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CONFIDENTIAL FINAL REPORT

SPONSOR: Chiaphua Industries Limited

SPONSOR'S REPRESENTATIVE: Ian Van Trump

STUDY TITLE: VIRUCIDAL HARD-SURFACE EFFICACY TEST – Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

STUDY IDENTIFICATION: Microbac Project No. 1025-101 (refer to signed Protocol No. CHIA.1.05.29.20)

TEST AGENT NAME	LOT NO.	ACTIVE INGREDIENTS	DATE RECEIVED	DS NO.
Germagic Thyme	GMTP-HKUST2020060201	Thyme Essential Oil, Polyethylenimine, Polyhexanide	06/18/20	K858
	GMTP-HKUST2020060901		06/18/20	K859

CHALLENGE ORGANISM: SARS-CoV-2 (COVID-19 Virus), Strain: USA-WA1/2020, Source: BEI Resources, NR-52281

HOST CELL LINE: Vero E6 cells, ATCC CRL-1586

DILUTION MEDIUM: Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS)

NEUTRALIZER: MEM + 10% NCS + 0.5% Lecithin + 1 mM EDTA

CONTACT TIME: 9 minutes 55 seconds

CONTACT TEMPERATURE: Room Temperature (20±1°C, Actual: 21°C)

RELATIVE HUMIDITY: 48-49% RH

NUMBER OF REPLICATES: 1 replicate (four wells per dilution)

Microbac Laboratories, Inc.
105 Carpenter Drive | Sterling, VA 20164 | 703.925.0100 p | 703.925.9366 f | www.microbac.com

INCUBATION TEMPERATURE: 36±2°C with 5±3% CO_2

INCUBATION TIME: 4 – 9 days (Actual: 7 days)

DILUTION OF TEST AGENT: Ready to use

ORGANIC LOAD: 5.0% serum

CARRIER INOCULATION AND DRY TIME: Glass Petri dishes with marked areas of 4-square inches were inoculated with 0.4 mL of the challenge organism and dried for 46 minutes at 21°C and 49-52% RH.

TEST APPLICATION: Carriers were sprayed with three pumps until thoroughly wet from a distance of 6 – 8 inches.

CALCULATION OF TITER: The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the Spearman-Karber method using the following formula:

$$m = x_k + \left(\frac{d}{2}\right) - d\sum p_i$$

where:

m = the logarithm of the dilution at which half the wells are infected relative to the test volume
x_k = the logarithm of the smallest dosage which induces infection in all cultures
d = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i
$\sum p_i$ = sum of p_i (starting with the highest dilution producing 100% infection)

The values were converted to $TCID_{50}$/mL using a sample inoculum of 1.0 mL.

RESULTS:

Results are presented in Tables 1– 6.

The Log_{10} Reduction Factor was calculated in the following manner:

Log_{10} Reduction Factor = Initial viral load (Log_{10} $TCID_{50}$, per assayed volume and per carrier) – Output viral load (Log_{10} $TCID_{50}$, per assayed volume and per carrier)

The Load (Log_{10} $TCID_{50}$) per carrier was calculated in the following manner:

Virus Load (Log_{10} $TCID_{50}$) = Virus Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [Volume per sample (mL)]

Key (for all tables):

T/y = Cytotoxicity observed in y wells inoculated; viral cytopathic effects (CPE) could not be determined

X/y = X wells out of y wells inoculated exhibited positive viral CPE

0/y = 0 out of y wells inoculated exhibited positive viral CPE; no cytotoxicity or bacterial contamination was observed in any of the wells inoculated

RESULTS (Continued):

Table 1
Plate Recovery Control (PRC)

Dilution*	PRC
	Replicate 1
10^{-3}	4/4
10^{-4}	4/4
10^{-5}	4/4
10^{-6}	3/4
10^{-7}	1/4
10^{-8}	0/4
Titer (Log_{10} $TCID_{50}$/mL)	6.50
Load (Log_{10} $TCID_{50}$)**	6.10

*Dilution refers to the fold of dilution from the virus inoculum.
**Per carrier (0.4 mL of Undilute [10^0])

Table 2
Test Agent

Dilution*	Germagic Thyme	
	Lot No. GMTP-HKUST2020060201	Lot No. GMTP-HKUST2020060901
10^{-2}	T/4	T/4
10^{-3}	T/4	T/4
10^{-4}	0/4	0/4
10^{-5}	0/4	0/4
10^{-6}	0/4	0/4
10^{-7}	0/4	0/4
Titer (Log_{10} $TCID_{50}$/mL)	≤ 3.50	≤ 3.50
Load (Log_{10} $TCID_{50}$)**	≤ 3.10	≤ 3.10
Log_{10} Reduction***	≥ 3.00	≥ 3.00

*Dilution refers to the fold of dilution from the virus inoculum.
**Per carrier (0.40 mL of Undilute [10^0])
***Per assayed volume and per carrier

RESULTS (continued):

Table 3
Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls

Dilution*	Germagic Thyme	
	Lot No. GMTP-HKUST2020060201	
	NE/VI	CT
10^{-2}	T/4	T/4
10^{-3}	T/4	T/4
10^{-4}	4/4	0/4

*Dilution refers to the fold of dilution from the mock inoculum.

Table 4
Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls

Dilution*	Germagic Thyme	
	Lot No. GMTP-HKUST2020060901	
	NE/VI	CT
10^{-2}	T/4	T/4
10^{-3}	T/4	T/4
10^{-4}	4/4	0/4

*Dilution refers to the fold of dilution from the mock inoculum.

Table 5
Cell Viability Control (CVC)

CVC
0/4
Cells were viable; media was sterile

RESULTS (continued):

Table 6
Virus Stock Titer Control (VST)

Dilution*	VST
10^{-4}	4/4
10^{-5}	4/4
10^{-6}	4/4
10^{-7}	3/4
10^{-8}	0/4
10^{-9}	0/4
Titer (Log_{10} $TCID_{50}$/mL)	7.25

*Dilution refers to the fold of dilution from the virus inoculum.

CONCLUSION:

According to the US Environmental Protection Agency, the test agent passes the Virucidal Hard-Surface Efficacy Test if the product demonstrates a ≥ 3 log_{10} reduction on each surface in the presence or absence of cytotoxicity. When cytotoxicity is present, the virus control titer should be increased, if necessary, to demonstrate a ≥ 3 log_{10} reduction in viral titer on each surface beyond the cytotoxic level.

- Germagic Thyme, Lot No. GMTP-HKUST2020060201: **passed**
- Germagic Thyme, Lot No. GMTP-HKUST2020060901: **passed**

The viral reductions for the test agent are presented in Table 2. All controls met the criteria for a valid test. These conclusions are based on observed data.

Study Director: _____ 10/08/2020
Cameron Wilde Date