Innovative Bioanalysis, Inc.
3188 Airway Ave Suite D
Costa Mesa, CA 92626
www.InnovativeBioanalysis.com
Email: Albert.Brockman@innovativebioanalysis.com

SARS-CoV-2 USA-CA1/2020

CLIENT: ULTRAVATION

PROJECT: LCI-4 SURFACE

PRODUCT: UV MATRIX LCI-4

CAP LIC NO: 886029801

CLIA LIC NO: O5D0955926

STATE ID: CLF 00324630

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

INNOVATIVE BIOANALYSIS
creating solutions | getting results

ABSTRACT: EFFICACY OF THE UV MATRIX LCI-4™ DEVICE AGAINST SURFACE SARS-CoV-2

Background: This in vitro study was designed to determine the efficacy of the UV Matrix LCI-4™ unit on surfaces. The product is a commercially available disinfection device manufactured by Ultravation. The UV MATRIX LCI-4™ unit is designed to deactivate viral pathogens in the airstream of a typical HVAC system and ductwork. The functional goal is for the system to be placed in an active HVAC system and decrease the concentration of pathogens in the HVAC airflow when it is operating, to prevent the spread of viral media though ductwork. For this challenge, the SARS-CoV-2 USA-CA1/2020 pathogen was used. There is a demand for disinfectant devices that have a proven ability to reduce infectious pathogens in the air thereby reducing the risk of human infection and transmission. Ultravation supplied a pre-packaged UV MATRIX LCI-4™ 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: ULTRAVATION

MODEL: UV MATRIC LCI-4™

SERIAL #: NA

LAMPS – P/N -AS-IH-0610

UV MATRIX LCI-4™ EQUIPMENT:

The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. Four mercury vapor UV-C lamps were provided by the manufacturer to install in the device upon arrival. The optics were installed, and the device was powered on to check for normal operations. Prior to starting the challenge, the UV MATRIX LCI-4™ unit was operated for over 100 hours for a burn in period to simulate normal working conditions of the equipment.

VIRAL CHALLENGE TESTING CHAMBER:

The testing chamber was a large BSL3 testing Lab consisting of treated walls and epoxy floor which complied with BSL3 standards. Due to the size of the lamps the main BSL3 lab was used and the lamps were placed on a stainless-steel table with adjustable stands to increase and lower the height of the lamps to the testing surface. The standard HVAC system was operating during the course of testing, which consisted of a negative pressure system and outfitted with HEPA filters on the exhaust line. There was an electronic control anti chamber with self-locking doors and key card access required. Air pressure was monitored throughout the test to confirm negative pressure was maintained.

All seals for the chamber were confirmed and all equipment used had a function test done to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

BSL-3 LAB:

INNOVATIVE BIOANALYSIS
creating solutions | getting results

EXPERIMENTAL SUMMARY:

- Each test time point used a 3" x 1.5" piece of sterile glass, 0.125" thick. Test slides were inoculated with the virus by directly applying 1mL of viral solution to the slide and splaying it out with a spatula.
- The UV MATRIX LCI-4™ was operated for 20 minutes in an adjacent room to warm up the UVC lamp prior to being placed in the test environment.
- Test was conducted in one phase with three different time points.
- Distances were measured from the centerline between the lower UV-C lamp to the face of the glass slide. Test slides were placed in the center orientation 6 inches below the lower plane of lamps.
- One slide sample was used for each time point and was placed on top of a wire rack over a stainless-steel surface.
- For accurate time point a pneumatic iris dampener was placed over the slides with an ATC 304 GX Solid State timer attached.
- Iris was checked visually for light pollution leaks and closing speeds were checked.
- ATC timer was checked visually and tested against a stopwatch to confirm timing speeds.
- Sample time points were the following for each phase.
    - .25 seconds
    - .5 seconds
    - 2 seconds
- Each glass test slide was placed in a 50ml sterile tube and rinsed with 1ml of preservation media in addition to swabbing with a sterile swab.
- Upon testing, completion samples were provided to lab staff for further review.

TESTING PROCEDURE:

A single glass slide was placed on the wire rack underneath the UV MATRIX LCI-4™ System. The Iris damper system was placed above the system to restrict exposure to the sample for the afore mentioned time points. The Iris dampener was manually opened for the start of the test and was automatically closed at the appropriate time point by the ATC timer. The UV-C light was turned off and the Iris opened allowing for the removal of the test slide.

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.

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:

For the control section the same testing environment was replicated in an adjacent room so atmospheric conditions were similar. Humidity in the control room and relative temperature were approximately the same as the viral testing room. For the control samples five 3” x 1.5” pieces of sterile glass, 0.125” thick were placed in the center of the bio safety cabinet. The glass slides were inoculated with the virus by directly applying viral solution with a pipette, distributed with a spatula, and allowed to air dry for 5 minutes. Each test slide was placed in a 50ml sterile tube and washed with viral preservation media and swabbed than removed from the testing chamber after each time point.

EFFICACY TESTING:

Viral media with a known concentration was applied via pipette and distributed by spatula to the slides in 1 location at a distance of 6 inches away from the UV MATRIX LCI-4™. Samples were exposed to UV-C for a period of .25, seconds, .50 seconds, and 2 seconds post inoculation. Samples were collected of all marked inoculation sites and all distances according to time point and cultured by the same means as the original viral titration performed on the BEI Resources provided SARS-CoV-2 USA-CA1/2020 viral culture.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

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

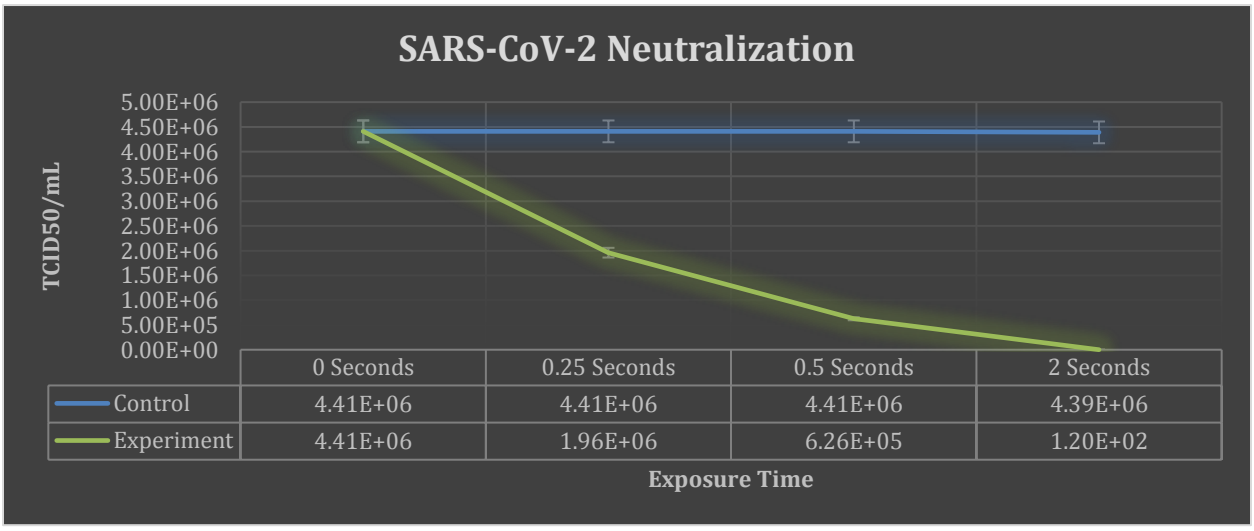
Inoculation of Surface Samples:

Surface inoculation consisted of applying exactly 1 ml of viral media to each coupon with a calibrated Eppendorf pipette utilizing filtered pipette tips. Coupons were standard sterile 25mm x 75mm slides. Once applied, the media was spread thin using a disposable spatula and allowed to dry for 10 minutes. The starting concentration of virus that was applied was 4.63 X 10^6 TCID50/mL. After several tests for recovery, it was determined that the most efficient method of recovering viable virus would be a 2 mL rinse in viral media followed by a swab of the inoculated area. The maximum recovery achieved was 4.41 X 10^6 TCID50/mL. This value was used as the "0 Minute" starting concentration for all surface testing to account for the recovery.

Test Results: Surface Inoculation:

Performed in the same manner as the control testing, the following deactivation rates were observed for direct surface inoculation in the three challenge trials. Collection at each time point was done by swab and rinse of the coupon. Samples were collected by a technician at specified time points inside the container and stored in viral media until testing and recovery were complete. Technicians were wearing full hazmat coveralls and had no direct exposure to the pathogen at any time. The graph below represents the data for the three experiments and the control, as it pertains to surface inoculation.

RESULTS:



	0 Seconds	0.25 Seconds	0.5 Seconds	2 Seconds
Control	4.41E+06	4.41E+06	4.41E+06	4.39E+06
Experiment	4.41E+06	1.96E+06	6.26E+05	1.20E+02

CONCLUSIONS FOR SURFACE INACTIVATION AT A GIVEN DISTANCE:

The UV MATRIX LCI-4™ performed as expected with an overall reduction in live viral count. This equipment has performed as claimed by the manufacturer and showed an overall 99.99% reduction of viral media in 2 seconds. For the purpose of this experiment, it can be deduced that given significant exposure time, the overall viral neutralization rate would be substantial.

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, or byproduct of any UV Matrix LCI-4™ device. Innovative BioAnalysis makes no claims to the overall efficacy of any UV Matrix LCI-4™. 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.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

DocuSigned by:
Dana Yee
7D5A69A0907947B...

3/8/2021

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

DocuSigned by:
Sam Kabbani
8B4B282DF4B34A3...

3/8/2021

Sam Kabbani, MS, BS, MT(ASCP), CLS **Date**
Chief Scientific Officer, Innovative Bioanalysis

DocuSigned by:
Albert Brockman
06DF5C77A0D2400...

3/8/2021

Albert Brockman **Date**
Chief Biosafety Officer, Innovative Bioanalysis

DocuSigned by:
Kevin Noble
5DF2797BAA78421...

3/8/2021

Kevin Noble **Date**
Chief Operating Officer, Innovative Bioanalysis