NON-GLP STUDY REPORT

STUDY TITLE

Evaluation of the Virucidal Efficacy of a UV Device for Use on Inanimate Environmental Surfaces

Virus: Human Coronavirus

TEST DEVICE IDENTITY

BLUEMORPH UVC EMITTER

TRF NUMBER

BLU003032320.COR

AUTHOR

Matt Cantin, B.S.
Senior Virologist

STUDY COMPLETION DATE

June 18, 2020

PERFORMING LABORATORY

Analytical Lab Group-Midwest
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

BlueMorph LLC
6318 Rocky Point Ct
Oakland, CA 94605

PROJECT NUMBER

A29600

This study was not performed under
EPA Good Laboratory Practice Regulations
(40 CFR Part 160)

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Evaluation of the Virucidal Efficacy of a UV Device for Use on Inanimate Environmental Surfaces

Project Number: A29600

TRF Number: BLU003032320.COR

TEST SUBSTANCE IDENTITY

Test Device Name: BLUEMORPH UVC EMITTER

STUDY DATES

Date Sample Received: March 31, 2020
Study Initiation Date: April 30, 2020
Experimental Start Date: May 8, 2020
Experimental End Date: June 15, 2020
Study Completion Date: June 18, 2020

TEST PARAMETERS

Carrier Type: Fabric (1" x 1")
Virus: Human Coronavirus, ATCC VR-740, Strain 229E
Exposure Time: 6 minutes and 12 minutes
Exposure Temperature: Room temperature (21.0°C)
Exposure Humidity: 50.45%
Organic Soil Load: 1% fetal bovine serum
Test Medium: Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-inactivated fetal bovine serum, 100 units/mL penicillin, 10 µg/mL gentamicin, and 2.5 µg/mL amphotericin B
Indicator Cell Cultures: WI-38 (human lung) cells

EXPERIMENTAL DESIGN

Input Virus Control

On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

Contamination of Carriers

For each replicate, a 100 µL aliquot of test virus was added to the surface of the carrier. The virus was air-dried at 21.0°C and 45.46% relative humidity until visibly dry (20 minutes).

Test Exposure

Following the completion of drying, the carriers were placed vertically, at a distance of 2 meters from the test device. The device was operated per the instructions and turned on cold at the start of the exposure period. A calibrated timer was used during the exposure.

A digital UV meter was allowed to record at 1 minute intervals, at the same distance/time as the test.

6 Minute Exposure	
Exposure Time Point	Digital UVC Light Meter Reading (µW/cm^2)
1 minute	99
2 minute	158
3 minute	181
4 minute	188
5 minute	191
6 minute	192

12 Minute Exposure	
Exposure Time Point	Digital UVC Light Meter Reading (µW/cm^2)
1 minute	21
2 minute	65
3 minute	111
4 minute	146
5 minute	165
6 minute	175
7 minute	181
8 minute	184
9 minute	186
10 minute	188
11 minute	189
12 minute	190

Recovery of Virus Following Exposure

Following exposure to the test device, each carrier was removed and added to a sterile 15 mL conical tube containing a 1.0 mL aliquot of test medium with 3 sterile glass beads (10^{-1} dilution). The conical tube was vortex mixed to resuspend the contents and then serial 10-fold dilutions were performed. Each dilution was then assayed for infectivity and/or cytotoxicity.

Dried Virus Control
The appropriate number of virus films (for each exposure time) were prepared as described previously and run in parallel to the test virus. Each virus control film was held uncovered in a sterile petri dish and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films. A calibrated timer was used for timing the exposure and the actual temperature was recorded. Immediately following the Sponsor requested exposure, each carrier was removed and added to a sterile 15 mL conical tube containing a 1.0 mL aliquot of test medium with 3 sterile glass beads (10^{-1} dilution). The conical tube was vortex mixed to resuspend the contents and then serial 10-fold dilutions were performed. Each dilution was then assayed for infectivity.

Cytotoxicity Control
A carrier was dried as above, however, an aliquot of test medium containing the requested organic soil load was used in lieu of virus. Following drying, the carrier was exposed to the test device in parallel with the test carriers (for the longest exposure time). Following exposure, the recovery was the same as indicated above in testing. Serial 10-fold dilutions were performed and each dilution was assayed for cytotoxicity.

Assay of Non-Virucidal Level of Test Substance (Neutralization Control)
Each dilution of the neutralized test substance (cytotoxicity control dilutions) was challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures was inoculated with a 100 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates.

Per Sponsor's direction, the study was not required to be conducted under US EPA 40 CFR Part 160 or US FDA 21 CFR Part 58.

UNFORESEEN CIRCUMSTANCES

The initial assay performed on May 8, 2020 was repeated on June 5, 2020 in order to recover at least 4 $\log_{10}$ of infectivity from the dried virus control films. For the fabric carriers exposed for 6 minutes, only 1.25 $\log_{10}$ was recovered from each replicate. For the fabric carriers exposed for 12 minutes, only 1.25 $\log_{10}$ was recovered from replicate 1 and 1.00 $\log_{10}$ from replicate 2. See Attachment I for the initial fabric data from the May 8, 2020 assay.

Repeat testing, of the fabric carriers only, was performed on June 5, 2020 utilizing sterile glass beads to aid in the recovery of the virus from the carriers. The results obtained from the repeat assay may be found in the body of this report.

CONCLUSION

Under the conditions of this investigation and in the presence of a 1% fetal bovine serum organic soil load, the BLUEMORPH UVC EMITTER, demonstrated an average ≥4.14 log_{10} reduction in titer of Human Coronavirus on fabric carriers following a 6 minute exposure time at room temperature (21.0°C) and 50.45% relative humidity as compared to the average titer of the 6 minute dried virus controls.

Under the conditions of this investigation and in the presence of a 1% fetal bovine serum organic soil load, the BLUEMORPH UVC EMITTER, demonstrated an average ≥3.57 log_{10} reduction in titer of Human Coronavirus on fabric carriers following a 12 minute exposure time at room temperature (21.0°C) and 50.45% relative humidity as compared to the average titer of the 12 minute dried virus controls.

In the opinion of the Author, there were no circumstances that may have affected the quality or integrity of the data.

STUDY RESULTS

TABLE 1: Virus Controls

Dilution	Input Virus Control	Dried Virus Control (Fabric Carriers)			
		6 minute exposure		12 minute exposure	
		Replicate 1	Replicate 2	Replicate 1	Replicate 2
Cell Control	0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	+ +	+ + + +	+ + + +	+ + + +	+ + + +
10^{-2}	+ +	+ + + +	+ + + +	+ + + +	+ + + +
10^{-3}	+ +	+ + + +	+ + + +	+ + + +	+ + + +
10^{-4}	+ +	+ + + 0	+ + 0 +	+ 0 + 0	0 + 0 0
10^{-5}	0 +	+ 0 0 +	0 0 0 +	0 + 0 0	0 0 0 0
10^{-6}	0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-7}	0 0	NT	NT	NT	NT
$TCID_{50}$/100 µL	$10^{5.00}$	$10^{4.75}$	$10^{4.50}$	$10^{4.25}$	$10^{3.75}$
Average $TCID_{50}$/100 µL	NA	$10^{4.64}$		$10^{4.07}$	

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(NA) = Not applicable
(NT) = Not tested

TABLE 2: Effects of BLUEMORPH UVC EMITTER Following a 6 Minute and 12 Minute Exposure to Human Coronavirus Dried on an Inanimate Surface

Dilution	Human Coronavirus + BLUEMORPH UVC EMITTER (Fabric Carriers)			
	6 minute exposure		12 minute exposure	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$\leq 10^{0.50}$
Average $TCID_{50}$/100 µL	$\leq 10^{0.50}$		$\leq 10^{0.50}$	
Average Log reduction*	≥ 4.14 $\log_{10}$		≥ 3.57 $\log_{10}$	

(0) = No test virus recovered and/or no cytotoxicity present
(*) = Calculated using the corresponding dried virus control

TABLE 3: Cytotoxicity Control and Neutralization Control

Dilution	Cytotoxicity Control BLUEMORPH UVC EMITTER (Fabric Carrier)	Neutralization Control BLUEMORPH UVC EMITTER (Fabric Carrier)
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	0 0 0 0	+ + + +
10^{-2}	0 0 0 0	+ + + +
10^{-3}	0 0 0 0	+ + + +
TCD_{50}/100 µL	$\leq 10^{0.50}$	See below

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present

Results of the non-virucidal level control (neutralization control) indicate that the test substance was neutralized at a $TCID_{50}$/100 µL of ≤0.50 log_{10}.

ATTACHMENT I: Initial Assay Data

See unforeseen circumstances on page 4.

Set-up date: May 8, 2020
Test Device: BLUEMORPH UVC EMITTER
Carrier Type: Fabric (1" x 1")
Virus: Human Coronavirus, ATCC VR-740, Strain 229E
Exposure Time: 6 minutes and 12 minutes
Exposure Temperature: Room temperature (18.0°C)
Exposure Humidity: 14.75%
Organic Soil Load: 1% fetal bovine serum

Virus Controls

Dilution	Input Virus Control	Dried Virus Control (Fabric Carriers)			
		6 minute exposure		12 minute exposure	
		Replicate 1	Replicate 2	Replicate 1	Replicate 2
Cell Control	0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	+ +	+ + + +	+ + + +	+ + + +	+ + + +
10^{-2}	+ +	+ 0 0 0	0 + 0 0	0 0 0 +	0 0 0 0
10^{-3}	+ +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-4}	+ +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-5}	0 +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-6}	0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-7}	0 0	NT	NT	NT	NT
$TCID_{50}$/100 µL	$10^{5.00}$	$10^{1.75}$	$10^{1.75}$	$10^{1.75}$	$10^{1.50}$
Average $TCID_{50}$/100 µL	NA	$10^{1.75}$		$10^{1.64}$	

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(NA) = Not applicable
(NT) = Not tested

Effects of BLUEMORPH UVC EMITTER Following a 6 Minute and 12 Minute Exposure to Human Coronavirus Dried on an Inanimate Surface

Dilution	Human Coronavirus + BLUEMORPH UVC EMITTER (Fabric Carriers)			
	6 minute exposure		12 minute exposure	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	0 0 0 +	0 0 0 +	0 0 0 0	0 0 0 0
10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$/100 µL	$10^{0.75}$	$10^{0.75}$	$\leq 10^{0.50}$	$\leq 10^{0.50}$
Average $TCID_{50}$/100 µL	$10^{0.75}$		$\leq 10^{0.50}$	
Average Log reduction*	1.00 $\log_{10}$		≥1.14 $\log_{10}$	

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(*) = Calculated using the corresponding dried virus control

Cytotoxicity Control and Neutralization Control

Dilution	Cytotoxicity Control BLUEMORPH UVC EMITTER (Fabric Carrier)	Neutralization Control BLUEMORPH UVC EMITTER (Fabric Carrier)
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	0 0 0 0	+ + + +
10^{-2}	0 0 0 0	+ + + +
10^{-3}	0 0 0 0	+ + + +
TCD_{50}/100 µL	$\leq 10^{0.50}$	See below

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present

Results of the non-virucidal level control (neutralization control) indicate that the test substance was neutralized at a $TCID_{50}$/100 µL of ≤0.50 $\log_{10}$.

PREPARED BY:

Matt Cantin, B.S.
Senior Virologist

6-18-2020
Date