



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

Mary J. Miller, M.T.
Principal Virologist

STUDY COMPLETION DATE

May 5, 2020

PERFORMING LABORATORY

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

SPONSOR

Exponent
980 9th Street
16th Floor
Sacramento, CA 95814

PROJECT NUMBER

A29403

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

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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

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



UNFORSEEN CIRCUMSTANCES

The initial assay performed on April 6, 2020, was repeated on April 21, 2020, to recover at least 4.8 log₁₀ 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 log₁₀/carrier for Replicate #1 and 3.55 log₁₀/carrier for Replicate #2. The average titer of the dried virus control replicates for the April 6, 2020 assay was 4.29 log₁₀/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.

EXPERIMENTAL DESIGN

For each replicate, an individual film of virus, dried on a glass surface, was exposed 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 9 minute 55 second Sponsor specified exposure time at room temperature (20.0°C) and 50% relative humidity. Following the exposure time, the virucidal and cytotoxic activities were removed from the virus-test substance mixtures utilizing individual Sephadex gel columns, and the mixtures were assayed for viral infectivity by an accepted assay method. 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 log₁₀ 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	00	0000	0000	0000	0000
10 ⁻¹	++	++++	++++	TTTT	TTTT
10 ⁻²	++	++++	++++	0000	0000
10 ⁻³	++	++++	++++	0000	0000
10 ⁻⁴	++	0+++	+++0	0000	0000
10 ⁻⁵	00	000+	0+00	0000	0000
10 ⁻⁶	00	0000	0000	0000	0000
10 ⁻⁷	00	NT	NT	NT	NT
TCID ₅₀ /100 µL	10 ^{4.50}	10 ^{4.50}	10 ^{4.50}	≤10 ^{1.50}	≤10 ^{1.50}
TCID ₅₀ /carrier	NA	10 ^{4.80}	10 ^{4.80}	≤10 ^{1.80}	≤10 ^{1.80}
Average TCID ₅₀ /100 µL	NA	10 ^{4.50}		≤10 ^{1.50}	
Average TCID ₅₀ /carrier	NA	10 ^{4.80}		≤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 TCID₅₀/100 µL of ≤1.50 log₁₀.



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



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	00	0000	0000	0000	0000
10 ⁻¹	++	++++	++++	0000	TTTT
10 ⁻²	++	++++	++++	0000	0000
10 ⁻³	++	++++	0+++	0000	0000
10 ⁻⁴	++	+++0	0000	0000	0000
10 ⁻⁵	++	0000	0000	0000	0000
10 ⁻⁶	00	0000	0000	0000	0000
10 ⁻⁷	00	NT	NT	NT	NT
TCID ₅₀ /100 µL	10 ^{5.50}	10 ^{4.25}	10 ^{3.25}	≤10 ^{0.50}	≤10 ^{1.50}
TCID ₅₀ /carrier	NA	10 ^{4.55}	10 ^{3.55}	≤10 ^{0.80}	≤10 ^{1.80}
Average TCID ₅₀ /100 µL	NA	10 ^{3.99}		≤10 ^{1.24}	
Average TCID ₅₀ /carrier	NA	10 ^{4.29}		≤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	+ + + +
TCID 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 TCID₅₀/100 µL of ≤1.50 log₁₀.



PREPARED BY:

Mary J. Miller

Mary J. Miller, M.T.
Principal Virologist

5-5-2020

Date

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