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EFFICACY OF THE LUMINAYRE 800 DEVICE AGAINST SARS-COV-2

PROJECT: LUMINAYRE 800 SARS-COV-2

PRODUCT: LUMINAYRE 800

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM(S):

SARS-COV-2 USA-CA1/2020

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Laboratory Project Number

1091

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Efficacy Study Summary

Study Title	EFFICACY OF THE LUMINAYRE 800 DEVICE AGAINST SARS-COV-2
Laboratory Project #	1091
Guideline:	Modified ISO standards and no international standards exist.
Testing Facility	Innovative Bioanalysis, Inc.
Study Dates:	
Study Initiation Date:	05/17/2021
Study Completion Date:	06/28/2021
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	SARS-CoV-2 USA-CA1/2020
Description	A two-part in vitro study to determine the efficacy of the Luminys System Corp Luminayre 800 against the known pathogen, SARS-CoV-2. Part 1 focuses on the effectiveness of the internal UV Reactor on surface SARS-CoV-2; and Part 2 focuses on the Luminayre 800 as a whole and its ability to reduce aerosolized SARS-CoV-2 concentrations in air.
Test Conditions	The surface efficacy testing was conducted in a certified biosafety hood inside a BSL3 laboratory under ambient conditions, approximately 74°F ±2°F and a relative humidity of 36%. The bioaerosol efficacy testing took place in a 20'x8'x8' BSL3 chamber that complied to BSL3 standards with an ambient temperature of 75°F ±2°F and a relative humidity of 42%.
Test Results	Active SARS-CoV-2 concentrations on the sample surface inside the UV-C core was reduced by 99.99% after 2 seconds of UV exposure. Aerosol testing with the Luminayre 800 device showed a 99.996% reduction after 20 minutes at high and half fan speeds and 99.93% for low.
Control Results	A control test was conducted for each part of the challenge to serve as a comparative baseline for viral reduction. A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 6.32 X 10^6 TCID50/mL was used for this experiment. This value was measured and confirmed upon sample collection at T-0 minutes.
Conclusion	The results from both parts of the study reveal the capabilities of the Luminayre 800 in reducing active SARS-CoV-2 concentrations in the air when used in conjunction with the internal UV-C Reactor unit.

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Study Report

Study Title: EFFICACY OF THE LUMINAYRE 800 DEVICE AGAINST SARS-COV-2

Sponsor: Luminys Systems Corp

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

Device Testing: A two-part study testing the efficacy of the Luminayre 800 system against the known pathogen, SARS-CoV-2.

Study Report Date: 06/28/2021
Experimental Start Date: 06/18/2021
Experimental End Date: 06/18/2021
Study Completion Date: 06/28/2021

Study Objective:

This two-part in vitro study was designed to determine the efficacy of the Luminys System Corp Luminayre 800 against the known pathogen, SARS-CoV-2. Part 1 will test the effects of direct exposure of the internal UV-C core Reactor unit on surface SARS-CoV-2; and part 2 will look at the Luminayre 800 device as a whole and its ability to reduce aerosolized SARS-CoV-2 concentrations in air.

Test Method:

Part 1: Surface Testing

Surface Inoculation:

For the control and viral challenge, each of the glass slides were equally subjected to a 1 mL inoculation of viral media containing a known titer of 6.32 X 10^6 TCID50/mL. The viral solution was spread with a spatula to ensure even distribution and saturation of all materials and left to air dry for 5 minutes.

Surface Sampling:

Swabs were moistened with viral media solution prior to collecting samples to maximize collection. Each slide was subjected to a 1 mL rinse in viral media and swabbed for residual pathogen material. After collection was completed, the swab and media were vortexed for 1 full minute. All samples collected were subjected to the same TCID50 assay protocol to determine viral concentration.

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Part 2: Aerosol Testing

Bioaerosol Generation:

For the control and the viral challenges, the nebulizer was filled with the same amount of viral stock (6.32 X 10^6 TCID50 per mL) in FBS-based viral media. The solution was nebulized at a flow rate of 1mL/min. The nebulizer was driven by untreated local atmospheric air. After each completion, the nebulizer's remaining viral stock volume was weighed to confirm that the same amount of viral stock was nebulized.

Bioaerosol Sampling:

For air sampling, four Gilian 10i programmable vacuum devices were used. The manufacturer calibrated the air samplers in September 2020, and the 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. The air samplers were operated in conjunction with removable sealed cassettes, which were manually removed after each sampling time point. The cassettes had a delicate internal filtration disc coated with a viral suspension media to aid in the collection of viral samples.

Test System Strains: SARS-CoV-2 USA-CA1/2020

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 USA-CA1/2020, NR-52382.

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Study Materials and Equipment:

Equipment Overview: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. The lamps and wiring were all installed prior to arrival at the laboratory. The device was powered on to confirm functionality prior to testing. Per manufacturer, the UV Reactor had at least 100 hours of burn-in time, as a result no burn in period was conducted in our lab prior to testing.

MANUFACTURER: Luminys Systems Corp

MODEL: Luminayre 800

SIZE: 50” x 38” x 20”

- UV Reactor: Length – 21”; Diameter – 10”

MAKE: N/A

SERIAL #: N/A

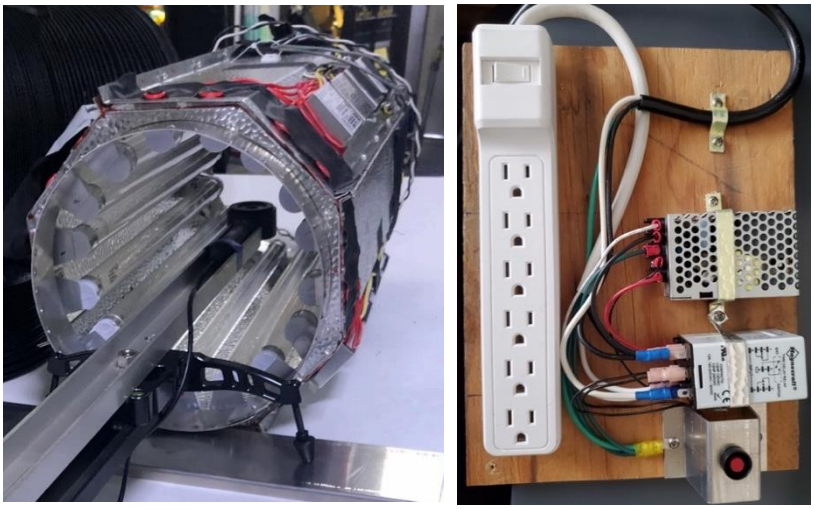

Figure 1. A UV Reactor(left) unit along with a switchboard(right) was provided by the manufacturer specifically for surface efficacy testing.

Equipment Specifics: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. The lamps and wiring were all installed prior to arrival at the laboratory. The cylindrical UV Reactor houses 16) UV-C G25T8 lamps. For this surface testing study, a switchboard and sliding platform were provided by the manufacturer as seen in Figure 1. The switchboard had an automatic switch timer and was pre-wired upon arrival with the pre-determined timepoints. The elevated sliding platform was used to move test samples in and out of the Reactor during testing.

Testing Chamber: Surface efficacy testing was conducted in a certified biosafety hood located inside a BSL3 laboratory while the aerosol testing was conducted in a 20’x8’x8’ bioaerosol chamber. Both laboratories complied to BSL3 standards and had active monitoring of testing conditions via calibrated wireless devices. Aerosol testing also had air sampling sensors and low volume mixing fans. The testing chamber was set up to allow all exhausted air after the test samples had been taken to be exhausted through a dual HEPA filtration system.

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Design Layout:

Prior to testing, all internal lab systems were reviewed and determined to be functioning. The chambers were pressure tested for leaks by visual inspections using a colored smoking device to ensure the chambers were completely sealed. Furthermore, all equipment used had a function test conducted to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

Part 1: Surface Testing

The test was conducted in a certified metal and glass biosafety hood inside a BSL3 laboratory, which maintained negative pressure greater than 12.00 Pa. The dimensions of the cabinet were 72”x32”x32”. The temperature during all test runs was approximately 74°F ±2°F, with a relative humidity of 36%. The device was placed in the center of the biosafety hood with a track sliding system going through the Reactor. The sample slides were placed on a sliding platform that was manually pushed to the center of the Reactor.

Part 2: Aerosol Testing

The testing chamber was a 20’x8’x8’ sealed chamber consisting of metal walls and epoxy flooring equipped with 4 sealed viewing windows and a lockable antechamber door complying with BSL-3 standards. The chamber was designed to maintain a negative pressure environment with multiple HEPA filters to prevent any release of testing media into the atmosphere. A monitoring system is set in place to confirm no loss of pressure occurred during testing. Calibrated wireless devices and air sampling sensors are strategically placed to provide active monitoring of testing conditions. Temperature and humidity throughout the testing process were approximately 75°F and 42%. The overall dimensions of the test chamber provided a displacement volume of 1,280 cubic feet and approximately 36,245.56 liters of air.

At each corner of the chamber, low volume mixing fans were positioned at 45-degree angles to ensure homogenous mixing of bioaerosol concentrations when nebulized into the chamber. Furthermore, the use of the mixing fans encouraged bioaerosol suspension and reduction in natural particle descent rates. The fans help circulate the air around the room, allowing a mixing of the nebulized viral media and air within the testing chamber. The testing chamber had HEPA-filtered inlets and exhaust, coupled with an active UV-C system in all ducting lines. The chamber was set up to allow all exhausted air after collection of test samples to be exhausted through this dual HEPA filtration system.

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 # 24320 made by Zefon International. A single bioaerosol nebulizing port was in the center of the 20’ wall. The dissemination port protruded from the wall 24” and was connected to a programmable compressor nebulizer system.

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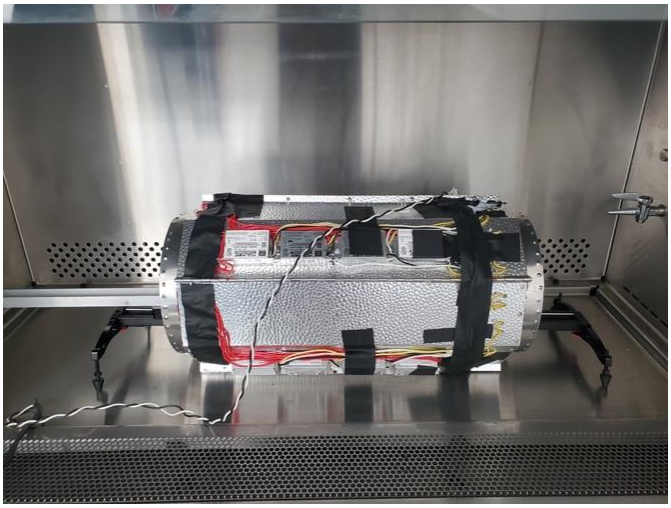

Figure 2. The UV Reactor unit placed in the center of the testing chamber for surface efficacy testing.

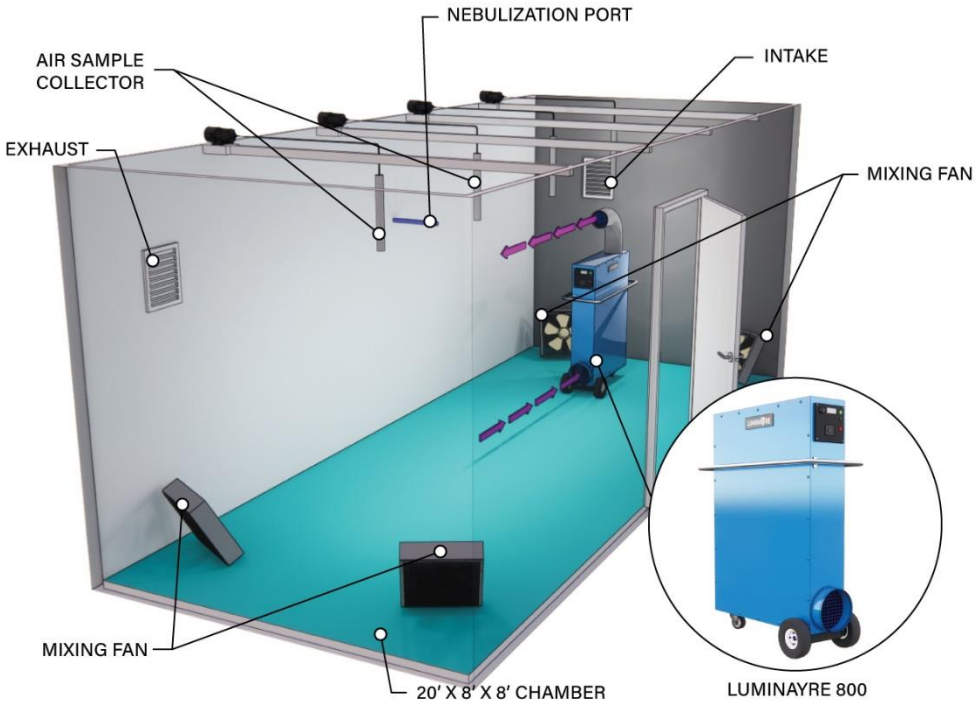

Figure 3. Room layout for aerosol control and experimental trial.

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Test Method:

Luminys System Corp supplied an internal UV Reactor unit and the Luminayre 800 for testing purposes to determine efficacy against a viral pathogen. Two test methods were utilized when conducting this study to evaluate the surface efficacy of the internal UV Reactor unit and the Luminayre 800 in its ability to inactivate the viral strain referred to as SARS-CoV-2 in the air.

Part 1: Surface Testing

Exposure Conditions:

1. Prior to the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. The temperature during all test runs was approximately 74°F ±2°F with a relative humidity of 36%.
3. The sample slides were inoculated with 1mL of a known concentration of viral media and left to air dry for 5 minutes.

Experimental Procedure:

1. The UV Reactor unit was turned on just prior to the start of testing at the 0-minute time point.
2. Sterile glass slides were inoculated with the viral media and labeled with a pre-determined time point.
3. Sample slide was placed on a sliding system and slid to the center of the UV Reactor.
4. A switchboard connected to an automatic switch timer was used to turn off the device after 2 seconds of UV exposure.
5. A swab and rinse were performed on each sample slide based on time point and cultured to determine microorganism recovery and overall efficacy.
6. All swabs were sealed after collection and provided to lab staff for analysis after study completion.

Post Decontamination:

At the conclusion of each viral challenge test, the UV system inside the biosafety chamber was activated for 30 minutes. After 30 minutes of UV exposure, all test equipment was cleaned at the end of each day with a 70% alcohol solution.

Part 2: Aerosol Testing

Exposure Conditions:

1. Prior to the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. The temperature during all test runs was approximately 75°F ±2°F with a relative humidity of 42%.
3. The 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.
4. The air sample collection volumes were set to 10-minute continual draws at the point of sampling.

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5. Low volume mixing fans were placed at each corner of the chamber at a 45-degree angle and turned on prior to nebulization.

Nebulization:

1. Nebulization for control and viral test challenges were performed in the same manner.
2. After nebulization of the pathogen, the Luminayre 800 system was turned on via remote control.
3. For the viral challenge, a known quantity of viral media was nebulized into the sealed environment from a dissemination port.
4. The viral media was nebulized at a constant rate for 25 minutes.
5. During the pathogen challenge, the Luminayre 800 was turned off after 5 minutes, 10 minutes, and 20 minutes of exposure for air sample collection to start.
6. The pre-determined timepoints were conducted at three fan settings (high, half, and low) on the Luminayre 800. The fan speed setting is a numerical dial and the speeds were labeled as 8 (high), 4 (half), and 1 (low).
7. Air sampling collection occurred at each time point for a total of 10 minutes for both the challenge and control tests.
8. The test condition had the Luminayre 800 in the center of the test box.
9. The sample cassettes were manually removed from the collection system after each control run and each air pass challenge and pooled.
10. Upon cassette removal after each challenge, cassette sets were taken to an adjacent biosafety cabinet for extraction and placement into viral suspension media.
11. One control was completed and a viral challenge was conducted for three different fan speeds using the same methodology.

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 there was a 30-minute air purge through the air filtration system. 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.

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Preparation of The Pathogen

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 the complete genome using Illumina® iSeq™ 100 Platform	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1	99.9% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1
Approx. 940 Nucleotides	≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1	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	2.8 X 10^5 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	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.

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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 7.5% 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.

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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.

Protocol Changes:

Protocol Amendments: None

Protocol Deviations: None

Control Protocol

One control test was conducted without the Luminayre 800 system in the testing chamber for both parts of the study with samples taken at the corresponding sample times used for the challenge trial. Control testing was used for the comparative baseline to assess the viral reduction when the Luminayre 800 device was operated in the challenge trials to enable net reduction calculations to be made. Furthermore, temperature and relative humidity were monitored and confirmed to be in relative range, ±5% compared to control testing conditions prior to running the viral challenges.

Part 1: Inoculation of Viral Media

A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 6.32 X 10^6 TCID50/mL was used for this experiment. Each of the testing sites were equally subjected to a 1 mL inoculation of viral media and was spread evenly on the slide using a spatula before letting it air dry for 5 minutes. The viral solution was splayed out on a sterile glass slide that was 3”x1.5” and 0.125” thick. Samples were collected using a swab moistened with viral media and rinsed to maximize collection. The control samples were prepared and collected in the same manner as the viral test regarding the time points and collection rate.

Part 2: Aerosolization of Viral Media

The control samples were performed in the same manner as the viral test regarding the time points and collection rate. A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 6.32 X 10^6 TCID50/mL was used for this experiment. Four low volume fans were operated in each corner to ensure homogenous mixing of the air and reduce particle drop rates. Nebulization of viral media and collection methods were the same for the control as the viral challenge.

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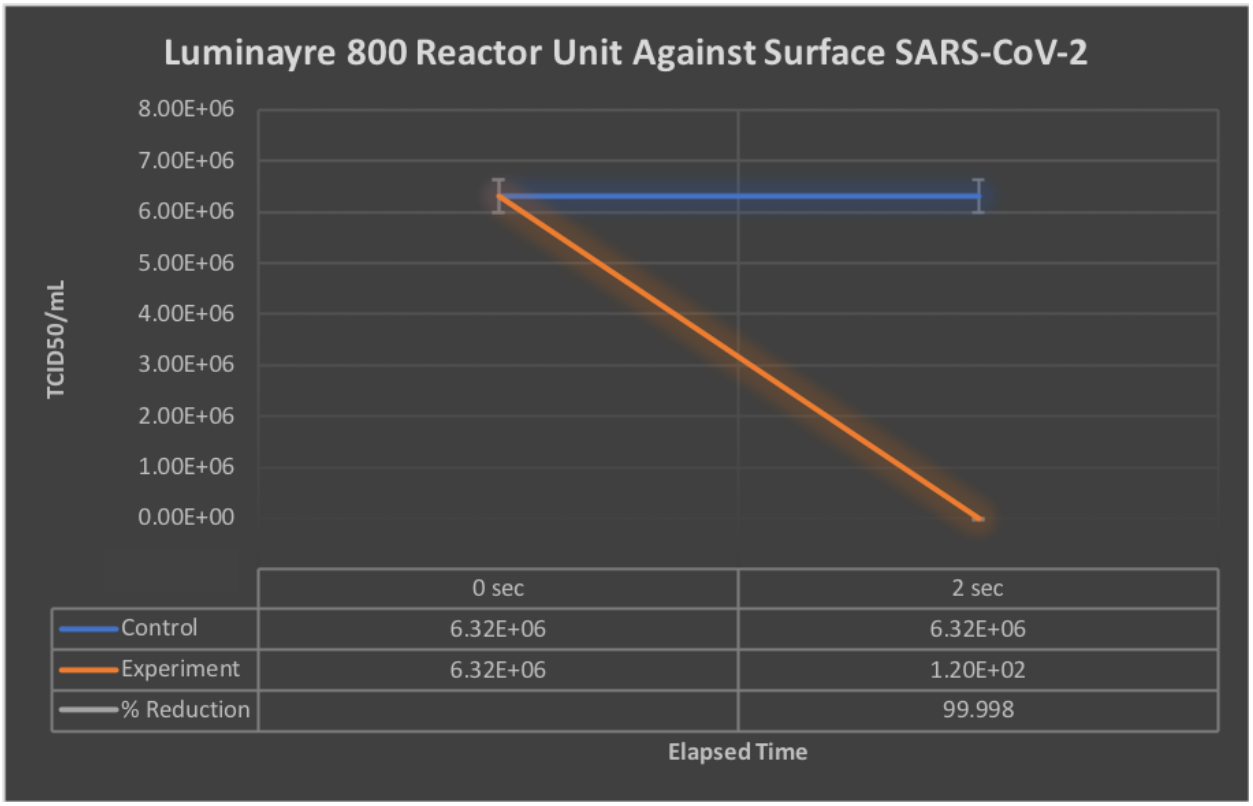
Study Results

Results from the control runs were graphed and plotted to show natural viability loss over time in the chamber for both surface and aerosol testing. The control data served as a basis to determine the reduction achieved with the internal UV Reactor unit and the Luminayre 800 device after operating for pre-determined time points.

RESULTS:

Part 1: Surface Testing

The UV Reactor unit was observed to have rapidly reduced active viral concentrations from 6.32 X 10^6 TCID50/mL to below the lower limits of quantitation of 1.2E+02 after 2 seconds of direct UV exposure.



	0 sec	2 sec
Control	6.32E+06	6.32E+06
Experiment	6.32E+06	1.20E+02
% Reduction		99.998

**As it pertains to data represented herein, the value of 1.2E+02 indicates a titer that is lower than the specified limit of quantitation. The limit of quantitation for this assay is 1.2E+02.

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

Part 2: Aerosol Testing

The Luminayre 800 device was observed to have reduced active SARS-CoV-2 in the air at all three fan speeds. The data shows a similar trend with the high, half, and low speed, but with varying rates of reduction. After 5 minutes of operation, the following active viral concentrations were quantified for the high, half, and low speeds: 1.66 X 10^6 TCID50/mL, 2.47 X 10^6 TCID50/mL, and 3.02 X 10^6 TCID50/mL. The results show that a higher

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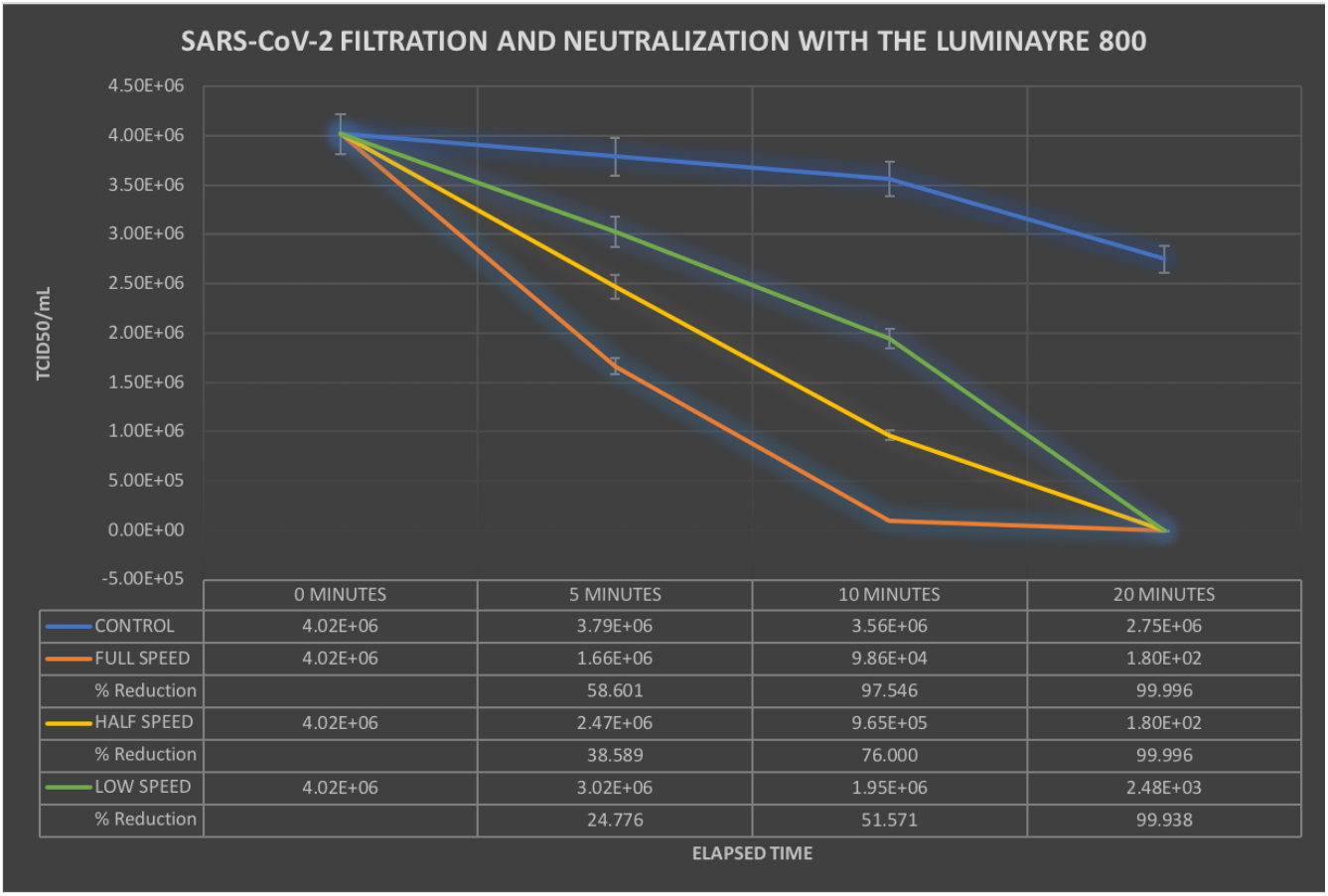
fan speed is directly correlated to a more rapid reduction of aerosolized pathogen. After 20 minutes of elapsed time, there was a 99.996% reduction seen for both the full and half speed and a 99.93% for the low fan speed.



	0 MINUTES	5 MINUTES	10 MINUTES	20 MINUTES
CONTROL	4.02E+06	3.79E+06	3.56E+06	2.75E+06
FULL SPEED	4.02E+06	1.66E+06	9.86E+04	1.80E+02
% Reduction		58.601	97.546	99.996
HALF SPEED	4.02E+06	2.47E+06	9.65E+05	1.80E+02
% Reduction		38.589	76.000	99.996
LOW SPEED	4.02E+06	3.02E+06	1.95E+06	2.48E+03
% Reduction		24.776	51.571	99.938

**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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Conclusion:

As seen in the first part of the study, the Luminys System Corp internal UV Reactor unit from the Luminayre 800 demonstrated the ability to reduce the concentration of the active pathogen, SARS-CoV-2 on a surface in a relatively short period of time. After 2 seconds of UV exposure in the sealed testing chamber, the collectable viral concentration on the surface was reduced by 99.998%.

Further testing was conducted in part 2 of the study to determine the efficacy of the Luminayre 800 against aerosolized SARS-CoV-2 at three different fan speeds: 8 (high), 4 (half), and 1 (low). The results also demonstrated the capabilities of the Luminayre 800 in reducing active, collectable SARS-CoV-2 in the air at all speeds, but at varying rates of reduction. With a higher fan speed, more air is being filtered through the device thus potentially increasing the percent reduction seen in the results. For example, at the lowest fan speed the percent reduction observed at 5, 10, and 20 minutes of operation was: 24.77%, 51.57%, and 99.93%. In comparison, at maximum fan speed the percent reduction doubled: 58.60%, 97.54%, and 99.996%. While at half speed, the reductions were 38.58% at 5 minutes, 76.00% at 10 minutes, and 99.996% at 20 minutes. As the test was designed to observe aerosol functions, it is unknown if any active pathogen remained on the surface areas inside the unit or on the testing chamber walls.

Overall, the reduction of collectable virus in the air was significant in the experiment design. The data shown in part 2 further validates the capabilities of the Luminayre 800 in reducing pathogens in the air. However, as this was conducted in a sealed chamber, it's effective use in a larger environment will be drastically different. This design was to initiate discussion and review efficacy of what the system would be capable of doing in a confined area. When the system is applied to a room environment the results will scale with variables present. Room size, occupancy, air flow dynamics and amount of pathogen in the air to list a few. These variables will all play a significant factor in the efficacy of the system which cannot be determined based on the outcome of this report. Air moves differently in all spaces and a complete analysis of airflow would be needed to understand the impact of the UV-C system in different enclosed spaces. Effort was made to simulate a real-life environment in the chamber while taking into consideration the special precautions needed when working with a Biosafety Level 3 Pathogen. Furthermore, when aerosolizing pathogens and collecting said pathogens even in a controlled environment, 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 nebulization, 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.

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Dana Yee M.D **Date**

Clinical Pathologist and Medical Director, Innovative Bioanalysis, Inc.

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

Chief Scientific Officer, Innovative Bioanalysis, Inc.

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Albert Brockman
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Albert Brockman **Date**

Chief Biosafety Officer, Innovative Bioanalysis, Inc.

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8/17/2021

Kevin Noble **Date**

Laboratory Director, Innovative Bioanalysis, Inc.

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