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SARS-CoV-2USA-CA1/2020

CLIENT: SIGNIFY

PROJECT: UV WALL SYSTEM AEROSOL

PRODUCT: WL345W UV WALL MOUNT

CAP LIC NO: 886029801

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

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

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ABSTRACT: EFFICACY OF A WALL MOUNTED UV DEVICE AGAINST AEROSOLIZED SARS-CoV-2

Background: This in vitro study was designed to determine the efficacy of a The Philips UV-C disinfection upper air luminaire with a Philips T5 TUV lamp installed. The wall mounted luminaire is designed to decrease the concentration of pathogens in the air within a room when it is operating to lower the risk of transferring pathogens. For this challenge, the SARS-CoV-2-CA1/2020 pathogen was used. Coronavirus and similar pathogens can be spread through the air and by touching contaminated surfaces. Signify supplied a pre-packaged UV-C disinfection upper air wall mounted system for testing purposes. For the testing, power was supplied through a power regulated 120v outlet with a 200w step up voltage transformer. Transformer was set to the needed 220v required to power the 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: SIGNIFY

MODEL: PHILIPS UV-C DISINFECTION UPPER AIR LUMINAIRE

WALL MOUNT – PHILIPS T5 TUV LAMP 25W INCLUDED

UV EQUIPMENT:

The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. The system came with all components installed including the UV lamps. The device was powered on to check for normal operations. A burn in period was not performed by the lab as the manufacturer advised they had already completed the required burn in on the mercury lamps.

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'x10'.

The testing chamber had HEPA filtered inlets and exhaust, coupled with an active UV-C system in all ducting lines. 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 # 19766 made by Sensidine. 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.

Four pedestal stands were placed near each corner of the room with a height of 36 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.

The center line of the Philips UV-C upper air system was mounted 2.1 meters from the floor. The Philips UV-C upper air unit was mounted in the center of the 8-foot side wall of the testing chamber adjacent to the entrance. To allow the Philips UV-C upper air to reach optimal operating temperatures a remote release was fabricated with a strip of rigid material which the UV could not penetrate. Remote release mechanism was placed in front of the Philips UV-C upper air wall and prior to starting the trial the release was activated to expose the environment to UV-C.

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.

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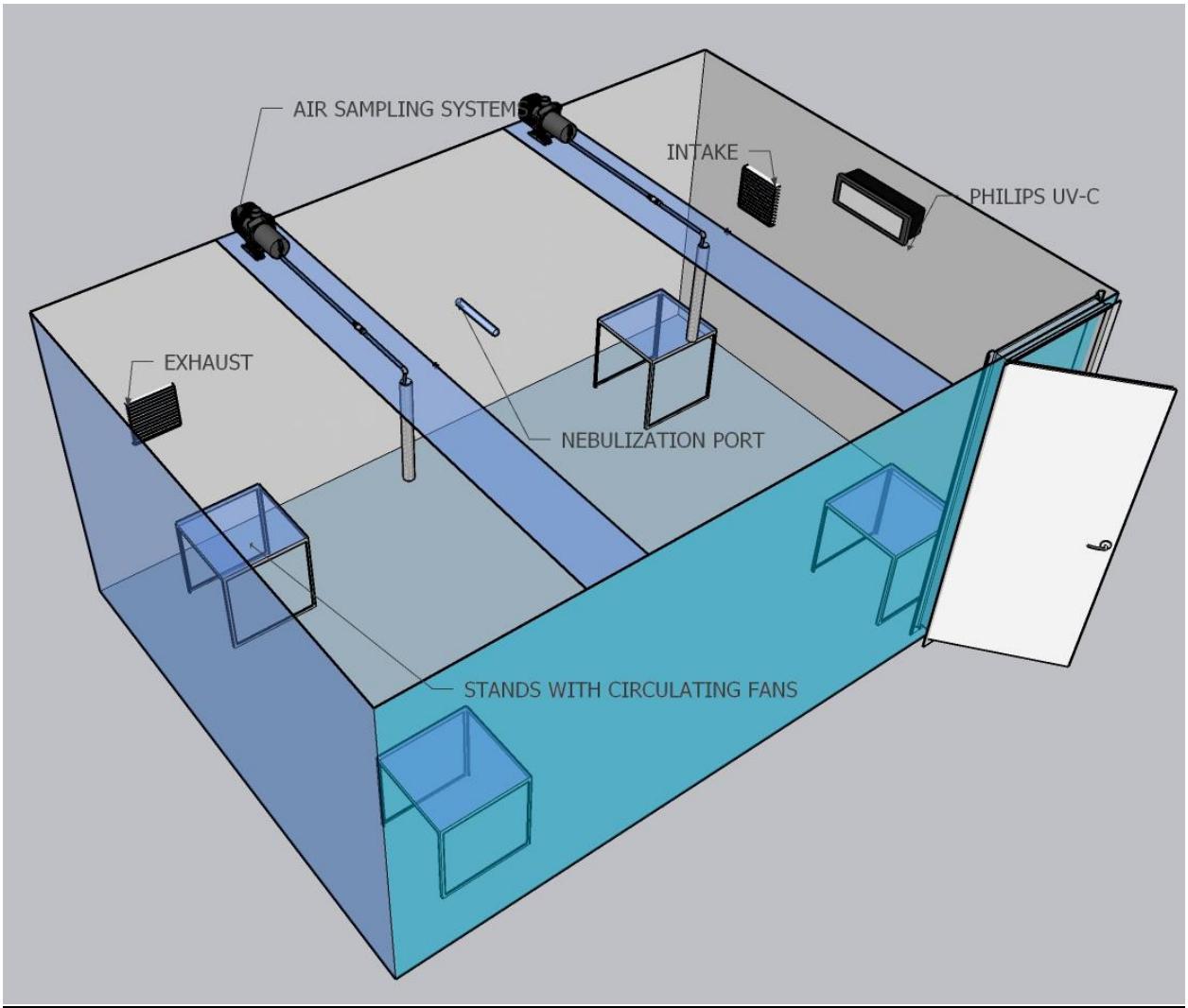
CHAMBER DESIGN:



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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 19.4c +/- 2F with a relative humidity of 51%.
- 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-2, T-5, T-10, T-20, measured in minutes from completed nebulization.
- Low volume mixing fans were turned on prior to nebulization to confirm homogenous concentrations in the test chamber.
- Mixing fans remained on and positioned at a 45-degree angle to encourage bioaerosol suspension and reduce natural particle descent rates.
- Nebulization for control and viral test challenges were performed in the same manner.
- After nebulization was completed the mechanical release for the material blocking the UV lamp dropped, exposing the atmosphere to UV, and was considered the zero-time point.
- 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 4 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 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. 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 +/- 5%.

VIRAL CHALLENGE:

The challenge pathogen, SARS-CoV-2-USA-CA1/2020, was used for testing the efficacy of the wall mounted UV system. 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 four distinct trials with the live pathogen to create a baseline of data. The wall mounted Philips upper air UV-C 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. UV emissions were blocked by a rigid material connected to an automatic drop system which prevented the UV from interacting with the virus during the nebulization period. Prior to starting the trial, the remote activated drop mechanism would be tripped, and the material would fall away from the UV system. Four low volume mixing fans were used throughout the entire control test and viral pathogen test. Sample times were as follows with T equal to minutes, T-2, T-5, T-10, T-20. 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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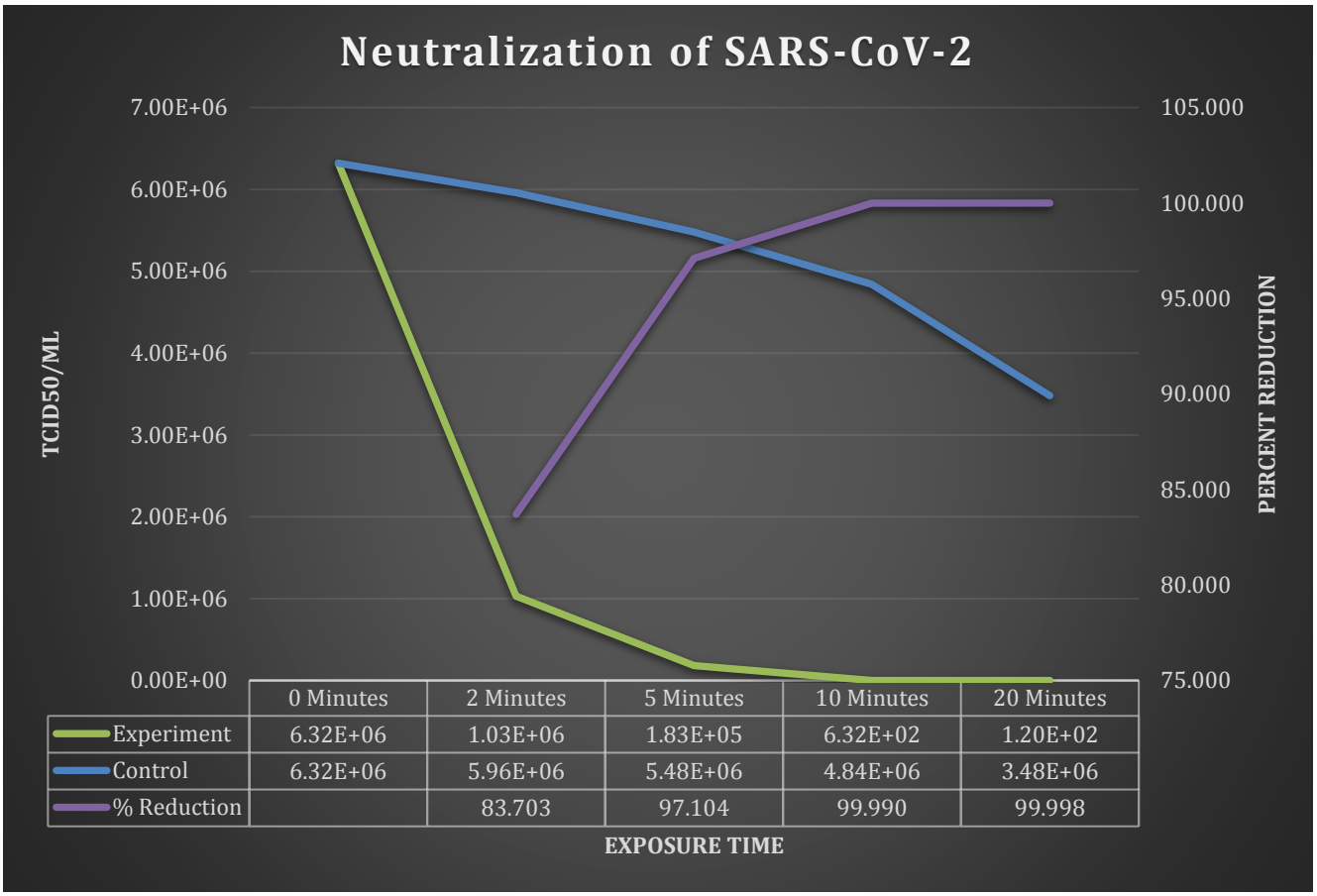
RESULTS:



	0 Minutes	2 Minutes	5 Minutes	10 Minutes	20 Minutes
Experiment	6.32E+06	1.03E+06	1.83E+05	6.32E+02	1.20E+02
Control	6.32E+06	5.96E+06	5.48E+06	4.84E+06	3.48E+06
% Reduction		83.703	97.104	99.990	99.998

CONCLUSIONS:

The Philips wall mounted UV-C system performed to manufacturer specifications and demonstrated a progressive reduction of active virus after 5 minutes of exposure in aerosol form. The live SARS-CoV-2 virus was not detectable at the 20-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 2 minutes there was an 83.6% reduction in recoverable active pathogens in the air. After 10 minutes of exposure in the chamber there was a 99.99% reduction of collectable active pathogen in the air.

Taking into consideration the starting concentration of active SARS-CoV-2 virus, the volume aerosolized, one could assume that the likelihood of entering an environment with this quantity of pathogen in a real-life circumstance to 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 UV system in the first 5 minutes. The reduction of collectable virus in the air was significant over the course of 10 minutes. Overall, the wall mounted UV system device showed efficacy in the destruction of SARS-CoV-2USA_CA1/2020 in air. Based on the understood method by which UV-C exposure deactivates pathogens it would be expected similar results on the various genetic mutations of SARS-CoV-2 would occur.

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