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EFFICACY OF THE GPS AIR IDF-2 AGAINST AEROSOLIZED SARS-COV-2

PROJECT: GPS AIR IDF-2 – SARS-COV-2 AEROSOL

TECHNOLOGY: Needlepoint Bipolar Ionization (NPBI™)

DEVICE: GPS AIR IDF-2

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM:

SARS-CoV-2

STUDY COMPLETION DATE:

01/11/2023

Medical Director

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Testing Facility

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Laboratory Project Number

1365S

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Efficacy Study Summary

Study Title	EFFICACY OF THE GPS AIR IDF-2 AGAINST AEROSOLIZED SARS-COV-2
Laboratory Project #	1365S
Guideline:	GCLP, modified ISO, and BSL-3 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
Description	Per the manufacturer, the GPS Air IDF-2 device incorporating GPS's NPBI™ technology is a ceiling fan designed to fit into commercial drop/suspended ceilings to reduce the concentration of pathogens within a room. Testing was conducted with the device set at two different fan speeds to evaluate the effectiveness of the IDF-2 against aerosolized SARS-CoV-2.
Test Conditions	Testing was conducted in a 22' x 11' x 8' chamber following BSL-3 standards. The temperature during testing was approximately 70 ±2°F, with a relative humidity of 41-43%. A 2.44 x 10^6 TCID50/mL of SARS-CoV-2 in suspension media was nebulized into the room with mixing fans before collection. Air sample collections occurred at 0, 10, and 20 minutes and were tested in duplicate at each device speed of 575 ± 10 RPM and 480 ± 10 RPM.
Test Results	The results at both fan speeds 575 ± 10 RPM and 480 ± 10 RPM, show increased reductions in viral concentration over the natural viability loss observed in the controls. The GPS Air IDF-2 functional sample incorporating GPS's NPBI™ technology decreased recoverable SARS-CoV-2 from 2.44 x 10^6 TCID50/mL to an average of 8.60 x 10^5 TCID50/mL after 10 minutes at a fan speed of 575 ± 10 RPM. After 20 minutes, an average of 4.13 x 10^4 TCID50/mL was recovered. With the fan set at 480 ± 10 RPM, an average of 9.87 x 10^5 TCID50/mL was observed after 10 minutes and 2.18 x 10^5 TCID50/mL after 20 minutes.
Control Results	A single control run was conducted for the two device speeds without the ion function activated, and samples were taken at the corresponding time points used for the challenge. The results displayed natural viability loss in the chamber and were used as a comparative baseline to calculate viral reduction.
Conclusion	The GPS AIR IDF-2 functional sample incorporating GPS's NPBI™ technology demonstrated the ability to reduce active SARS-CoV-2 in the air. At a fan speed of 480 ± 10 RPM, a 49.21% (0.29 log) net reduction after 10 minutes and 84.72% (0.82 log) net reduction within 20 minutes. At a speed of 575 ± 10 RPM, the device demonstrated a 51.27% (0.31 log) net reduction after 10 minutes and a 96.71% (1.48 log) net reduction after 20 minutes of operation.

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Study Report

Study Title: EFFICACY OF THE GPS AIR IDF-2 AGAINST AEROSOLIZED SARS-COV-2

Sponsor: Global Plasma Solutions (dba GPS Air)

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

Technology Tested: NPBI™

Device Testing: GPS Air IDF-2

Study Dates:

Study Report Date: 01/18/2023
Experimental Start Date: 12/06/2022
Experimental End Date: 12/11/2022
Study Completion Date: 01/11/2023

Study Objective:

The GPS AIR IDF-2 fan incorporating GPS's NPBI™ technology was provided by Global Plasma Solutions (dba GPS Air) for testing to evaluate the device's efficacy against aerosolized viral pathogens. The study was conducted to determine the device's effectiveness in reducing the viral strain SARS-CoV-2 within the air 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 to confirm the average particle size distribution of approximately 0.8 µm. See Table 1 and Appendix B for particle distribution specifics from the sampling of aerosolized solution in particle testing prior to this study. The nebulizer was filled with 2.44 x 10^6 TCID50/mL of SARS-CoV-2 in suspension media and nebulized at a flow rate of 1mL/min with untreated local atmospheric air. After nebulization, the nebulizer's remaining viral stock volume was weighed to confirm that approximately the same amount was nebulized during each run. Bioaerosol procedures for the controls and viral challenges were performed in the same manner with corresponding time points and collection rates.

Figure 1: BLAM Nebulizer

Table 1: Particle Size Distribution Table

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

Bioaerosol Sampling:

This study used 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. 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. Cassettes had an internal filtration disc (Fig. 2) to collect virus samples, which was moistened with a suspension media to aid collection. Filtration discs from Zefon International, Lot# 28875, were used for testing. All sample discs were pooled into one collection tube at each time point to provide an average across the four sampling locations.

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

Test System Strains:

The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate hCoV-19/USA-WA1/2020, NR-52281.

TCID50 Procedure:

Materials and Equipment:

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

Procedure:

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

Additions of virus dilutions to cells:

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

Study Materials and Equipment:

Equipment Overview: The Alaska Fan incorporating GPS's NPBI™ technology (Fig. 3) arrived at the laboratory pre-assembled 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 ionizer. Before testing, the test device was powered on and operated for a 10-minute period in a dry run to confirm correct operations. Alpha Lab AIC2 ion polarity meters were used at 6 locations in a grid on both sides of the chamber prior to testing for each fan speed, as shown in Figure 4. It should be noted that due to the nature of ions, there were fluctuations in concentrations around the entire room.

MANUFACTURER: GPS Air

FUNCTIONAL SAMPLE NAME: IDF-2

TECHNOLOGY: GPS NPBI™

- KEY COMPONENTS
  - ELECTRIC FAN:
    - BRAND: Alaska
    - MODEL: SA-398
    - SERIAL #: 21090447
    - SIZE: 23.75" x 23.75" x 7.1"
  - IONIZER:
    - BRAND: GPS Air
    - MODEL: CI-2

Figure 3. IDF-2 functional sample device tested with a closeup of the installed GPS's CI-2 needlepoint bipolar ionizer.

Testing Layout:

Testing was conducted in a sealed 22' x 11' x 8' chamber (Fig. 4) per Biosafety Level 3 (BSL3) standards. The room had a displacement volume of 1,936 ft^3 (54,821.42 L) of air. The chamber remained closed during testing, with no air entering or leaving the room. A nebulizing port connected to a programmable compressor system was located in the center of the 22 ft wall. At each chamber corner, low-volume mixing fans (approx. 30 cfm each) were positioned at 45-degree angles to ensure homogenous mixing of bioaerosol concentrations when nebulized into the chamber. The room was equipped with four sampling ports equally spaced around the chamber located between 48-60 inches off the chamber floor, at least 6ft away from the device, and at least 2ft from the walls. The device was mounted in the center of the chamber as close to the ceiling as possible and was operated at the fan speed setting of 480 ± 10 RPM and 575 ± 10 RPM. The chamber was visually inspected, and pressure tested, and all internal lab systems and equipment were reviewed before testing.

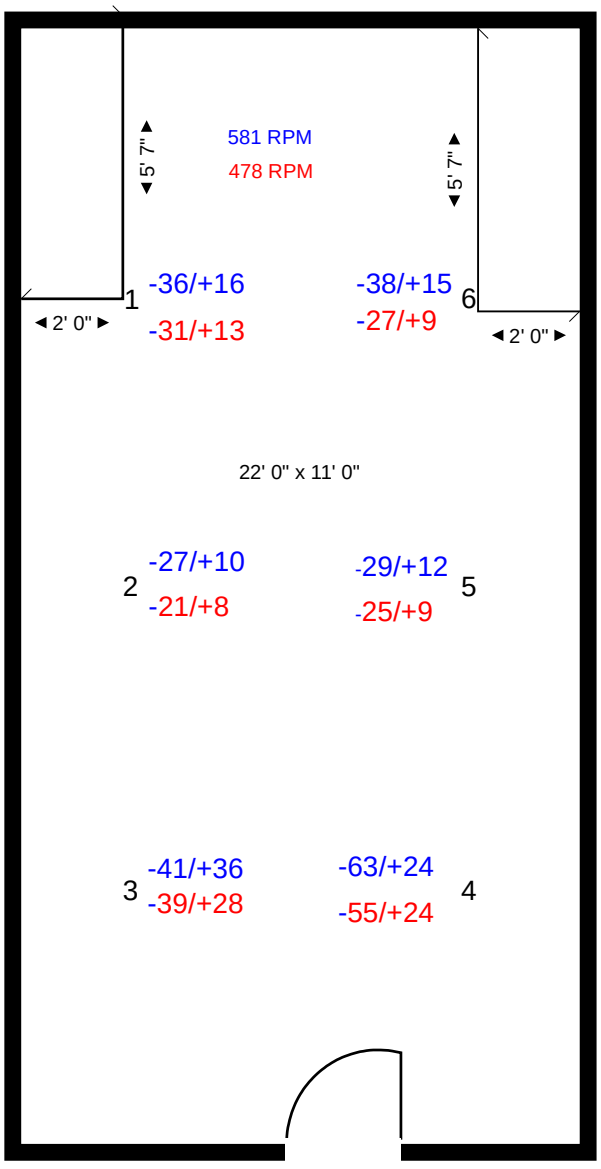

Figure 4. Ion concentration measurements from a dry run for each fan speed—575 ± 10 RPM (blue) and 480 ± 10 RPM (red)—across six locations in the chamber.

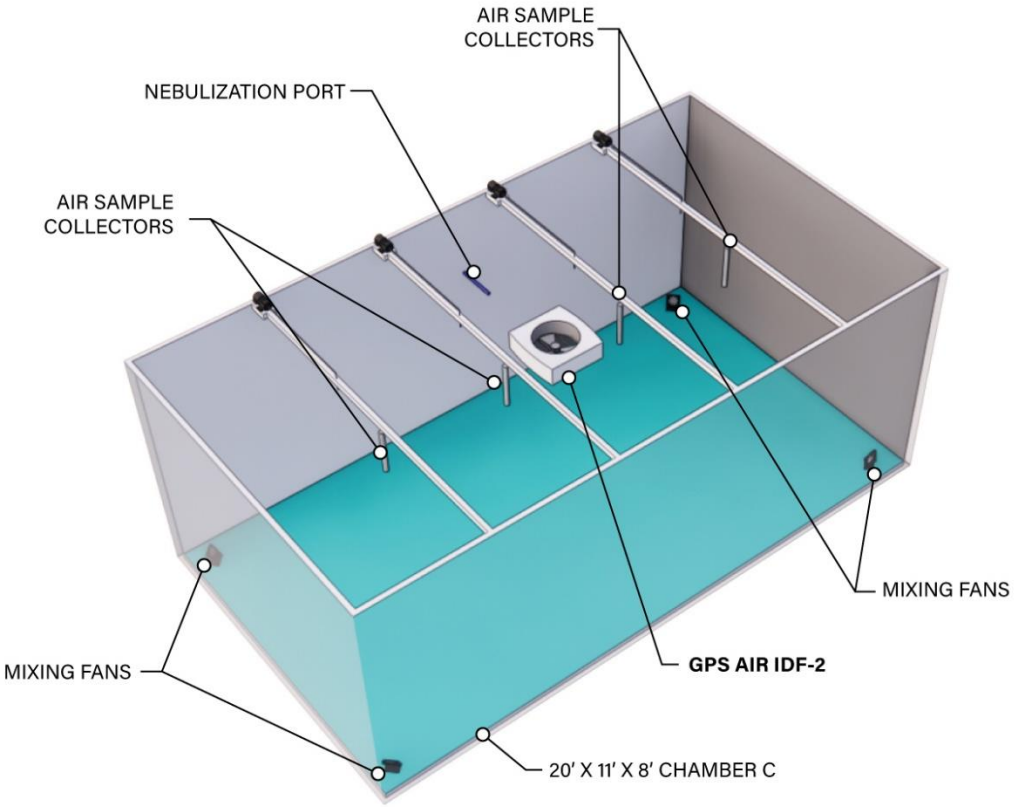

Figure 5. Room layout for control and experimental testing.

Control Protocol:

Control testing was conducted for each device speed without the ionizer turned on in the testing chamber to accurately assess the GPS Air IDF-2 functional sample incorporating GPS's NPBI™ technology. The samples were taken in the same manner and at the corresponding time points used for the challenge trial to serve as a comparative baseline to assess viral reduction when the device was operating.

Test Procedures:

Exposure Conditions:

1. The temperature during all test runs was approximately 70 ±2°F, with a relative humidity of 41-43%.
2. Two device fan speeds were tested: 575 ± 10 RPM and 480 ± 10 RPM.
3. Testing time points were as follows, with T equal to minutes: T-0, T-10, and T-20.
4. A single control and two challenges for each fan speed was conducted using the same methodology.

Experimental Procedure:

1. Before the initial control test and following each trial, the testing area was decontaminated and prepped per internal procedures.
2. The device was turned on and ran at the designated speed for 5 minutes before starting each ion test.
3. 10 mL of a 2.44 x 10^6 TCID50/mL of SARS-CoV-2 in suspension media was nebulized via a dissemination port into the room.
4. The device was turned off at each predetermined time point for sample collection.
5. Air sampling collection was set to 10-minute continuous draws at the point of sampling occurring after nebulization ceased.
6. Sample cassettes were manually removed from the collection system and taken to an adjacent biosafety cabinet to be pooled.
7. All samples were sealed after 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 underwent a 30-minute air purge. All test equipment was cleaned with a 70% isopropyl alcohol solution at the end of each day. Collection lines were soaked in a bleach bath mixture for 30 minutes and then rinsed repeatedly with DI water. The nebulizer and vacuum collection pumps were decontaminated with hydrogen peroxide mixtures.

Preparation of The Pathogen

Viral Stock: SARS-CoV-2 USA-WA1/2020 (BEI NR-52281)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 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, isolate USA-WA1/2020 GenBank: MN985325.1	99.9% identity with SARS-CoV 2, isolate USA-WA1/2020 GenBank: MN985325.1
Sequencing of Species-Specific Region (~ 930 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate USA-WA1/2020 GenBank: MN985325.1	100% identity with SARS-CoV 2, isolate USA-WA1/2020 GenBank: MN985325.1
(~ 930 nucleotides)	≥ 98% identity with SARS-CoV-2, strain FDAARGOS_983 isolate USA-WA1/2020 GenBank: MT246667.1	100% identity with SARS-CoV-2, strain FDAARGOS_983 isolate USA-WA1/2020 GenBank: MT246667.1
Titer by TCID50 in Vero E6 Cells by Cytopathic Effect	Report Results	1.6 X 10^6 TCID50 per mL in 5 days at 37°C and 5% CO_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, aerobic	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 is representative of the titer provided by BEI Resources. These viruses are grown on VeroE6 cells either in-house or at a partner lab to the concentrations listed within the experiment design.

Study Results:

Test 1: 575 ± 10 RPM Fan Speed Setting

The results were plotted below (Fig. 6) to display collectible active SARS-CoV-2 with and without the GPS AIR IDF-2 functional sample incorporating GPS's NPBI™ technology operating in the chamber at a fan speed of 575 ± 10 RPM. The controls showed a natural loss of aerosolized SARS-CoV-2 for 20 minutes under controlled conditions. Across two runs, a starting concentration of 2.44 x 10^6 TCID50/mL SARS-CoV-2 decreased to 8.17 x 10^5 and 9.04 x 10^5, averaging 8.60 x 10^5 TCID50/mL after 10 minutes of device operation. The data showed that an increased device operation time resulted in a higher reduction, as presented by the average 4.13 x 10^4 TCID50/mL of active SARS-CoV-2 recovered after 20 minutes.

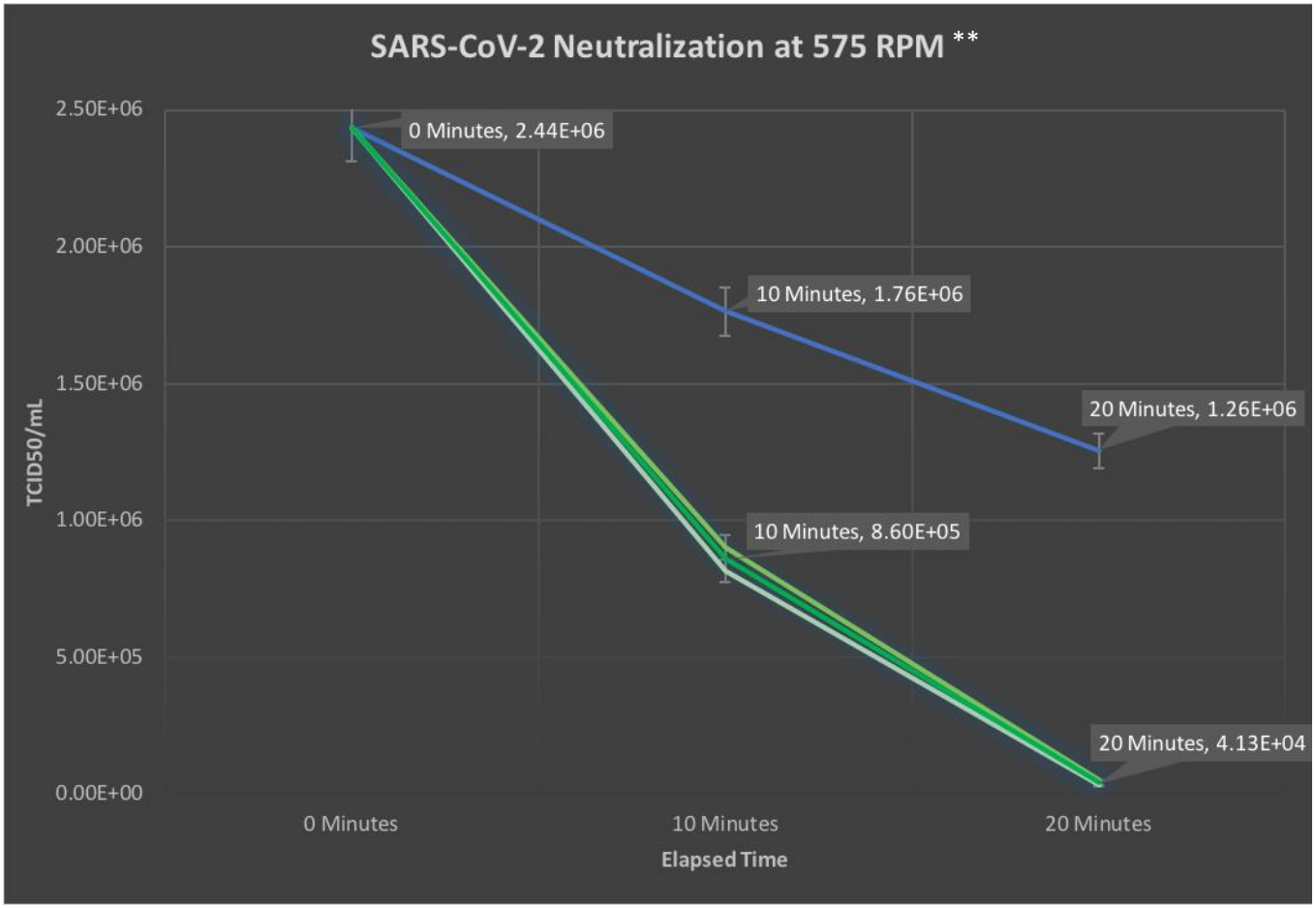

Figure 6: SARS-CoV-2 neutralization using the IDF-2 functional sample over 20 minutes under controlled conditions at a fan speed of 575 ± 10 RPM.

***As it pertains to data represented herein; the percentage error equates to an average ±5% of the final concentration.*

Table 2: Results Data and Calculated Percentage Reductions for SARS-CoV-2 at a fan speed of 575 ± 10 RPM

Time (min)	0	10	20
Control (TCID50/mL)	2.44E+06	1.76E+06	1.26E+06
% Gross Reduction - Control		27.54%	48.45%
Gross Log Reduction - Control		0.14	0.29
Experiment 1 (TCID50/mL)	2.44E+06	8.17E+05	3.28E+04
Experiment 2 (TCID50/mL)	2.44E+06	9.04E+05	4.99E+04
Experiment Average (TCID50/mL)	2.44E+06	8.60E+05	4.13E+04
% Gross Reduction - Experiment		64.69%	98.30%
Gross Log Reduction - Experiment		0.45	1.77
% Net Reduction		**51.27%**	**96.71%**

Test 2: 480 ± 10 RPM Fan Speed Setting

The results were plotted below (Fig. 7) to display collectible active SARS-CoV-2 with and without the GPS AIR IDF-2 functional sample incorporating GPS's NPBI™ technology operating in the chamber at a fan speed of 480 ± 10 RPM. The controls showed a natural loss of aerosolized SARS-CoV-2 for 20 minutes under controlled conditions. Across two runs, a starting concentration of 2.44 x 10^6 TCID50/mL SARS-CoV-2 decreased to 9.67 x 10^5 and 1.01 x 10^6, averaging 9.87 x 10^5 TCID50/mL after 10 minutes of device operation. The data showed that an increased device operation time resulted in a higher reduction, as presented by the average active SARS-CoV-2 recovered after 20 minutes, 2.18 x 10^5 TCID50/mL.

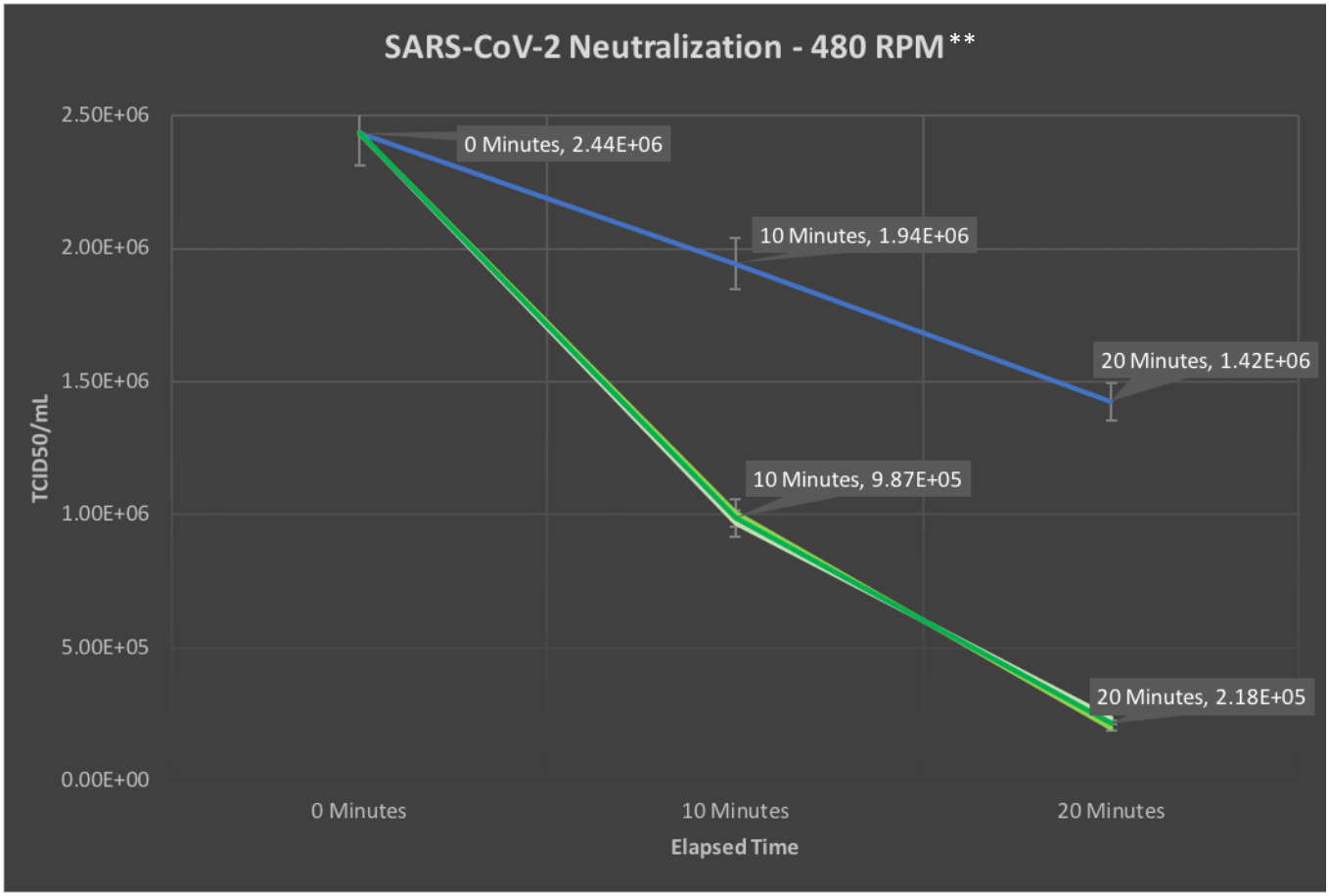

Figure 7: SARS-CoV-2 neutralization using the IDF-2 functional sample over 20 minutes under controlled conditions at a fan speed of 480 ± 10 RPM.

***As it pertains to data represented herein; the percentage error equates to an average ±5% of the final concentration.*

Table 3: Results Data and Calculated Percentage Reductions for SARS-CoV-2 at a fan speed of 480 ± 10 RPM

Time (min)	**0**	**10**	**20**
Control (TCID50/mL)	2.44E+06	1.94E+06	1.42E+06
% Gross Reduction - Control		20.25%	41.52%
Gross Log Reduction - Control		0.10	0.23
Experiment 1 (TCID50/mL)	2.44E+06	9.67E+05	2.36E+05
Experiment 2 (TCID50/mL)	2.44E+06	1.01E+06	2.00E+05
Experiment Average (TCID50/mL)	2.44E+06	9.87E+05	2.18E+05
% Gross Reduction - Experiment		59.49%	91.06%
Gross Log Reduction - Experiment		0.39	1.05
% Net Reduction		**49.21%**	**84.72%**

Conclusion:

The GPS AIR IDF-2 functional sample incorporating GPS's NPBI™ technology demonstrated the ability to reduce active, aerosolized SARS-CoV-2 across all time points at both fan settings compared to the natural loss rate observed in the temperature-controlled room. After 10 minutes of operation, the device achieved a 51.27% net reduction of active SARS-CoV-2 at 575 ± 10 RPM and a 49.21% net reduction at 480 ± 10 RPM. With more prolonged exposure and a higher fan speed, the device has more time to interact with the air reducing the amount of pathogen recovered. This was observed with the 96.71% net reduction achieved at 575 ± 10 RPM and 84.72% net reduction at 480 ± 10 RPM after 20 minutes.

When aerosolizing pathogens and collecting said pathogens, some variables cannot be fully accounted for, namely, placement of pathogen, collection volume, collection points, drop rate, surface saturation, viral destruction upon 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 the virus in the control test.

Considering the variables, the GPS AIR IDF-2 functional sample displayed measurable reduction at fan speeds of 575 ± 10 RPM and 480 ± 10 RPM. The results observed were consistent with the manufacturer's claim that the device can decrease concentrations of active pathogens in the air. Overall, the unit resulted in a 1.48 net log reduction after 20 minutes, with the fan running at 575 ± 10 RPM and 0.82 net log reduction when running at 480 ± 10 RPM.

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Chief Biosafety Officer, Innovative Bioanalysis, Inc.

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Kevin Noble **Date**

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.

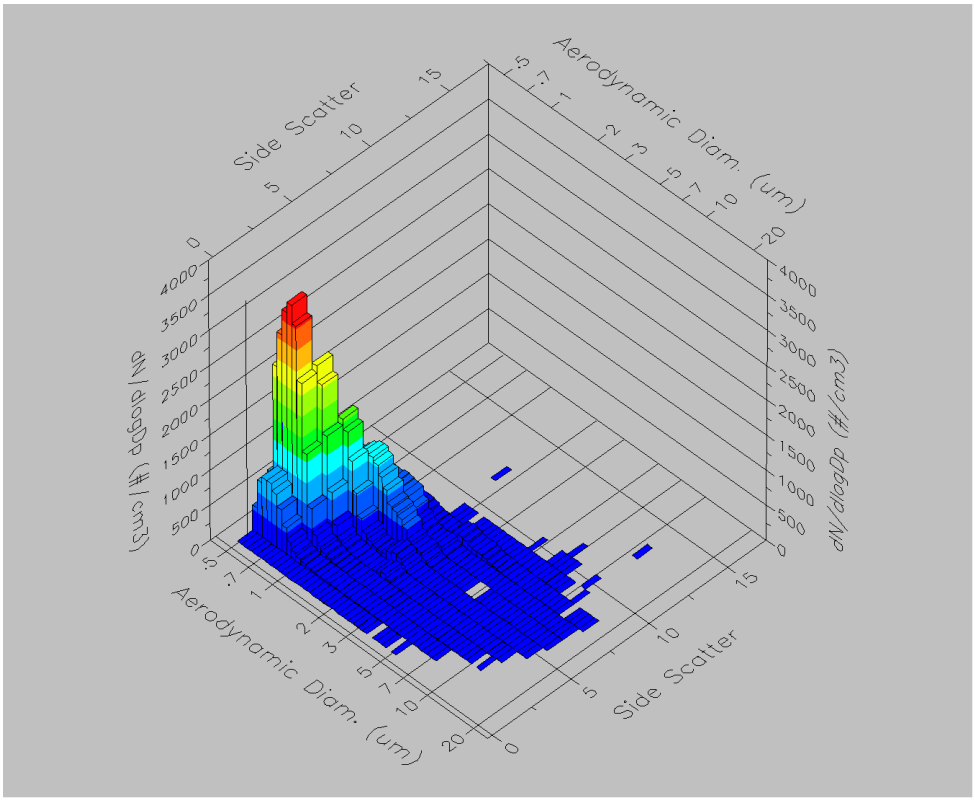
TCID50/mL: The number of infectious virus particles is frequently quantified using the Median Tissue Culture Infectious Dose (TCID50) assay. The assay works by adding a serial dilution of the virus sample to cells in a 96-well plate format. The cell type is specifically selected to show a cytopathic 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.

Vero E6: Vero cells are a lineage of cells used in cell cultures. Vero E6, also known as Vero C1008 (ATCC No. CRL-1586) This line is a clone from Vero 76. Vero E6 cells show some contact inhibitions, so are suitable for propagating viruses that replicate slowly.

APPENDIX B: Particle Size Distribution

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

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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 15 22	7.600 7.600 7.600	8.400 8.400 8.400	√ √ √
10	2 6 12	9.500 9.500 9.500	10.500 10.500 10.500	√ √ √

Technician Stamp 10

091-1015-01C

APPENDIX E: BEI Resources - Certificate of Authenticity

b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52281

SARS-Related Coronavirus 2, Isolate USA-WA1/2020

Catalog No. NR-52281

Product Description:
Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020 was isolated from an oropharyngeal swab from a patient with a respiratory illness who had recently returned from travel to the affected region of China and developed clinical disease (COVID-19) in January 2020 in Washington, USA. Deposited and labeled as 2019 Novel Coronavirus (2019 nCoV) prior to the determination of the official name. NR-52281 lot 70036318 was produced by infecting *Cercopithecus aethiops* kidney cells (Vero E6; ATCC® CRL-1586™) with the deposited material in Eagle's Minimum Essential Medium (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 6 days at 37°C with 5% CO_2.

Passage History:
V(3)/VE6(1) (Centers for Disease Control and Prevention/BEI Resources); V = Vero cells; VE6 = Vero E6 cells

Lot: 70036318 **Manufacturing Date: 28MAY2020**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with SARS-CoV-2, isolate USA-WA1/2020 (GenBank: MN985325.1)	99.99% identity with SARS-CoV-2, isolate USA-WA1/2020 (GenBank: MN985325.1)
Sequencing of Species-Specific Region		
(~ 930 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate USA-WA1/2020 (GenBank: MN985325.1)	100% identity with SARS-CoV-2, isolate USA-WA1/2020 (GenBank: MN985325.1)
(~ 930 nucleotides)	≥ 98% identity with SARS-CoV-2, strain FDAARGOS_983 isolate USA-WA1/2020 (GenBank: MT246667.1)	100% identity with SARS-CoV-2, strain FDAARGOS_983 isolate USA-WA1/2020 (GenBank: MT246667.1)
Titer by $TCID_{50}$ Assay in Vero E6 Cells by Cytopathic Effect[1] (5 days at 37°C and 5% CO_2)	Report results	1.6×10^6 $TCID_{50}$ per mL
Sterility (33-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[2]	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, aerobic	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 extracted Test Article nucleic acid	None detected	None detected

[1]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.
[2]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52281

/Heather Couch/
Heather Couch 21 AUG 2020
Program Manager or designee, ATCC Federal Solutions

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.

Support Provided by NIAID

APPENDIX I: NGS Information for NR-52281 lot 70036318

Sequence analysis resulted in the discovery of two SNPs when compared to the reference sequence from GenBank MN985325.1. Additionally, both the reference sequence GenBank MN985325.1 and NR-52281_70036318 contained three SNPs when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52281_ 70036318 Sequence	Position in MN985325.1 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported MN985325.1 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
8771	8782	8782	C	T	T	n/a	SNP	1	1.0000000
18049	18060	18060	C	T	T	n/a	SNP	1	1.0000000
22471	22482	22482	C	C	T	111	SNP	1	0.1718464
23596	23607	23607	G	G	T	142	SNP	1	0.1593870
28133	28144	28144	T	C	C	n/a	SNP	1	1.0000000

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