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EFFICACY OF AIRGLE AG300 AGAINST SARS-CoV-2 USA-CA1/2020 PATHOGEN

PROJECT: BIOAEROSOL EFFICACY

PRODUCT: AIRGLE AG300 AIR PURIFIER

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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Medical Director

Study Completion Date:

6/2/2021

Testing Facility

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Laboratory Project Number

1041

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Efficacy Study Summary

Study Title	EFFICACY OF AIRGLE AG300 AGAINST SARS-CoV-2 USA-CA1/2020 PATHOGEN
Laboratory Project #	1041
Guideline:	Modified ISO standards as no international standards exist.
Testing Facility	Innovative Bioanalysis, Inc.
Study Dates:	
Study Initiation Date:	04/26/2021
Study Completion Date:	06/02/2021
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	SARS-CoV-2 USA-CA1/2020
Description	The Airgle AG300 system is a commercially available mobile air purifier designed to be placed free standing in a room to decrease concentration of pathogens in the air when operating. An in vitro study to determine the efficacy of the Airgle AG300 air purifier against the known pathogen SARS-CoV-2.
Test Conditions	The test was conducted in a large, sealed environment that complied to BSL-3 standards and inspected for any leaks prior to usage. The temperature during all test runs was approximately 76°F ±2°F, with a relative humidity of 42%. Air samplers were calibrated by the manufacturer on September 3, 2020 and set at a standard flow of 5.02L/min. Calibration records indicate a 0.20% tolerance. The nebulizer was filled with the same amount of viral stock (6.32×10^6 TCID50 per mL) in FBS-based viral media and nebulized at a constant rate while four mixing fans were running simultaneously to ensure homogenous air.
Test Results	When tested against SARS-CoV-2 USA-CA1/2020, the Airgle AG300 air purifier showed a significant reduction of active pathogens, resulting in a reduction below 1.20×10^2 TCID50 per mL after one hour of operation.
Control Results	Two control tests were conducted without the air purifying unit in the test chamber and samples were taken at the corresponding sample times used for the challenge trial. Results show a linear decline of active pathogens over time.
Conclusion	The Airgle AG300 air purification device performed to manufacturer's specifications and demonstrated a reduction to below testing equipment sensitivity of greater than a 99.998% reduction of recoverable active SARS-CoV-2 virus in the air after 60 minutes of operation. There was no cytopathic effect in the lowest concentration of vero e6 cells, indicating a SARS-CoV-2 virus concentration lower than the limit of detection after a 21-day incubation.

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Study Report

Study Title: SARS-CoV-2 USA-CA1/2020 PATHOGEN AIR PASS ON THE AIRGLE AG300

Sponsor: Airgle Corporation

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

Device Testing: Testing the efficacy of the Airgle AG300 system against a known pathogen, SARS-CoV-2

Study Report Date: 06/11/2021
Experimental Start Date: 05/19/2021
Experimental End Date: 05/19/2021
Study Completion Date: 06/02/2021

Study Objective:

This in vitro study was designed to determine the efficacy of the Airgle AG300 air purifier against the airborne transmission of the known pathogen, SARS-CoV-2.

Test Method:

Bioaerosol Generation:

For the control and the viral challenges, the nebulizer was filled with the same amount of viral stock (6.32×10^6 TCID50 per mL*) of SARS-CoV USA-CA1/2020 and nebulized at a flow rate of 1mL/min. The nebulizer was driven by untreated local atmospheric air. After each completion, the nebulizer's remaining viral stock volume was weighed to confirm that the same amount of viral stock was nebulized. Control samples were performed in the same manner as the viral test at the timepoints and rate of collection.

Bioaerosol Sampling:

For air sampling, two different Gilian 10i programmable vacuum devices were used. Air samplers were calibrated by the manufacturer in September 2020, and the certificates were inspected prior to use. Air sample volume collections were confirmed prior to use with a Gilian Gilibrator 2, SN-200700-12 and a high flow bubble generator SN-2009012-H. The air samplers were operated in conjunction with removable sealed cassettes, which were manually removed after each sampling time point. Cassettes had a delicate internal filtration disc to collect viral samples which were coated with a viral suspension media to aid collections.

Aerosolization of Viral Media:

Control samples were performed in the same manner as the viral test at the timepoints and rate of collection. A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 6.32×10^6 TCDI50/mL* was used for this experiment.

Test System Strains: SARS-CoV-2

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.

Study Materials and Equipment:

Equipment Overview: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. All filtration systems were installed prior to arrival at the laboratory. The device was powered on to confirm functionality prior to testing.

MANUFACTURER: AIRGLE CORPORATION

MODEL: AG300

SIZE: H13.3in X W11.7in X D9.7in

MAKE: AIRGLE

SERIAL #: N/A

Equipment Specifics: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. The system has an internal fan system with a cHEPA and activated carbon 2-in-1 filters, and a UV-C photocatalytic oxidization system.

Testing Chamber: The test was conducted inside a large, sealed chamber with active monitoring of testing conditions via calibrated wireless devices and air sampling sensor. At each corner of the chamber, low volume mixing fans were positioned to ensure homogenous, bioaerosol concentrations. The testing chamber was set up to allow all air to be exhausted after the test samples had been taken. After samplers were taken air was treated with vaporized mixture and exposed to UV-C prior to being exhausted through a dual HEPA filtration system.

Design Layout:

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 any potential release of testing media into the atmosphere. The testing chamber was equipped with 4 sealed viewing windows and lockable chamber door for entry and exit. The overall dimensions of the test chamber were approximately 10'x8'x8' with a displacement volume of 640 cubic feet. Based on cubic foot volume the chamber has 18,122.8 liters of air.

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. For air sample testing, the chamber was equipped with 2 probes that were along the centerline of the room and protruded down from the ceiling 24". Each probe tube was connected to a Gilian 10i programmable system with sampling cassettes from lot #23166 made by Zefon International. A single bioaerosol nebulizing port was in the center of the 10' wall opposite of the entry doors. The dissemination port protruded from the wall 24' and was connected to a programmable compressor nebulizer system.

An active air sampling sensor was used to confirm operations of the equipment and O_3 measurements were taken only for verification that the system was operating. Test scenario captures O_3 data, but the conditions are not designed to be compared to EPA requirements and cannot be used for O_3 claims as the sensors and test parameters are not designed to meet O_3 certification requirements.

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.

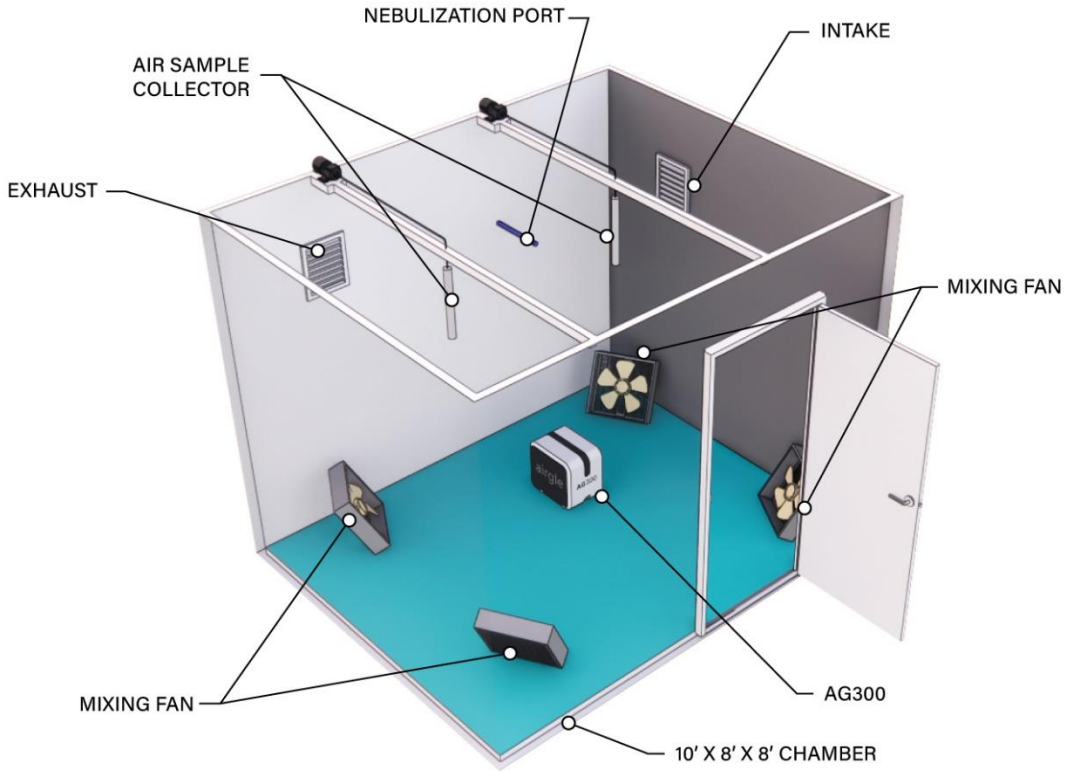


Figure 1. Room layout for control and experimental trial.

Test Method:

Airgle Corp. supplied an AG300 system for testing purposes to determine efficacy against viral pathogens. This study evaluated the efficacy of the Airgle AG300 in its ability to inactivate the viral strain referred to as SARS-CoV-2.

Exposure Conditions:

1. Prior to the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. The temperature during all test runs was approximately 76°F ±2°F with a relative humidity of 42%.
3. The air samplers were calibrated by the manufacturer on September 3, 2020 and set at a standard flow of 5.02L/min. Calibration records indicate a 0.20% tolerance.
4. The air sample collection volumes were set to 10-minute continual draws at the point of sampling.
5. Low volume mixing fans were placed at each corner of the chamber at a 45-degree angle and turned on prior to nebulization.
6. Each timepoint was treated as an individual test and the chamber was reset after sample collection.
7. Testing timepoints were as follows with T equals to minutes: T-0, T-15, T-30, T-45, T-60

Nebulization:

1. Nebulization for control and viral test challenges were performed in the same manner.
2. After nebulization of the pathogen, the Airgle AG300 system was turned on via remote control provided by the manufacturer.
3. Fan speed on the Airgle AG300 was set on high for the test conditions and was turned off at the defined timepoint for sample collection.
4. For the viral challenge, a known quantity of viral media was nebulized into the sealed environment from a dissemination port.
5. The viral media was nebulized at a constant rate for 25 minutes.
6. During the pathogen challenge, the Airgle AG300 was turned off at the defined timepoints for sample collection to start.
7. Air sample collection occurred for both the challenge and the control test at the defined sample timepoints after nebulization ceased for a total of 10 minutes.
8. The test condition had the Airgle AG300 in the center of the test box.
9. Sample cassettes were manually removed from the collection system after each control run and each air pass challenge.
10. Upon cassette removal after each challenge, cassette sets were taken to an adjacent biosafety cabinet for extraction and placement into viral suspension media.
11. Two controls were completed and three viral challenges were conducted using the same methodology.

Post Decontamination:

At the conclusion of each viral challenge test, the UV system inside the testing chamber was activated for 30 minutes. After 30 minutes of UV exposure there was a 30-minute air purge through the air filtration system. 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.

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.

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 7.5% 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.

Protocol Changes:

Protocol Amendments: None

Protocol Deviations: None

Control Protocol

Two control tests were conducted without the Airgle AG300 unit in the testing chamber. Control samples were taken at the corresponding sample times used for the challenge trial. Nebulization of viral media and collection methods were the same for the control as the viral challenge. Control testing was used for the comparative baseline to assess the viral reduction when the Airgle AG300 device was operated in the challenge trials to enable net reduction calculations to be made. During the control test, four low volume fans were operated in each corner of the testing chamber to ensure homogenous mixing of the air. During the control, temperature and relative humidity were monitored. Prior to running the viral challenges, temperature and humidity were confirmed to be in the relative range to the control ±5%.

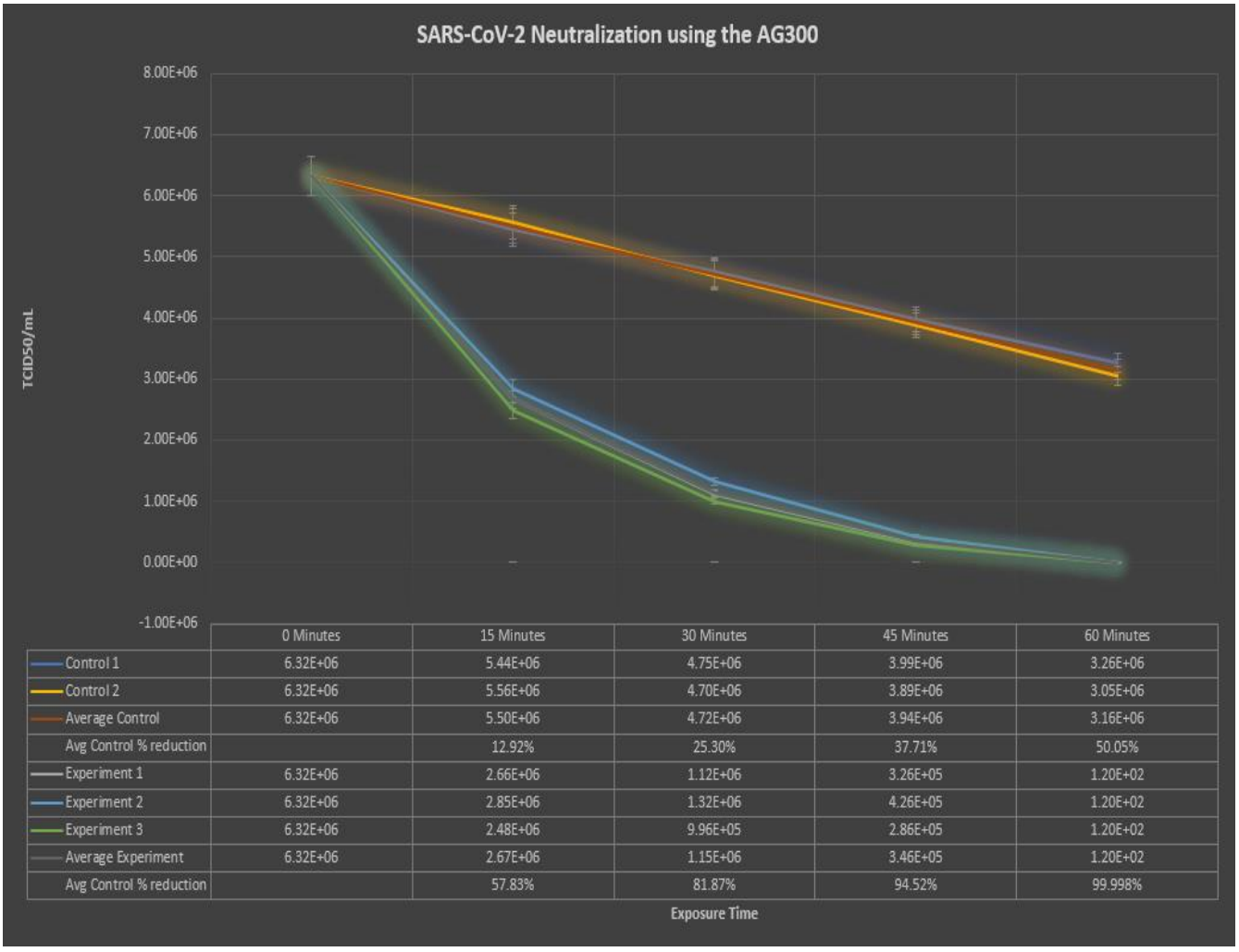
Aerosolization of Viral Media:

The control samples were performed in the same manner as the viral test regarding the time points and collection rate. A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 6.32 X 10^6 TCID50/mL* was used for this experiment.

Study Results

RESULTS:

When tested against the SARS-CoV-2 virus, the Airgle AG300 unit showed a reduction of active pathogens during the time it was operated resulting in a reduction below 1.20 x 10^2 TCID50 per mL after one hour of continuous operation. The Airgle AG300 showed the ability to reduce collectable the collectable pathogen in the air below the lower limits of detection. This would result in a 99.998% reduction of collectable virus in the air after 60 minutes of operation.



	0 Minutes	15 Minutes	30 Minutes	45 Minutes	60 Minutes
Control 1	6.32E+06	5.44E+06	4.75E+06	3.99E+06	3.26E+06
Control 2	6.32E+06	5.56E+06	4.70E+06	3.89E+06	3.05E+06
Average Control	6.32E+06	5.50E+06	4.72E+06	3.94E+06	3.16E+06
Avg Control % reduction		12.92%	25.30%	37.71%	50.05%
Experiment 1	6.32E+06	2.66E+06	1.12E+06	3.26E+05	1.20E+02
Experiment 2	6.32E+06	2.85E+06	1.32E+06	4.26E+05	1.20E+02
Experiment 3	6.32E+06	2.48E+06	9.96E+05	2.86E+05	1.20E+02
Average Experiment	6.32E+06	2.67E+06	1.15E+06	3.46E+05	1.20E+02
Avg Control % reduction		57.83%	81.87%	94.52%	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.

Conclusion:

The Airgle AG300 air purification device performed to manufacturer's specifications and demonstrated a reduction to below testing equipment sensitivity of greater than a 99.998% reduction of recoverable active SARS-CoV-2 virus in the air after 60 minutes of operation. There was no cytopathic effect in the lowest concentration of vero e6 cells, indicating a SARS-CoV-2 virus concentration lower than the limit of detection after a 21-day incubation.

Effort was made to simulate a real-life environment in the chamber while taking into consideration the special precautions needed when working with a Biosafety Level 3 Pathogen. Taking into consideration the starting concentration of active SARS-CoV-2 virus, the volume aerosolized, and the volume inoculated, one could assume that the likelihood of entering an environment with this quantity of pathogen in a real-life circumstance to be unlikely.

When aerosolizing pathogens and collecting said pathogens, there are variables that cannot be fully accounted for, namely, placement of pathogen, collection volume, collection points, drop rate, surface saturation, viral destruction on collection, viral destruction on nebulization 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.

Testing confirmed the Airgle AG300 unit showed a high-level reduction of pathogens in the air during the time it was operated resulting in a reduction below 1.20×10^2 TCID50 per mL after 60 minutes.

Considerations:

Different volume environments will create different efficacy reductions. Airflow/ACH/location and a multitude of other factors will have effects on overall performance of the equipment.

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Disclaimer

The Innovative Bioanalysis, Inc. ("Innovative Bioanalysis") laboratory is not certified or licensed by the United States Environmental Protection Agency and makes no equipment emissions claims pertaining to ozone or byproduct of any Airgle device. Innovative Bioanalysis, Inc. makes no claims to the overall efficacy of any Airgle AG300 in different environments. The experiment results are solely applicable to the device used in the trial and space used. The results are only representative of the experiment design described in this report. Innovative Bioanalysis, Inc. makes no claims as to the reproducibility of the experiment results given the possible variation of experiment results even with an identical test environment, viral strain, collection method, inoculation, nebulization, viral media, cell type, and culture procedure. Innovative Bioanalysis, Inc. makes no claims to third parties and takes no responsibility for any consequences arising out of the use of, or reliance on, the experiment results by third parties.

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