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AURA TECHNOLOGIES ION BAR EFFICACY IN SARS-COV-2 DEACTIVATION

PROJECT: AURA TECHNOLOGIES – ION BAR – SARS-COV-2

PRODUCT: ION BAR

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM(S):

SARS-CoV-2 USA-CA1/2020

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Laboratory Project Number

1140

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Efficacy Study Summary

Study Title	AURA TECHNOLOGIES ION BAR EFFICACY IN SARS-COV-2 DEACTIVATION
Laboratory Project #	1140
Guideline	Custom design based on ISO principles as no international standards exist.
Testing Facility	Innovative Bioanalysis, Inc.
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations per the Clinical Laboratory Improvement Amendments of 1988 (CLIA) Regulations Standards and Certification: Laboratory Requirements (42 CFR 493) and the College of American Pathologists (CAP) All Common Checklist.
Test Substance	SARS-CoV-2 USA-CA1/2020
Description	The AURA Technologies Ion Bar is an overhead airflow curtain system with an integrated negative ion generator. The Ion Bar is typically mounted above a doorway or portal, creating a high-negative-air-ion germicidal partition between the air masses on each side of the threshold. The germicidal partition is intended to eliminate or significantly reduce the transfer of airborne particles between the air masses and to kill or inactivate pathogens in the air and on surfaces proximate to the doorway or portal. This in-vitro study consists of aerosol and surface testing to determine the efficacy of the AURA Tech Ion Bar against a known pathogen, SARS-CoV-2.
Test Conditions	Testing was conducted in a sealed 20 ft × 8 ft × 8 ft chamber that complied with Biological Safety Level 3 (BSL-3) standards per NIH guidelines. The temperature was maintained at 77.2 °F ± 2 °F (25 °C ± 1 °C), with a relative humidity of 49%. A simulated wall with a doorway/portal was constructed such that the chamber was partitioned into two 10 ft × 8 ft × 8 ft sections. The Ion Bar was mounted above the doorway, approximately 7 ft above the floor, on the "clean" side of the door. A 6.32×10^6 TCID50/mL of SARS-CoV-2 fetal bovine serum (FBS) based viral media was inoculated onto glass slides as controls and challenge samples for surface testing. The identical concentration of SARS-CoV-2 was nebulized for aerosol testing. Surface samples were placed on the floor approximately 2 ft from the doorway (on the clean side) and exposed for 10, 20, and 30 minutes. For aerosol testing, nebulization occurred 5 ft above the floor, 1.5 ft away from the doorway, and pointed towards the door while air samples were collected at the same distance from the clean side for 2 minutes.

Test Results	During surface testing, SARS-CoV-2 was reduced to below quantification limits (≥ 4.57 log reduction) after 30 minutes of operation. With aerosol testing, the germicidal barrier blocked an average of 99.98% (3.6 log) of active SARS-CoV-2 from entering during device operation, which was quantified at approximately 1.16×10^3 TCID50/mL.
Control Results	A control was conducted for each subtest with the device fan and ionizer off. Samples taken at the corresponding time points used for each challenge. The results displayed a natural viability loss and served as a comparative baseline to calculate viral reduction and evaluate device efficacy.
Conclusion	AURA Technologies Ion Bar demonstrated significant efficacy as a germicidal partition, reducing the transfer of SARS-CoV-2 aerosols by 99.98% and neutralizing surface SARS-CoV-2 to below quantification limits (≥ 99.998%) after 30 minutes of exposure.

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Study Report

Study Title: AURA TECHNOLOGIES ION BAR EFFICACY IN SARS-COV-2 DEACTIVATION

Sponsor: AURA Technologies, LLC

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

Device Testing: Ion Bar

Study Report Date: 11/10/2021
Experimental Start Date: 08/26/2021
Experimental End Date: 10/09/2021
Study Completion Date: 11/01/2021

Study Objective:

AURA Technologies supplied the Ion Bar for testing purposes to determine efficacy against viral pathogens. This study evaluated the effectiveness of the Ion Bar in its ability to reduce the viral strain referred to as SARS-CoV-2 USA-CA1/2020.

Test Method:

Part 1: Surface Testing

Surface Inoculation:

12 glass slides were equally subjected to a 1 mL inoculation of SARS-CoV-2 containing a known titer of 6.32×10^6 TCID50/mL for the control and viral challenge. The viral solution was spread onto a sterile AmScope glass slide (ASIN: B00T5OM5C) measuring 3 in × 1.5 in and 0.125 in thick. A clean spatula was used to ensure even distribution and saturation of all materials and left to air dry for 5 minutes.

Surface Sampling:

Swabs were moistened with viral media solution before collecting samples to maximize collection. After swabbed, each slide was subjected to a 1 mL rinse in viral suspension media and swabbed for any residual pathogen material to optimize recovery. The swab was placed into a universal transport medium vial, Lot# 200929, manufactured by Puritan Medical Products. After collection, the swab and media were vortexed for one full minute.

Part 2: Aerosol Testing

Bioaerosol Generation:

The CH Technologies BLAM 4 jet nebulizer was filled with 6.32×10^6 TCID50/mL SARS-CoV-2 in viral media and nebulized at a flow rate of 1 mL/min with untreated local atmospheric air. Prior to testing, the nebulizer was checked for proper functionality by nebulizing the FBS solution without the test virus present to confirm average particle size distribution (approximately 0.1-5 um with the average around 0.8 um). 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.

Bioaerosol Sampling:

This study used four probes connected to calibrated Gilian 10i vacuum devices set at a standard flow of 5.00 L/min with a 0.20% tolerance and were inspected for functionality before being used. Air sample volume collections were confirmed with a Gilian Gilibrator 2 SN-200700-12 and a high flow bubble generator SN-2009012-H with calibrations performed in September 2020. 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 time point. The 37 mm cassettes and filtration disc, Lot # 26338, used for testing was manufactured by Zefon International Inc. The delicate internal filtration disc used to collect viral samples was moistened with a viral suspension media to aid in the collection.

Test System Strains: SARS-CoV-2 USA/CA-1/2020

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.

TCID50 Procedure:

Materials and Equipment:

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips—20 µL, 200 µL, 1000 µL
- 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 7.5% fetal bovine serum, 4 mmol 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 1.8 mL.
4. Vortex the viral samples, then transfer 20 µL of the virus to the first tube, vortex, discard tip.
5. With a new tip, serial dilute subsequent tips transferring 200 µL.

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 by 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. Two Alpha Lab AIC2 ion polarity meters confirmed negative ion concentrations between 600 k to 700 k negative ions/cm^3 at 2 ft from the doorway on the clean side.

MANUFACTURER: AURA Technologies, LLC.

MODEL: Ion Bar

MAKE: AURA Blue

SERIAL #: NA

Testing Layout:

All testing was conducted in a sealed 20 ft × 8 ft × 8 ft chamber following BSL-3 standards. The room had a displacement volume of 1,280 ft^3 (36,245.56 L) of air. The chamber remained closed to prevent any air from entering and leaving the room during testing.

A nebulizing port connected to a programmable compressor system was set 5 ft off the floor and 1.5 ft from the simulated door opening during aerosol testing; air samples were collected 5 ft off the floor and 1.5 ft inside the simulated doorway. The Ion Bar was positioned to replicate the device being mounted above a door, clear of any obstructions.

The same testing chamber was used for surface and aerosol testing with slight modifications to accommodate sampling. Glass slides were placed on the floor 2 ft from the door's opening.

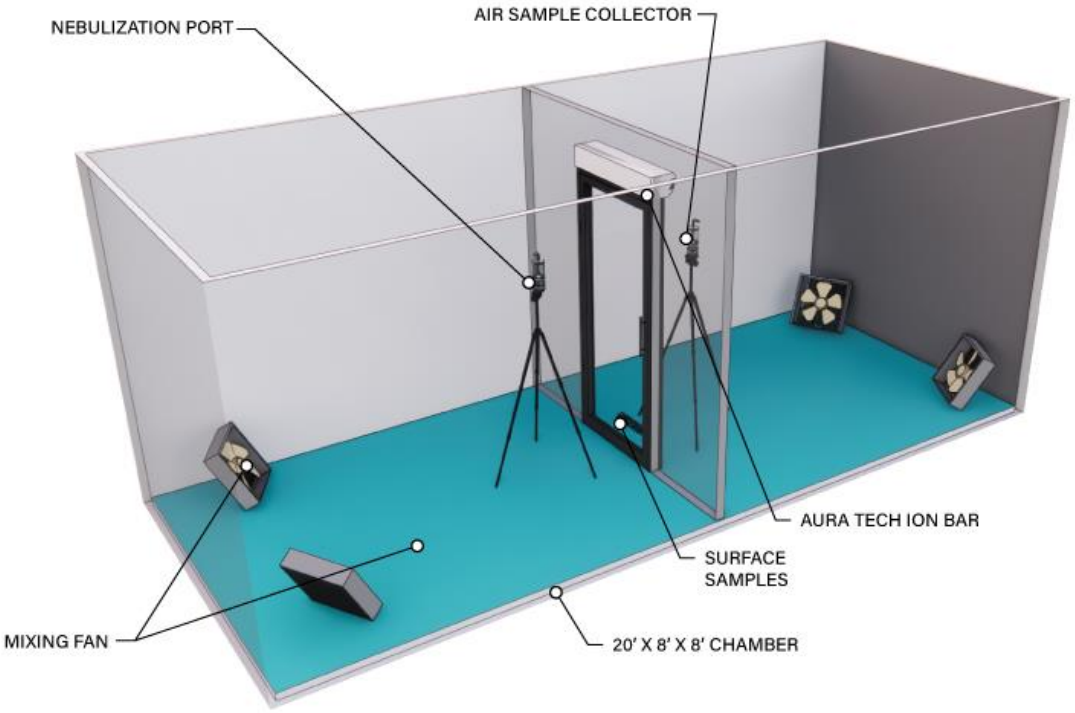


Figure 1. Testing layout for control and experimental trials.

Figure 2. Testing setup.

Figure 3. Visual light on Ion Bar indicating fan and ionizer on.

Test Method:

General Testing Conditions:

1. Before the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. The Ion Bar ran for the duration of testing unless stated otherwise.
3. The temperature during all test runs was approximately 77.2 °F ± 2 °F with a relative humidity of 49%.

Part 1: Surface Testing

Exposure Conditions:

1. Inoculated glass slides were placed under the device 2 ft from the simulated door opening on the "clean" side.
2. Surface test samples were taken at 10, 20, and 30 minutes of ionized air exposure.

Experimental Procedures:

1. Sterile glass slides were inoculated with 6.32×10^6 TCID50/mL viral media and labeled with a pre-determined time point
2. Sample slides were centered under the device 2 ft from the simulated doorway.
3. The slide was removed at the corresponding time points to be swabbed and rinsed with viral suspension media.
4. After collection, all swabs 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, a 30-minute air purge was performed. All test equipment was cleaned with a 70% isopropyl alcohol solution at the end of each day. The Ion Bar system had no direct contact with the pathogen and was not decontaminated after testing.

Part 2: Aerosol Testing

Exposure Conditions:

1. Test sample collection occurred between 0-2 minutes and was conducted in triplicate.
2. Nebulization of viral pathogen occurred at the height of 5 ft and distance of 1.5 ft outside the simulated door opening.
3. Collection occurred 5 ft off the floor and 1.5 ft from the simulated doorway on the chamber's inside (clean) side.

Experimental Procedures:

1. 10 mL of 6.32×10^6 TCID50/mL SARS-CoV-2 viral media was nebulized from 0-2 minutes to distribute viral stock into the room.
2. Air samples were taken for 2 minutes with the Gilian 10i programmable vacuum devices.
3. The sample cassettes were manually removed from the collection system after each run.
4. Upon cassette removal after each run, cassette sets were taken to an adjacent biosafety cabinet for extraction and placement into viral suspension media.
5. 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 with a 70% isopropyl alcohol solution at the end of each day. 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.

Control Protocol:

A control was conducted for aerosol and surface testing with the fan and ionizer off to assess the AURA Technologies Ion Bar accurately. Control samples were taken in the same manner and at the corresponding time points used for the challenge trials to serve as a comparative baseline to assess the viral reduction when the device was operating.

Preparation of The Pathogen

Viral Stock: SARS-CoV-2 USA-CA1/2020 (BEI NR-52382)

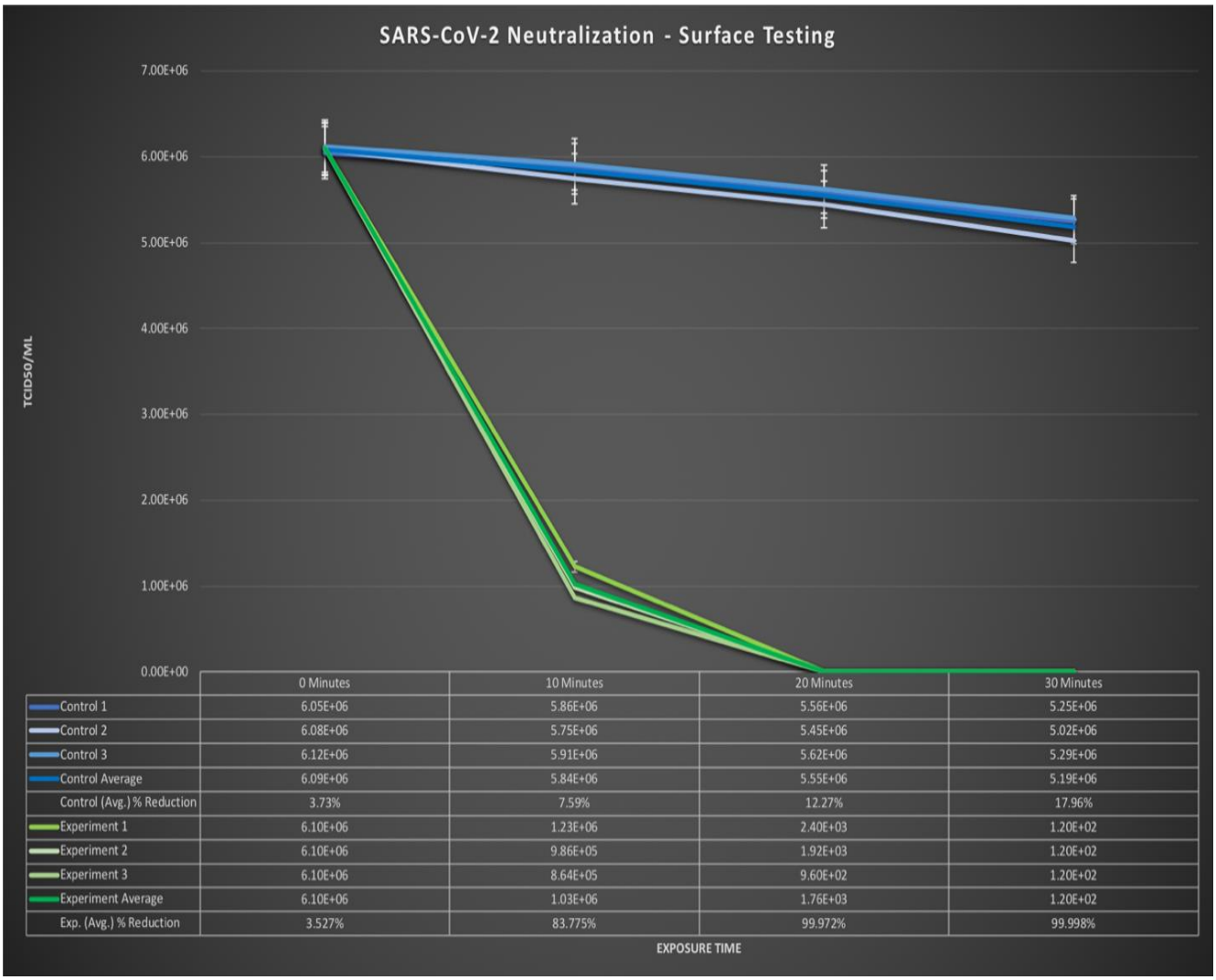
Test	Specifications	Results
Identification by Infectivity in Vero 6 cells	Cell Rounding and Detachment	Cell Rounding and Detachment
Next-Generation Sequencing (NGS) of the complete genome using Illumina® iSeq 100 Platform	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1	99.9% 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	2.8×10^5 TCID50/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.

Study Results

Part 1: Surface Testing

Surface testing was conducted to determine the time needed to inactivate pathogens. Controls were plotted to show natural viability loss with the device (fan off, ion off) not operating in the chamber. Against SARS-CoV-2, the Ion Bar lowered a starting concentration of 6.32×10^6 TCID50/mL to (1.23×10^6, 9.86×10^5, and 8.64×10^5) TCID50/mL averaging to approximately 1.03×10^6 TCID50/mL (83.775%) after 10 minutes. After 20 minutes, the concentration of active SARS-CoV-2 collected was 1.76×10^3 TCID50/mL (99.972%) and was reduced to below the assay quantitation limit after 30 minutes (≥99.998%).



	0 Minutes	10 Minutes	20 Minutes	30 Minutes
Control 1	6.05E+06	5.86E+06	5.56E+06	5.25E+06
Control 2	6.08E+06	5.75E+06	5.45E+06	5.02E+06
Control 3	6.12E+06	5.91E+06	5.62E+06	5.29E+06
Control Average	6.09E+06	5.84E+06	5.55E+06	5.19E+06
Control (Avg.) % Reduction	3.73%	7.59%	12.27%	17.96%
Experiment 1	6.10E+06	1.23E+06	2.40E+03	1.20E+02
Experiment 2	6.10E+06	9.86E+05	1.92E+03	1.20E+02
Experiment 3	6.10E+06	8.64E+05	9.60E+02	1.20E+02
Experiment Average	6.10E+06	1.03E+06	1.76E+03	1.20E+02
Exp. (Avg.) % Reduction	3.527%	83.775%	99.972%	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. The percentage error equates to an average of ±5% of the final concentration.

Part 2: Aerosol Testing

Part 2 was conducted to determine any pass-through of aerosolized SARS-CoV-2 through the Ion Bar air curtain within 2 minutes of device operation. From the inside of the air barrier, the concentration of active SARS-CoV-2 collected at 2 minutes from three test runs were (1.20×10^2, 2.40×10^3, and $9.60 \times 10^{2)}$ TCID50/mL, averaging to approximately 1.16×10^3 TCID50/mL. The presence of SARS-CoV-2 indicated about 0.018% of nebulized SARS-CoV-2 passed through the barrier after 2 minutes resulting in a 99.982% reduction of nebulized SARS-CoV-2 on the protected side. The control observed a 71.07% pass-through of nebulized SARS-CoV-2 after 2 minutes with the Ion Bar fan and ionizer off.

	Starting Concentraiton	Final Concentration
Control 1	6.32E+06	4.26E+06
Control 2	6.32E+06	4.47E+06
Control 3	6.32E+06	4.87E+06
Control Average	6.32E+06	4.53E+06
Control (Avg.) % collected		71.707
Experiment 1	6.32E+06	1.20E+02
Experiment 2	6.32E+06	2.40E+03
Experiment 3	6.32E+06	9.60E+02
Experiment Average	6.32E+06	1.16E+03
Exp (Avg.) % collected		0.018

**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. The percentage error equates to an average of ±5% of the final concentration.

Conclusion

The AURA Technologies Ion Bar demonstrated a significant ability to neutralize surface SARS-CoV-2 USA-CA1/2020 and prevent the pathogen from entering the chamber with the test unit running. Aerosolized SARS-CoV-2 transfer was significantly restricted through the Ion Bar germicidal partition with a 0.018% of collected SARS-CoV-2 after 2 minutes from a starting concentration of 6.32×10^6 TCID50/mL; equivalently, 99.98% (3.6 log) of active pathogen was blocked by the air barrier. The Ion Bar reduced surface SARS-CoV-2 by 83.77% after 10 minutes, 99.972% after 20 minutes, and 99.998% (4.5 log) after 30 minutes. Overall, the Ion Bar utilized a combination of methods (laminar air curtain and negative ion generation) to reduce the transfer of pathogens through a doorway/portal if installed correctly.

It should be noted that testing was designed to observe the ability of Ion Bar (controlled airflow and ionizing function ability) to reduce potential exposure to a pathogen. The study focused on the Ion Bar being mounted above a doorway without obstructions. Air moves differently in all spaces, and human interactions within the environment can change subtle airflow movements. Every effort was made to simulate a real-life situation and address constraints with experimental design and execution while taking the proper precautions when working with a BSL-3 pathogen.

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