

## AURA TECHNOLOGIES ION BAR MARK IV EFFICACY AGAINST ASPERGILLUS FUMIGATUS

#### **PROJECT: AURA TECHNOLOGIES – ION BAR – FUNGI**

PRODUCT: AURA Ion Bar<sup>™</sup> Mark IV

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

#### **CHALLENGE ORGANISM:**

Aspergillus Fumigatus

#### STUDY COMPLETION DATE:

9/20/2023

#### **Medical Director:**

Dana Yee, M.D.

#### **Testing Facility:**

Innovative Bioanalysis, Inc.

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#### Laboratory Project Number:

1394A

Innovative Bioanalysis, Inc.

AURA TECH ION BAR MARK IV / FUNGI

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## Efficacy Study Summary

Study Title	AURA TECHNOLOGIES ION BAR MARK IV EFFICACY AGAINST ASPERGILLUS FUMIGATUS
Laboratory Project #	1394A
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	Aspergillus Fumigatus
Description	The AURA Technologies AURA Ion Bar <sup>™</sup> Mark IV is an overhead airflow curtain system with an integrated negative ion generator. The AURA Ion Bar <sup>™</sup> 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 testing to determine the efficacy of the AURA Ion Bar <sup>™</sup> Mark IV against Aspergillus Fumigatus.
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 was maintained at 71 °F $\pm$ 2 °F (~21.7 °C $\pm$ 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 Io AURA Ion Bar <sup>TM</sup> Mark IV was mounted above the doorway, approximately 7 ft (2.15 m) above the floor, on the "clean" side of the door. A 5.43 × 10 <sup>4</sup> CFU/mL of Aspergillus Fumigatus fetal bovine serum (FBS) based fungi media was nebulized for aerosol testing. Aerosol samples were
	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.
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Test Results	During aerosol testing, the germicidal barrier blocked an average of 99.982 % (3.75 log) of active Aspergillus Fumigatus from entering through the simulated door opening during device operation, which was quantified at approximately 9.67 CFU/mL.
Control Results	A control was conducted for each subtest with the device fan and ionizer off. Samples taken at the corresponding time points used for each challenge. The results displayed a natural viability loss and served as a comparative baseline to calculate fungi reduction and evaluate device efficacy.
Conclusion	AURA Technologies AURA Ion Bar <sup>™</sup> Mark IV demonstrated significant efficacy as a germicidal partition, reducing the transfer of Aspergillus Fumigatus aerosols to below quantification limits ( <u>&gt;</u> 99.982 %) after 2 minutes.



## Study Report

Study Title: AURA TECHNOLOGIES ION BAR MARK IV EFFICACY AGAINST ASPERGILLYS FUMIGATUS

Sponsor: AURA Technologies, LLC

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

Device Testing: AURA Ion Bar<sup>™</sup> Mark IV

#### Study Dates:

Study Report Date: 9/18/2023 Experimental Start Date: 7/26/2023 Experimental End Date: 8/6/2023 Study Completion Date: 9/1/2023

## Study Objective:

AURA Technologies supplied the AURA Ion Bar<sup>™</sup> Mark IV for testing purposes to determine efficacy against Aspergillus Fumigatus. This study evaluated the effectiveness of the AURA Ion Bar<sup>™</sup> Mark IV in its ability to reduce the pathogen strain referred to as Aspergillus Fumigatus.

## 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. A calibrated nebulizer is 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.

The nebulizer was filled with a  $5.43 \times 10^4$  CFU/mL of Aspergillus Fumigatus in suspension media and nebulized at a flow rate of 1mL/min with untreated local atmospheric air. After nebulization, the nebulizer's remaining fungi stock volume was weighed to confirm that approximately the same amount was nebulized during each run. Bioaerosol procedures for the controls and fungi challenges were performed in the same manner with corresponding time points and collection rates.



Figure 1: BLAM Nebulizer

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# INNOVATIVE BICANALYSIS creating solutions getting results

#### Table 1: Particle Size Distribution Table

	Number	Surface	Mass
	Particle Size	Particle Size	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(#/cm <sup>3</sup> )	7.22e+03(µm²/cm³)	2.38(mg/m <sup>3</sup> )

#### **Bioaerosol Sampling:**

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

#### Test System Strains: Aspergillus fumigatus, Isolate 2

The following reagent was obtained through BEI Resources, NIAID, NIH: Aspergillus fumigatus, Isolate 2, NR-41312.



Colony Forming Unit (CFU) Assay Procedure:

Materials and Equipment:

- Certified Biological Safety Cabinet
- CO<sub>2</sub> Incubator set at 37 °C or 34 °C, or other temperature as indicated
- Inverted Microscope
- Micropipette and sterile disposable aerosol resistant tips—20 uL, 200 uL, 1000 uL
- Tubes for dilution
- Growth media appropriate for fungi growth
- Overlay medium
- Lint-free wipes saturated with 70% isopropyl alcohol

Procedure:

- Prepare a series of 1:10 dilutions of the fungi sample in sterile distilled water filling each tube with 9.0 mL distilled water. Vortex the test sample, then transfer 1.0 mL of the virus to the first tube, vortex, and discard tip.
- 2. With a new tip, serial dilute the subsequent sample transferring 1.0 mL.
- 3. Inoculate the labeled empty Petri dish with each diluted sample, using one plate per dilution.
- 4. Pour overlay medium into the Petri dish, ensuring aseptic techniques are used to prevent contamination.
- 5. Gently swirl the plate to mix culture and medium. Ensure that the medium covers the plate evenly and does not slip over the edge of the dish.
- 6. Wait 10 minutes to allow time for the agar to set.
- 7. Seal and incubate in the appropriate conditions to allow for fungi growth, which may vary depending on the microorganism being cultured.
- 8. Record the number of colonies observed for each dilution. A log drop should be noted between serial dilutions and will vary by 10 % for every 100 colonies counted when comparing sample replicates.
- Identify the fungi dilution factor that yields 30 to 300 colonies per dish. Calculate Virus Titer by counting the number of colonies formed on the plate. Then use the following formula to determine the titer (CFU/mL) of the virus stock:

No. of colonies/
$$(D \times V) = CFU/mL$$

D = Dilution factor

V = Volume of culture plated on to dish

Sample calculation:

- An average of 50 colonies formed in the 1:10,000 dilution wells
- Volume of culture added: 0.2mL

$$\frac{50}{10^{-4} \times 0.2} = 2.50 \times 10^{6} \text{ CFU/mL}$$

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## Study Materials and Equipment:

**Equipment Overview:** The equipment arrived at the laboratory pre-packaged by the manufacturer and was inspected for damage upon arrival. Due to the closed design, no assessment was conducted on the inner components of the device. The device was powered on to confirm functionality before testing. Two Alpha Lab AIC2 ion polarity meters confirmed average ion concentrations of ~960,000 negative ions/cm<sup>3</sup> 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<sup>™</sup> 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<sup>3</sup> (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<sup>™</sup> Mark IV was positioned to replicate the device mounted above a door, clear of any obstructions.

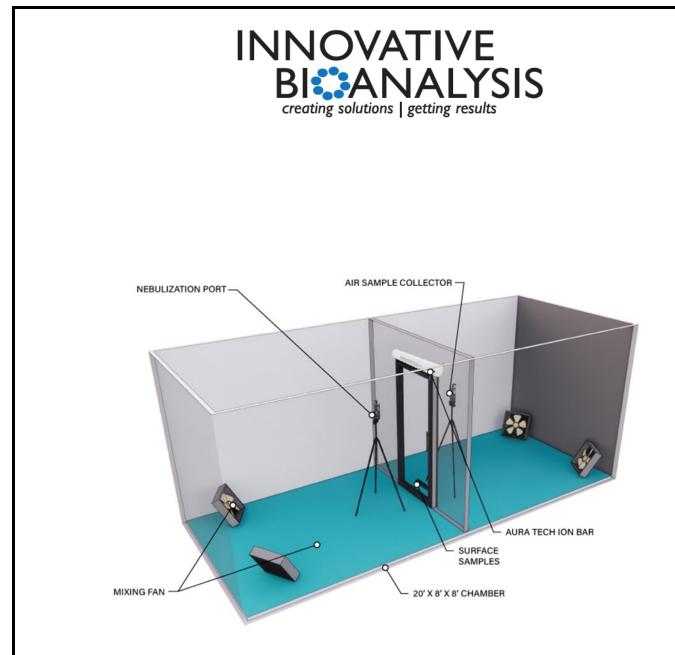


Figure 3. Testing layout for control and experimental trials.



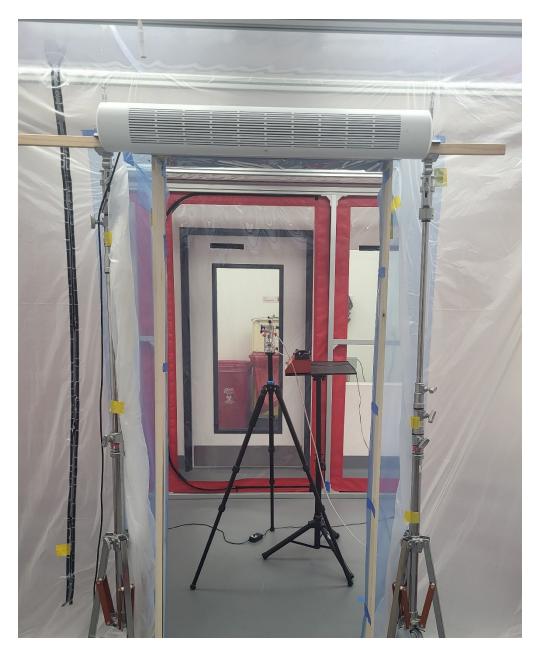


Figure 4. Testing setup.

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## 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<sup>™</sup> 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 %.

#### **Exposure Conditions:**

- 1. Test sample collection occurred between 0 to 2 minutes and was conducted in triplicate.
- 2. Nebulization of fungi 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  $5.43 \times 10^4$  CFU/mL Aspergillus Fumigatus fungi media was nebulized from 0 to 2 minutes to distribute 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 fungi suspension media.
- 5. All samples were sealed and provided to lab staff for analysis after study completion.

#### **Post Decontamination:**

After each fungi 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 testing with the fan and ionizer off to assess the AURA Ion Bar<sup>TM</sup> 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 fungi reduction when the device was operating.

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#### **Preparation of The Pathogen**

Microorganism Stock: Aspergillus fumigatus, Isolate 2 (NR-41312, Lot #: 61697597)

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Colony morphology <sup>2</sup>	Report results	Gray to green with white margins (Figure 1A)
Conidial morphology <sup>2</sup> Conidia, conidial heads	Report results Report results	Globose to ovoid and smooth Columnar strips, smooth-walled, expanding gradually into pyriform (Figure 1B)
Genotypic Analysis Sequencing of internal transcribed spacer (ITS) 1 (~ 560 base pairs)	Consistent with A. fumigatus	Consistent with <i>A. fumigatus</i> <sup>3</sup>
Sequencing of beta-tubulin gene (~ 450 base pairs)	Consistent with A. fumigatus	Consistent with <i>A. fumigatus</i> <sup>4</sup>
Purity⁵		
Nutrient broth with 0.1% Yeast Extract at 25°C	No fungi growth	No fungi growth
Nutrient broth with 0.1% Yeast Extract at 37°C	No fungi growth	No fungi growth
Viability (post-freeze) <sup>2</sup>	Growth	Growth

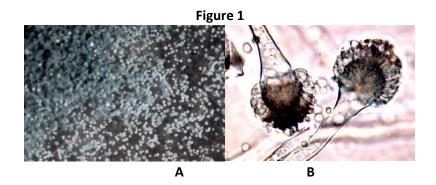
1NR-41312 was produced by growth of the deposited material on Yeast Mold agar in an aerobic atmosphere at 25°C for 7 days.

24 days at 25°C in an aerobic atmosphere on Yeast Mold agar

3Sequence aligns with 100% similarity to *A. fumigatus* type strain 118 (ATCC₀ 1022™)

₄Sequence aligns with >99.7% similarity to *A. fumigatus* type strain 118 (ATCC<sub>®</sub> 1022<sup>™</sup>)

sClarity of broth was determined by visual inspection after 3 days at 25°C and 37°C in an aerobic atmosphere.



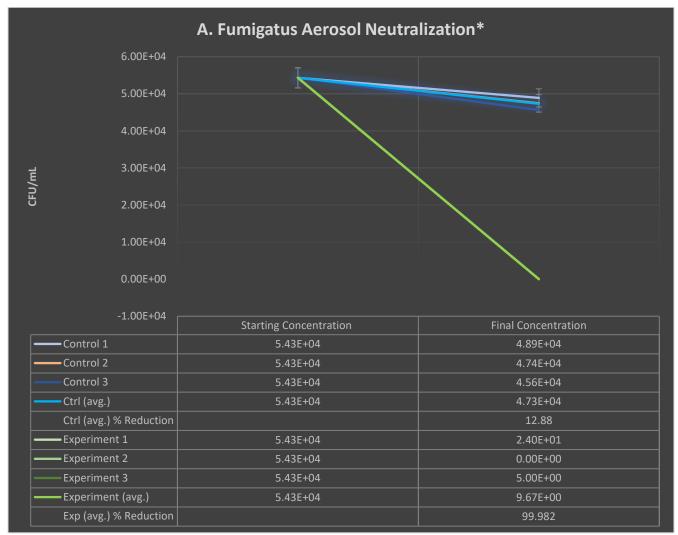
The fungi were prepared at Innovative Bioanalysis using a proprietary procedure, identified internally as SOP# MICRO 108.01. In summary, a host fungus was rehydrated with molecular-grade water.

\*The Certificate of Analysis represents the titer provided by ATCC, on behalf of BEI Resources. See Appendix E for more details.



## Study Results

Testing was conducted to determine any pass-through of aerosolized Aspergillus Fumigatus through the AURA Ion Bar<sup>™</sup> Mark IV air curtain within 2 minutes of device operation. From the inside of the air barrier, the concentration of active Aspergillus Fumigatus collected at 2 minutes from three test runs were (24, 0, and 5) CFU/mL, averaging to approximately 9.67 CFU/mL. The presence of Aspergillus Fumigatus indicated about 0.018% of nebulized Aspergillus Fumigatus passed through the barrier after 2 minutes resulting in a 99.982 % reduction of nebulized Aspergillus Fumigatus on the protected side. The control observed an 87.12 % passthrough of nebulized Aspergillus Fumigatus after 2 minutes with the AURA Ion Bar<sup>™</sup> Mark IV fan and ionizer off.



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

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## Conclusion:

The AURA Ion Bar<sup>TM</sup> Mark IV demonstrated a significant ability to prevent the pathogen from entering the chamber with the test unit running. Aerosolized Aspergillus Fumigatus transfer was significantly restricted through the AURA Ion Bar<sup>TM</sup> Mark IV germicidal partition with a 0.003% of collected Aspergillus Fumigatus after 2 minutes from a starting concentration of  $5.43 \times 10^4$  CFU/mL; equivalently, 99.982 % (3.75 log) of active pathogen was blocked by the air barrier. Overall, the AURA Ion Bar<sup>TM</sup> Mark IV utilized a combination of methods (laminar air curtain and negative ion generation) to reduce the transfer of pathogens through a doorway/portal if installed correctly.

It should be noted that testing was designed to observe the ability of AURA Ion Bar<sup>™</sup> Mark IV (controlled airflow and ionizing function ability) to reduce potential exposure to a pathogen. The study focused on the AURA Ion Bar<sup>™</sup> 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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Dana Yee M.D	Date
Clinical Pathologist and Medical Director, Innovative Bioanalysi	s, Inc.
Sam tahbani 88482820E483443	10/16/2023
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Chief Scientific Officer, Innovative Bioanalysis, Inc.	
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Albert Brockman	Date
Chief Biosafety Officer, Innovative Bioanalysis, Inc.	
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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 AURA Technologies' units. Innovative Bioanalysis, Inc. makes no claims to the overall efficacy of any Ion Bar. 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, fungi strain, collection method, inoculation, nebulization, fungi 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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## 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).

CFU/mL: A CFU or colony-forming unit is a unit for measuring microorganism concentration in a test sample. The total count of observable colonies on an agar plate is multiplied with the dilution factor to provide the resulting CFU/mL.

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<sub>2</sub>HPO<sub>4</sub>, and 2 mM KH<sub>2</sub>PO<sub>4</sub>.

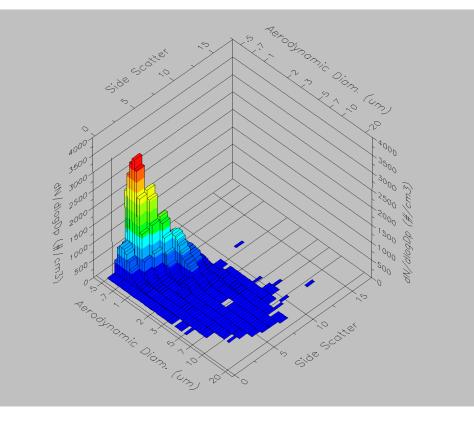


## APPENDIX B: Particle Size Distribution

The TSi Aerodynamic Particle Sizer<sup>®</sup> (APS<sup>™</sup>) 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 fungi 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(#/cm <sup>3</sup> )	7.22e+03(µm²/cm³)	2.38(mg/m <sup>3</sup> )



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## APPENDIX C: Calculation equations

## CFU/mL calculation method:

No. of colonies/ $(D \times V) = CFU/mL$ 

D = Dilution factor V = Volume of culture plated on to dish

## 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

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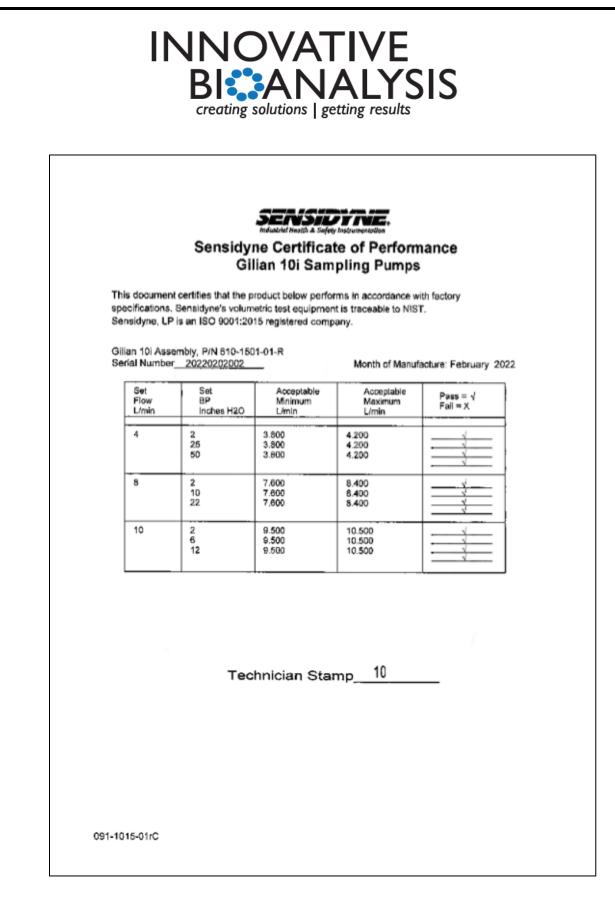
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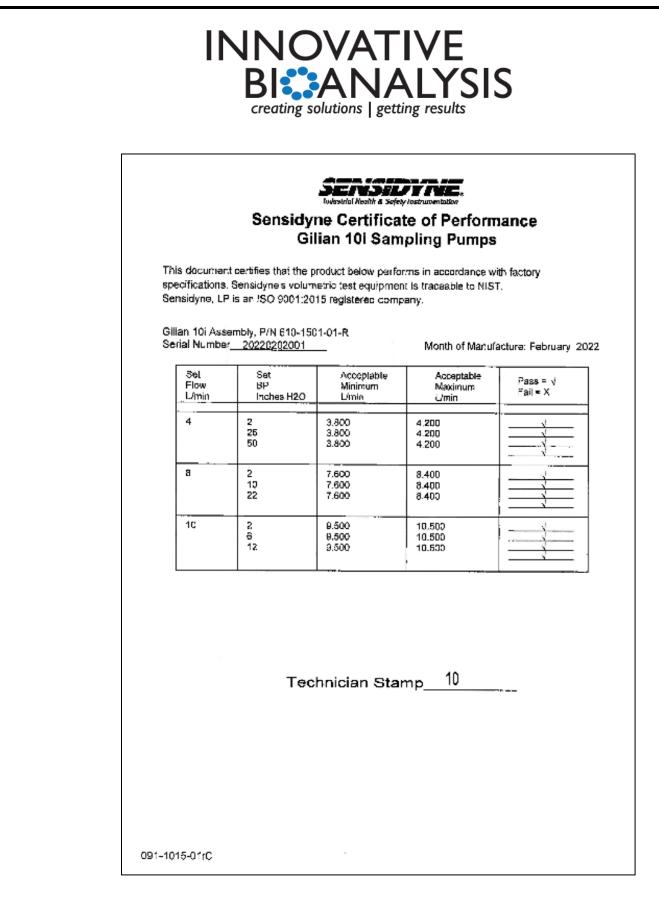
## APPENDIX D: Equipment Calibration Certificates

	Sanalda	Industrial Health & Sufe		
			te of Perform pling Pumps	
This documen	t certifies that the p	roduct below perfo	rms in accordance w	ith factory
specifications. Sensidyne, LP	Sensidyne's volum is an ISO 9001:20	etric test equipment 15 registered comp	nt is traceable to NI\$1 pany.	r.
Gilian 10i Asse	embly, P/N 610-150	)1-01-R		
Serial Number	20220202003	-	Month of Manufa	acture: February 2022
Set Flow L/min	Şət 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 4.200	
8	2 10 22	7.600 7.600 7.600 7.600	8.400 8.400 8.400	
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