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SARS-CoV-2 USA-CA1/2020

CLIENT: PURIFI LABS

PROJECT: BI-POLAR COLD PLASMA WITH O2 CATALYST, IONIZATION AEROSOL TEST

PRODUCT: PURIFI IAI-100

CAP LIC NO: 886029801

CLIA LIC NO: O5D0955926

STATE ID: CLF 00324630

CHALLENGE VIRUS: SARS-CoV-2 USA-CA1/2020

LABORATORY PROJECT NUMBER (STUDY FILE): 1025

STUDY TEST DATE: 02/19/2021

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ABSTRACT: EFFICACY OF THE PURIFI LABS IAI-100™ DEVICE AGAINST AEROSOLIZED SARS-CoV-2

Background: This in vitro study was designed to determine the efficacy of the PuriFi Labs IAI-100™ air purification unit. The product is a commercially available HVAC mounted disinfection device manufactured by PuriFi Labs. The PuriFi Labs IAI-100™ system tested is designed for installation and use in central heating and ventilating air conditioning systems to decrease the concentration of pathogens in the air and on surfaces when operating. The PuriFi technology is designed to produce positively and negatively charged ions during operation. For this challenge, the SARS-CoV-2 USA-CA1/2020 pathogen was used. There is a demand for disinfectant devices that have a proven ability to reduce infectious pathogens in the air and on surfaces thereby reducing the risk of human infection and transmission. PuriFi Labs supplied a pre-packaged IAI-100™ unit for testing purposes. For the testing, power was supplied through a power regulated 120v outlet with surge protector and backup battery system. Test procedures were followed using internal SOPs for aerosolized viral pathogen challenges and subsequent decontamination. All internal SOPs and processes follow GCLP guidelines and recommendations.

EQUIPMENT PROVIDED:

MANUFACTURER: PURIFI LABS

MODEL: PURIFI IAI-100™

SERIAL #: 9454935D9913



PURIFI LABS IAI-100™ EQUIPMENT:

The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. Prior to starting the challenge, the PuriFi Labs IAI-100™ unit was operated for multiple hours of dry runs in a sealed bioaerosol chamber to confirm correct operation of the system. Chamber was the same BSL3 chamber used for the viral challenge testing.



VIRAL CHALLENGE TESTING CHAMBER:

The testing chamber was a large, sealed air volume testing chamber consisting of metal walls and epoxy floor which complied with BSL3 standards. The chamber was designed to be completely sealed from the outside environment to prevent any potential release of testing media into the atmosphere. The testing chamber was equipped with 4 sealed viewing windows and a lockable chamber door for entry and exit. The overall dimensions of the test chamber were approximately 8'x8'x20' with a displacement volume of 1280 cubic feet.

The testing chamber had HEPA filtered inlets and exhaust which were used for decontamination purposes after the test and were coupled with an active UV-C system in all ducting lines. Humidity and temperature were monitored inside the chamber using a calibrated wireless device. For air sample testing, the chamber was equipped with 4 probes that were along the centerline of the room and protruded down from the ceiling 24". Each probe tube was connected to a Gilian 10i programmable system with sampling cassettes from lot 23166 made by Zefon International. A single bioaerosol nebulizing port was in the center of the 20' wall opposite of the entry doors. The dissemination port protruded from the wall 24" and was connected to a programmable compressor nebulizer system.

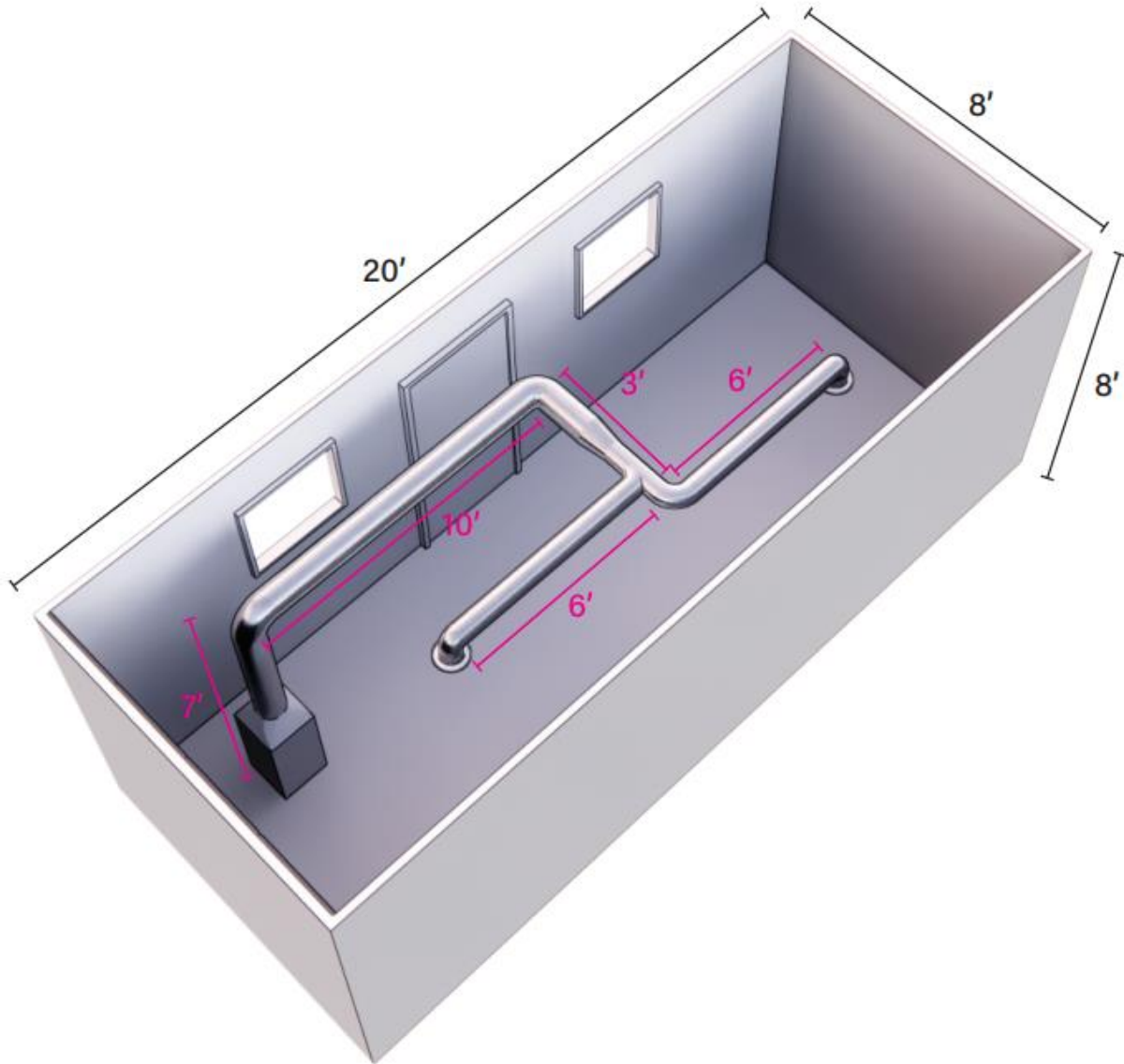
Prior to testing, the chamber was pressure tested for leaks and visual inspections were made using a colored smoking device. All seals for the chamber were confirmed and all equipment used had a function tests done to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

To test in conditions like a realistic scenario under the constraints, a modified HVAC system was built in the interior of the testing chamber. The modified HVAC consisted of an air handler box where the testing unit was placed on the downstream side of a fan unit. The air handling box was placed in one corner of the room with a vertical stack of ducting then traveled along the ceiling of the bioaerosol chamber. This created an internal duct line which had two diffusers almost in the center line of the room, positioned in similar interval distances to that of a typical indoor environment being served by a central HVAC system (please refer to the chamber layout diagram). With the test conditions, air inside the bioaerosol chamber was moved through the room through the HVAC system and the secondary function delivered consistent ion concentrations across the room. An airflow of approximately 8 air changes per hour was created through the modified HVAC system through the circular ducting.

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CHAMBER LAYOUT:



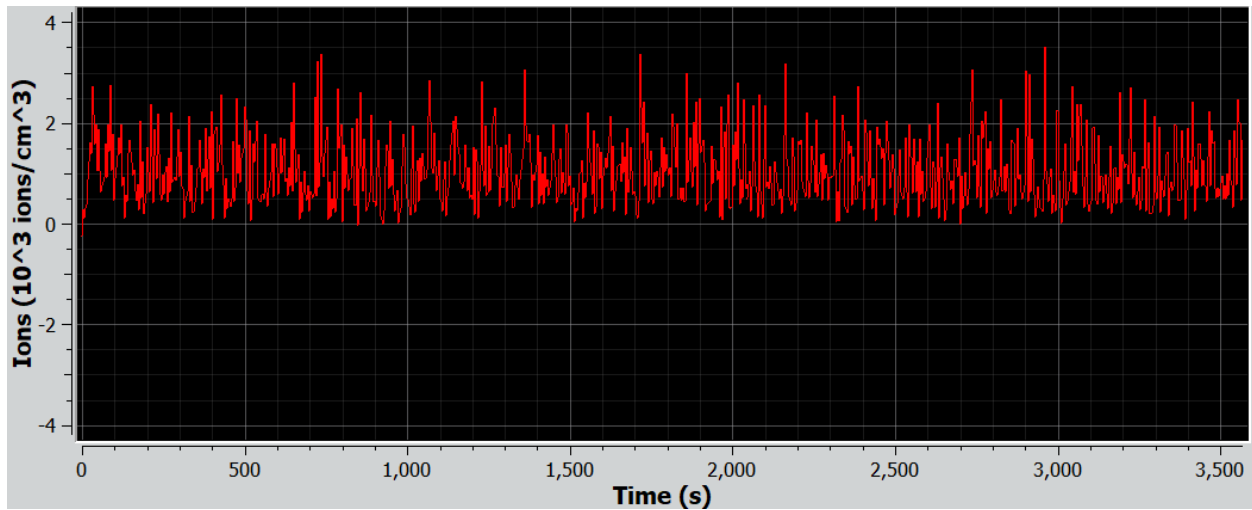
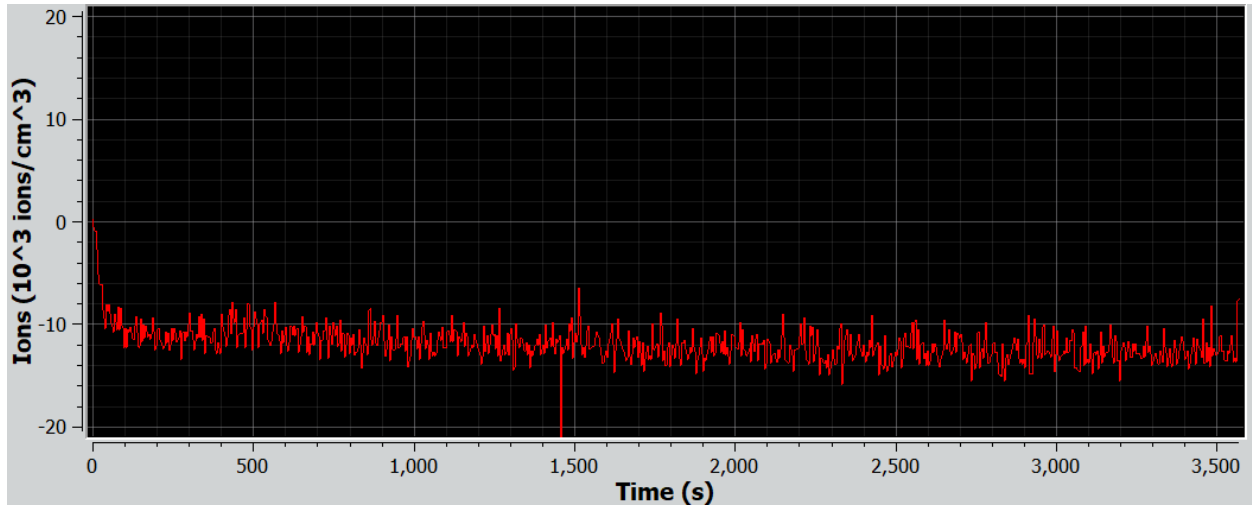


EXPERIMENTAL SUMMARY:

- Prior to the initial control test and following each trial run the testing area was decontaminated and prepped per internal procedures.
- Temperature during all test runs was approximately 75F +/- 2F with a relative humidity of 43%.
- Relative humidity and temperature were taken in two sections of the chamber during all tests to confirm there was no more than a 3% deviation from each side.
- Air samplers were calibrated by the manufacturer on September 3, 2020 and set at a standard flow of 5.02L/min. Calibration records indicate a 0.20% tolerance.
- All sample collection volumes were set to 10-minute draws per time point.
- Test conditions were a "Hot Start" scenario where the Ion and modified internal HVAC system were turned on 15 minutes prior to the introduction of the pathogen into the environment.
- Dry run testing was conducted in the bioaerosol test chamber prior to introducing the pathogen to determine average ion concentrations.
- Positive and negative Ion levels were measured using multiple Alpha Labs AIC2 devices.
- Average negative ion density per cubic centimeter was 1,900.
- Test condition was a high viral load efficacy test in a simulated indoor environment with a central HVAC system, where a known amount of pathogen was aerosolized into the air through a central nebulization system.
- A single bioaerosol nebulizing port was in the center of the 20' wall opposite of the entry doors. The dissemination port protruded from the wall 24" and was connected to a programmable compressor nebulizer system that dispensed a predefined amount of viral media.
- Air sampling was taken at four different time points, 3 minutes, 7 minutes, 14 minutes, and 29 minutes of elapsed exposure time.
- Each time point was completed as an independent test scenario to maximize collection values.
- Aerosolization for control and viral test challenges were performed in the same manner.
- Sample cassettes were manually removed from the collection system and stored after each time point and replaced with new cassettes.
- Upon cassette removal at each time point, cassette sets were taken to an adjacent bio safety cabinet and pooled.
- Four total sample times were collected per bioaerosol time point.
- 1 static control and 1 airflow control were completed for each time point, and 1 viral challenge for each time point were completed using the same methodology.

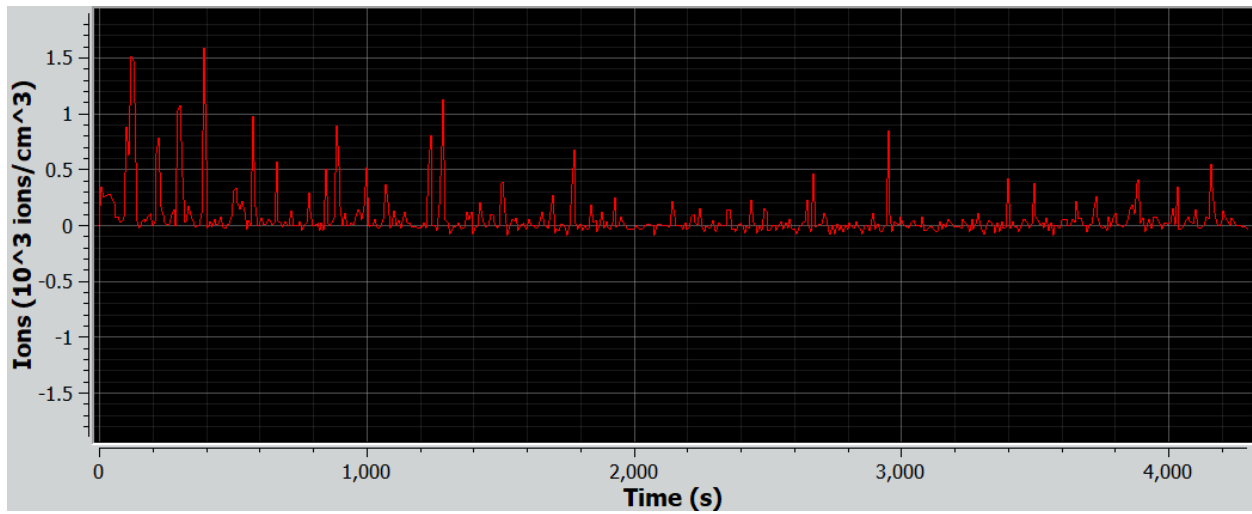
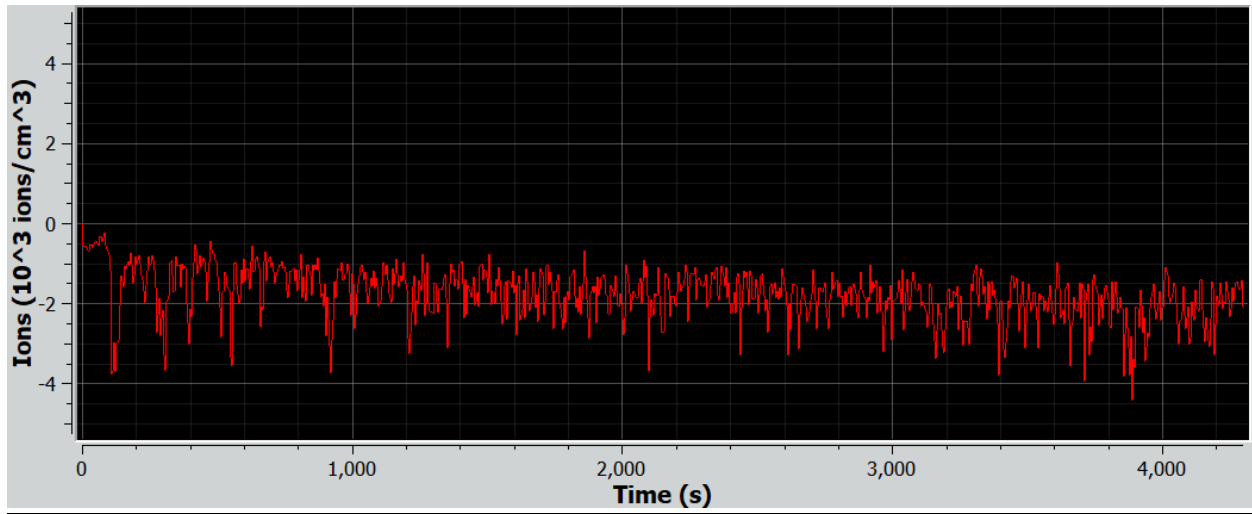


ION CONCENTRATIONS DIRECTLY UNDER HVAC DIFFUSER VENTS:





ION CONCENTRATIONS IN SIMULATED BREATHING ZONE, 2 FEET AWAY FROM DIFFUSER VENTS:



**BIOAEROSOL GENERATION:**

Controls samples were performed in the same manner as the viral test at the time-points and rate of collection. A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 4.50×10^6 TCID₅₀/mL* was used for this experiment. For the purposes of this experiment, and to simulate a high viral load in an indoor environment with a central HVAC system, exactly 15 mL of virus was aerosolized and homogeneously mixed from the central nebulization port of the containment unit. The concentration of pathogen was chosen to saturate that space and limit environment variables that would make collecting a true sample less likely.

BIOAEROSOL SAMPLING:

For air sampling, 4 different Gillian 10i programmable vacuum devices were used. Air samplers were calibrated by the manufacturer in September 2020 and certificates were inspected prior to use. Air sample volume collections were confirmed prior to use with a Gilian Gilibrator 2 SN- 200700-12 and a high flow bubble generator SN-2009012-H. Air samplers were operated in conjunction with removable sealed cassettes, which were manually removed after each sampling time point. Cassettes had a delicate internal filtration disc to collect viral samples.

VIRUS STRAIN BACKGROUND:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-CA1/2020 (also referred to as USA_CA_NP530_F11/2020) was isolated from a nasopharyngeal swab from patient in California, USA on January 23, 2020. The pathogen was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-CA1/2020, NR-52382.”

POST DECONTAMINATION:

At the conclusion of each viral challenge test the UV system inside the testing chamber was activated for 30 minutes. After 30 minutes of UV exposure the chamber was fogged with a Hydrogen Peroxide gas mixture followed by a 30-minute air purge. 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. Nebulizer and Vacuum collection pumps were decontaminated with Hydrogen Peroxide mixtures.

**The viral titer listed in the Certificate of Analysis is representative of the titer provided by BEI Resources. These viruses are grown on VeroE6 cells either inhouse or at a partner lab to the concentrations listed within the experiment design.*



TCID50 PROCEDURE:

Materials and Equipment:

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips – 20uL, 200uL, 1000uL
- Inverted Microscope
- Tubes for dilution
- Hemocytometer with cover slip
- Cell Media for infection
- Growth Media appropriate for cell line
- 0.4 % Trypan Blue Solution
- Lint Free Wipes saturated with 70% isopropyl alcohol.
- CO₂ Incubator set at 37°C or 34°C or other temperature indicated.

Procedure:

1. One day prior to infection, prepare 96 well dishes by seeding each well with Vero E6 cells in DMEM plus 7.5 % fetal bovine serum, 4mM Glutamine, and antibiotics.
2. On the day of infection, make dilutions of virus sample in PBS.
3. Make a series of dilutions at 1:10 of the original virus sample. First tube with 2.0 mL PBS and subsequent tubes with 1.8mL.
4. Vortex Viral samples, transfer 20 uL of virus to first tube, vortex, discard tip.
5. With new tip, serial dilute subsequent tips transferring 200 uL.

Additions of virus dilutions to cells

1. Label lid of 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 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 4 wells per dilution, working backward.
6. Allow the virus to absorb to cells at 37°C for 2 hours.
7. After absorption, remove virus inoculum. Start with the most dilute and work backwards.
8. Add 0.5 mL infection medium to each well being careful to not 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.

**CONTROL:**

Two Control tests were conducted without the PuriFi Labs IAI-100™ unit operating in the testing chamber: One static decay control to simulate a HVAC system in standby mode, and one airflow control used to simulate active HVAC system air circulation/ventilation without the air purification device in the chamber. The airflow control was conducted at the same airflow as the test challenge. Control samples were taken at each of the corresponding sample times used for the challenge for each of the control scenarios. Aerosolization of viral media and collection methods were the same for the controls as the viral challenge. Control testing was used for the comparative baseline to assess the viral reduction when the PuriFi Labs IAI-100™ device was operated versus simulated scenarios of a HVAC system in standby mode (static control) and a HVAC system actively operating and circulating air (airflow control). During the control tests and the challenge, four low volume fans were operated in each corner of the testing chamber to simulate occupant activity and to ensure homogenous mixing of the air. An emergency O3 alarm was outfitted to the room and calibrated to 1ppm. During the control tests and the challenge test, no O3 alarms were observed. During the controls and the challenge, temperature and relative humidity were monitored. Prior to running the viral challenges temperature and humidity were confirmed to be in relative range to the control +/- 5%.

VIRAL CHALLENGE:

The challenge pathogen, SARS-CoV-2 USA-CA1/2020, was used for testing the efficacy of the PuriFi Labs IAI-100™ device. During the challenge tests the pressure in the challenge chamber was monitored to confirm no portion of the chamber was leaking. The bioaerosol efficacy challenge was completed in three distinct trials with the live pathogen to create a baseline of data. The PuriFi Labs IAI-100™ device was placed in the same position for each viral challenge time point and operated in the same manner. Prior to aerosolizing the viral pathogen, the PuriFi Labs IAI-100™ device was turned on and allowed to run for 15 minutes to simulate a real-world environment and allow the device to reach standard operating conditions. Sample times were as follows with T equal to minutes, T-3, T-7, T-14, T-29. Sampling occurred using 4 automatic air volume samplers that operated simultaneously for each collection. Samplers were pre-set to automatically shut off after 10 minutes of collection. Collections were made via the equipment utilizing viral media coated filters for maximum pathogen trapping and stability. Collection samples were provided to lab staff for pooling after each collection time point.

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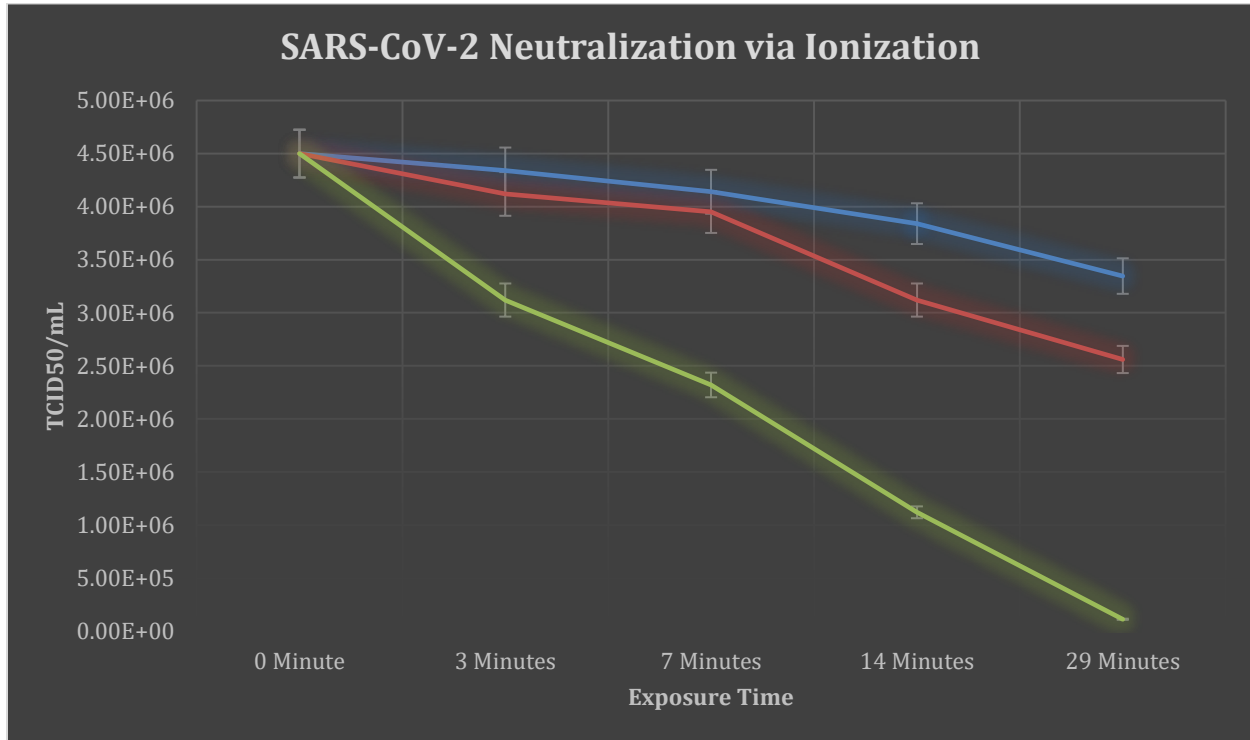
VIRAL STOCK: SARS-CoV-2 USA-CA1/2020 (BEI NR-52382)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero 6 cells	Cell Rounding and Detachment	Cell Rounding and Detachment
Next Generation Sequencing (NGS) of complete genome using Illumina® iSeq™ 100 Platform (Approx. 940 Nucleotides)	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1 ≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1	99.9% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1 100% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1
Titer by TCID50 in Vero E6 Cells by Cytopathic effect	Report Results	4.50 X 10 ⁶ TCID50 per mL in 5 days at 37°C and 5% CO ₂
Sterility (21-Day Incubation) Harpos HTYE Broth, aerobic Trypticase Soy Broth, aerobic Sabourad Broth, aerobic Sheep Blood Agar, aerobic Sheep Blood Agar, anaerobic Thioglycollate Broth, anaerobic DMEM with 10% FBS	No Growth No Growth No Growth No Growth No Growth No Growth No Growth	No Growth No Growth No Growth No Growth No Growth No Growth No Growth
Sterility (21-Day Incubation) Harpos HTYE Broth, aerobic Trypticase Soy Broth, aerobic Sabourad Broth, aerobic Sheep Blood Agar, aerobic Sheep Blood Agar, anaerobic Thioglycollate Broth, anaerobic DMEM with 10% FBS	No Growth No Growth No Growth No Growth No Growth No Growth No Growth	No Growth No Growth No Growth No Growth No Growth No Growth No Growth
Mycoplasma Contamination Agar and Broth Culture DNA Detection by PCR of extracted Test Article nucleic acid.	None Detected None Detected	None Detected None Detected

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RESULTS:



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

VIRAL VOLUME REDUCTION OF SARS-COV-2 (SCIENTIFIC) – TCID50/mL			
	Static Control	Airflow Control	PuriFi
0 Minutes	4.50E+06	4.50E+06	4.50E+06
3 Minutes	4.34E+06	4.12E+06	3.12E+06
7 Minutes	4.14E+06	3.95E+06	2.32E+06
14 Minutes	3.84E+06	3.12E+06	1.12E+06
29 Minutes	3.35E+06	2.56E+06	1.12E+05

VIRAL VOLUME REDUCTION OF SARS-COV-2 (NUMERICAL) – TCID50/mL			
	Static Control	Airflow Control	PuriFi
0 Minutes	4,500,000	4,500,000	4,500,000
3 Minutes	4,340,000	4,120,000	3,120,000
7 Minutes	4,140,000	3,950,000	2,320,000
14 Minutes	3,840,000	3,120,000	1,120,000
29 Minutes	3,350,000	2,560,000	112,000

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VIRAL LOG REDUCTION OF SARS-COV-2			
	Static Control	Airflow Control	PuriFi
0 Minutes	0	0	0
3 Minutes	0.039	0.093	0.341
7 Minutes	0.088	0.135	0.538
14 Minutes	0.163	0.34	0.834
29 Minutes	0.284	0.479	1.834

PURIFI VIRAL INACTIVATION OF SARS-COV-2			
	PuriFi vs Static Control	PuriFi vs Airflow Control	PuriFi Total Reduction
0 Minutes	0.00%	0.00%	0.00%
3 Minutes	28.11%	24.27%	30.67%
7 Minutes	43.96%	41.27%	48.44%
14 Minutes	70.83%	64.10%	75.11%
29 Minutes	96.66%	95.63%	97.51%

CONCLUSIONS:

The PuriFi Labs IAI-100™ device performed to manufacturer specifications and demonstrated a consistent, progressive reduction of active virus after 29 minutes of exposure in aerosol form. The amount of active SARS-CoV-2 in the atmosphere was reduced over the control samples by approximately 96.66% vs the static control and 95.63% vs the airflow control, with a total reduction of 97.51%

When aerosolizing pathogens and collecting said pathogens, there are variables that 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.

Taking variables into account there was a measurable amount of reduction achieved by the PuriFi Labs IAI-100™ device at each of the T-3, T-7, T-14, and T-29 time points. The reduction in air was consistent with the manufacturer's claims that the PuriFi Labs IAI-100™ device can decrease the concentration of active pathogens in the air. Overall, the PuriFi Labs IAI-100™ device showed efficacy in the reduction of SARS-CoV-2 USA-CA1/2020 from air samples collected.

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DISCLAIMER:

The Innovative BioAnalysis, LLC. ("Innovative BioAnalysis") laboratory is not certified or licensed by the United States Environmental Protection Agency and makes no equipment emissions claims pertaining to ozone, hydrogen peroxide gas, reactive oxygen species, volatile organic compounds, or byproduct of any PURIFI LABS IAI-100™ device. Innovative BioAnalysis makes no claims to the overall efficacy of any IAI-100™. The experiment results are solely applicable to the device used in the trial, serial number: 945935D9913. The results are only representative of the experiment design described in this report.

Innovative BioAnalysis 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 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.

Dr. Dana Yee M.D
Clinical Pathologist and Medical Director

Date

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