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EFFICACY OF THE AURA TECHNOLOGIES ION BAR MARK IV AGAINST RESPIRATORY SYNCYTIAL VIRUS (RSV)

PROJECT: AURA TECHNOLOGIES – ION BAR – RSV

PRODUCT: AURA Ion Bar™ Mark IV

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM(S):

Respiratory syncytial virus *(RSV)*

STUDY COMPLETION DATE:

09/15/2023

Medical Director:

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Testing Facility:

Innovative Bioanalysis, Inc.

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Laboratory Project Number:

1394R

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Efficacy Study Summary

Study Title	EFFICACY OF THE AURA TECHNOLOGIES ION BAR MARK IV AGAINST RSV
Laboratory Project #	1394R
Guideline	Custom design based on ASHRAE and 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	Respiratory Syncytial Virus *(*RSV*)*
Description	The AURA Technologies AURA Ion Bar™ Mark IV is an overhead airflow curtain system with an integrated negative ion generator. The AURA Ion Bar™ Mark IV 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 Ion Bar™ Mark IV against Respiratory syncytial virus *(*RSV*)*.
Test Conditions	Testing was conducted in a sealed 20 ft (~6.1 m) x 8 ft (~2.44 m) x 8 ft (~2.44 m) chamber that complied with Biological Safety Level 3 (BSL-3) standards per NIH guidelines. The temperature during testing was approximately 71 °F ± 2 °F (~21.7 °C ± 1.1 °C), with a relative humidity of 35 %. A simulated wall with a doorway/portal was constructed such that the chamber was partitioned into two 10 ft (~3.05 m) x 8 ft (~2.44 m) x 8 ft (~2.44 m) sections. The AURA Ion Bar™ Mark IV was mounted above the doorway, approximately 7 ft (2.15 m) above the floor, on the "clean" side of the door. A 5.65×10^6 TCID50/mL of RSV fetal bovine serum (FBS) based viral media was inoculated onto glass slides as controls and challenge samples for surface testing. A known concentration of RSV was nebulized for aerosol testing. Surface samples were placed on the floor approximately 2 ft (~0.61 m) from the doorway (on the clean side) and exposed for (10, 20, 30) minutes. For aerosol testing, nebulization occurred 5 ft (~1.52 m) above the floor, 1.5 ft (~0.46 m) 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, RSV was reduced to below quantification limits ($\geq$ 4.57 log reduction) after 20 minutes of operation. With aerosol testing, the germicidal barrier blocked or deactivated an average of 99.997% (4.5 log reduction) of active RSV during device operation, which was quantified at approximately 1.60×10^2 TCID50/mL.
Control Results	Control testing was conducted for each subtest with the device fan and ionizer off and samples were 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	The AURA Technologies AURA Ion Bar™ Mark IV demonstrated a measurable efficacy as a germicidal partition, reducing the transfer of RSV aerosols by 99.997% over 2 minutes and neutralizing surface RSV to below quantification limits ($\geq$ 99.998%) after 20 minutes of exposure.

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Study Report

Study Title: EFFICACY OF THE AURA TECHNOLOGIES ION BAR MARK IV AGAINST RSV

Sponsor: AURA Technologies, LLC.

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

Device Testing: AURA Ion Bar™ Mark IV

Study Dates:

Study Report Date: 9/15/2023

Experimental Start Date: 7/26/2023

Experimental End Date: 8/6/2023

Study Completion Date: 9/15/2023

Study Objective:

AURA Technologies supplied the AURA Ion Bar™ Mark IV for testing purposes to determine efficacy against Respiratory Syncytial Virus (RSV). This study evaluated the effectiveness of the AURA Ion Bar™ Mark IV in its ability to reduce the pathogen strain referred to as Respiratory syncytial virus *(*RSV*)*.

Test Method:

Part 1: Surface Testing

Surface Inoculation:

Glass slides were equally subjected to a 1 mL inoculation of RSV containing a known titer of 5.65×10^6 TCID50/mL for the control and viral challenge. The viral solution was spread onto a sterile AmScope glass slide (ASIN: B00T5OM5C) measuring 3 in (76.2 mm) × 1.5 in (38.1 mm) and 0.125 in (3.175 mm) thickness. 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 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:

Nebulization occurred using a Blaustein Atomizing Module (BLAM), as shown in Figure 1, with a pre-set PSI and computer-controlled liquid delivery system. A calibrated nebulizer was used to check for proper functionality by nebulizing a solution without the test virus to confirm the average particle size distribution of approximately 0.8 um. See Table 1 and Appendix B for particle distribution specifics from sampling of aerosolized solution from the functionality particle testing prior to this study.

Figure 1: BLAM Nebulizer

The nebulizer was filled with a 5.65 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.

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 four probes connected to calibrated Gilian 10i vacuum devices and 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 # 28875 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: Respiratory Syncytial Virus, A2000/3-4

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

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 (Fig. 2) 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 an average ion concentration of ~960,000 negative ions/cm^3 in the doorway six inches above the ground. Ion air sampling was taken every few inches along the width of the doorway to confirm all sections were producing negative ions.

MANUFACTURER: AURA Technologies, LLC.

MODEL: Mark IV pre-production prototype

MAKE: AURA Blue

SERIAL #: NA

Figure 2. AURA Ion Bar™ Mark IV

Testing Layout:

All testing was conducted in a sealed 20 ft (~6.1 m) x 8 ft (~2.44 m) x 8 ft (~2.44 m) 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 (~1.52 m) off the floor and 1.5 ft (~0.46 m) from the simulated door opening during aerosol testing; air samples were collected 5 ft (~1.52 m) off the floor and 1.5 ft (~0.46 m) inside the simulated doorway. The AURA Ion Bar™ Mark IV 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 (~0.61 m) from the door's opening.

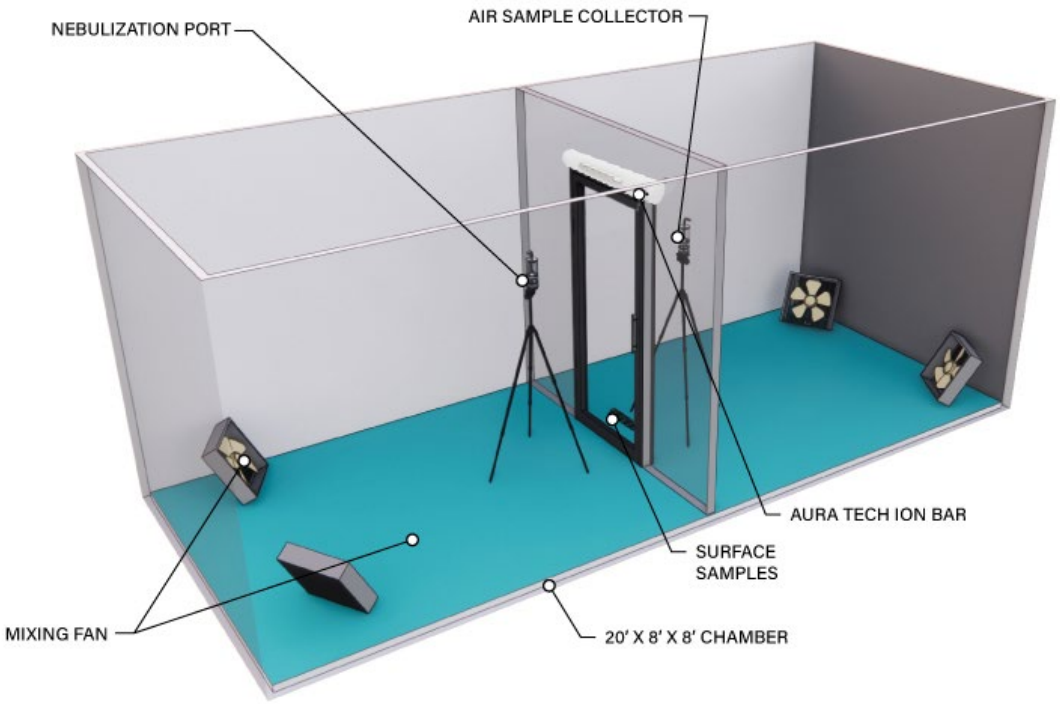


Figure 3. Testing layout for control and experimental trials

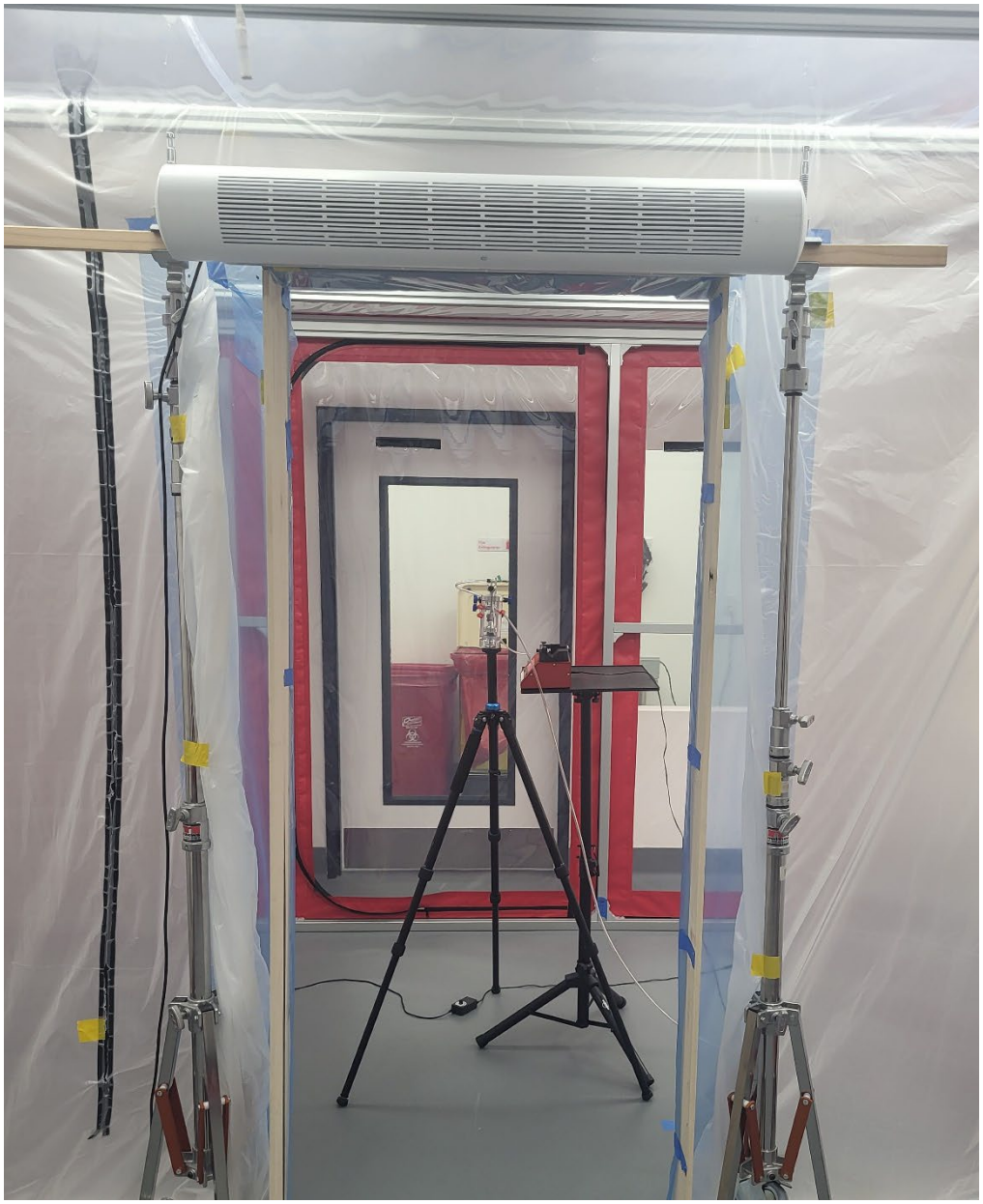


Figure 4. Testing setup

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 AURA Ion Bar™ Mark IV ran for the duration of testing unless stated otherwise.
3. The temperature during all test runs was approximately 71 °F ± 2 °F (~21.7 °C ± 1.1 °C) with a relative humidity of 35 %.

Part 1: Surface Testing

Exposure Conditions:

1. Inoculated glass slides were placed under the device 2 ft (~0.61 m) from the simulated door opening on the "clean" side.
2. Surface test samples were taken at (10, 20, 30) minutes of ionized air exposure.

Experimental Procedures:

1. Sterile glass slides were inoculated with 5.65×10^6 TCID50/mL viral media and labeled with a pre-determined time point.
2. Sample slides were centered under the device 2 ft (~0.61 m) 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 AURA Ion Bar™ Mark IV 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 to 2 minutes and was conducted in triplicate.
2. Nebulization of viral pathogen occurred at the height of 5 ft (~1.52 m) and distance of 1.5 ft (~0.46 m) outside the simulated door opening.
3. Collection occurred 5 ft (~1.52 m) off the floor and 1.5 ft (~0.46 m) from the simulated doorway on the chamber's inside (clean) side.

Experimental Procedures:

1. 2 mL of 5.65×10^6 TCID50/mL RSV viral media was nebulized from 0 to 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 Ion Bar™ Mark IV 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 net viral reduction when the device was operating.

Preparation of The Pathogen

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

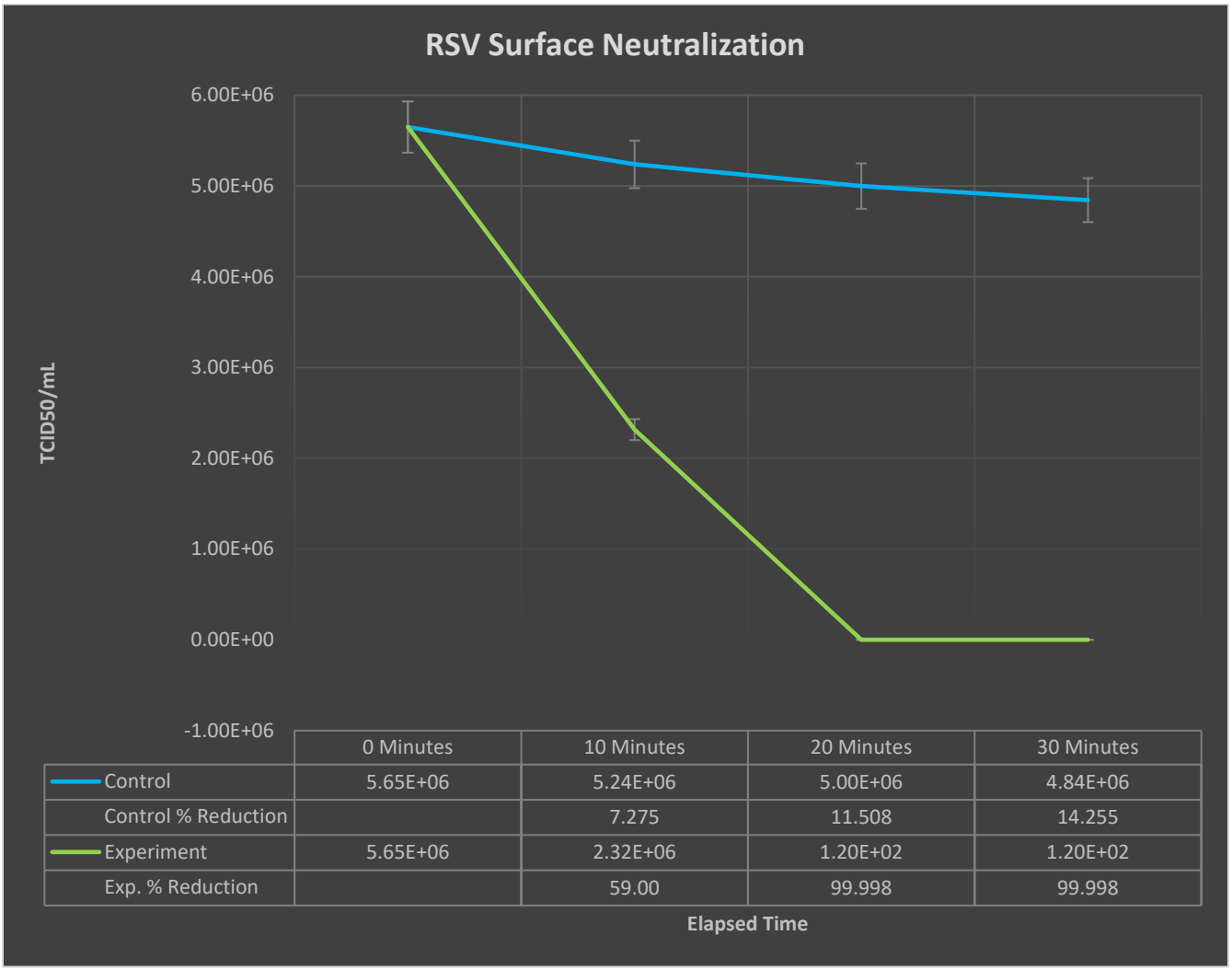
TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HEp-2 Cells	Cell rounding, syncytia formation and detachment	Cell rounding, syncytia formation and detachment
Identification by Direct Fluorescent Antibody (DFA) Assay	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific 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 E for more details.

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 RSV, the AURA Ion Bar™ Mark IV lowered a starting concentration of 5.65×10^6 TCID50/mL to approximately 2.32×10^6 TCID50/mL (59.00 %) after 10 minutes. After 20 minutes, the concentration of active RSV collected was reduced to below the assay quantitation limit (≥ 99.998 %).

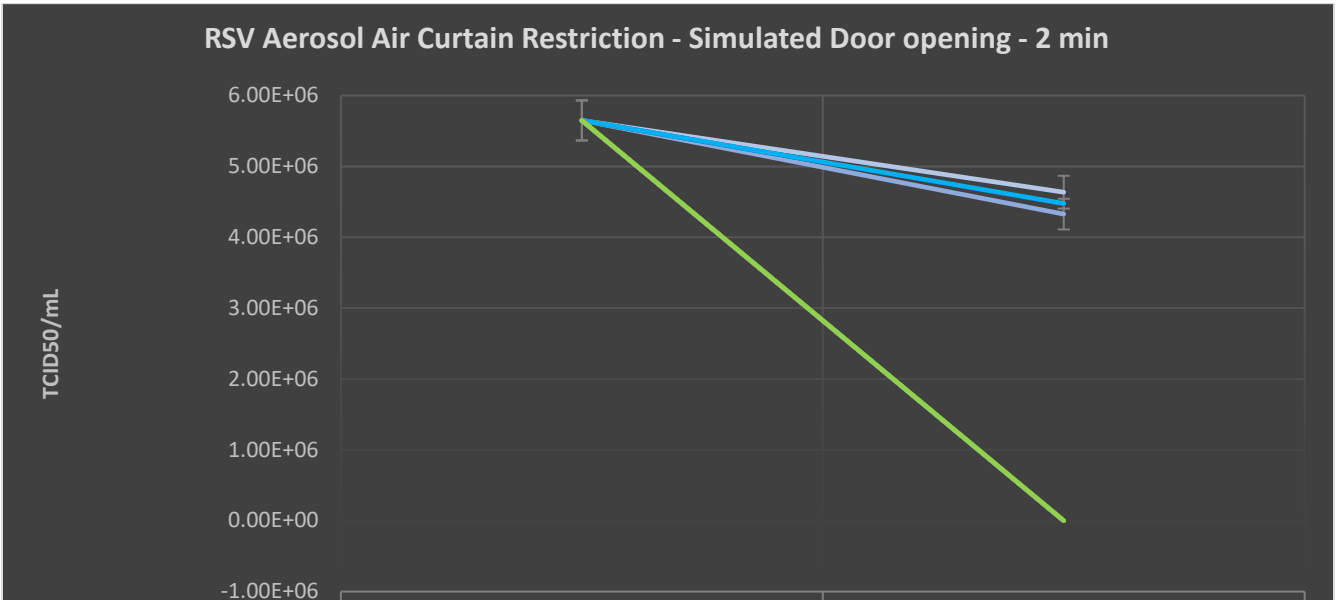


	0 Minutes	10 Minutes	20 Minutes	30 Minutes
Control	5.65E+06	5.24E+06	5.00E+06	4.84E+06
Control % Reduction		7.275	11.508	14.255
Experiment	5.65E+06	2.32E+06	1.20E+02	1.20E+02
Exp. % Reduction		59.00	99.998	99.998

**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 presence of aerosolized RSV through the AURA Ion Bar™ Mark IV air curtain within 2 minutes of device operation. From the inside of the air barrier, the concentration of active RSV collected at 2 minutes from three test runs were (1.20×10^2, 1.20×10^2, and 2.40×10^2) TCID50/mL, averaging to approximately 1.60×10^2 TCID50/mL. The presence of RSV indicated that 0.003 % of the nebulized RSV passed through the barrier and was collected at the specified location after 2 minutes resulting in a 99.997% reduction of nebulized RSV on the protected side. The control observed a 79.26 % pass-through of nebulized RSV after 2 minutes with the AURA Ion Bar™ Mark IV device off.



	Starting Concentration	Final Concentration
Control 1	5.65E+06	4.64E+06
Control 2	5.65E+06	4.33E+06
Control 3	5.65E+06	4.47E+06
Ctrl (avg.)	5.65E+06	4.48E+06
Ctrl (avg.) % Reduction		20.74
Experiment 1	5.65E+06	1.20E+02
Experiment 2	5.65E+06	1.20E+02
Experiment 3	5.65E+06	2.40E+02
Experiment (avg.)	5.65E+06	1.60E+02
Exp (avg.) % Reduction		99.997

**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 Ion Bar™ Mark IV demonstrated the ability to neutralize surface Respiratory Syncytial Virus (RSV) and reduced the presence of active RSV on the clean side of the simulated doorway with device running. Aerosolized Respiratory Syncytial Virus (RSV) transfer was lower inside the chamber compared to the controls with the AURA Ion Bar™ Mark IV with a 0.003% of collected RSV after 2 minutes from a starting concentration of 5.65×10^6 TCID50/mL; equivalently, 99.997 % (~4.5 log) of active pathogen. The AURA Ion Bar™ Mark IV reduced surface RSV by 59.00% after 10 minutes and to below the assay quantitation limit or $\geq$ 99.998 % ($\geq$ 4.67 log) after 20 minutes. Overall, the AURA Ion Bar™ Mark IV utilizing a combination of methods (laminar air curtain and negative ion generation) reduced the transfer of pathogens through a doorway/portal in the controlled chamber test scenario.

It should be noted that testing was designed to observe the ability of AURA Ion Bar™ Mark IV (controlled airflow and ionizing function ability) to reduce potential exposure to a pathogen. The study focused on the AURA Ion Bar™ Mark IV 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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Laboratory Director, Innovative Bioanalysis, Inc.

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.

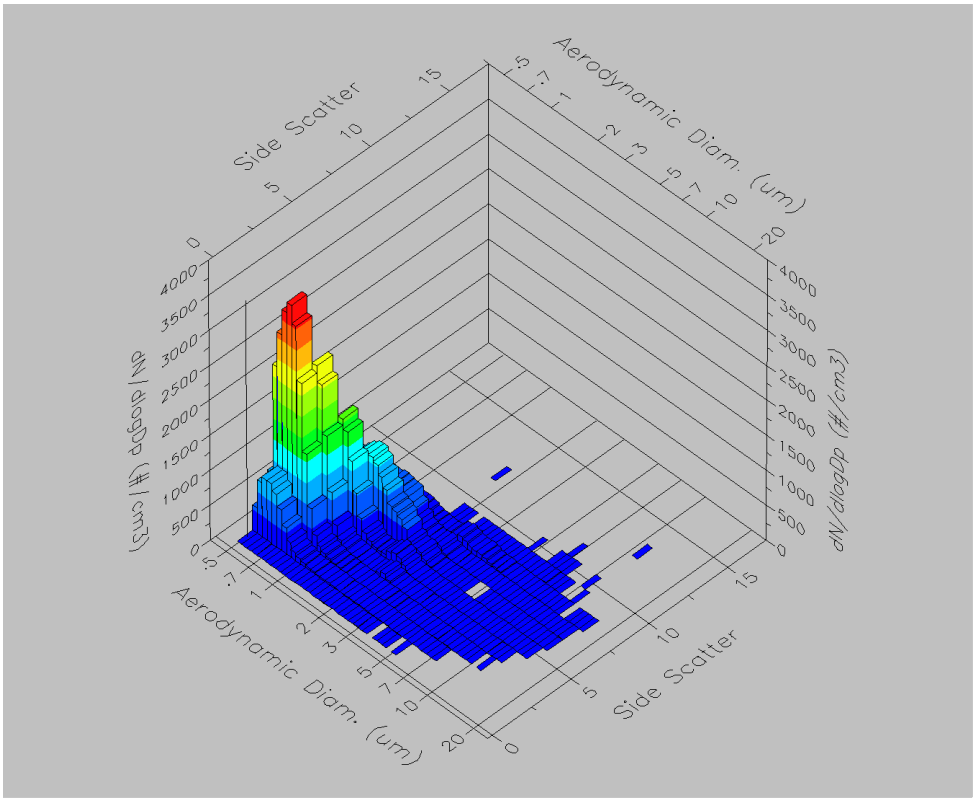
TCID50/mL: The number of infectious virus particles is frequently quantified using the Median Tissue Culture Infectious Dose (TCID50) assay. The assay works by adding a serial dilution of the virus sample to cells in a 96-well plate format. The cell type is specifically selected to show a cytopathic 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 functionality 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 are positive
d = $\log_{10}$ of the dilution factor
n_i = number used in each dilution
r_i = number of positive ls (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
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Gilian 10i Sampling Pumps

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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 10 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

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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