NON-GLP STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Human Coronavirus

PRODUCT IDENTITY

GERMAGIC Thyme (GMTP)

TRF NUMBER

EXN01030220.COR

AUTHOR

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STUDY COMPLETION DATE

May 5, 2020

REVISED REPORT DATE

October 2, 2020

PERFORMING LABORATORY

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SPONSOR REPRESENTATIVE

Exponent
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PROJECT NUMBER

A29403

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

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Project Number: A29403

TRF Number: EXN01030220.COR

TEST SUBSTANCE IDENTITY

Test Substance Name: GERMAGIC Thyme (GMTP)

STUDY DATES

Date Sample Received: March 5, 2020
Study Initiation Date: March 30, 2020
Experimental Start Date: April 6, 2020
Experimental End Date: May 1, 2020
Study Completion Date: May 5, 2020
Revised Report Date: October 2, 2020

TEST PARAMETERS

Dilution: Received as a liquid, applied as a trigger spray
Virus: Human Coronavirus, ATCC VR-740, Strain 229E
Exposure Time: 9 minutes 55 seconds
Exposure Temperature: Room temperature (20.0°C)
Exposure Humidity: 50%
Spray Conditions: 3 sprays, until thoroughly wet, at a distance of 6 to 8 inches
Organic Soil Load: 5% 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
Neutralizer: To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel. Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

UNFORSEEN CIRCUMSTANCES

The initial assay performed on April 6, 2020, was repeated on April 21, 2020, to recover at least 4.8 $\log_{10}$ of infectivity per carrier from the dried virus control film, as required for a valid study. The titer of the dried virus control for the April 6, 2020 assay was 4.55 $\log_{10}$/carrier for Replicate #1 and 3.55 $\log_{10}$/carrier for Replicate #2. The average titer of the dried virus control replicates for the April 6, 2020 assay was 4.29 $\log_{10}$/carrier.

See Attachment I for the invalid data from the April 6, 2020 assay.

Valid results were obtained from the assay performed on April 21, 2020 and may be found in the body of this report.

VIRUS

The 229E strain of Human Coronavirus used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-740). Stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at ≤ -70°C until the day of use. On the day of use, an aliquot of stock virus was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contained 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Coronavirus on WI-38 cells.

EXPERIMENTAL DESIGN

All testing was performed following ASTM E1053-20.

For each replicate, one dried virus film was individually exposed for 9 minute 55 seconds at room temperature (20.0°C) and 50% relative humidity to the amount of spray released under use conditions. The carriers were sprayed using 3 sprays, until thoroughly wet, at a distance of 6 to 8 inches, and held covered for the exposure time. The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

The WI-38 cell line, which exhibits cytopathic effect (CPE) in the presence of Human Coronavirus, was inoculated with 100 µL of the dilutions prepared from test and control groups. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were held for ten days and microscopically scored for the absence or presence of CPE, cytotoxicity, and for viability.

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.

CONCLUSION

Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, GERMAGIC Thyme (GMTP), a ready to use trigger spray, demonstrated complete inactivation of Human Coronavirus following a 9 minute 55 second exposure time at room temperature (20.0°C) and 50% relative humidity.

Taking the cytotoxicity and neutralization control results into consideration, a ≥3.00 $\log_{10}$ average reduction in viral titer was demonstrated, per volume inoculated per well and per carrier.

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: Effects of GERMAGIC Thyme (GMTP) Following a 9 Minute 55 Second Exposure to Human Coronavirus Dried on an Inanimate Surface

Dilution	Input Virus Control	Dried Virus Control		Human Coronavirus + GERMAGIC Thyme (GMTP)	
		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}	+ +	+ + + +	+ + + +	T T T T	T T T T
10^{-2}	+ +	+ + + +	+ + + +	0 0 0 0	0 0 0 0
10^{-3}	+ +	+ + + +	+ + + +	0 0 0 0	0 0 0 0
10^{-4}	+ +	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 0 0
10^{-7}	0 0	NT	NT	NT	NT
$TCID_{50}$/100 µL	$10^{4.50}$	$10^{4.50}$	$10^{4.50}$	$\leq 10^{1.50}$	$\leq 10^{1.50}$
$TCID_{50}$/carrier	NA	$10^{4.80}$	$10^{4.80}$	$\leq 10^{1.80}$	$\leq 10^{1.80}$
Average $TCID_{50}$/100 µL	NA	$10^{4.50}$		$\leq 10^{1.50}$	
Average $TCID_{50}$/carrier	NA	$10^{4.80}$		$\leq 10^{1.80}$	
Average Log Reduction*	NA	NA		≥ 3.00 $\log_{10}$	

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present
(NA) = Not applicable
(NT) = Not tested
(*) = This reduction is both per volume inoculated per well and per carrier.

TABLE 2: Cytotoxicity and Neutralization Controls

Dilution	Cytotoxicity Control GERMAGIC Thyme (GMTP)	Neutralization Control GERMAGIC Thyme (GMTP)
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	T T T T	T T T T
10^{-2}	0 0 0 0	+ + + +
10^{-3}	0 0 0 0	+ + + +
10^{-4}	0 0 0 0	+ + + +
10^{-5}	0 0 0 0	+ + + +
10^{-6}	0 0 0 0	+ + + +
TCD_{50}/100 µL	$10^{1.50}$	See below

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(T) = 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 ≤1.50 $\log_{10}$.

ATTACHMENT I: Invalid Data

See Unforeseen Circumstances section on page 3.

Set-up date:	April 6, 2020
Date Sample Received:	March 5, 2020
Test Substance:	GERMAGIC Thyme (GMTP)
Dilution:	Received as a liquid, applied as a trigger spray
Virus:	Human Coronavirus, ATCC VR-740, Strain 229E
Exposure Time:	9 minutes 55 seconds
Exposure Temperature:	Room temperature (20.0°C)
Exposure Humidity:	50%
Spray Conditions:	3 sprays, until thoroughly wet, at a distance of 6 to 8 inches
Organic Soil Load:	5% 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
Neutralizer:	To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel. Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

Virus Controls and Assay Results

Effects of GERMAGIC Thyme (GMTP) Following a 9 Minute 55 Second Exposure to Human Coronavirus Dried on an Inanimate Surface

Dilution	Input Virus Control	Dried Virus Control		Human Coronavirus + GERMAGIC Thyme (GMTP)	
		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}	+ +	+ + + +	+ + + +	0 0 0 0	T T T T
10^{-2}	+ +	+ + + +	+ + + +	0 0 0 0	0 0 0 0
10^{-3}	+ +	+ + + +	0 + + +	0 0 0 0	0 0 0 0
10^{-4}	+ +	+ + + 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 0 0
10^{-7}	0 0	NT	NT	NT	NT
$TCID_{50}$/100 µL	$10^{5.50}$	$10^{4.25}$	$10^{3.25}$	$\leq 10^{0.50}$	$\leq 10^{1.50}$
$TCID_{50}$/carrier	NA	$10^{4.55}$	$10^{3.55}$	$\leq 10^{0.80}$	$\leq 10^{1.80}$
Average $TCID_{50}$/100 µL	NA	$10^{3.99}$		$\leq 10^{1.24}$	
Average $TCID_{50}$/carrier	NA	$10^{4.29}$		$\leq 10^{1.54}$	
Average Log Reduction*	NA	NA		≥ 2.49 $\log_{10}$	

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present
(NA) = Not applicable
(NT) = Not tested
(*) = This reduction is both per volume inoculated per well and per carrier and takes the cytotoxicity and neutralization control results into consideration.

Cytotoxicity and Neutralization Controls

Dilution	Cytotoxicity Control GERMAGIC Thyme (GMTP)	Neutralization Control GERMAGIC Thyme (GMTP)
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	T T T T	T T T T
10^{-2}	0 0 0 0	+ + + +
10^{-3}	0 0 0 0	+ + + +
10^{-4}	0 0 0 0	+ + + +
10^{-5}	0 0 0 0	+ + + +
10^{-6}	0 0 0 0	+ + + +
TCD_{50}/100 µL	$10^{1.50}$	See below

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(T) = 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 ≤1.50 log_{10}.

PREPARED BY:

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10-2-2020
Date

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