

## 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)

<b>INCUBATION TEMPERATURE:</b>	36±2°C with 5±3% CO <sub>2</sub>
<b>INCUBATION TIME:</b>	4 – 9 days (Actual: 7 days)
<b>DILUTION OF TEST AGENT:</b>	Ready to use
<b>ORGANIC LOAD:</b>	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<sub>50</sub>/mL) was determined using the Spearman-Kärber 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<sub>50</sub>/mL using a sample inoculum of 1.0 mL.

## RESULTS:

Results are presented in Tables 1– 6.

The Log<sub>10</sub> Reduction Factor was calculated in the following manner:

Log<sub>10</sub> Reduction Factor = Initial viral load (Log<sub>10</sub> TCID<sub>50</sub>, per assayed volume and per carrier) – Output viral load (Log<sub>10</sub> TCID<sub>50</sub>, per assayed volume and per carrier)

The Load (Log<sub>10</sub> TCID<sub>50</sub>) per carrier was calculated in the following manner:

Virus Load (Log<sub>10</sub> TCID<sub>50</sub>) = Virus Titer (Log<sub>10</sub> TCID<sub>50</sub>/mL) + Log<sub>10</sub> [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 <sup>-3</sup>	4/4
10 <sup>-4</sup>	4/4
10 <sup>-5</sup>	4/4
10 <sup>-6</sup>	3/4
10 <sup>-7</sup>	1/4
10 <sup>-8</sup>	0/4
Titer (Log <sub>10</sub> TCID <sub>50</sub> /mL)	6.50
Load (Log <sub>10</sub> TCID <sub>50</sub> )**	6.10

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

\*\*Per carrier (0.4 mL of Undilute [10<sup>0</sup>])

**Table 2  
Test Agent**

Dilution*	Germagic Thyme	
	Lot No. GMTP-HKUST2020060201	Lot No. GMTP-HKUST2020060901
10 <sup>-2</sup>	T/4	T/4
10 <sup>-3</sup>	T/4	T/4
10 <sup>-4</sup>	0/4	0/4
10 <sup>-5</sup>	0/4	0/4
10 <sup>-6</sup>	0/4	0/4
10 <sup>-7</sup>	0/4	0/4
Titer (Log <sub>10</sub> TCID <sub>50</sub> /mL)	≤ 3.50	≤ 3.50
Load (Log <sub>10</sub> TCID <sub>50</sub> )**	≤ 3.10	≤ 3.10
<b>Log<sub>10</sub> Reduction***</b>	<b>≥ 3.00</b>	<b>≥ 3.00</b>

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

\*\*Per carrier (0.40 mL of Undilute [10<sup>0</sup>])

\*\*\*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 <sup>-2</sup>	T/4	T/4
10 <sup>-3</sup>	T/4	T/4
10 <sup>-4</sup>	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 <sup>-2</sup>	T/4	T/4
10 <sup>-3</sup>	T/4	T/4
10 <sup>-4</sup>	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 <sup>-4</sup>	4/4
10 <sup>-5</sup>	4/4
10 <sup>-6</sup>	4/4
10 <sup>-7</sup>	3/4
10 <sup>-8</sup>	0/4
10 <sup>-9</sup>	0/4
Titer (Log <sub>10</sub> TCID <sub>50</sub> /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  $\geq 3 \log_{10}$  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  $\geq 3 \log_{10}$  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:   
Cameron Wilde

10/08/2020  
Date