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EFFICACY OF THE AURA AIR AGAINST AEROSOLIZED RESPIRATORY SYNCYTIAL VIRUS

PROJECT: AURA AIR AEROSOL RSV

PRODUCT: AURA AIR

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM(S):

RESPIRATORY SYNCYTIAL VIRUS (RSV)

STUDY COMPLETION DATE:

12/27/2022

Medical Director

Dana Yee, M.D.

Testing Facility

Innovative Bioanalysis, Inc.

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Laboratory Project Number

1361

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Efficacy Study Summary

Study Title	EFFICACY OF THE AURA AIR AGAINST AEROSOLIZED RESPIRATORY SYNCYTIAL VIRUS
Laboratory Project #	1361
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	Respiratory Syncytial Virus
Description	Per the manufacturer, the Aura Air purification and disinfection system was designed as a wall or ceiling mount utilizing a multi-cascading filtration and disinfection technology to reduce active airborne pathogens. This study sought to determine the device's efficacy in lowering active, aerosolized RSV in a controlled environment.
Test Conditions	Testing was conducted in a 10'x8'x8' chamber following BSL-3 standards. The temperature during testing was approximately 69 ±2°F, with a relative humidity of 41%. A 6.86 x 10^6 TCID50/mL of RSV in 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 increased reductions in viral concentration over the natural viability loss observed in the controls. The Aura Air device decreased recoverable active RSV from 6.86 x 10^6 TCID50/mL to 2.36 x 10^6 TCID50/mL after 15 minutes. A longer operation time resulted in higher reductions as observed by the 9.55 x 10^5 TCID50/mL recovered after 30 minutes. After 60 minutes recovered RSV fell below the limit of assay quantitation of 2.40 x 10^2 TCID50/mL.
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. At 15 minutes, recoverable RSV was 5.53 x 10^6 TCID50/mL and was 2.94 x 10^6 TCID50/mL after 60 minutes.
Conclusion	The wall-mounted Aura Air demonstrated the ability to reduce active RSV in the air, as shown by the 65.56% (0.46 log) gross reduction after 15 minutes, 86.09% (0.86 log) reduction, and reaching a minimum 99.997% (4.46 log) gross reduction after 60 minutes.

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Study Report:

Study Title: EFFIACY OF THE AURA AIR AGAINST AEROSOLIZED RESPIRATORY SYNCYTIAL VIRUS

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: 12/20/2022

Experimental Start Date: 12/02/2022

Experimental End Date: 12/02/2022

Study Completion Date: 12/27/2022

Study Objective:

Aura Air supplied a smart air device designed to be mounted on the ceiling or wall 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 Respiratory Syncytial Virus (RSV).

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 to confirm the average particle size distribution of approximately 0.8 µm. See Table 1, and Appendix B, for particle distribution specifics from sampling of aerosolized solution in particle testing prior to this study. The nebulizer was filled with 6.86 x 10^6 TCID50/mL of RSV 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 that approximately 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

Table 1: Particle Size Distribution Table

	Number Particle Size	Surface Particle Size	Mass Particle Size
Median (µm)	0.783	1.2	2.66
Mean (µm)	0.911	2	4.56
Geo. Mean (µm)	0.845	1.43	2.98
Mode (µm)	0.723	0.777	12
Geo. St. Dev.	1.42	2.06	2.57
Total Conc.	2.45e+03(#/cm³)	7.22e+03(µm²/cm³)	2.38(mg/m³)

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. 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 after each sampling time point. Cassettes had an internal filtration disc (Fig. 2) to collect virus samples, which was moistened with a suspension media to aid collection. Filtration discs from Zefon International, Lot# 29114, were used for testing. All sample discs were pooled into one collection tube at each time point to provide an average across the two sampling locations.

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

Test System Strains: Respiratory Syncytial Virus (RSV)

The following reagent was obtained through BEI Resources, NIAID, NIH: Human Respiratory Syncytial Virus, A2000/3-4, NR-28530.

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’ x 8’ x 8’ chamber per Biosafety Level 3 (BSL3) standards, as shown in Figure 5. The room had 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. 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.

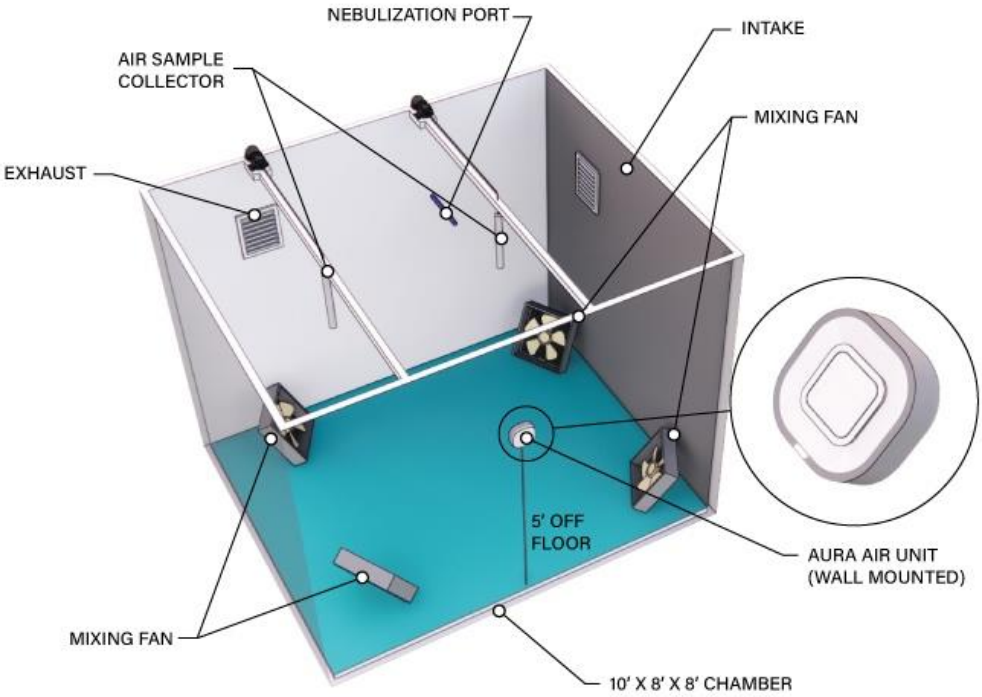

Figure 5. 3D model of the room layout for control and experimental testing.

Control Protocol:

A set of controls were conducted without the device operating in the testing chamber to accurately assess the Aura Air unit. 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 viral reduction when the device was operating.

Test Procedures:

Exposure Conditions:

1. The temperature during all test runs was approximately 69 ±2°F, with a relative humidity of 41%.
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. 5mL of a 6.86 x 10^6 TCID50/mL RSV 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 test was completed, 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.

Preparation of The Pathogen

Viral Stock: Human Respiratory Syncytial Virus, A2000/3-4 (NR-28530)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HEp-2 Cells	Cell rounding, syncytia formation and detachment	Cell rounding, syncytia formation and detachment
Identification by Direct Fluorescent Antibody (DFA) Assay	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (851 nucleotides)	Consistent with human respiratory syncytial virus, A2000/3-4	99% identity with human respiratory syncytial virus, A2000/3-4 GenBank: JX069803
Titer by TCID50 in HEp-2 Cells	Report Results	2.8 X 10^6 TCID50 per mL
Sterility (21-Day Incubation)		
Harpo's 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 test article nucleic acid	None Detected	None Detected

*The viral titer listed in the Certificate of Analysis represents the titer provided by BEI Resources. See Appendix F for more details.

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, and 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 (Figure 7) to display collectible active Respiratory Syncytial Virus (RSV) with and without the Aura Air device operating in the chamber. The controls showed a 0.09 log natural loss of aerosolized RSV after 15 minutes, 0.23 log reduction after 30 minutes, and a 0.37 log natural loss after 60 minutes under controlled conditions. With the Aura Air unit in operation against RSV, a starting concentration of 6.86 x 10^6 TCID50/mL decreased to 2.36 x 10^6 TCID50/mL after 15 minutes for a log reduction of 0.46. With longer exposure, the data showed after 30 minutes, the concentration of recoverable RSV was 9.55 x 10^5 TCID50/mL equating to a 0.86 log reduction. The data showed that at 60 minutes of device operation, the concentration of the test article was below the quantifiable limit of detection of 2.40 x 10^2 TCID50/mL. This represents a minimum of 4.46 log reduction of the initial challenge sample concentration. Table 3 provides the complete test results.

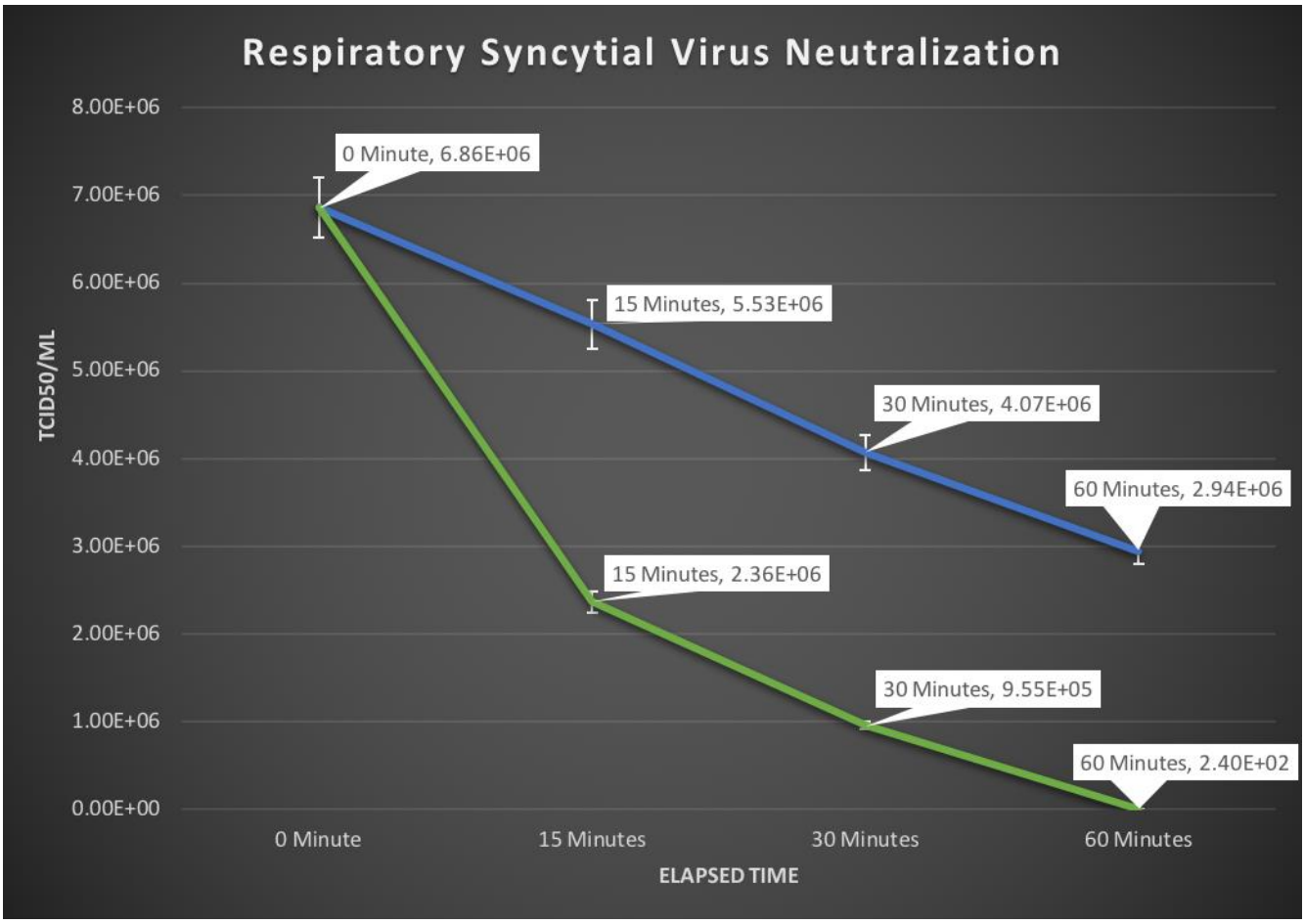

Figure 7: Respiratory Syncytial Virus Study Results

*** 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.*

Table 3: RSV Results Data and Calculated Percentage Reductions

Time (min)	0 Minute	15 Minutes	30 Minutes	60 Minutes
Control 1 (TCID50/mL)	6.86E+06	5.53E+06	4.07E+06	2.94E+06
% Gross Reduction - Control		-19.38	-40.75	-57.12
Gross Log Reduction - Control		0.09	0.23	0.37
Experiment 1 (TCID50/mL)	6.86E+06	2.36E+06	9.55E+05	2.40E+02
% Gross Reduction - Experiment		-65.56	-86.09	-99.997
Gross Log Reduction - Experiment		0.46	0.86	4.46

Conclusion:

The Aura Air demonstrated the ability to reduce active, aerosolized Respiratory Syncytial Virus (RSV) across all time points compared to the natural loss rate observed in the temperature-controlled room. Against RSV, the device achieved a 65.56% gross reduction after 15 minutes. At 30 minutes, an increase in reduction capabilities was observed at 86.09% and 99.997% gross reduction after 60 minutes. Thus, the Aura Air, when operated at the manufacturers recommended condition for typical operation, resulted in a 0.46 log reduction of RSV at 15 minutes and a minimum of 4.46 log reduction at 60 minutes.

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 Respiratory Syncytial Virus from the air samples collected under controlled conditions.

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12/30/2022

Dana Yee M.D **Date**

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Chief Biosafety Officer, Innovative Bioanalysis, Inc.

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12/29/2022

Kevin Noble **Date**

Laboratory Director, Innovative Bioanalysis, Inc.

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 Aura Air devices. Innovative Bioanalysis, Inc. makes no claims to the overall efficacy of any Aura Air devices. The experiment results are solely applicable to the device used in the trial. 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.

APPENDIX A: Glossary of Terms

CAP: The College of American Pathologists (CAP), the leading organization of board-certified pathologists, serves patients, pathologists, and the public by fostering and advocating excellence in the practice of pathology and laboratory medicine worldwide. A laboratory can pursue a higher level of quality by becoming accredited by The College of American Pathologists (CAP).

CLIA: The Clinical Laboratory Improvement Amendments of 1988 (CLIA) are federal regulations for the United States-based clinical laboratories to provide industry standards for testing human samples for diagnostic purposes.

COA: A Certificate of Analysis refers to an authenticated document that is issued by BEI or ATCC Quality Assurance Department that ascertains that a product has met its predetermined pathogen specifications and preparations.

DMEM: Dulbecco's Modified Eagle Medium (DMEM) is a widely used basal medium for supporting the growth of many different mammalian cells.

FBS: Fetal bovine serum (FBS) is derived from the blood drawn from a bovine fetus via a closed collection system at the slaughterhouse. Fetal bovine serum is the most widely used serum supplement for the in vitro cell culture of eukaryotic cells. This is because it has an extremely low level of antibodies and contains more growth factors, allowing for versatility in many different cell culture applications.

The globular protein, bovine serum albumin (BSA), is a major component of fetal bovine serum. The rich variety of proteins in fetal bovine serum maintains cultured cells in a medium where they can survive, grow, and divide.

Because it is a biological product, FBS is not a fully defined media component and varies in composition between batches. As a result, serum-free and chemically defined media (CDM) have been developed to minimize the possibility of transferring adventitious agents. However, the effectiveness of serum-free media is limited, as many cell lines still require serum to grow, and many serum-free media formulations can only support the growth of narrowly defined types of cells.

LLOQ: The ULOQ and LLOQ are the highest and lowest standard curve points that can still be used for quantification; they are the values below and above which, respectively, quantitative results may be obtained with a specified degree of confidence, or the highest/lowest concentration of an analyte that can be accurately measured. Together, the ULOQ and LLOQ define the range of quantification for the assay. Limits of quantitation are matrix, method, and analyte-specific, and can be calculated as follows:

Equation 1.

(Calculation used in Q-View): ULOQ & LLOQ = Highest or Lowest Standard, respectively, with a %backfit of 120%-80%, a %CV of < 30%, and a positive mean pixel intensity difference between it and the negative control.

Equation 2.

(Commonly used in science to estimate the LLOQ): LLOQ = (Mean negative control pixel intensity) + 10 * (StDev of negative control pixel intensities).

PBS: Phosphate buffered saline (PBS) is a pH-adjusted blend of ultrapure-grade phosphate buffers and saline solutions which, when diluted to a 1X working concentration, contains 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 2 mM KH_2PO_4.

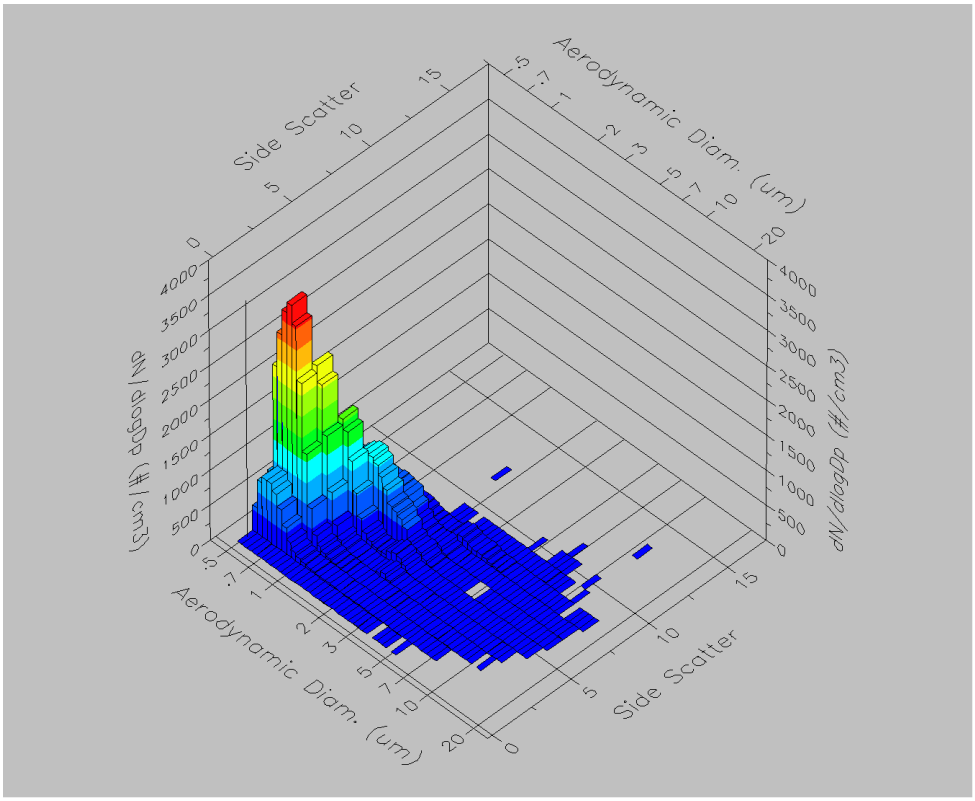
TCID50/mL: The number of infectious virus particles is frequently quantified using the Median Tissue Culture Infectious Dose (TCID50) assay. The assay works by adding a serial dilution of the virus sample to cells in a 96-well plate format. The cell type is specifically selected to show a cytopathic effect (CPE), i.e., morphological changes upon infection with the virus or cell death. After an incubation period, the cells are inspected for CPE or cell death, and each well is classified as infected or not infected. Colorimetric or fluorometric readouts are also possible, which can increase assay sensitivity. The dilution, at which 50% of the wells show a CPE, is used to calculate the TCID50 of the virus sample. Virus titer is expressed as TCID50/mL. See Appendix E for Spearman-Karber method calculation details.

HEp-2: A cell line initially thought to have originated from laryngeal cancer cells but was subsequently found to have been derived via HeLa contamination. These cells are invaluable in the analysis of autoantibodies and are one of the most common substrates for antinuclear antibody detection by immunofluorescence.

APPENDIX B: Particle Size Distribution

The TSi Aerodynamic Particle Sizer® (APS™) 3321 spectrometer is a device designed to collect high-resolution, real-time aerodynamic measurements of particles from 0.5 to 20 microns. The APS was used during pre-study testing and validation for particle dispersion with the Blaustein Atomizing Module (BLAM) bioaerosol-generating nebulizer. All test equipment, suspension solution, and setup were the same as what was used in this viral study.

	Number Particle Size	Surface Particle Size	Mass Particle Size
Median (µm)	0.783	1.2	2.66
Mean (µm)	0.911	2	4.56
Geo. Mean (µm)	0.845	1.43	2.98
Mode (µm)	0.723	0.777	12
Geo. St. Dev.	1.42	2.06	2.57
Total Conc.	2.45e+03(#/cm³)	7.22e+03(µm²/cm³)	2.38(mg/m³)



APPENDIX C: Calculation equations

Spearman-Karber TCID50 calculation method:

$\log_{10}$ 50% endpoint dilution = - (x_0 - d/2 + d $\sum$ r_i/n_i)

x_0 = $\log_{10}$ of the reciprocal of the highest dilution (lowest concentration) at which all animals are positive
d = $\log_{10}$ of the dilution factor
n_i = number of animals used in each dilution (after discounting accidental deaths)
r_i = number of positive animals (out of n_i)
Summation is started at dilution x_0.

Percent Reduction calculation:

Percent Reduction = (A-B) * 100 / A

A = initial number of viable microorganisms
B = final number of viable microorganisms

Log Reduction calculation:

Log Reduction = $\log_{10}$ (A/B)

A = initial number of viable microorganisms
B = final number of viable microorganisms

APPENDIX D: Equipment Calibration Certificates

SENSIDYNE
Industrial Health & Safety Instrumentation

Sensidyne Certificate of Performance
Gilian 10i Sampling Pumps

This document certifies that the product below performs in accordance with factory specifications. Sensidyne's volumetric test equipment is traceable to NIST. Sensidyne, LP is an ISO 9001:2015 registered company.

Gilian 10i Assembly, P/N 610-1501-01-R
Serial Number 20220202003 Month of Manufacture: February 2022

Set Flow L/min	Set BP Inches H2O	Acceptable Minimum L/min	Acceptable Maximum L/min	Pass = √ Fail = X
4	2	3.800	4.200	√
	25	3.800	4.200	√
	50	3.800	4.200	√
8	2	7.600	8.400	√
	10	7.600	8.400	√
	22	7.600	8.400	√
10	2	9.500	10.500	√
	6	9.500	10.500	√
	12	9.500	10.500	√

Technician Stamp 10

091-1015-01rC

SENSIDYNE
Industrial Health & Safety Instrumentation

Sensidyne Certificate of Performance
Gilian 10i Sampling Pumps

This document certifies that the product below performs in accordance with factory specifications. Sensidyne's volumetric test equipment is traceable to NIST. Sensidyne, LP is an ISO 9001:2015 registered company.

Gilian 10i Assembly, P/N 610-1501-01-R
Serial Number 20220202002 Month of Manufacture: February 2022

Set Flow L/min	Set BP Inches H2O	Acceptable Minimum L/min	Acceptable Maximum L/min	Pass = √ Fail = X
4	2	3.800	4.200	√
	25	3.800	4.200	√
	50	3.800	4.200	√
8	2	7.600	8.400	√
	10	7.600	8.400	√
	22	7.600	8.400	√
10	2	9.500	10.500	√
	6	9.500	10.500	√
	12	9.500	10.500	√

Technician Stamp 10

091-1015-01rC

SENSIDYNE
Industrial Health & Safety Instrumentation

Sensidyne Certificate of Performance
Gilian 10i Sampling Pumps

This document certifies that the product below performs in accordance with factory specifications. Sensidyne's volumetric test equipment is traceable to NIST. Sensidyne, LP is an ISO 9001:2015 registered company.

Gilian 10i Assembly, P/N 610-1501-01-R
Serial Number 20220202001 Month of Manufacture: February 2022

Set Flow L/min	Set BP Inches H2O	Acceptable Minimum L/min	Acceptable Maximum L/min	Pass = √ Fail = X
4	2 25 50	3.800 3.800 3.800	4.200 4.200 4.200	√ √ √
8	2 15 22	7.600 7.600 7.600	8.400 8.400 8.400	√ √ √
10	2 6 12	9.500 9.500 9.500	10.500 10.500 10.500	√ √ √

Technician Stamp 10

091-1015-01rC

APPENDIX E: BEI Resources - Certificate of Authenticity

b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-28530

Human Respiratory Syncytial Virus, A2000/3-4

Catalog No. NR-28530

Product Description: Cell lysate and supernatant from HEp-2 cells[1] infected with human respiratory syncytial virus, A2000/3-4

Lot[2]: 63339791 **Manufacturing Date: 22MAY2015**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HEp-2 Cells[1]	Cell rounding and sloughing	Cell rounding and sloughing
Identification by Direct Fluorescent Antibody (DFA) Assay[3]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (851 nucleotides)	Consistent with human respiratory syncytial virus, A2000/3-4	99% identity with human respiratory syncytial virus, A2000/3-4 (GenBank: JX069803)
Titer by $TCID_{50}$ Assay[4,5] in HEp-2 Cells[1] by Cytopathic Effect	Report results	2.8×10^6 $TCID_{50}$ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth[6], 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO_2	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA Detection by PCR of Test Article nucleic acid	None detected	None detected

[1]HEp-2 cells: ATCC® CCL-23™
[2]Grown in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 4 days at 37°C and 5% CO_2
[3]Using Light Diagnostics™ Respiratory Syncytial Virus FITC Reagent (Millipore 5022)
[4]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[5]8 days at 37°C and 5% CO_2
[6]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 19 OCT 2015 **Signature:** Michael R. Gyrla

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NR-28530_63339791_19OCT2015