INNOVATIVE BIOANALYSIS
creating solutions | getting results

EFFICACY OF THE ANTHEM ONE A-PW-UVC-001 AGAINST SURFACE SARS-COV-2

PROJECT: ANTHEM ONE – A-PW-UVC-001 – SURFACE SARS-COV-2

PRODUCT: A-PW-UVC-001

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM (S):

SARS-COV-2 USA-CA1/2020

Medical Director

Dana Yee, M.D.

Testing Facility

Innovative Bioanalysis, Inc.

3188 Airway Ave Suite D

Costa Mesa CA, 92626

www.InnovativeBioanalysis.com

Email: info@innovativeBioanalysis.com

Laboratory Project Number

1279

INNOVATIVE BIOANALYSIS
creating solutions | getting results

Table of Contents

INNOVATIVE BIOANALYSIS
creating solutions | getting results

Efficacy Study Summary

Study Title	EFFICACY OF THE ANTHEM ONE A-PW-UVC-001 AGAINST SURFACE SARS-COV-2
Laboratory Project #	1279
Guideline:	No standard exists; GLP and modified ISO 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 A-PW-UVC-001 is a portable, handheld UV-C disinfection device used to reduce active surface pathogens. The device was provided by Anthem One for an in vitro study to evaluate the efficacy of the UV-C device against surface SARS-CoV-2.
Test Conditions	All testing was conducted in a sealed plexiglass glovebox inside a BSL-3 laboratory. The temperature during all test runs was approximately 77 ±2°F, with a relative humidity of 29%. A 6.33×10^6 TCID50/mL of SARS-CoV-2 in suspension media was dried onto a glass slide placed 2-inches from the direct line of the UV light. Surface collection occurred after 10 seconds of operation.
Test Results	After 10 seconds, the device reduced a starting concentration of 6.33×10^6 TCID50/mL of SARS-CoV-2 to below the specified limit of quantitation represented by the value 1.20×10^2 TCID50/mL for all three test trials.
Control Results	The control was conducted in triplicate without the device, and samples were taken at corresponding time points as the challenge. The results displayed a natural viability loss in 10 seconds within the chamber and was used as a comparative baseline to calculate viral reduction.
Conclusion	The Anthem One A-PW-UVC-001 handheld UV-C device demonstrated it could reduce active SARS-CoV-2 on non-porous surfaces located 2-inches away from the device within 10 seconds of operation. The data showed the device achieved a 99.998% gross reduction after 10 seconds.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

Study Report

Study Title: EFFICACY OF THE ANTHEM ONE A-PW-UVC-001 AGAINST SURFACE SARS-COV-2

Sponsor: Anthem One

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

Device Testing: A-PW-UVC-001

Study Dates:

Study Report Date: 03/21/2022

Experimental Start Date: 03/19/2022

Experimental End Date: 03/19/2022

Study Objective:

Anthem One supplied a A-PW-UVC-001 designed as a handheld, portable UV-C device to decrease the concentration of active pathogens on surfaces. This study evaluated the A-PW-UVC-001's efficacy against surface SARS-CoV-2 under controlled conditions.

Test Method:

Surface Inoculation:

Each glass slide was equally subjected to a 1mL inoculation of viral media containing a known titer of 6.33 X 10^6 TCID50/mL for the control and viral challenge. 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. The slides were visibly dry before being used for testing.

Surface Sampling:

Slides were rehydrated with 1mL viral media before collecting samples. Each slide was subjected to a 1mL rinse in viral media and swabbed for residual pathogen material. After collection, the swab and media were vortexed for one full minute.

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.

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 Vero E6 cells 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 2.0 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, 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.
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 0.5 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.

Study Materials and Equipment:

Equipment Overview: The equipment (Fig. 1) arrived at the laboratory pre-packaged 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 device. Before testing, the A-PW-UVC-001 handheld UV-C device was powered on to confirm correct operations.

MANUFACTURER: Anthem One

MODEL: A-PW-UVC-001

DIMENSIONS: 5.5-inch cube

Figure 1. Anthem One A-PW-UVC-001D as tested.

Testing Layout:

Testing was conducted in a sealed plexiglass glovebox inside a lab conforming to Biosafety Level 3 (BSL3) standards. The room remained closed to prevent any air from entering and leaving the room during testing. The device was placed in the center of the test chamber and elevated. The inoculated slide was placed 2 inches away from the device on top of a black matte non-reflective mat in direct line of the UV light. The chamber was visually inspected, pressure tested, and all internal lab systems and equipment were reviewed before testing.

Figure 2. Control and experimental testing layout.

Control Protocol:

Surface testing control was conducted in triplicate without the device operating in the testing chamber to assess the Anthem One A-PW-UVC-001 accurately. The collection was taken at corresponding time points used for the challenge trial in the same manner, to serve as a comparative baseline to evaluate aerosolized viral reduction when the device was operating.

Test Procedures:

Exposure Conditions:

1. The temperature during all test runs was approximately 77 ±2°F, with a relative humidity of 29%.
2. The surface sample was collected at 0 and 10 seconds of operation.
3. Three controls and viral challenges were conducted using the same methodology.

Experimental Procedures:

1. Before the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. Sterile glass slides were labeled and inoculated with 1mL of 6.33×10^6 TCID50/mL SARS-CoV-2 suspension media inside a biosafety cabinet.
3. The sample slide was placed 2 inches away from the device in direct line of the UV light.
4. The slide was removed at the corresponding time points to be swabbed and rinsed with viral suspension media.
5. After collection, all swabs were sealed and provided to lab staff for analysis after study completion.

Post Decontamination:

After 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.

Preparation of The Pathogen

Viral Stock: SARS-CoV-2 USA-CA1/2020 (BEI NR-52382)

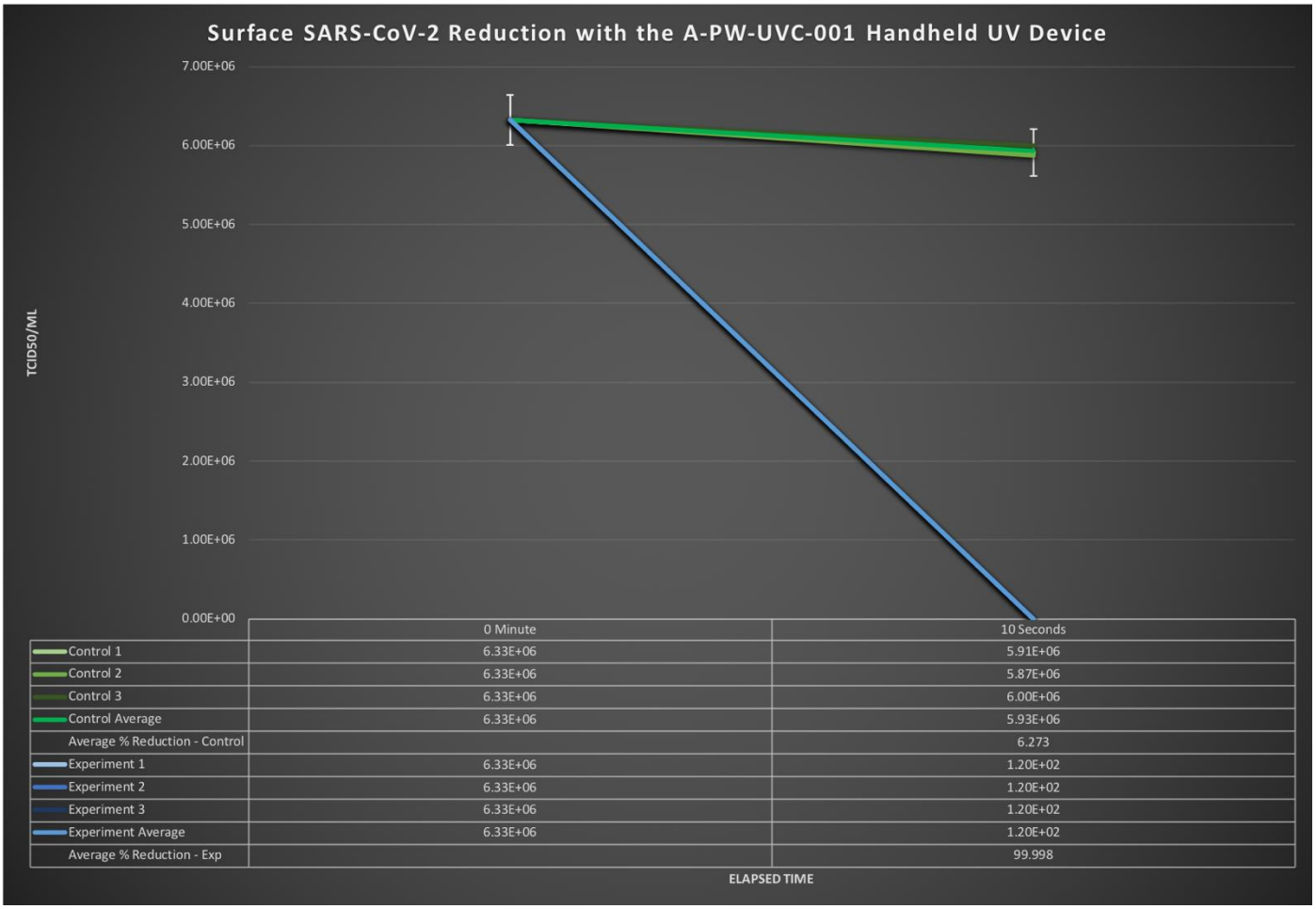
TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero 6 Cells	Cell Rounding and Detachment	Cell Rounding and Detachment
Next-Generation Sequencing (NGS) of the complete genome using Illumina® iSeq™ 100 Platform	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1	99.9% 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.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

Study Results

The results were plotted to display active SARS-CoV-2 recovered on the surface with and without A-PW-UVC-001 operating inside the chamber. The controls showed an average viability loss of 6.273% in 10 seconds. With the A-PW-UVC-001, a reduction from an initial starting concentration of 6.33 x 10^6 TCID50/mL to 1.20 x 10^2 TCID50/mL, indicating a titer below levels of quantification, for all three runs was observed.



	0 Minute	10 Seconds
Control 1	6.33E+06	5.91E+06
Control 2	6.33E+06	5.87E+06
Control 3	6.33E+06	6.00E+06
Control Average	6.33E+06	5.93E+06
Average % Reduction - Control		6.273
Experiment 1	6.33E+06	1.20E+02
Experiment 2	6.33E+06	1.20E+02
Experiment 3	6.33E+06	1.20E+02
Experiment Average	6.33E+06	1.20E+02
Average % Reduction - Exp		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.

Conclusion

The Anthem One A-PW-UVC-001 significantly reduced active surface SARS-CoV-2 virus within 10 seconds of exposure. The results showed at 2-inches, the device could decrease surface pathogens by a gross reduction of 99.998%.

When inoculating pathogens and collecting said pathogens, some variables cannot be fully accounted for, namely, placement of pathogen, collection volume, surface saturation, viral destruction on inoculation, viral destruction on collection, 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.

Considering the variables, there was a measurable reduction achieved by the Anthem One A-PW-UVC-001 at 10 seconds of exposure. Overall, the UV lamp significantly reduced SARS-CoV-2 from the surface samples collected.

INNOVATIVE BIOANALYSIS
creating solutions | getting results

DocuSigned by:
Dana Yee
52F0012FC10C43F...

4/6/2022

Dana Yee M.D **Date**

Clinical Pathologist and Medical Director, Innovative Bioanalysis, Inc.

DocuSigned by:
Sam Kabbani
8B4B282DF4B34A3...

4/6/2022

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

Chief Scientific Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Albert Brockman
42751B72F2FF40A...

4/6/2022

Albert Brockman **Date**

Chief Biosafety Officer, Innovative Bioanalysis, Inc.

DocuSigned by:
Kevin Noble
5DE2797BAA78421...

4/6/2022

Kevin Noble **Date**

Laboratory Director, Innovative Bioanalysis, Inc.

Disclaimer

The Innovative Bioanalysis, Inc. ("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 Anthem One devices. Innovative Bioanalysis, Inc. makes no claims to the overall efficacy of any A-PW-UVC-001. 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, Inc. 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, nebulization, viral media, cell type, and culture procedure. Innovative Bioanalysis, Inc. 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.