experimental trials.

Control Protocol:

Controls were conducted in duplicate without the device operating in the testing chamber to accurately assess the AutoClean 1500. 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 Method:

Exposure Conditions:

1. The temperature during all test runs was approximately 72 ±2°F (22.22 ±1.1°C) with a relative humidity of 37%.
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 experimental time point run, the testing area was reset, decontaminated, and prepped per internal procedures.
2. 10 mL of 7.01 x 10^6 TCID50/mL SARS-CoV-2 in viral media was nebulized via the dissemination port into the room.
3. After nebulization, the AutoClean 1500 ionizer was turned on via remote.
4. The device was turned off at each pre-determined time point for air sample collection.
5. Air sample collections began after the device was turned off and set to 10-minute continuous draws from the time of sampling.
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.
8. Each time point and control were completed as separate tests and followed procedures 1 through 8 for each one.

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 USA-CA1/2020 (BEI NR-52382)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero 6 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, isolate USA-CA1/2020 GenBank: MN994467.1	99.9% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1
Approx. 940 Nucleotides	≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1	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	2.8 X 10^5 TCID50 per mL in 5 days at 37°C and 5% CO_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 is representative of the titer provided by BEI Resources. These viruses are grown on VeroE6 cells either in-house or at a partner lab to the concentrations listed within the experiment design.

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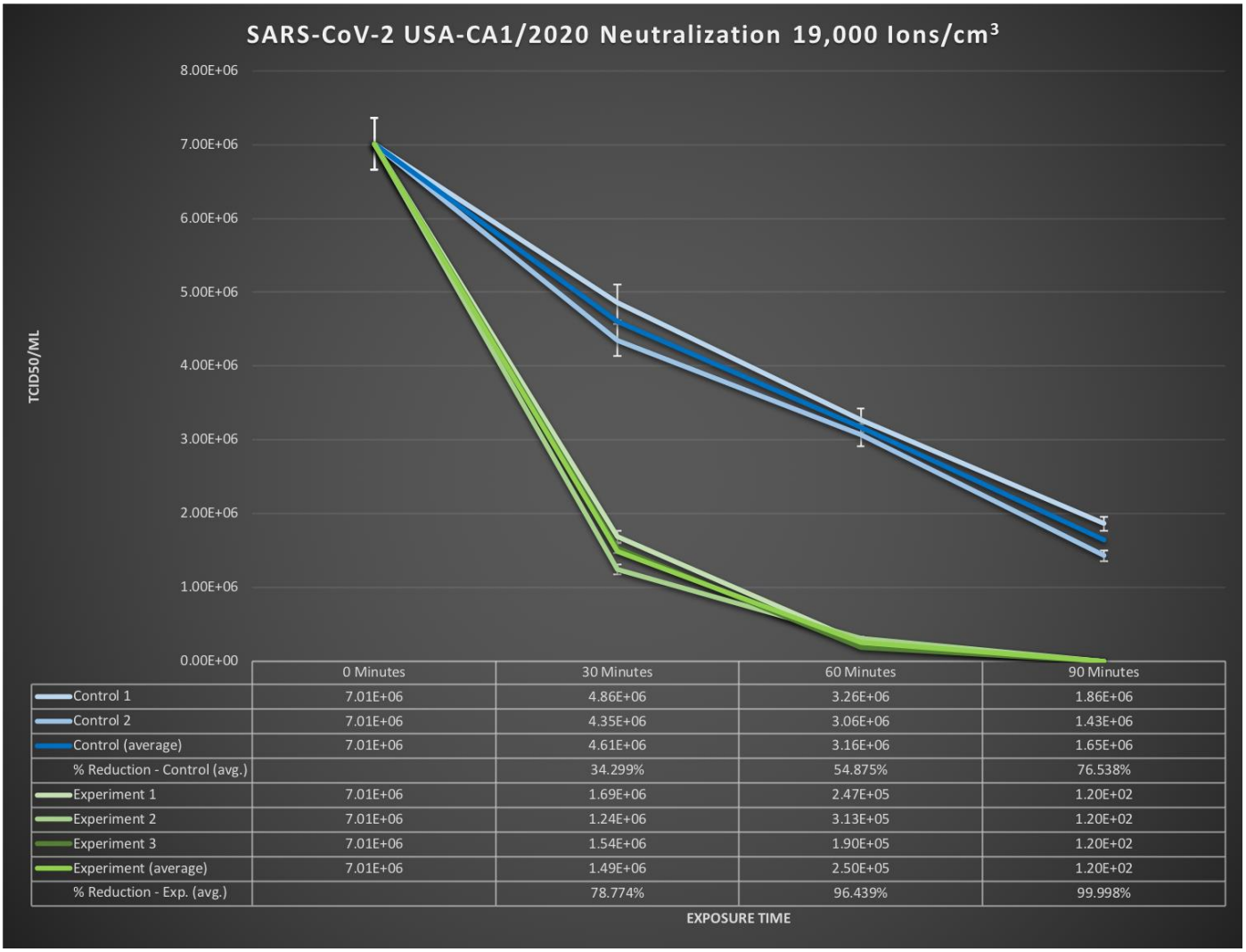
Study Results

The below graph displayed recoverable active SARS-CoV-2 with and without the AutoClean 1500 module operating. The control showed a natural viability loss of aerosolized SARS-CoV-2 for 90 minutes within the chamber under controlled conditions. For the three trials against SARS-CoV-2, an initial concentration of 7.01 x 10^6 TCID50/mL was reduced to 1.69 x 10^6, 1.24 x 10^6, and 1.54 x 10^6 TCID50/mL averaging to 1.49 x 10^6 TCID50/mL at 30 minutes. After 60 minutes of device operation, recoverable SARS-CoV-2 reduced to 2.47 x 10^5, 3.13 x 10^5, and 1.90 x 10^5 TCID50/mL averaging to 2.50 x 10^5 TCID50/mL. Aerosolized SARS-CoV-2 was reduced to below levels of quantitation, as represented by the value 1.20 x 10^2 TCID50/mL after 90 minutes of operation.



	0 Minutes	30 Minutes	60 Minutes	90 Minutes
Control 1	7.01E+06	4.86E+06	3.26E+06	1.86E+06
Control 2	7.01E+06	4.35E+06	3.06E+06	1.43E+06
Control (average)	7.01E+06	4.61E+06	3.16E+06	1.65E+06
% Reduction - Control (avg.)		34.299%	54.875%	76.538%
Experiment 1	7.01E+06	1.69E+06	2.47E+05	1.20E+02
Experiment 2	7.01E+06	1.24E+06	3.13E+05	1.20E+02
Experiment 3	7.01E+06	1.54E+06	1.90E+05	1.20E+02
Experiment (average)	7.01E+06	1.49E+06	2.50E+05	1.20E+02
% Reduction - Exp. (avg.)		78.774%	96.439%	99.998%

**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 USA-CA1/2020 Neutralization 19,000 ions/cm^3 (TCID50/mL)				
Time (min)	**0**	**30**	**60**	**90**
Control 1	7.01E+06	4.86E+06	3.26E+06	1.86E+06
Control 2	7.01E+06	4.35E+06	3.06E+06	1.43E+06
Test 1	7.01E+06	1.69E+06	2.47E+05	1.20E+02
Test 2	7.01E+06	1.24E+06	3.13E+05	1.20E+02
Test 3	7.01E+06	1.54E+06	1.90E+05	1.20E+02
Control (average)	**7.010E+06**	**4.605E+06**	**3.160E+06**	**1.645E+06**
Test (average)	**7.010E+06**	**1.490E+06**	**2.500E+05**	**1.200E+02**
Net Reduction (average)	**0.000%**	**-67.644%**	**-92.089%**	**-99.993%**

Conclusion

The AutoClean 1500 demonstrated the ability to reduce aerosolized SARS-CoV-2 USA-CA1/2020 across all time points compared to the natural loss rate observed in the controlled setting. The device achieved a gross 78.77% reduction of active viruses after 30 minutes and reached a gross 99.998% reduction after 90 minutes of exposure.

The study focused on the impact the ionizer would have on a specific volume of space. Therefore, when applied to a different sized room, the results will scale and vary due to variables present, such as room size, occupancy rating, air movement, and more. Every effort was made to simulate a real-life situation and address constraints with the experimental design and execution while taking the proper precautions when working with a BSL-3 pathogen. These efforts are reflected in the meaningful recovery of the virus in the control test.

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DocuSigned by:
Dana Yee
7D5A69A0907947B...

2/1/2022

Dana Yee M.D **Date**

Clinical Pathologist and Medical Director, Innovative Bioanalysis, Inc.

DocuSigned by:
Sam Kabbani
8B4B282DF4B34A3...

2/1/2022

Sam Kabbani, MS, BS, MT(ASCP), CLS **Date**

Chief Scientific Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Albert Brockman
06DF5C77A0D2400...

2/1/2022

Albert Brockman **Date**

Chief Biosafety Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Kevin Noble
5DF2797BAA78421...

1/27/2022

Kevin Noble **Date**

Laboratory Director, Innovative Bioanalysis, Inc.

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