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EFFICACY OF THE AURA AIR AGAINST AEROSOLIZED INFLUENZA A

PROJECT: AURA AIR - AEROSOL - INFLUENZA A

PRODUCT: AURA AIR

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM:

INFLUENZA A

Medical Director:

Dana Yee, M.D.

Study Completion Date:

04/11/2022

Testing Facility:

Innovative Bioanalysis, Inc.

3188 Airway Ave Suite D

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Laboratory Project Number

1245

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Efficacy Study Summary

Study Title	EFFICACY OF THE AURA AIR AGAINST AEROSOLIZED INFLUENZA A
Laboratory Project #	1245
Guideline:	Modified ISO standards as no international standards exist.
Testing Facility	Innovative Bioanalysis, Inc.
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	Influenza A
Description	Per the manufacturer, the Aura Air purification and disinfection system was designed as for wall or ceiling mount utilizing a multi-cascading filter system, UV-C, and ionization technology to reduce active airborne pathogens. This study sought to determine the device's efficacy in lowering active, aerosolized Influenza A in a sealed controlled environment.
Test Conditions	The test was conducted in a 10'x8'x8' chamber following BSL-3 standards. The temperature during testing was approximately 68 ±2°F, with a relative humidity of 30%. A 2.47 x 10^7 CEID50/mL concentration Influenza A viral suspension media was nebulized into the room with mixing fans before collection. Air sample collections occurred at 0, 15, 30, and 60 minutes of device operation.
Test Results	The experiment displayed an accelerated reduction in viral concentration than the natural viability loss observed in the controls. After 30 minutes of operation, 5.17 x 10^6 CEID50/mL of active Influenza A was recovered in the air. The device reduced active Influenza A concentrations to 4.80 x 10^2 CEID50/mL after 60 minutes.
Control Results	Control testing 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 viral reduction.
Conclusion	The wall-mounted Aura Air demonstrated a significant ability in reducing active Influenza A in the air. At 15 minutes, the device displayed a 63.69% gross reduction. At 30 minutes, a 79.04% gross reduction was observed which increased to 99.998% after 60 minutes of operation.

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Study Report:

Study Title: EFFICACY OF THE AURA AIR AGAINST AEROSOLIZED INFLUENZA A

Sponsor: Aura Air

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

Device Testing: Aura Air

Study Dates:

Study Report Date: 04/18/2022
Experimental Start Date: 03/04/2022
Experimental End Date: 03/05/2022
Study Completion Date: 04/11/2022

Study Objective:

Aura Air supplied a smart air device designed to be mounted on the ceiling or wall in a room to decrease the concentration of pathogens and particulates in the air of an occupied space. This study evaluated the Aura Air unit's effectiveness in its ability to reduce aerosolized Influenza A.

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 FBS solution without the test virus present to confirm the average particle size distribution. The nebulizer was filled with 2.47 x 10^7 CEID50/mL of Type A Influenza virus in 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 two 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 (Fig. 2) had a delicate internal filtration disc to collect virus samples, which was moistened with a virus suspension media to aid in the collection. Filtration discs from Zefon International, Lot# 28144, were used for testing. At each time point, all the sample discs were pooled into one collection tube to provide an average across the two sampling locations.

Figure 2: Sensidyne 37mm directionnel air flow sample cassette.

Test System Strains: Influenza A

The following reagent was obtained through BEI Resources, NIAID, NIH: Influenza A Virus, A/mallard/Wisconsin/2785/2009 (H2N3), NR-31132.

Study Materials and Equipment:

Equipment Overview: The equipment (Fig. 3) 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.

MANUFACTURER: Aura Smart Air Ltd.

MODEL: Aura Air Rev 1.0

SIZE: 15" x 15" x 6"

MAKE: Aura Air

Figure 3. Aura Air unit as tested.

Testing Layout:

Testing was conducted in a sealed 10'x8'x8' chamber per Biosafety Level 3 (BSL3) standards, as shown in Figure 5. The overall dimensions of the test chamber provided a displacement volume of 640 ft^3 (approximately 18,122.78 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 10 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 two probes for air sampling positioned along the room's centerline and located 6 feet off the chamber floor. The device was mounted in the center of the 10-foot wall opposite the dissemination port, approximately 5 ft above the floor (Figure 4). The chamber was visually inspected, and pressure tested, and all internal lab systems and equipment were reviewed before testing.

Figure 4. Device mounted 5ft off the floor on a stand located in the center of the 10ft wall.

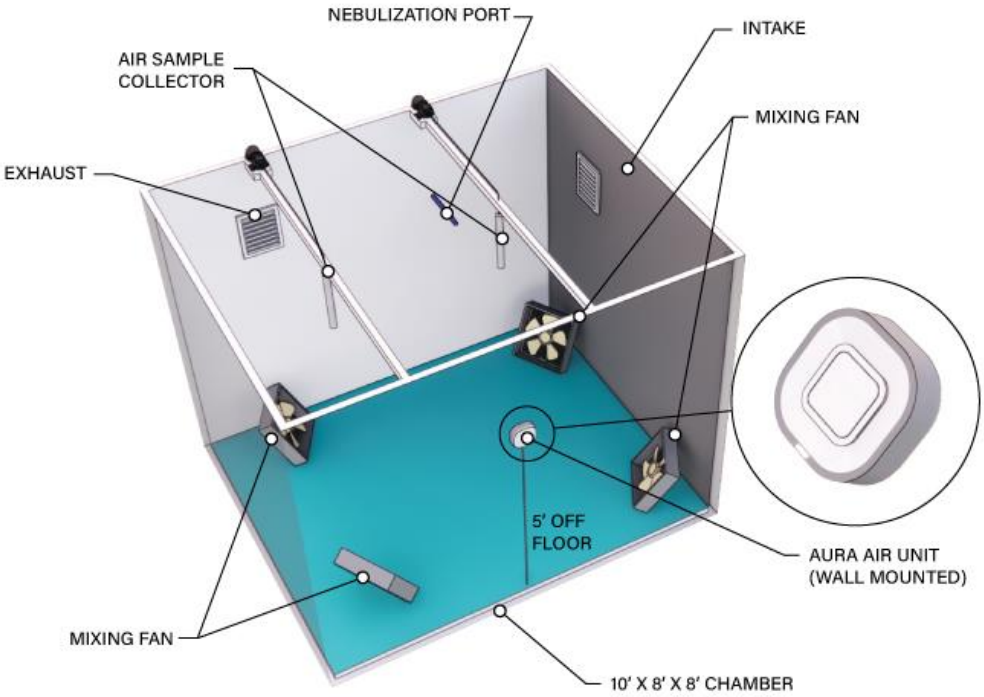


Figure 5. 3D model of the room layout for control and experimental testing.

Test Procedure:

Exposure Conditions:

1. The temperature during all test runs was approximately 68 ±2°F, with a relative humidity of 30%.
2. Testing time points were as follows, with T equal to minutes: T-0, T-15, T-30, and T-60.

Experimental Procedure:

1. Before the initial control test and following each trial, the testing area was decontaminated and prepped per internal procedures.
2. 5 mL of a 2.47 x 10^7 CEID50/mL Influenza A viral suspension was nebulized via a dissemination port into the room.
3. After nebulization, the mounted Aura Air unit was turned on via remote control.
4. At each predetermined time point, the device was turned off for sample collection.
5. Air sampling collections were set to 10-minute continuous draws at the point 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. All samples were sealed after collection 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 and then rinsed repeatedly with DI water. The nebulizer and vacuum collection pumps were decontaminated with hydrogen peroxide mixtures.

Control Protocol:

A set of control was conducted without the device operating in the testing chamber to assess the Aura Air accurately. Control samples were taken in the same manner and at the corresponding time points used for the challenge trial to serve as a comparative baseline to assess the device's viral reduction when active.

Preparation of The Pathogen

Viral Stock: Influenza A Virus, A/mallard/Wisconsin/2785/2009 (H2N3) (BEI NR-31132)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs		
Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Hemagglutinin and Matrix Coding Regions		
Hemagglutinin (619 nucleotides)	Consistent with A/mallard/ Wisconsin/2785/2009 (H2N3)	100% identity with A/mallard/ Wisconsin/2785/2009 (H2N3) (GenBank: CY097374)
Matrix (937 nucleotides)	Consistent with A/mallard/ Wisconsin/2785/2009 (H2N3)	100% identity with A/mallard/ Wisconsin/2785/2009 (H2N3) (GenBank: CY097375)
Titer by $CEID_{50}$ in Embryonated Chicken Eggs	Report Results	8.9 X 10^8 $CEID_{50}$ per mL
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
Blood Agar, aerobic	No Growth	No Growth
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. These viruses are grown on 10- to 11-day-old SPF Embryonated Chicken Eggs 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, 200uL, 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:

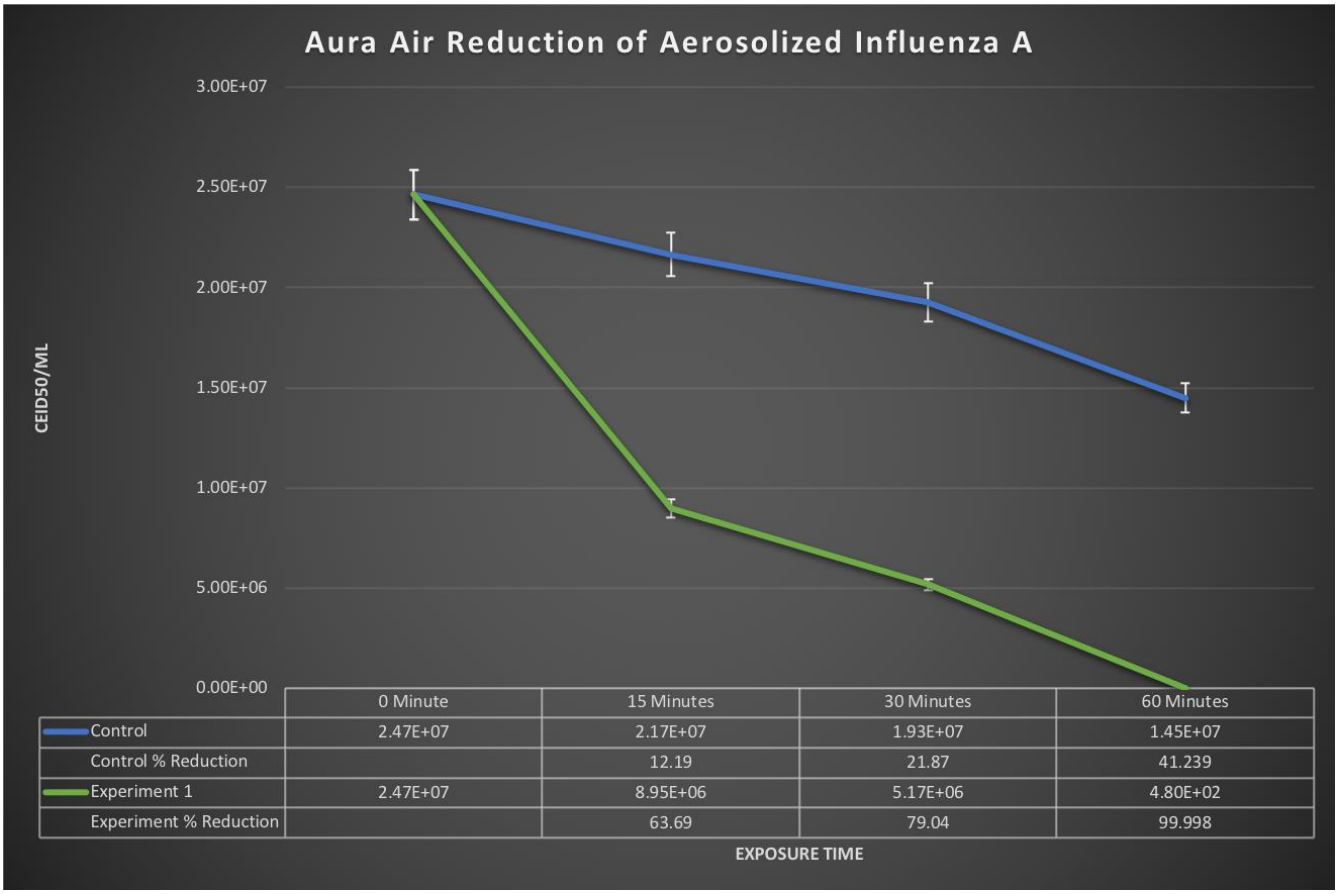
1. One day before infection, prepare 96-well dishes by seeding each well with Vero E6 cells in DMEM plus fetal bovine serum, 4mM Glutamine, and antibiotics.
2. On the day of infection, make dilutions of virus samples in PBS.
3. Make a series of dilutions at 1:10 of the original virus sample. Fill the first tube with 2.0mL PBS and the subsequent tubes with 1.8mL.
4. Vortex the viral samples, then transfer 20uL of the virus to the first tube, vortex, discard tip.
5. With a new tip, serial dilute subsequent tips transferring 200uL.

Additions of virus dilutions to cells:

1. Label the lid of a 96-well dish by drawing grid lines to delineate quadruplicates, 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 0.1mL of media from each well by vacuum aspiration.
4. Starting from the most dilute sample, add 0.1mL 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 into 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 0.5mL 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 Results:

The results were plotted to show the amount of active Influenza A collected with and without the Aura Air operating for 60 minutes. The control displayed a gradual viability loss over time, while the Aura Air displayed a more rapid reduction. Against aerosolized Influenza A, an initial concentration of 2.47 x 10^7 CEID50/mL decreased to 8.95 x 10^6 CEID50/mL after 15 minutes of exposure. More prolonged exposure resulted in a more significant reduction in collectible, active Influenza A virus, as shown by the 5.17 x 10^6 CEID50/mL collected at 30 minutes and 4.80 x 10^2 CEID50/mL after 60 minutes.



	0 Minute	15 Minutes	30 Minutes	60 Minutes
Control	2.47E+07	2.17E+07	1.93E+07	1.45E+07
Control % Reduction		12.19	21.87	41.239
Experiment 1	2.47E+07	8.95E+06	5.17E+06	4.80E+02
Experiment % Reduction		63.69	79.04	99.998

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

Conclusion:

The wall-mounted Aura Air device demonstrated significant reduction of aerosolized Influenza A virus in a controlled environment. At 15 minutes of operation, a 63.69% gross reduction of active Influenza A was observed. Reduction capabilities increased with elapsed time with a 79.04% gross reduction after 30 minutes and a 99.998% gross reduction after 60 minutes of operation.

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, virus destruction on collection, virus 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.

Considering the variables, the Aura Air device achieved a measurable amount of reduction at each time point (T-15, T-30, and T-60), demonstrating the device efficiently reduced Influenza A from the air samples collected under controlled conditions.

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