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SARS-CoV-2 USA-CA1/2020

CLIENT: WAC LIGHTING

PROJECT: UV-C AEROSOL

PRODUCT: UVF7IN-120V-R1-MOD/UVF7IN-120V-R1

CAP LIC NO: 886029801

CLIA LIC NO: O5D0955926

STATE ID: CLF 00324630

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

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ABSTRACT: EFFICACY OF THE WAC LIGHTING FAN UVF7IN-120V-R1-MOD AGAINST AEROSOL SARS-CoV-2

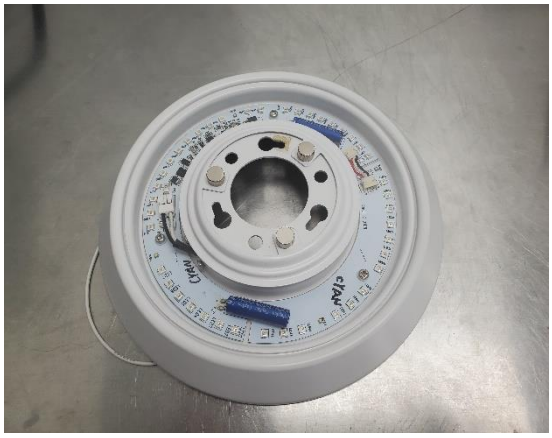
Background: This in vitro study was designed to determine the efficacy of a combination fan and UV-C ring unit **(UVF7IN-120V-R1-MOD)** on an aerosolized pathogen. The product is designed to be commercially available in conjunction with the overhead fan assembly tested. The UV-C unit is designed to deactivate viral pathogens in the air above a ceiling fan. The functional goal is for the completed system to be placed in rooms and decrease the concentration of pathogens in the air when it is operating to prevent the spread of pathogens. For this challenge, the SARS-CoV-2-CA1/2020 pathogen was used. There is a demand for disinfectant devices that have a proven ability to reduce infectious pathogens in the air thereby reducing the risk of human infection and transmission. WAC Lighting supplied a pre-packaged UV-C ring and fan 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 surface viral pathogen challenges and subsequent decontamination. All internal SOPs and processes follow GCLP guidelines and recommendations.

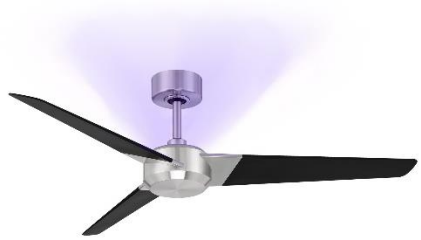
EQUIPMENT PROVIDED:

MANUFACTURER: WAC LIGHTING

MODEL: UVF7IN-120V-R1-MOD/UVF7IN-120V-R1

SERIAL #: TESTING UNITS N/A





WAC LIGHTING EQUIPMENT:

The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. The equipment needed no installation by lab staff and all LED diodes came installed and functioning. The UV-C ring and fan housing were operated to confirm irradiance output and the system was functioning as intended.

VIRAL SAFETY 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 2 sealed viewing windows and a lockable chamber door for entry and exit. The overall dimensions of the test chamber were approximately 8'x8'x10'.

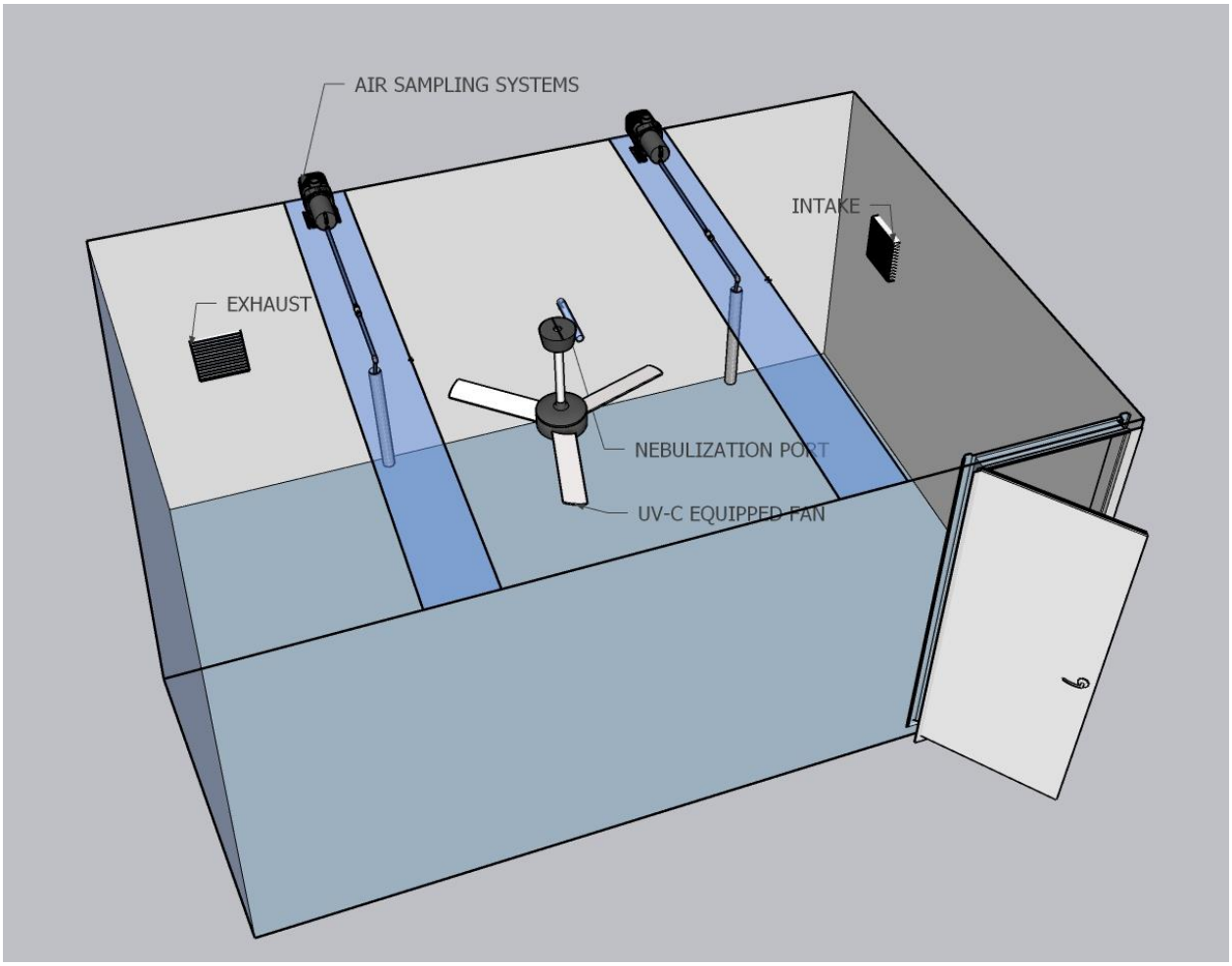
For this test no outside air was brought in during testing and intake remained sealed. Humidity and temperature were monitored inside the chamber using a calibrated wireless device. For air sample testing, the chamber was equipped with 2 probes that were along the centerline of the room and protruded down from the ceiling 24". Each probe tube was connected to a Gilian 10i programmable system with sampling cassettes from lot #2316 made by Zefron International. A single bioaerosol nebulizing port was in the center of the 10' wall. The dissemination port protruded from the wall 24" and was connected to a programmable compressor nebulizer system.

The UV-C fan combination provided by WAC Lighting was affixed to a mobile stainless-steel tubular stand inside the test chamber. A 10" down rod was provided by WAC Lighting and was affixed to the fan body. The main fan motor was approximately 12 inches below the ceiling line of the testing chamber. The fan operated in the standard forward rotation on a speed setting of 6.

Four pedestal stands were placed near each corner of the room with a height of 12 inches. On top of each table a single low volume mixing fan was placed to assist homogeneous mixing of the nebulized virus. Mixing fans had an approximately CFM of 32 and were angled up at approximately 45 degrees. Mixing fan speed were confirmed prior to testing with a vane anemometer.

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 test to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

BIOAEROSOL SETUP:



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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 72F +/- 2F with a relative humidity of 48%.
- 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.
- Sampling time points were T-20, T-30, T-40, measured in minutes from completed nebulization.
- Low volume mixing fans were turned on prior to nebulization to confirm homogenous concentrations in the test chamber. Once the overhead fan system was turned on the low volume mixing fans were turned off.
- Mixing fans were positioned at a 45-degree angle to encourage bioaerosol suspension prior to starting the UV-C fan system.
- Nebulization for control and viral test challenges were performed in the same manner with the variable being the UV-C fan unit.
- After nebulization was completed the UV-C fan system was engaged at the highest speed setting of 6. UV-C light ring was visually confirmed to be operational during testing.
- After each time point collection was completed the chamber was decontaminated and the air evacuated.
- 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.
- 1 control was completed, and 3 viral challenges were completed. Each time point was its own viral challenge, and the chamber was prepped and decontaminated the same way each time.

BIOAEROSOL GENERATION:

For the control and the viral challenges, the nebulizer was filled with the same amount of viral stock 6.23 x 10^6 TCID50 per mL in FBS based viral media. Solution was nebulized at a flow rate of 1ml/min. Nebulizer was driven by untreated local atmospheric air. The nebulizer's remaining viral stock volume was weighed after each completion to confirm the same amount of viral stock that was nebulized. Nebulizer was calibrated prior to testing and a particle counter confirmed average size disbursement to be .8 microns.

BIOAEROSOL SAMPLING:

For air sampling 2 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. The low volume mixing fans stayed active throughout all testing scenarios and conditions.

VIRUS STRAIN BACKGROUND:

The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through the BEI Resources, BIAID, NIH SARS-Related Coronavirus 2, Isolate USA-CA1/2020, NR-52382. This was the chosen pathogen strain because it was taken from a 38-year-old subject with severe acute respiratory syndrome in California as was part of the A lineage. This was a non-fatal case which represents most of the cases in the United State. The age group the patient belonged to was not elderly or juvenile, which was determined to be a good medium average.

POST DECONTAMINATION:

At the conclusion of each viral challenge timepoint/ 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.

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.
- CO_2 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:

One Control test was conducted without the UV-C fan system activated in the testing chamber. Control samples were taken at each of the corresponding sample times for the viral challenge. Nebulization of viral media was the same for the control as the viral challenge. Control testing was used for the comparative baseline to assess the viral reduction when the UV system was operated in the challenge trials, to enable net reduction calculations to be made. During the control test, four low volume fans were operated in each corner of the testing chamber to ensure homogenous mixing of the pathogen and shut off at the start of the trial which would be indicated by the 0-time point. During the control 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 +/- 3%.

VIRAL CHALLENGE:

The challenge pathogen, SARS-CoV-2-USA-CA1/2020, was used for testing the efficacy of the fan system equipped with the UV-C ring technology. 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 active pathogen to create a baseline of data. The UV-C fan combination system was in the same position for each viral challenge and operated in the same manner. Prior to nebulizing the viral pathogen, the UV system 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 and turned off just prior to starting nebulization. Four low volume mixing fans were used during the nebulization period of the control test and viral pathogen test. Sample times were as follows with T equal to minutes, T-20, T-30, T-40. Sampling occurred using 2 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	2.8 X 10^5 TCID50 per mL in 5 days at 37°C and 5% CO2
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

CONCLUSIONS:

The WAC Lighting Fan and UV-C combined technology performed similar to manufacturer specifications and showed a reduction of active virus after 20 minutes of exposure in aerosol form. The active SARS-CoV-2 virus was not detectable in the breathable air at the 30-minute timepoint, (levels were below the 120 TCID50 / ml limit of quantification. This would equate to a 4-log reduction compared to the control values. Within 20 minutes there was an 84.71% reduction in recoverable active pathogens in the air. After 30 minutes of exposure in the chamber there was a 99.99% reduction of collectable active pathogen in the air. After 40 minutes of exposure the results were the same as the 30-minute time point with a 99.99% reduction of collectable active pathogen in the air.

Taking into consideration the starting concentration of active SARS-CoV-2 virus used in the testing environment, the volume aerosolized, one could assume that the likelihood of entering a naturally occurring environment with this quantity of active pathogen in the air would be unlikely.

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 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.

There was a large amount of sterilization achieved by the fan UV-C combination system in the first 20 minutes. The reduction of collectable virus in the air was significant over the course of 40 minutes. Overall, the ceiling mountable fan with UV-C installed showed efficacy in the destruction and removal of SARS-CoV-2USA_CA1/2020 from the breathable air zones. Based on the understood method by which UV-C exposure deactivates pathogens and effects cell structure results would likely not be confined to s single genetic variation of the pathogen. Results would be expected to be similar on the various genetic mutations of SARS-CoV-2 which currently occur if the structures and sizes of the pathogens were consistent with test samples.

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 WAC Lighting device. Innovative Bioanalysis makes no claims to the overall efficacy of any WAC Lighting UV-C system. The experiment results are solely applicable to the device used in the trial. The results are only representative of the experiment design described in this report. Innovative Bioanalysis 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, 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.

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