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EFFICACY OF THE NUVAWAVE HANDHELD UV DEVICE AGAINST SURFACE MONKEYPOX

PROJECT: WELLAIR – NUVAWAVE UV – MONKEYPOX SURFACE

PRODUCT: NUVAWAVE HANDHELD UV DEVICE

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CDF 00324630

CHALLENGE ORGANISM:

MONKEYPOX VIRUS

STUDY COMPLETION DATE:

09/01/2022

Medical Director

Dana Yee, M.D.

Testing Facility

Innovative Bioanalysis, Inc.

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Laboratory Project Number

1336

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Efficacy Study Summary

Study Title	EFFICACY OF THE NUVAWAVE HANDHELD UV DEVICE AGAINST SURFACE MONKEYPOX
Laboratory Project #	1336
Guidelines:	GCLP, modified ISO, and BSL-3 standards were used.
Testing Facility	Innovative Bioanalysis, Inc.
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	Monkeypox Virus
Description	Per the manufacturer, the LED array on the NuvaWave Handheld UV device was designed to reduce surface pathogens when exposed to the UV emitted from the diodes. WellAir Solutions supplied a customized rig with the UV LED array installed for an in vitro study to evaluate the efficacy of the NuvaWave Handheld UV Device prototype against the Monkeypox virus on a surface.
Test Conditions	Testing was conducted in a customized rig provided by WellAir in an 11'x11'x8' chamber following BSL-3 standards. The temperature during testing was approximately 75 ±2 (23.9 ±1°C), with a relative humidity of 41%. A hydrophilic glass slide was inoculated with 0.1 mL of 3.65×10^7 TCID50/mL Monkeypox in viral media and left to air dry for 40 minutes before being placed on the customer-provided rig for testing. Surface collection occurred at 1 and 2 seconds of exposure, with irradiance recorded during each trial.
Test Results	After 1 second against the Monkeypox virus, the NuvaWave Handheld UV device reduced a starting concentration of 3.65×10^7 TCID50/mL to an average of 1.42×10^7 TCID50/mL. After 2 seconds of UV exposure, recoverable active Monkeypox decreased to 5.71×10^6 TCID50/mL.
Control Results	Control was conducted in duplicate without UV exposure from the device, and samples were taken at corresponding time points as the challenge. The data was used as a comparative baseline to calculate viral reduction.
Conclusion	The NuvaWave Handheld UV device demonstrated the ability to reduce the Monkeypox virus on a surface, as shown by the 61.01% (0.41 log reduction) gross reduction after 1 second. More prolonged exposure resulted in a higher drop, as observed by the gross reduction of 84.35% (0.81 log reduction) after 2 seconds.

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Study Report

Study Title: EFFICACY OF THE NUVAWAVE HANDHELD UV DEVICE AGAINST SURFACE MONKEYPOX

Sponsor: WellAir Solutions

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

Device Testing: NuvaWave Handheld UV device & "Corona Coaster" test rig

Study Dates:

Study Report Date: 11/01/2022
Experimental Start Date: 09/15/2022
Experimental End Date: 09/15/2022
Study Completion Date: 10/25/2022

Study Objective:

WellAir Solutions provided a NuvaWave Handheld UV prototype device designed for ease of use in disinfecting contaminated surfaces. This study sought to determine the device's ability to reduce surface Monkeypox virus under controlled conditions.

Test Method:

Surface Inoculation:

Each hydrophilic glass slide was equally subjected to a 0.1 mL inoculum of viral media containing a known Monkeypox virus titer of 3.65×10^7 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 40 minutes. The slides were visibly inspected for dryness before being used for testing.

Surface Sampling:

Slides were rehydrated with 1mL of viral media before collecting samples. Each slide was subjected to a rinse into 10mL viral media and swabbed for residual pathogen material. After collection, the swab and media were vortexed for one full minute.

Test System Strains: Monkeypox Virus – hMPXV/USA/MA001/2022 (Lineage B.1, Clade IIb)

The following reagent was deposited by Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: Monkeypox Virus, hMPXV/USA/MA001/2022 (Lineage B.1, Clade IIb), NR-58622.

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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 temperatures as indicated

Procedure:

1. One day before infection, prepare a 96-well plate by seeding each well with BSC-40 cells in DMEM plus fetal bovine serum, 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 2.0mL PBS and the subsequent tubes with 1.8mL.
4. Vortex the viral samples, then transfer 20uL of the virus to the first tube, vortex, and discard tip.
5. With a new tip, serial dilute subsequent tips transferring 200uL.

Additions of virus dilutions to cells:

1. Label the lid of a 96-well plate 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 0.1mL of media from each well by vacuum aspiration.
4. Starting from the most dilute sample, add 0.1mL 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 0.5mL 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. The customized rig provided by WellAir Solutions was pre-assembled and had the NuvaWave Handheld UV prototype device installed. Confirmation of functionality prior to testing was performed following an operation video, "Corona Coaster Test Fixture," and a Quick Start Guide Verification Protocol provided by WellAir.

MANUFACTURER: WellAir Solutions

PRODUCT: NuvaWave Handheld UV (prototype) & Corona Coaster test rig

DIMENSIONS: 4" x 4" Disinfection area

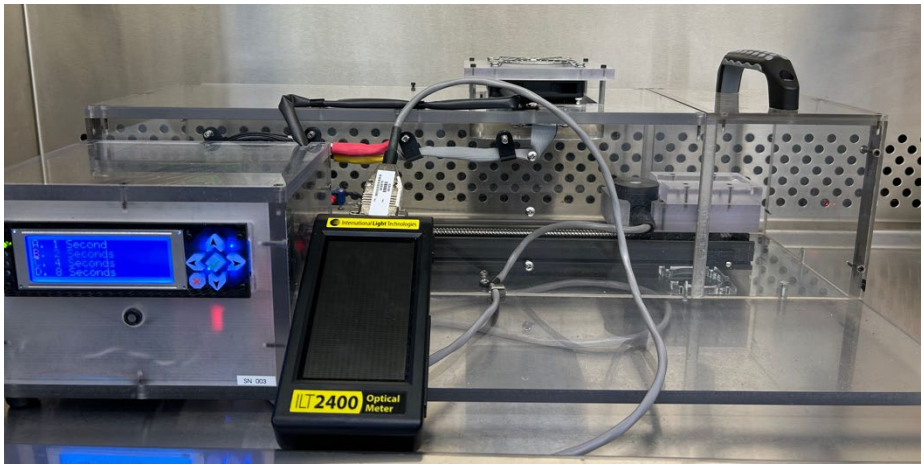

Figure 1. The NuvaWave Handheld UV prototype pre-installed at the top of the customized Corona Coaster rig as tested.

Testing Layout:

Testing was conducted in a customer-provided rig called the Corona Coaster, placed in an 11' x 11' x 8' chamber following Biosafety Level 3 (BSL3) standards, as shown in Figure 2. The Corona Coaster Test Fixture was placed in the center of the biosafety cabinet and set up according to the Quick State Guide Verification Protocol. Before each test, the glass slide was placed on the stage for automated exposure to the device. The chamber was visually inspected, pressure tested, and all internal lab systems and equipment were reviewed before testing.

Control Protocol:

The Surface testing control was conducted in duplicate without UV exposure to the surface to assess the NuvaWave Handheld UV prototype device 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 assess viral reduction when the device was operating.

Test Procedures:

Exposure Conditions:

1. The temperature during all test runs was approximately 70 ±2°F (21.1 ±1°C).
2. Surface samples were collected after 1 second and 2 seconds of exposure.
3. Two controls and three challenges were conducted using the same methodology.

Experimental Procedures:

1. Sterile hydrophilic glass slides were labeled and inoculated with 0.1 mL of 3.65 x 10^7 TCID50/mL Monkeypox viral media inside a biosafety cabinet and left to dry.
2. The slide was placed on the stage of the sliding platform inside the customer-provided test rig. The NuvaWave Handheld UV device, light meter, and test rig were operated per instructions and video.
3. The predetermined time dosage was selected before pressing the green button.
4. Once the stage irradiates the sample and returns the sample to the home position, the radiant exposure will be recorded. Refer to Appendix C for measurements.
5. The slide was then removed at the corresponding time points to be rehydrated, rinsed with suspension media, and vortexed.
6. After collection, all swabs were sealed and provided to lab staff for analysis after study completion.

Post Decontamination:

After each viral challenge test was completed, the UV system inside the biosafety chamber was activated for 30 minutes for decontamination. In addition to the 30 minutes of UV exposure between tests, all test equipment was cleaned at the end of each day with a 70% alcohol solution.

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Preparation of The Pathogen

Viral Stock: Monkeypox Virus, hMPXV/USA/MA001/2022 (NR-58622)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in BSC-40 Cells	Cell rounding and detachment	Cell rounding and detachment
Titer by $TCID_{50}$ Assay in BSC-40 Cells by Cytopathic Effect	Report Results	1.8×10^6 $TCID_{50}$ per mL
Sterility (21-Day Incubation)		
Trypticase Soy Broth, aerobic	No Growth	No Growth
Sabouraud Agar, aerobic	No Growth	No Growth
Trypticase Soy Agar, aerobic	No Growth	No Growth
Trypticase Soy Agar, anaerobic	No Growth	No Growth
Thioglycollate Broth, anaerobic	No Growth	No Growth
DMEM with 10% FBS	No Growth	No Growth
Mycoplasma Contamination		
MycoSensor QPCR Assay Kit	Negative	Negative

*The viral titer listed in the Certificate of Analysis represents the titer provided by BEI Resources. See Appendix D for more details.

Study Results:

The graph (Fig. 2) below displays recovered active Monkeypox virus with and without 1 second UV exposure from the NuvaWave Handheld UV prototype on the test surface. The controls showed natural viability loss of surface Monkeypox after 1 second under controlled conditions. Across three surface testing trials, the device lowered a starting concentration of 3.65 x 10^7 TCID50/mL to 1.66 x 10^7, 1.36 x 10^7, and 1.25 x 10^7, averaging approximately 1.42 x 10^7 TCID50/mL after 1 second of exposure. See Table 1 for the data table and Appendix E for the $\log_{10}$ reduction data.

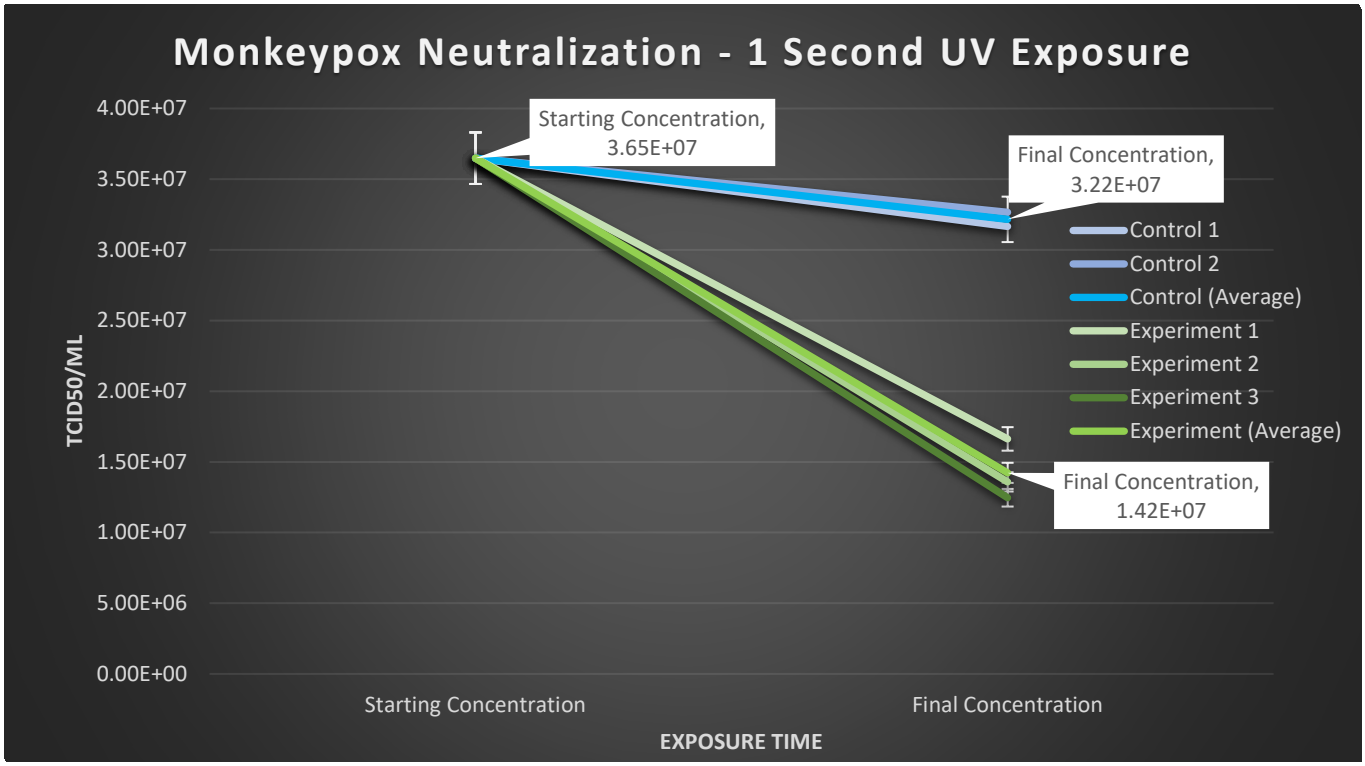

Figure 2: Monkeypox neutralization using the NuvaWave prototype for 1-second UV exposure under controlled conditions.
*** As it pertains to data represented herein; the percentage error equates to an average of ±5% of the final concentration.*

Table 1: Results Data and Calculated Percentage Reductions after 1 second of UV exposure

	Starting Concentration	Final Concentration
Control 1 (TCID50/mL)	3.65E+07	3.17E+07
Control 2 (TCID50/mL)	3.65E+07	3.27E+07
Control Average (TCID50/mL)	3.65E+07	3.22E+07
% Gross Reduction – Control		11.86%
Experiment 1 (TCID50/mL)	3.65E+07	1.66E+07
Experiment 2 (TCID50/mL)	3.65E+07	1.36E+07
Experiment 3 (TCID50/mL)	3.65E+07	1.25E+07
Experiment Average (TCID50/mL)	3.65E+07	1.42E+07
% Gross Reduction – Experiment		61.01%
% Net Reduction		**55.77%**

The graph (Fig. 3) below displays recovered active Monkeypox virus with and without 2 seconds of UV exposure from the NuvaWave prototype on the test surface. The controls showed natural viability loss of surface Monkeypox after 2 seconds under controlled conditions. Across three surface testing trials, the device lowered a starting concentration of 3.65 x 10^6 TCID50/mL to 5.89 x 10^6, 8.25 x 10^6, and 3.00 x 10^6, averaging approximately 5.71 x 10^7 TCID50/mL after 1 second of exposure. See Table 2 for the data table and Appendix E for the $\log_{10}$ reduction data.

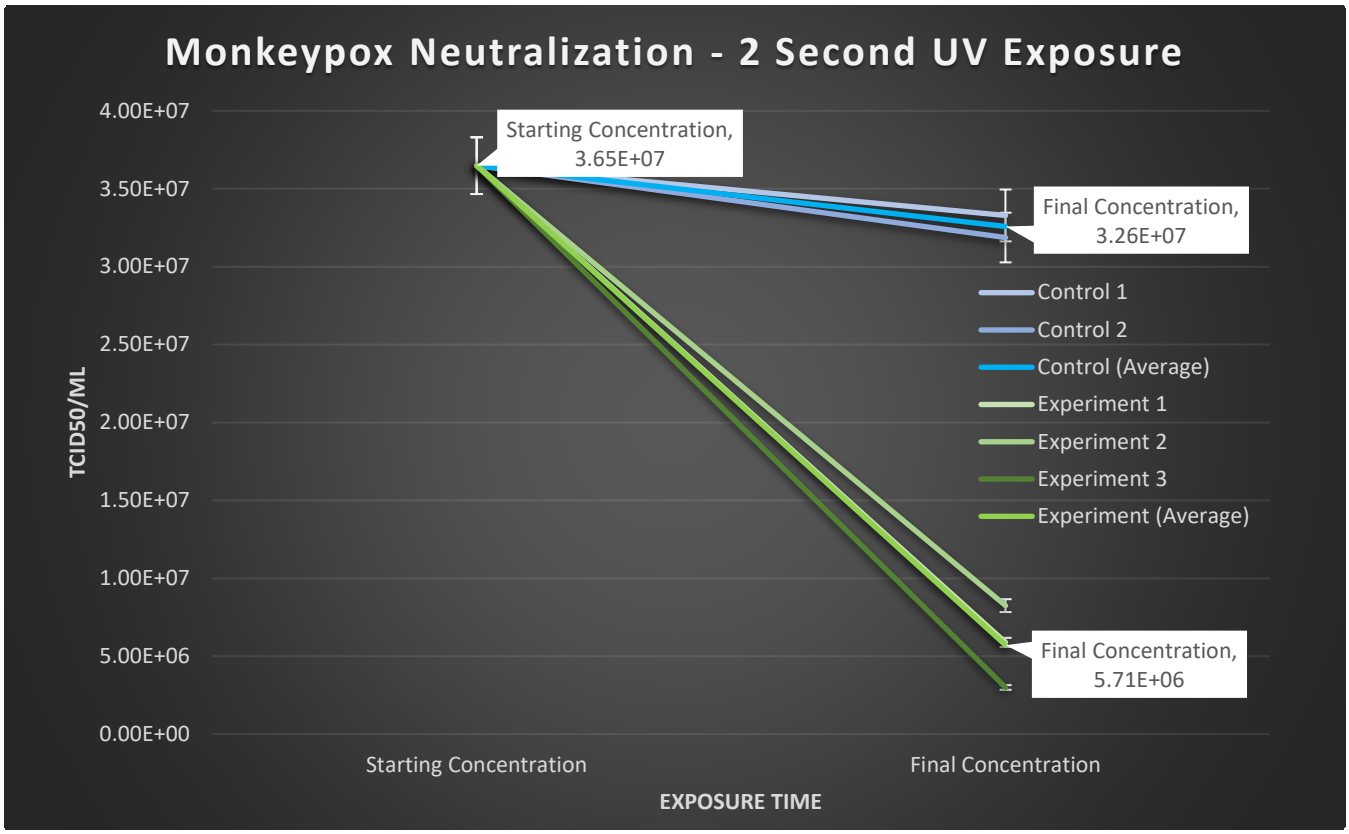

Figure 3: Monkeypox neutralization using the NuvaWave prototype for a 2-second UV exposure under controlled conditions.
*** As it pertains to data represented herein; the percentage error equates to an average of ±5% of the final concentration.*

Table 2: Results Data and Calculated Percentage Reductions after 2 seconds of UV exposure

	Starting Concentration	Final Concentration
Control 1 (TCID50/mL)	3.65E+07	3.33E+07
Control 2 (TCID50/mL)	3.65E+07	3.19E+07
Control Average (TCID50/mL)	3.65E+07	3.26E+07
% Gross Reduction – Control		10.71%
Experiment 1 (TCID50/mL)	3.65E+07	5.89E+06
Experiment 2 (TCID50/mL)	3.65E+07	8.25E+06
Experiment 3 (TCID50/mL)	3.65E+07	3.00E+06
Experiment Average (TCID50/mL)	3.65E+07	5.71E+06
% Gross Reduction – Experiment		84.35%
% Net Reduction		**82.47%**

Conclusion:

The NuvaWave Handheld UV device demonstrated the ability to reduce active surface Monkeypox virus more rapidly than the natural loss rates observed under controlled conditions. The device displayed an average 55.77% net reduction after 1 second of UV exposure. With 2 seconds of exposure from the prototype, the average net reduction increased to 82.47%.

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 collection, viral destruction on inoculation, 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 viruses in the control test.

Considering the variables, the NuvaWave Handheld UV prototype device displayed measurable reduction after 1 and 2 seconds of UV exposure. The results observed were consistent with the manufacturer's claim that the device can decrease concentrations of active pathogens on surfaces. Overall, the NuvaWave Handheld UV prototype resulted in a 0.41 log reduction after 1 second and 0.81 log reduction after 2 seconds as compared to a 0.05 log reduction observed in the control group.

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Laboratory Director, Innovative Bioanalysis, Inc.

APPENDIX A: Glossary of Terms

CAP: The College of American Pathologists (CAP), the leading organization of board-certified pathologists, serves patients, pathologists, and the public by fostering and advocating excellence in the practice of pathology and laboratory medicine worldwide. A laboratory can pursue a higher level of quality by becoming accredited by The College of American Pathologists (CAP).

CLIA: The Clinical Laboratory Improvement Amendments of 1988 (CLIA) are federal regulations for the United States-based clinical laboratories to provide industry standards for testing human samples for diagnostic purposes.

COA: A Certificate of Analysis refers to an authenticated document that is issued by BEI or ATCC Quality Assurance Department that ascertains that a product has met its predetermined pathogen specifications and preparations.

DMEM: Dulbecco's Modified Eagle Medium (DMEM) is a widely used basal medium for supporting the growth of many different mammalian cells.

FBS: Fetal bovine serum (FBS) is derived from the blood drawn from a bovine fetus via a closed collection system at the slaughterhouse. Fetal bovine serum is the most widely used serum supplement for the in vitro cell culture of eukaryotic cells. This is because it has an extremely low level of antibodies and contains more growth factors, allowing for versatility in many different cell culture applications.

The globular protein, bovine serum albumin (BSA), is a major component of fetal bovine serum. The rich variety of proteins in fetal bovine serum maintains cultured cells in a medium where they can survive, grow, and divide.

Because it is a biological product, FBS is not a fully defined media component and varies in composition between batches. As a result, serum-free and chemically defined media (CDM) have been developed to minimize the possibility of transferring adventitious agents. However, the effectiveness of serum-free media is limited, as many cell lines still require serum to grow, and many serum-free media formulations can only support the growth of narrowly defined types of cells.

LLOQ: The ULOQ and LLOQ are the highest and lowest standard curve points that can still be used for quantification; they are the values below and above which, respectively, quantitative results may be obtained with a specified degree of confidence, or the highest/lowest concentration of an analyte that can be accurately measured. Together, the ULOQ and LLOQ define the range of quantification for the assay. Limits of quantitation are matrix, method, and analyte-specific and can be calculated as follows:

Equation 1.

(Calculation used in Q-View): ULOQ & LLOQ = Highest or Lowest Standard, respectively, with a %backfit of 120%-80%, a %CV of < 30%, and a positive mean pixel intensity difference between it and the negative control.

Equation 2.

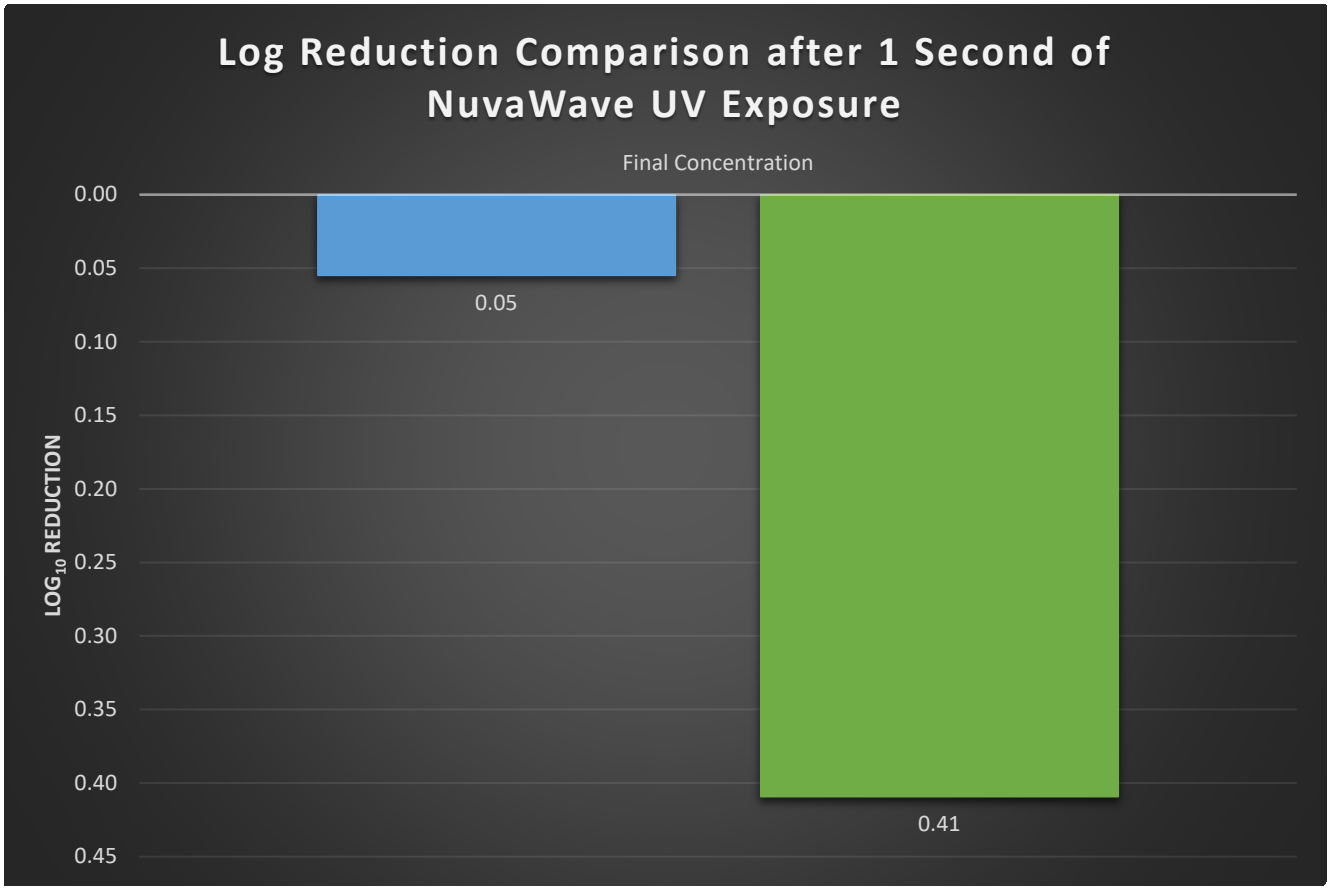
(Commonly used in science to estimate the LLOQ): LLOQ = (Mean negative control pixel intensity) + 10 * (StDev of negative control pixel intensities).

PBS: Phosphate buffered saline (PBS) is a pH-adjusted blend of ultrapure-grade phosphate buffers and saline solutions which, when diluted to a 1X working concentration, contains 137mM NaCl, 2.7mM KCl, 8mM Na_2HPO_4, and 2mM KH_2PO_4.

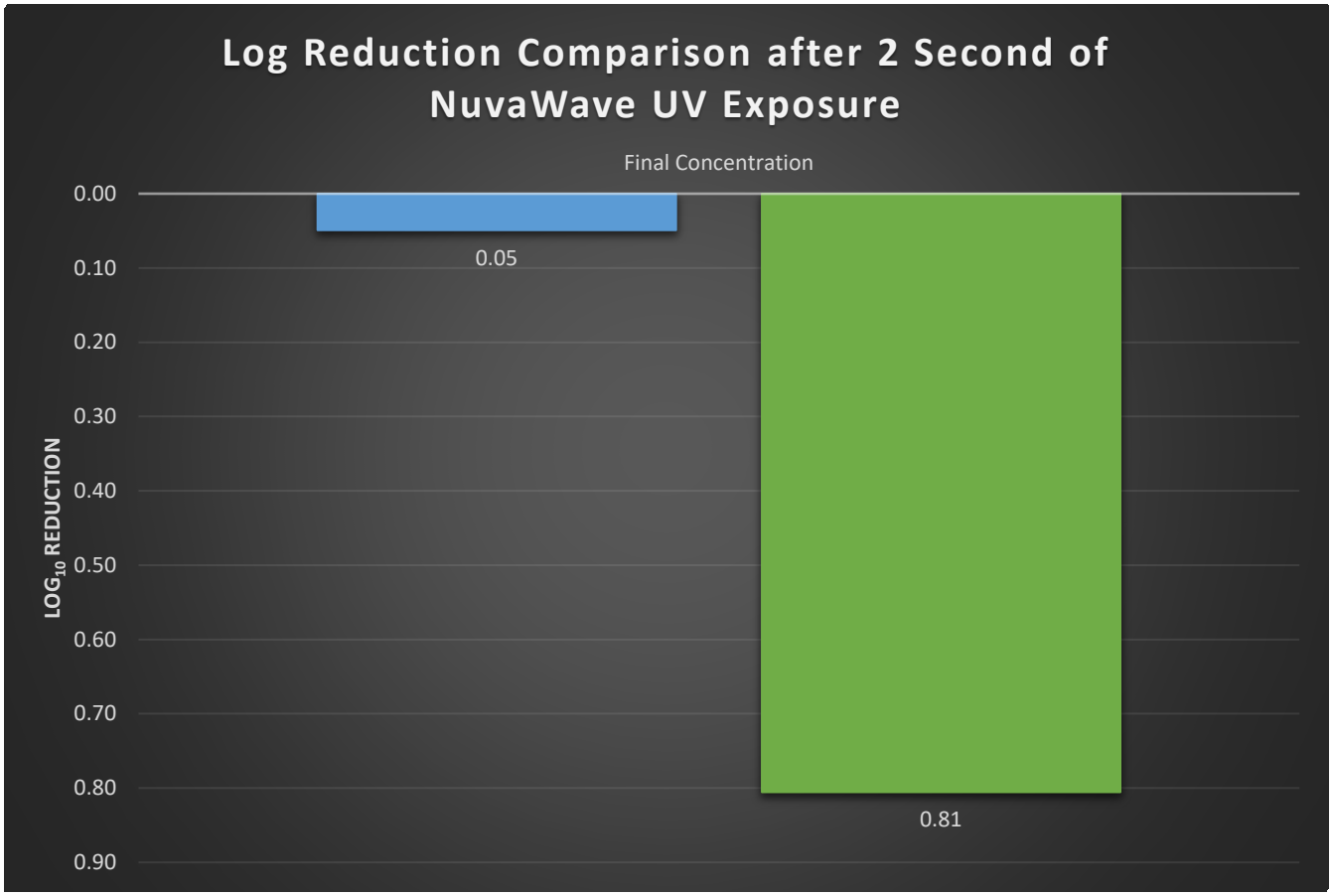
TCID50/mL: The number of infectious virus particles is frequently quantified using the Median Tissue Culture Infectious Dose (TCID50) assay. The assay works by adding a serial dilution of the virus sample to cells in a 96-well plate format. The cell type is specifically selected to show a cytopathic effect (CPE), i.e., morphological changes upon infection with the virus or cell death. After an incubation period, the cells are inspected for CPE or cell death, and each well is classified as infected or not infected. Colorimetric or fluorometric readouts are also possible, which can increase assay sensitivity. The dilution, at which 50% of the wells show a CPE, is used to calculate the TCID50 of the virus sample. Virus titer is expressed as TCID50/mL. See Appendix D for Spearman-Karber method calculation details.

BSC-40: A continuous line of African green monkey cells derived from BSC-1 cells for growth at high temperatures, approximately 31-40°C.

APPENDIX B: Additional Results Graph – Log_{10} Reduction

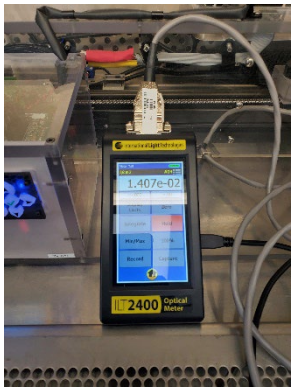


	Final Concentration
Control Average (TCID50/mL)	3.22E+07
Log Reduction – Control	0.05
Experiment Average (TCID50/mL)	1.42E+07
Log Reduction – Experiment	0.41

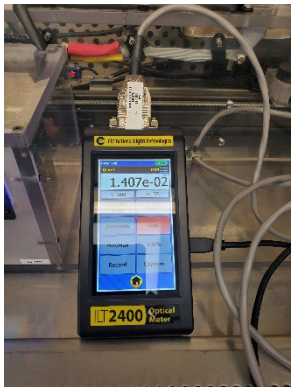


	Final Concentration
Control Average (TCID50/mL)	3.26E+07
Log Reduction – Control	0.05
Experiment Average (TCID50/mL)	5.71E+06
Log Reduction – Experiment	0.81

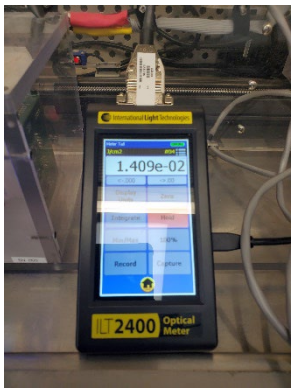
IRRADIANCE DURING 1 SECOND EXPOSURE:



1 Second Experiment 1



1 Second Experiment 2

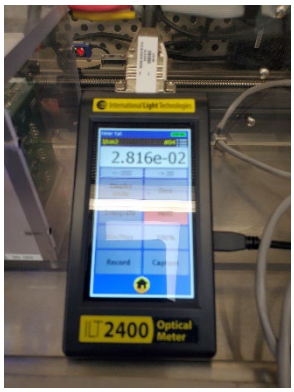


1 Second Experiment 3

IRRADIANCE DURING 2 SECOND EXPOSURE:

2 Second Experiment 1

2 Second Experiment 2



2 Second Experiment 3

APPENDIX C: Calculation Equations

Spearman-Karber TCID50 calculation method:

$$\log_{10} 50\% \text{ endpoint dilution} = -(x_0 - \frac{d}{2} + d\sum\frac{r_i}{n_i})$$

x_0 = $\log_{10}$ of the reciprocal of the highest dilution (lowest concentration) at which all are positive
d = $\log_{10}$ of the dilution factor
n_i = number used in each dilution
r_i = number of positives (out of n_i)
Summation is started at dilution x_0.

Percent Reduction calculation:

$$\text{Percent Reduction} = \frac{(A - B)}{A} \times 100$$

A = initial number of viable microorganisms
B = final number of viable microorganisms

Log Reduction calculation:

$$\text{Log Reduction} = \log_{10}\left(\frac{A}{B}\right)$$

A = initial number of viable microorganisms
B = final number of viable microorganisms

APPENDIX D: BEI Resources - Certificate of Authenticity

b|e|i RESOURCES
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Certificate of Analysis for NR-58622

Monkeypox Virus, hMPXV/USA/MA001/2022 (Lineage B.1, Clade IIb)

Catalog No. NR-58622
This reagent is the property of the U.S. Government.

Product Description:
Monkeypox virus, hMPXV/USA/MA001/2022 was isolated from a human in Massachusetts, USA in May 2022, during an epidemic of mpox. NR-58622 lot 70054242 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (BSC-40; ATCC® CRL-2761™) with the deposited material and incubating in Eagle's Minimum Essential Medium (EMEM; HyClone) supplemented with 2% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin solution for 4 days at 37°C. The harvested cells were freeze/thawed three times and recombined with the spin-clarified supernatant.

Passage History:
B(1)/B(1) (Centers for Disease Control and Prevention/The University of Texas Medical Branch); B = BSC-40 cells

Lot: 70054242 **Manufacturing Date: 15JUL2022**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in BSC-40 Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with isolate hMPXV/USA/MA001/2022 (GenBank: ON563414.3)	99.99% identity with isolate hMPXV/USA/MA001/2022 (GenBank: ON563414.3)
Titer by $TCID_{50}$ Assay in BSC-40 Cells by Cytopathic Effect[1]	Report results	1.8×10^6 $TCID_{50}$ per mL
Sterility (14-day incubation)		
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud agar, 37°C and 26°C, aerobic	No growth	No growth
Trypticase Soy agar, 37°C, aerobic	No growth	No growth
Trypticase Soy agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Mycoplasma Contamination		
MycoSensor QPCR Assay Kit	Negative	Negative

[1]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.

/Sonia Bjorum Brower/
Sonia Bjorum Brower 06 MAR 2023
Technical Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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Certificate of Analysis for NR-58622

APPENDIX I: NGS Information for NR-58622 lot 70054242

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of four SNPs, one insertion (INS) and two deletions (DEL) when compared to GenBank ON563414.3 (see Table I below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-58622 lot 70054242 and reference sequence GenBank ON563414.3

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
INS	G28540insA	193	1	5.6995%	MPXVgp032	Amino acids148-149 MN → amino acids 148-150 NEL
SNP	G34325A	69	1	68.1159%	MPXVgp041	S128L
SNP	C55125T	86	1	32.5581%	MPXVgp059	A12T
SNP	G104962A	43	1	6.9767%	MPXVgp108	Silent mutation
SNP	G119451A	170	1	63.5294%	MPXVgp121	L52F
DEL	Δ136523-136525	129	-3	6.9767%	MPXVgp138	ΔD (amino acid 385)
DEL	Δ173267-173268	42	-2	7.1429%	Intergenic	Untranslated

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