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EFFICACY OF THE PURIFI LABS™ IAI-100 AGAINST AEROSOLIZED SARS-COV-2 OMICRON

PROJECT: PURIFI IAI-100 AEROSOL SARS-COV-2 OMICRON VARIANT

PRODUCT: PURIFI LABS™ IAI-100

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM:

SARS-COV-2 OMICRON VARIANT

STUDY COMPLETION DATE:

01/26/2022

Medical Director

Dana Yee, M.D.

Testing Facility

Innovative Bioanalysis, Inc.

3188 Airway Ave Suite D

Costa Mesa CA, 92626

www.InnovativeBioanalysis.com

Email: info@innovativeBioanalysis.com

Laboratory Project Number

1236

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Efficacy Study Summary

Study Title	EFFICACY OF THE PURIFI LABS™ IAI-100 AGAINST AEROSOLIZED SARS-COV-2 OMICRON VARIANT
Laboratory Project #	1236
Guideline:	No standard exists; GLP and modified ISO standards were used.
Testing Facility	Innovative Bioanalysis, Inc.
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	SARS-CoV-2 Omicron Variant
Description	Per the manufacturer, the PuriFi IAI-100 Generator is a commercially available HVAC mounted disinfection device for installation and use within central heating and ventilation systems. The device was provided by PuriFi Labs™ for an in vitro study to evaluate the efficacy of the IAI-100 against aerosolized SARS-CoV-2 Omicron variant.
Test Conditions	A modified HVAC ducting housing the device in a 20'x8'x8' BSL-3 chamber was used for testing. The temperature during testing was 73 ±2°F, with a relative humidity of 37%. A 6.83 x 10^6 TCID50/mL of SARS-CoV-2 Omicron variant in viral media was nebulized into the room with mixing fans before collection. Air sample collections occurred after 0, 14, 29, and 59 minutes of device operation.
Test Results	The device continually reduced collectable active SARS-CoV-2 Omicron variant more rapidly than the natural loss rates. After 59 minutes of operation, a concentration below the specified limit of quantitation (1.20 x 10^2 TCID50/mL) was observed.
Control Results	A static and dynamic airflow control were conducted and plotted to show the observable natural loss rate over 59 minutes. The static control simulates the device on standby while the airflow control replicates a running HVAC system without the introduction of the ionization device. The data was used to assess the device's ability to reduce aerosolized pathogens.
Conclusion	The PuriFi Labs™ IAI-100 demonstrated a consistent and progressive reduction of active aerosolized SARS-CoV-2 Omicron variant. After 59 minutes of exposure, a 99.998% gross reduction was observed, indicative of a 4.7 log reduction.

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Study Report

Study Title: EFFICACY OF THE PURIFI LABS™ IAI-100 AGAINST AEROSOLIZED SARS-COV-2 OMICRON

Sponsor: PuriFi Labs™

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

Device Testing: IAI-100

Study Dates:

Experimental Start Date: 12/29/2021
Experimental End Date: 01/02/2022
Study Completion Date: 01/26/2022
Study Report Date: 01/26/2022

Study Objective:

An ion generating device, IAI-100, was provided by PuriFi Labs™ for testing to determine its effectiveness against an aerosolized virus, SARS-CoV-2 Omicron variant, under controlled conditions.

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 present to confirm average particle size distribution. The nebulizer was filled with 6.83 x 10^6 TCID50/mL of SARS-CoV-2 Omicron variant in viral 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 four 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 had a delicate internal filtration disc (Fig. 2A) to collect virus samples, which was moistened with a virus suspension media to aid in the collection. Filtration discs from Zefon International, Lot# 26338, were used for testing.

Test System Strains: SARS-CoV-2 Omicron Variant

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

TCID50 Procedure:

Materials and Equipment:

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips—20uL, 200 uL, 1000uL
- Inverted Microscope
- Tubes for dilution
- Hemocytometer with coverslip
- Cell media for infection
- Growth media appropriate for the cell line
- 0.4% Trypan Blue Solution
- Lint-free wipes saturated with 70% isopropyl alcohol
- CO_2 Incubator set at 37°C or 34°C, or other temperature as indicated

Procedure:

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

Additions of virus dilutions to cells:

1. Label the lid of a 96-well dish by drawing grid lines to delineate quadruplicates and number each grid to correspond to the virus sample and label the rows of the plate for the dilution, which will be plated.
2. Include four (4) negative wells on each plate which will not be infected.
3. Remove all but 0.1 mL of media from each well by vacuum aspiration.
4. Starting from the most dilute sample, add 0.1 mL of virus dilution to each of the quadruplicate wells for that dilution.
5. Infect four wells per dilution, working backward.
6. Allow the virus to absorb to the cells at 37°C for 2 hours.
7. After absorption, remove the virus inoculum. Start with the most dilute and work backward.
8. Add 0.5 mL infection medium to each well, being careful not to touch the wells with the pipette.
9. Place plates at 37°C and monitor CPE using the inverted microscope over a period of 1 to 4 weeks.
10. Record the number of positive and negative wells.

Study Materials and Equipment:

Equipment Overview: The IAI-100 (Fig. 3) arrived at the laboratory pre-packaged from 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. Before testing, the PuriFi Labs™ IAI-100 device was powered on and operated for 1 hour in a dry run to confirm correct operations.

MANUFACTURER: PuriFi Labs™

MODEL: IAI-100

DIMENSIONS: 12” x 8.5” x 14” (with antenna)

MAKE: PuriFi Generator

SERIAL #: 9454935D9913

Figure 3. PuriFi Labs™ IAI-100 device tested.

Testing Layout:

Testing was conducted in a sealed 20'x8'x8' chamber per Biosafety Level 3 (BSL3) standards. The overall dimensions of the test chamber provided a displacement volume of 1,280 cubic feet (approximately 36,245.56 liters) of air. The room remained closed to prevent any air from entering and leaving the room during testing. A nebulizing port connected to a programmable compressor system was located in the center of the 20 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 four probes for air sampling positioned along the room's centerline and protruded down from the ceiling 24-inches.

A modified HVAC system with an air handler box housing the PuriFi IAI-100™ device was used. The air handling box was located downstream from a fan unit and was situated in one corner of the room with a vertical stack of ducting. The ducting traveled along the chamber ceiling, creating an internal duct line with two diffusers in the room's centerline, as shown in Figure 4. The modified HVAC system made an airflow of approximately eight air changes per hour through the circular ducting. Before testing, the chamber was visually inspected, pressure tested, and all internal lab systems and equipment were reviewed.

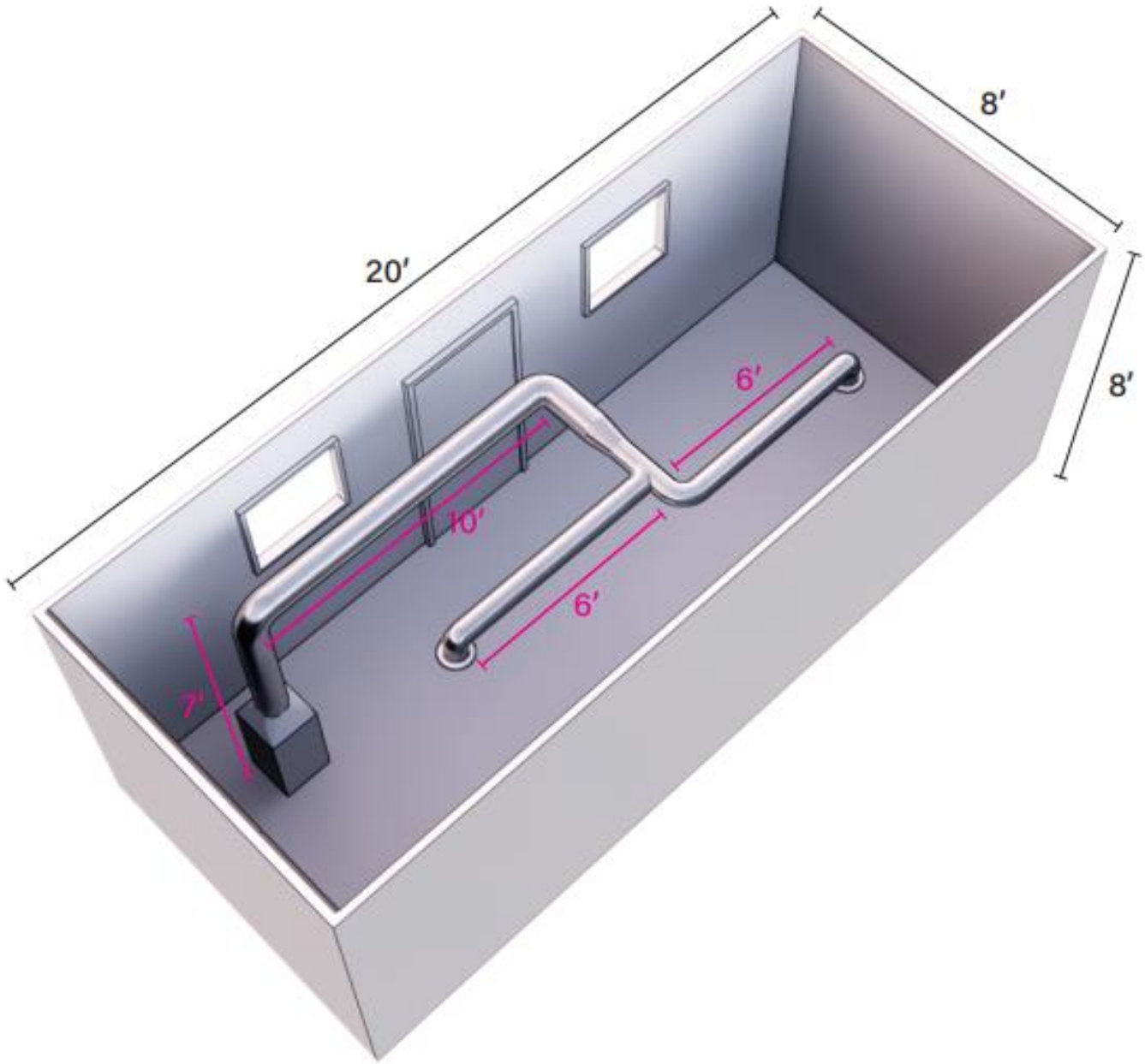


Figure 4. Testing layout for control and experimental trials.

Test Method:

Exposure Conditions:

1. The temperature during all test runs was approximately 73 ±2°F, with a relative humidity of 37%. Testing conditions were taken in two sections of the chamber to confirm a deviation of less than ±3%.
2. Test conditions were a "Hot Start" scenario where the ion and modified internal HVAC system were turned on 15 minutes before the introduction of the pathogen.
3. Testing time points were as follows, with T equal to minutes: T-0, T-14, T-29, and T-59.

Experimental Procedures:

1. 10mL of a 6.83 x 10^6 TCID50/mL SARS-CoV-2 Omicron variant viral media was nebulized into the sealed environment via the dissemination port.
2. Air sampling collection occurred after nebulization ceased for the challenges and control test.
3. After each run, sample cassettes were manually removed from the collection system and taken to an adjacent biosafety cabinet to be pooled.
4. All samples were sealed upon 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 was purged for 30-minutes. All test equipment was cleaned at the end of each day with a 70% alcohol solution. Collection lines were soaked in a bleach bath mixture for 30 minutes then rinsed repeatedly with DI water. The nebulizer and vacuum collection pumps were decontaminated with hydrogen peroxide mixtures.

Control Protocol:

To accurately assess the PuriFi IAI-100, a static decay control and airflow control was conducted without the device operating in the testing chamber. The static decay control was performed to simulate an HVAC system in standby mode, and the airflow control acts as an active HVAC system without an air purification device. The collection was taken at corresponding time points used for the challenge trial, in the same manner, to serve as a comparative baseline to assess aerosolized viral reduction when the device was operating.

Preparation of The Pathogen

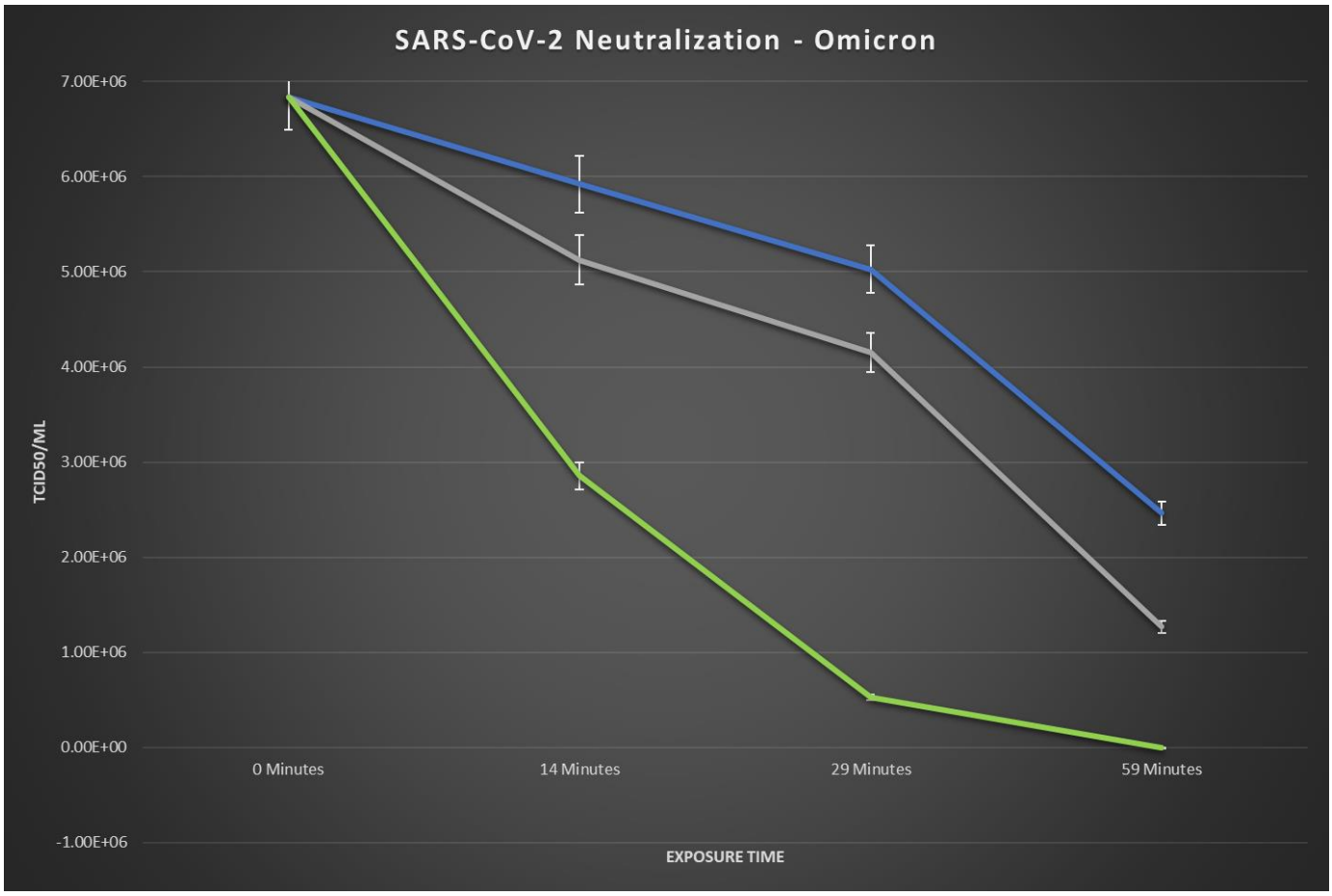
Viral Stock: SARS-CoV-2; Omicron Variant (BEI NR-56461)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Calu-3 Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of the complete genome using Illumina® iSeq™ 100 Platform	≥ 98% identity with SARS-CoV-2, hCoV-19/USA/MD-HP20874/2021 (GISAID: EPI_ISL_7160424)	99.99% identity with SARS-CoV-2, hCoV-19/USA/MD-HP20874/2021 (GISAID: EPI_ISL_7160424)
Titer by $TCID_{50}$ in Calu-3 Cells by Cytopathic Effect	Report Results	4.4 X 10^5 $TCID_{50}$ per mL^2
Sterility (21-Day Incubation)		
Harpos HTYE Broth, aerobic	No Growth	No Growth
Trypticase Soy Broth, aerobic	No Growth	No Growth
Sabourad Broth, aerobic	No Growth	No Growth
Sheep Blood Agar, aerobic	No Growth	No Growth
Sheep Blood Agar, anaerobic	No Growth	No Growth
Thioglycollate Broth, anaerobic	No Growth	No Growth
DMEM with 10% FBS	No Growth	No Growth
Mycoplasma Contamination		
Agar and Broth Culture	None Detected	None Detected
DNA Detection by PCR of extracted test article nucleic acid	None Detected	None Detected

*The viral titer listed in the Certificate of Analysis represents the titer provided by BEI Resources.

Study Results:

The below graph displayed recoverable active aerosolized SARS-CoV-2 Omicron variant with and without the IAI-100 device operating. The static and dynamic controls showed natural viability loss of aerosolized SARS-CoV-2 Omicron variant for 59 minutes within the chamber under controlled conditions. With the IAI-100 unit running for 14 minutes, an initial concentration of 6.83 x 10^6 TCID50/mL was reduced to 2.85 x 10^6 TCID50/mL and was further reduced to 5.25 x 10^5 TCID50/mL after 29 minutes. After 59 minutes, aerosolized SARS-CoV-2 Omicron variant was reduced to below levels of quantification represented by the value 1.20 x 10^2 TCID50/mL.



**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.

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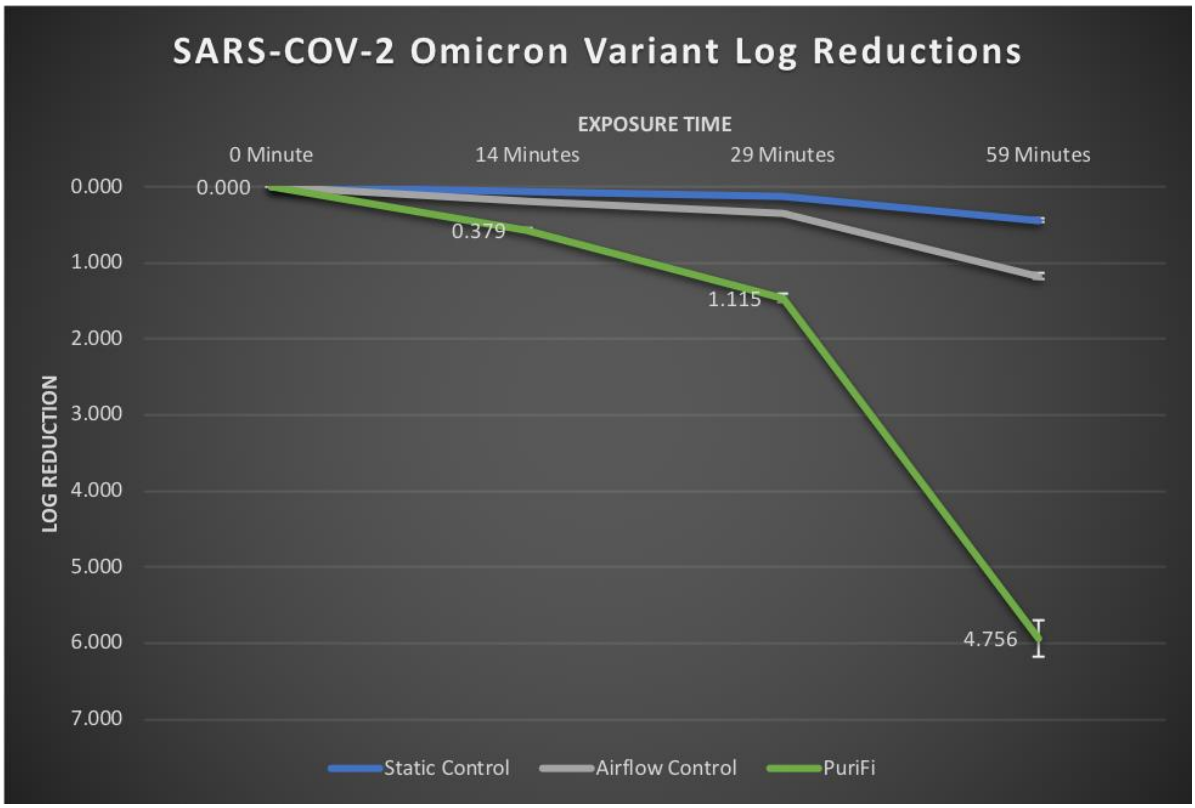
VIRAL VOLUME REDUCTION OF SARS-COV-2 OMICRON (SCIENTIFIC)**			
	Static Control	**Airflow Control**	**PuriFi**
0 Minutes	6.83E+06	6.83E+06	6.83E+06
14 Minutes	5.92E+06	5.13E+06	2.85E+06
29 Minutes	5.03E+06	4.15E+06	5.25E+05
59 Minutes	2.46E+06	1.27E+06	1.20E+02

VIRAL VOLUME REDUCTION OF SARS-COV-2 OMICRON (NUMERICAL)**			
	Static Control	**Airflow Control**	**PuriFi**
0 Minutes	6,830,000	6,830,000	6,830,000
14 Minutes	5,920,000	5,130,000	2,850,000
29 Minutes	5,030,000	4,150,000	525,000
59 Minutes	2,460,000	1,270,000	120

VIRAL LOG REDUCTION OF SARS-COV-2 OMICRON***			
	Static Control	**Airflow Control**	**PuriFi**
0 Minutes	0.000	0.000	0.000
14 Minutes	0.062	0.124	0.379
29 Minutes	0.133	0.216	1.114
59 Minutes	0.442	0.732	4.755

PURIFI VIRAL INACTIVATION OF SARS-COV-2 OMICRON***			
	PuriFi vs Static Control	**PuriFi vs Airflow Control**	**PuriFi Gross Reduction**
0 Minutes	0.000%	0.000%	0.000%
14 Minutes	51.819%	44.338%	58.250%
29 Minutes	89.562%	87.372%	92.324%
59 Minutes	99.995%	99.990%	99.998%



Conclusion:

The Purifi Labs™ IAI-100 device performed to manufacturer specifications and demonstrated a consistent progressive reduction of the active virus at 14 minutes, 29 minutes, and 59 minutes of exposure in aerosol form. The amount of active SARS-CoV-2 Delta variant in the atmosphere was reduced over the control samples by approximately 99.995% vs the static control and 99.990% vs the airflow control, with a total reduction of 99.998%.

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, viral destruction on collection, viral 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, there was a measurable amount of reduction achieved by the PuriFi Labs™ IAI-100 device at each of the T-14, T-29, and T-59 time points. The decline in air was consistent with indications that the PuriFi Labs™ IAI-100 device can decrease the concentration of active pathogens in the air. Overall, the IAI-100 device showed efficacy in reducing SARS-CoV-2 Omicron variant from air samples collected.

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DocuSigned by:
Dr. Dana Yee MD.
52F0012FC10C43F...

6/2/2022

Dana Yee M.D **Date**

Clinical Pathologist and Medical Director, Innovative Bioanalysis, Inc.

DocuSigned by:
Sam Kabbani
8B4B282DF4B34A3...

6/2/2022

Sam Kabbani, MS, BS, MT(ASCP), CLS **Date**

Chief Scientific Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Albert Brockman
42751B72F2FF40A...

6/2/2022

Albert Brockman **Date**

Chief Biosafety Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Kevin Noble
5DE2797BAA78421...

6/2/2022

Kevin Noble **Date**

Laboratory Director, Innovative Bioanalysis, Inc.

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