NON-GLP STUDY REPORT

STUDY TITLE

Evaluation of Antiviral Activity of a Modular Ionization Device

Virus: Human Coronavirus

PRODUCT IDENTITY

iWave-R

TRF NUMBER

NUC002030920.COR

AUTHOR

Mary J. Miller, M.T.
Principal Virologist

STUDY COMPLETION DATE

April 14, 2020

REVISED REPORT DATE

April 17, 2020

PERFORMING LABORATORY

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

SPONSOR

Global Plasma Solutions
3101 Yorkmont Road Suite 400
Charlotte, NC 28208

PROJECT NUMBER

A29381

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

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title:	Evaluation of Antiviral Activity of a Modular Ionization Device
Project Number:	A29381
TRF Number:	NUC002030920.COR

TEST SUBSTANCE IDENTITY

Device Name:	iWave-R

STUDY DATES

Date Sample Received:	March 3, 2020
Study Initiation Date:	March 24, 2020
Experimental Start Date:	April 1, 2020
Experimental End Date:	April 10, 2020
Study Completion Date:	April 14, 2020
Revised Report Date:	April 17, 2020

TEST PARAMETERS

iWave-R Preparation:	Ready to use
Virus:	Human Coronavirus, ATCC VR-740, Strain 229E
Exposure Times:	1 minute, 5 minutes, 15 minutes, 30 minutes and 60 minutes
Exposure Temperature:	Room temperature (22.0°C)
Exposure Humidity:	22.83% for the tests and cytotoxicity control 23.38% for the dried virus controls
Exposure Conditions:	Each carrier was 1" from the carbon fiber brushes of the iWave-R device during each exposure time.
Organic Soil Load:	1% fetal bovine serum
Test Medium:	Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-inactivated, 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

Prior to use in testing, the Air Ion counter (serial number 4296) was prepared following the Sponsor's directions. The dial was turned to the first position and the polarity switch was set to the neutral position. The offset dial was turned until the display read "0" or as close as possible and the polarity switch was turned to the negative setting. The measure switch was turned to "on".

For each exposure time, an individual film of virus, dried on a glass surface, was individually exposed to the iWave-R needlepoint bipolar ionization device. One carrier at a time was placed 1" from the carbon fiber brushes of the device with the dried virus film facing up. The carriers were exposed to the device with the petri dish lids off. Individual carriers were exposed to the iWave-R device for the 1 minute, 5 minute, 15 minute, 30 minute and 60 minute Sponsor specified exposure times at room temperature (22.0°C) and 22.83% relative humidity. The Air Ion counter was next to the iWave-R during the exposure times. Following each exposure time, the carrier was removed from underneath the iWave-R device and a 2.0 mL aliquot of test medium was added to the petri dish and the plate was scraped with a cell scraper to resuspend the contents (10^{-1} dilution). The 10^{-1} dilution was titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. Appropriate virus, test substance cytotoxicity, and neutralization controls were run concurrently.

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.

Below are the approximate ion/cm^3 readings taken by Air Ion counter during the exposure times. The cytotoxicity control carrier, which was held for the longest exposure time of 60 minutes, was the first carrier that was exposed to the iWave-R.

Cytotoxicity control:	-0.36
1 Minute Exposure:	0.34
5 Minute Exposure:	0.46
15 Minute Exposure:	1.07
30 Minute Exposure:	0.86
60 Minute Exposure:	1.48

CONCLUSION

Under the conditions of this investigation and in the presence of a 1% fetal bovine serum organic soil load, the iWave-R device did not demonstrate complete inactivation of Human Coronavirus following a 1 minute, 5 minute, 15 minute, 30 minute or 60 minute exposure time at room temperature (22.0°C) and 22.83% relative humidity. Taking the cytotoxicity and neutralization control results into consideration, the following reductions in viral titer was demonstrated:

1 Minute Exposure
No reduction in viral titer was demonstrated, per volume inoculated per well and per carrier, compared to the titer of the 1 minute dried virus control.

5 Minute Exposure
A 0.25 $\log_{10}$ reduction in viral titer was demonstrated, per volume inoculated per well and per carrier, compared to the titer of the 5 minute dried virus control.

15 Minute Exposure
A 0.50 $\log_{10}$ reduction in viral titer was demonstrated, per volume inoculated per well and per carrier, compared to the titer of the 15 minute dried virus control.

30 Minute Exposure
A 0.75 $\log_{10}$ reduction in viral titer was demonstrated, per volume inoculated per well and per carrier, compared to the titer of the 30 minute dried virus control.

60 Minute Exposure
A 1.00 $\log_{10}$ reduction in viral titer was demonstrated, per volume inoculated per well and per carrier, compared to the titer of the 60 minute dried virus control.

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 Control Results

Dilution	Input Virus Control	Dried Virus Controls				
		1 Minute Exposure	5 Minute Exposure	15 Minute Exposure	30 Minute Exposure	60 Minute Exposure
Cell Control	0 0	0 0 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 +
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	0 0 0 0
10^{-7}	0 0	NT	NT	NT	NT	NT
$TCID_{50}$/100 µL	$10^{4.50}$	$10^{4.50}$	$10^{4.50}$	$10^{4.50}$	$10^{4.50}$	$10^{4.25}$
$TCID_{50}$/carrier	NA	$10^{4.80}$	$10^{4.80}$	$10^{4.80}$	$10^{4.80}$	$10^{4.55}$

(+) = 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: Test Results

Effects of the iWave-R Device Following a 1 Minute, 5 Minute, 15 Minute, 30 Minute and 60 Minute Exposure to Human Coronavirus Dried on an Inanimate Surface

Dilution	Human Coronavirus + iWave-R				
	1 Minute Exposure	5 Minute Exposure	15 Minute Exposure	30 Minute Exposure	60 Minute Exposure
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
10^{-2}	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +	+ + + +	+ + + +	+ 0 + +
10^{-4}	+ + + +	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 0 0
10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$/100 µL	$10^{4.50}$	$10^{4.25}$	$10^{4.00}$	$10^{3.75}$	$10^{3.25}$
$TCID_{50}$/carrier	$10^{4.80}$	$10^{4.55}$	$10^{4.30}$	$10^{4.05}$	$10^{3.55}$
Log Reduction*	No Reduction	0.25 log_{10}	0.50 log_{10}	0.75 log_{10}	1.00 log_{10}

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(*) = This is the log reduction, per volume inoculated per well and per carrier, based on the titer of the corresponding dried virus control.

TABLE 2: Cytotoxicity and Neutralization Controls

Dilution	Cytotoxicity Control iWave-R	Neutralization Control iWave-R
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	+ + + +
10^{-4}	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:

Mary J. Miller

Mary J. Miller, M.T.
Principal Virologist

4-17-2020
Date