Test Report

Analytical Report: AAE74431
Eurofins Sample Number: NJ19AA3912-2
Version: 3

TGA Licence No: MI - 15112007 - LI - 002191 - 11
APVMA Licence No: 6139

Felton Grimwade & Bosisto's Pty Ltd
61-81 Clarinda Road
Oakleigh South, VIC 3167
AU

Client Account Number: A006354285R1
Eurofins Quote Number: AGW32018015504

Eurofins Sample Number NJ19AA3912-2	
Original Received Date:	02-Apr-2019
Description:	Germagic Disinfectant (Thyme), Exp: 28-Mar-2022
Lot Number:	DB17-148-04
Containers Submitted:	1 Bottle(s)

Analysis	Result
# TMCV 006 Testing of virucidal activity of disinfectants by surface carrier technique	See Attached Report for Result
Refer to Attachment # 1	----
Method: TMCV 006 Analysis Date: 02-Jul-2019	
*** TMCV 005 Testing of virucidal activity of disinfectants by direct inoculation technique**	See Attached Report for Result
Refer to Attachment # 2	----
Method: TMCV 005 * Test is NOT Accredited Analysis Date: 11-Jul-2019	
*** TMCV 005 Testing of virucidal activity of disinfectants by direct inoculation technique**	See Attached Report for Result
Refer to Attachment # 3	----
Method: TMCV 005 * Test is NOT Accredited Analysis Date: 11-Jul-2019	
*** TMCV 005 Testing of virucidal activity of disinfectants by direct inoculation technique**	See Attached Report for Result
Refer to Attachment # 4	----
Method: TMCV 005 * Test is NOT Accredited Analysis Date: 30-Jul-2019	

TGA Licence No: MI - 15112007 - LI - 002191 - 11

APVMA Licence No: 6139

Analysis	Result
*** Protocol or Final Report Writing**	
Refer to Attachment # 5	----
Method: N/A * Test is NOT Accredited Analysis Date: 02-Jul-2019	
*** Protocol or Final Report Writing**	
Refer to Attachment # 6	----
Method: N/A * Test is NOT Accredited Analysis Date: 11-Jul-2019	
*** Protocol or Final Report Writing**	
Refer to Attachment # 7	----
Method: N/A * Test is NOT Accredited Analysis Date: 11-Jul-2019	
*** Protocol or Final Report Writing**	
Refer to Attachment # 8	----
Method: N/A * Test is NOT Accredited Analysis Date: 30-Jul-2019	

TGA Licence No: MI - 15112007 - LI - 002191 - 11
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Supplemental Information

PO: PO013377

"The test item(s) has/ have been tested in full compliance with current GMP guidelines. Sample(s) meet(s) the requirement(s) for all listed test(s) where specifications were applied."

Samples tested as received

Specifications (if) reported are as provided by the client.

TGA Licence No: MI-15112007-LI-002191-11
APVMA Licence No: 6139

* Test is NOT Accredited

Contracted Company: Eurofins ams Laboratories Sydney

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amslabs@eurofins.com

TGA Licence No: MI-15112007-LI-002191-11 APVMA Licence No: 6139
Questions about this report should be directed to your project manager or the general email listed above.

Reviewed and electronically signed for Data Reviewer Approval by
Yirong Zhang, Senior Analyst
for Eurofins ams Laboratories Pty Ltd, on 17-Oct-2019 10:35:19 UTC+11:00
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for Eurofins ams Laboratories Pty Ltd, on 17-Oct-2019 10:46:54 UTC+11:00

Examination	**Virucidal Activity – Suspension**
Virus Strain	**Measles ATCC VR-24**
Test Method	**Suspension**
Test Parameters	
Cell Substrate	Vero ATCC CCL-81
Test Concentration	Neat
Contact Time	10 minutes
Neutraliser	FBS
Test Temperature	Room Temperature
Test Condition	Dirty (5% FBS)
Method used for Virus Titre end point	Reed & Muench LD50

RESULTS:

Virus Dilution	Test Virus Control	Cyto- toxicity	Neutralisation	Sample Results	Cell Control
10^{-2}	4+/4	C	C	C	4 wells with healthy cell monolayer
10^{-3}	4+/4	0/4	4+/4	0/4	
10^{-4}	4+/4	0/4	4+/4	0/4	
10^{-5}	4+/4	N/A	N/A	0/4	
10^{-6}	4+/4	N/A	N/A	N/A	
10^{-7}	N/A	N/A	N/A	N/A	
10^{-8}	N/A	N/A	N/A	N/A	
Log_{10}	6.5	2.5	2.5	2.5	
Log_{10} Reduction of Virus after Treatment: 4.0					

Note: Presence of virus in each response is recorded as "+"
Absence of virus in each response is recorded as "0"
Cytotoxic response is recorded as "C"

CONCLUSION:

The sample "Germagic" showed a virucidal efficacy against Measles virus of 4.0 log_{10} reduction; when tested under the conditions described above.

Examination	**Virucidal Activity –Suspension**
Virus Strain	**Mumps ATCC 1379**
Test Method	**Suspension**
Test Parameters	
Cell Substrate	Vero ATCC - CCL - 81
Test Concentration	Neat
Contact Time	10 mins
Neutraliser	FBS
Test Temperature	Room Temperature
Test Condition	Dirty (5% FBS)
Method used for Virus Titre end point	Reed & Muench LD50

RESULTS:

Virus Dilution	Virus Control	Cyto- toxicity	Neutralisation	Sample Results	Cell Control
10^{-2}	4+/4	0/4	4+/4	0/4	0/4
10^{-3}	4+/4	0/4	4+/4	0/4	
10^{-4}	2+/4	N/A	N/A	0/4	
10^{-5}	2+/4	N/A	N/A	0/4	
10^{-6}	1+/4	N/A	N/A	N/A	
10^{-7}	N/A	N/A	N/A	N/A	
10^{-8}	N/A	N/A	N/A	N/A	
Log_{10}	**4.8**	**1.5**	**1.5**	**1.5**	
Log_{10} Reduction of Virus after Treatment = 3.3					

Note: Presence of virus in each response is recorded as "+"
Absence of virus in each response is recorded as "0"
Cytotoxic response is recorded as "C"

CONCLUSION: The sample "Germagic" showed a virucidal efficacy against Mumps virus of 3.3 log_{10} reduction; when tested under the conditions described above.

Examination	**Virucidal Activity – Carrier**
Virus Strain	**FCV**
Test Method	**Carrier**
Test Parameters	
Cell Substrate	CRFK ATCC-CCL-94
Test Concentration	Neat
Contact Time	10 mins
Neutraliser	FBS
Test Temperature	Room Temperature
Test Condition	Dirty
Method used for Virus Titre end point	Reed & Muench LD50

RESULTS:

<table>
<tr><th>Virus Dilution</th><th>Test Virus Control</th><th>Cyto- toxicity</th><th>Neutralisation</th><th>Sample Results</th><th>Cell Control</th></tr>
<tr><td>10^{-2}</td><td>4+/4</td><td>C</td><td>C</td><td>C</td><td rowspan="8">0/4</td></tr>
<tr><td>10^{-3}</td><td>4+/4</td><td>0+/4</td><td>4+/4</td><td>0+/4</td></tr>
<tr><td>10^{-4}</td><td>4+/4</td><td>N/A</td><td>N/A</td><td>0+/4</td></tr>
<tr><td>10^{-5}</td><td>4+/4</td><td>N/A</td><td>N/A</td><td>0+/4</td></tr>
<tr><td>10^{-6}</td><td>3+/4</td><td>N/A</td><td>N/A</td><td>0+/4</td></tr>
<tr><td>10^{-7}</td><td>2+/4</td><td>N/A</td><td>N/A</td><td>N/A</td></tr>
<tr><td>10^{-8}</td><td>N/A</td><td>N/A</td><td>N/A</td><td>N/A</td></tr>
<tr><td>Log_{10}</td><td>**6.8**</td><td>**2.5**</td><td>**2.5**</td><td>**2.5**</td></tr>
<tr><td colspan="6">Log_{10} Reduction of Virus after Treatment = 4.3</td></tr>
</table>

Note: Presence of virus in each response is recorded as “+”
Absence of virus in each response is recorded as “0”
Cytotoxic response is recorded as “C”

CONCLUSION: The sample “Germagic” showed a virucidal efficacy against FCV virus of 4.3 log_{10} reduction; when tested under the conditions described above.

Examination	**Virucidal Activity – Suspension**
Virus Strain	**Rubella ATCC-1359 (VR)**
Test Method	**Suspension**
Test Parameters	
Cell Substrate	LLC-MK2
Test Concentration	Neat
Contact Time	10 mins
Neutraliser	FBS
Test Temperature	Room Temperature
Test Condition	Dirty
Method used for Virus Titre end point	Reed & Muench LD50

RESULTS:

Virus Dilution	Test Virus Control	Cyto- toxicity	Neutralisation	Sample Results	Cell Control
10^{-1}	4+/4	C	C	-	0/4
10^{-2}	4+/4	C	C	C	
10^{-3}	4+/4	0+/4	4+/4	0+/4	
10^{-4}	4+/4	N/A	N/A	0+/4	
10^{-5}	3+/4	N/A	N/A	0+/4	
10^{-6}	2+/4	N/A	N/A	0+/4	
10^{-7}	1+/4	N/A	N/A	N/A	
10^{-8}	N/A	N/A	N/A	N/A	
Log_{10}	**5.5**	**2.5**	**2.5**	**2.5**	
Log_{10} Reduction of Virus After Treatment: 3.0					

Note: Presence of virus in each response is recorded as "+"
Absence of virus in each response is recorded as "0"
Cytotoxic response is recorded as "C"

CONCLUSION: The sample "Germagic" showed a virucidal efficacy against Rubella virus of 3.0 log_{10} reduction; when tested under the conditions described above.

SUMMARY

This study was designed to evaluate the virucidal properties of “Germagic disinfectant (Thyme)” by a quantitative suspension method based upon ASTM E1052-97. In this test the solution reduced the Measles virus titre by 4.0 logs after the 10 minute exposure period at room temperature.

INTRODUCTION

A study was required by Felton Grimwade & Bosisto’s to evaluate a disinfectant product for virucidal properties against Measles virus. A contact time of 10 minutes was designated by the sponsor.

OBJECTIVE

This study was conducted to determine the virucidal activity of “Germagic disinfectant (Thyme)” against Measles virus using accepted criteria for making virucidal claims.

REFERENCES

Therapeutic Goods (Standard for Disinfectants and Sanitary Products) (TGO104) Order 2019.

Eurofins I ams Labs. - SOP TMCV-005 – Testing of Virucidal Activity of Disinfectants by Suspension Test Method.

ASTM E1052-97 Standard Test Method to Assess the Activity of Microbiocides against Viruses in Suspension

MATERIALS AND METHODS

A. VIRUS STRAIN

The test virus used in this study was Measles virus (ATCC VR-24) which was obtained from the ATCC. The virus was stored in liquid nitrogen prior to use.

B. CELL SUBSTRATE

The host cells used in this study were Vero (ATCC CCL-81) cells, which were obtained from the ATCC. The host cells were stored in liquid nitrogen prior to use. The cells were thawed and sub-cultured in EMEM cell growth medium.

C. TEST PRODUCT

Germagic Disinfectant (Thyme) Lot: DB17-148-04, Exp: 28/03/2022, with the active ingredients of Thyme Oil BP – 1.49% w/w. Certificate of Analysis was released as “Germagic”.

D. REAGENTS AND SUPPLIERS

1. Eagles MEM (EMEM), supplied by Lonza (Lot # 0000736166 / Exp: 03/08/2020)
2. L-glutamine, supplied by Gibco (Lot # 1952633 (Exp: 30/09/2020)
3. Foetal Bovine Serum (FBS), supplied by Gibco (Lot # 1947369 Exp: 30/12/2022)
4. Trypsin/Versene-EDTA, supplied by Gibco (Lot # 1997839 Exp: 30/11/2019)
5. Flat-bottom microtiter plates, supplied by Crown Scientific
6. Hepes Buffer supplied by Gibco (Lot # 1952649 Exp: 30/10/2020)
7. Phosphate Buffer Solution (PBS), supplied by Eurofins I ams Labs (Exp: 19/08/2019)
8. Non-Essential Amino Acid by Gibco ((Lot # 1964490 Exp: 30/10/2019)

E. TEST CONDITION

Test Commencement Date	02/07/2019
Test Virus	Measles virus ATCC VR-24
Test Disinfectant	Germagic disinfectant (Thyme) DB17-148-04 (Exp: 28/03/2022)
Contact time and test temperature	10 minutes and room temperature
Test Condition	Neat

F. PREPARATION OF CELL SUBSTRATE

1. All work was carried out in a class 2 biosafety cabinet.
2. Growth media was prepared by combining the following reagents in EMEM in their specified proportions; FBS (5%), L-glutamine (1%), NEAA (1%) and Hepes Buffer (1%).
3. A 25 cm^2 flask was prepared containing a monolayer of Vero (ATCC CCL-81).
4. Once the monolayer reached confluency the growth media was discarded and the cell monolayer washed twice with 2 mL PBS, with the remaining PBS being discarded.
5. A volume of 0.5 ml Trypsin was then added to the flask, which was subsequently incubated at 37°C ± 2°C for approximately 5 minutes until cells were visibly lifting from the flask. Progress was checked using an inverted microscope.
6. When all the cells were detached, 5 mL of growth media was added and the flask was shaken gently to resuspend the cells.
7. A volume of 1 ml of the cell suspension was removed and diluted in 10 mL of fresh growth media. Using a multichannel pipette, 100 µL of this cell suspension was dispensed into each well of a 96 well microtiter plate.
8. The plates were incubated in the CO_2 incubator with an atmosphere of 5% CO_2 in air at a temperature of 37°C ± 2°C for 18-24 hours.

G. PREPARATION OF VIRUS/DISINFECTANT TEST

1. A vial of Measles virus was removed from liquid nitrogen storage and thawed in a 37°C ±1°C water bath for approximately 5 minutes. A volume of 0.2 mL of virus suspension was transferred into 2 mL of sample.
2. After 10 minutes contact time; 0.2 mL of the disinfectant-virus mixture was then transferred to maintenance media and further diluted. Dilutions of the disinfectant-virus mixture were plated out onto the cell monolayer. The surviving virus particles were then assayed by serial dilution with quadruplicate.

H. PREPARATION OF POSITIVE VIRUS CONTROL

The positive control was prepared by transferring 0.2 mL of virus into 2 mL of EMEM maintenance media. The suspension was serially diluted and assayed for infectivity with quadruplicate.

I. PREPARATION OF CYTOTOXICITY CONTROL

An aliquot of 0.2 mL of maintenance media was added to 2mL of disinfectant and subjected to the same treatment as test sample. At the end of the contact time, 0.2 mL of the liquid was added to 1.8 mL maintenance media containing 50% FBS (10^{-1}) and serially diluted up to 10^{-3} dilutions. Infectivity was assayed by plating out an aliquot of 100 µL of each dilution into quadruplicate wells.

J. PREPARATION OF NEUTRALISATION CONTROL

Dilutions of neutralised sample filtrate from (10^{-2} to 10^{-3}) of the Cytotoxicity control (I) were spiked with 0.1 mL of low titre of virus suspension to determine the dilution (s) at which the disinfectant virucidal activity is completely neutralised. A volume of 0.1 mL of spiked neutralisation dilutions were plated out in quadruplicate along with the test and virus control plate.

K. METHOD FOR VIRAL ASSAY

1. Maintenance medium was prepared by combining the following reagents in EMEM in their specified proportions; FBS (2%), L-glutamine (1%), NEAA (1%) and Hepes Buffer (1%).
2. The virus was diluted in series from 10^{-2} to 10^{-6}.

L. INOCULATION OF THE TEST SAMPLES AND CONTROLS

1. Commencing with the highest dilution of the test samples, 100 μl from each dilution was dispensed into quadruplicate wells.
2. When all wells were filled, the plate was incubated at 37°C ± 2°C in a humidified atmosphere of 5% - 7% CO_2.

M. READING VIRUS TITRATION RESULTS

1. The test plate was periodically examined over seven days for presence / absence of cytopathic effect (CPE) and cytotoxicity.
2. The positive wells and negative wells at each dilution were recorded on the Virus Titration Worksheet

N. CALCULATION OF VIRUS TITRE

The Reed & Muench LD50 Method was used for determining the virus titre endpoint.

RESULTS

The titre of the test Measles virus control was 6.5 $\log_{10}$. There was no virus infectivity observed in the disinfectant-virus test mixture at any dilution assayed after the 10 minutes contact time.

There was cytotoxicity observed at 10^{-2} dilution tested (2.5 $\log_{10}$). The neutralisation control shows no viral inhibition at 10^{-3} dilution, which means the sample was neutralised at 2.50 $\log_{10}$. All cell substrate controls were normal.

Considering the cytotoxicity and neutralisation test results, the sample "Germagic disinfectant (Thyme)" has shown significant virucidal efficacy against Measles virus by achieving 4.0 log reduction in virus concentration after 10 minutes exposure period at room temperature.

TABLE 1: Virus Control Results

Virus Dilution	Number Inoculated	Virus Control	Cytotoxicity	Neutralisation	Germagic
10^{-1}	N/A	N/A	N/A	N/A	N/A
10^{-2}	4	$4^{+}/4$	C/4	C/4	C/4
10^{-3}	4	$4^{+}/4$	$0^{+}/4$	$4^{+}/4$	$0^{+}/4$
10^{-4}	4	$4^{+}/4$	$0^{+}/4$	$4^{+}/4$	$0^{+}/4$
10^{-5}	4	$4^{+}/4$	N/A	N/A	$0^{+}/4$
10^{-6}	4	$4^{+}/4$	N/A	N/A	N/A
Total host	N/A	**20**	**12**	**12**	**16**
Log_{10}	-	**6.5**	**2.5**	**2.5**	**2.5**
Log_{10} Reduction of Virus after Treatment				**4.0**	

Calculated virus titre = $10^{6.5}$ $TCID_{50/0.1ml}$ (6.5 log_{10})

Note: Presence of virus in each response is recorded as "+"
Absence of virus in each response is recorded as "0"
Cytotoxic response is recorded as "C"

CONCLUSION:

The sample "Germagic Disinfectant (Thyme)" has demonstrated significant virucidal efficacy against Measles virus (ATCC VR-24), after 10 minutes contact time at room temperature. The sample has achieved 4.0 log reduction in virus titre.

DATA STORAGE

The raw data and a copy of this report will be archived in the records section at Eurofins I ams Laboratories for a minimum period of 5 years.

SUMMARY

This study was designed to evaluate the virucidal properties of "Germagic Disinfectant (Thyme)" by a quantitative carrier test method based upon ASTM E1053-97. In this test the solution reduced the Feline Calicivirus (FCV) virus titre by 4.3 logs after the 10 minute exposure period at room temperature.

INTRODUCTION

A study was required by Felton Grimwade & Bosisto's to evaluate a disinfectant product for virucidal properties against FCV virus. A contact time of 10 minutes was designated by the sponsor.

OBJECTIVE

This study was conducted to determine the virucidal activity of "Germagic Disinfectant (Thyme)" against FCV virus using accepted criteria for making virucidal claims.

REFERENCES

Therapeutic Goods (Standard for Disinfectants and Sanitary Products) (TGO104) Order 2019.

Eurofins I ams Labs. - SOP TMCV-005 – Testing of Virucidal Activity of Disinfectants by Carrier Test Method.

ASTM E1053-97 Standard Test Method to Assess the Activity for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces.

MATERIALS AND METHODS

A. VIRUS STRAIN

The test virus used in this study was Feline Calicivirus (FCV) which was obtained from the Burnet Institute (Australia). The virus was stored in liquid nitrogen prior to use.

B. CELL SUBSTRATE

The host cells used in this study were CRFK (ATCC CCL-94) cells, which were obtained from the Burnet Institute (Australia). The host cells were stored in liquid nitrogen prior to use. Cells were thawed and sub-cultured in EMEM cell growth medium.

C. TEST PRODUCT

Germagic Disinfectant (Thyme) Lot: DB17-148-04, Exp: 28/03/2022, with the active ingredients of Thyme Oil BP – 1.49% w/w. Certificate of Analysis was released as "Germagic".

D. REAGENTS AND SUPPLIERS

1. Eagles MEM (EMEM), supplied by Lonza (Lot # 0000736166 / Exp: 03/08/2020)
2. L-glutamine, supplied by Gibco (Lot # 1952633 Exp: 30/01/2020)
3. Foetal Bovine Serum (FBS), supplied by Gibco (Lot # 1799178 Exp: 05/2021)
4. Trypsin/Versene-EDTA, supplied by Gibco (Lot # 1997839 Exp: 30/11/2019)
5. Flat-bottom microtiter plates, supplied by Crown Scientific
6. Hepes Buffer supplied by Gibco (Lot # 1952649 Exp: 30/01/2020)
7. Antibiotic-Antimycotic by Gibco (Lot # 2041557Exp: 30/10/2019)
8. Phosphate Buffer Solution (PBS), supplied by Eurofins I ams Labs (Exp: 09/08/2019)
9. Non-Essential Amino Acid by Gibco (Lot # 1964490 Exp: 30/10/2019)

E. TEST CONDITION

Test Commencement Date	11/07/2019
Test Virus	FCV (surrogate of Norovirus)
Test Disinfectant	Germagic Disinfectant (Thyme) DB17-148-04 (Exp: 28/03/2022)
Contact time and test temperature	10 minutes and room temperature
Test Condition	Neat

F. PREPARATION OF CELL SUBSTRATE

1. All work was carried out in a class 2 biosafety cabinet.
2. Growth media was prepared by combining the following reagents in EMEM in their specified proportions; FBS (5%), L-glutamine (1%), NEAA (1%), Antibiotic-Antimycotic (1%) and Hepes Buffer (1%).
3. A 25 cm^2 flask was prepared containing a monolayer of CRFK (ATCC CCL-94).
4. Once the monolayer reached confluency the growth media was discarded and the cell monolayer washed twice with 2 mL PBS, with the remaining PBS being discarded.
5. A volume of 0.5 mL Trypsin was then added to the flask, which was subsequently incubated at 37°C ± 2°C for approximately 5 minutes until cells were visibly lifting from the flask. Progress was checked using an inverted microscope.
6. When all the cells were detached, 5 mL of growth media was added and the flask was shaken gently to resuspend the cells.
7. A volume of 1 mL of the cell suspension was removed and diluted in 10 mL of fresh growth media. Using a multichannel pipette, 100 μL of this cell suspension was dispensed into each well of a 96 well microtiter plate.
8. The plates were incubated in the CO_2 incubator with an atmosphere of 5% CO_2 in air at a temperature of 37°C ± 2°C for 18-24 hours.

G. PREPARATION OF VIRUS/DISINFECTANT TEST

1. A vial of FCV was removed from liquid nitrogen storage and thawed in a 37°C water bath for approximately 5 mins. A volume of 0.2 mL of virus suspension was transferred into 2 sterile pyrex petri dishes. Even distribution of the virus suspension was achieved with the aid of the sterile plastic spreader. The pyrex petri dishes were then air dried for 20 - 30 minutes in the biosafety cabinet.

2. A volume of 2 mL of the Germagic solution was applied to the test petri dish containing the virus film with exposure time of 10 minutes at room temperature. Even distribution of the disinfectant was achieved with the aid of the sterile plastic spreader.
3. After 10 minutes contact time; 0.2 mL of the disinfectant-virus mixture was then transferred and plating out onto the cell monolayer. The surviving virus particles were then assayed by serial dilution with quadruplicate.

H. PREPARATION OF POSITIVE VIRUS CONTROL

The positive control was prepared by transferring 2 mL of EMEM maintenance media onto the virus control film. Following the contact time, the suspension was serially diluted and assayed for infectivity with quadruplicate.

I. PREPARATION OF CYTOTOXICITY CONTROL

An aliquot of 0.2 mL of maintenance media was spread onto sterile pyrex petri dish. The medium was air-dried for 20 - 30 minutes and subjected to the same treatment as test carrier. At the end of the contact time, 0.2 mL of the liquid was added to 1.8

mL maintenance media containing 50% FBS, then serially diluted up to 10^{-3}. Infectivity was assayed by plating out an aliquot of 100 µL of each dilution into quadruplicate wells.

J. PREPARATION OF NEUTRALISATION CONTROL

Dilutions of neutralised sample filtrate from (10^{-2} to 10^{-3}) of the Cytotoxicity control (I) were spiked with 0.1 mL of low titre of virus suspension to determine the dilution (s) at which the disinfectant virucidal activity is completely neutralised. A volume of 0.1 mL of spiked neutralisation dilutions were plated out in quadruplicate along with the test and virus control plate.

K. METHOD FOR VIRAL ASSAY

1. Maintenance medium was prepared by combining the following reagents in EMEM in their specified proportions; FBS (2%), L-glutamine (1%), NEAA (1%), Antibiotic-Antimycotic (1%) and Hepes Buffer (1%).
2. The virus was diluted in series from 10^{-2} to 10^{-7}.

L. INOCULATION OF THE TEST SAMPLES AND CONTROLS

1. Commencing with the highest dilution of the test samples, 100 µl from each dilution was dispensed into quadruplicate wells.
2. When all wells were filled, the plate was incubated at 37°C ± 2°C in a humidified atmosphere of 5% - 7% CO_2.

M. READING VIRUS TITRATION RESULTS

1. The test plate was periodically examined over seven days for presence / absence of cytopathic effect (CPE) and cytotoxicity.
2. The positive wells and negative wells at each dilution were recorded on the Virus Titration Worksheet

N. CALCULATION OF VIRUS TITRE

The Reed & Muench LD50 Method was used for determining the virus titre endpoint.

RESULTS

The titre of the test FCV virus control was 6.8 $\log_{10}$. There was no virus infectivity observed in the disinfectant-virus test mixture at any dilution assayed after the 10 minutes contact time.

There was cytotoxicity observed at 10^{-2} dilution tested (2.5 $\log_{10}$). The neutralisation control shows no viral inhibition at 10^{-3} dilution, which means the sample was neutralised at 2.50 $\log_{10}$. All cell substrate controls were normal.

Considering the cytotoxicity and neutralisation test results, the sample “Germagic Disinfectant (Thyme)” has shown significant virucidal efficacy against FCV virus by achieving 4.3 log reduction in virus concentration after 10 minutes exposure period at room temperature.

TABLE 1: Virus Control Results

Virus Dilution	Number Inoculated	Virus Control	Cytotoxicity	Neutralisation	Germagic
10^{-1}	4	N/A	N/A	N/A	N/A
10^{-2}	4	$4^{+}/4$	C/4	C/4	C/4
10^{-3}	4	$4^{+}/4$	$0^{+}/4$	$4^{+}/4$	$0^{+}/4$
10^{-4}	4	$4^{+}/4$	$0^{+}/4$	$4^{+}/4$	$0^{+}/4$
10^{-5}	4	$4^{+}/4$	N/A	N/A	$0^{+}/4$
10^{-6}	4	$3^{+}/4$	N/A	N/A	N/A
10^{-7}	4	$2^{+}/4$	N/A	N/A	N/A
Total host	N/A	**24**	**12**	**12**	**16**
Log_{10}	-	**6.8**	**2.5**	**2.5**	**2.5**
Log_{10} Reduction of Virus after Treatment				**4.3**	

Calculated virus titre = $10^{6.8}TCID_{50/0.1ml}$ (6.8 log_{10})

Note: Presence of virus in each response is recorded as “+”
Absence of virus in each response is recorded as “0”
Cytotoxic response is recorded as “C”

CONCLUSION:

The sample “Germagic Disinfectant (Thyme)” has demonstrated significant virucidal efficacy against FCV, after 10 minutes contact time at room temperature. The sample has achieved a 4.3 log reduction in virus titre.

DATA STORAGE

The raw data and a copy of this report will be archived in the records section at Eurofins I ams Laboratories for a minimum period of 5 years.

SUMMARY

This study was designed to evaluate the virucidal properties of "Germagic Disinfectant (Thyme)" by a quantitative suspension method based upon ASTM E1052-97. In this test the solution reduced the Mumps virus titre by 3.3 logs after the 10 minute exposure period at room temperature.

INTRODUCTION

A study was required by Felton Grimwade & Bosisto's to evaluate a disinfectant product for virucidal properties against Mumps virus. A contact time of 10 minutes was designated by the sponsor.

OBJECTIVE

This study was conducted to determine the virucidal activity of "Germagic Disinfectant (Thyme)" against Mumps virus using accepted criteria for making virucidal claims.

REFERENCES

Therapeutic Goods (Standard for Disinfectants and Sanitary Products) (TGO104) Order 2019.

Eurofins l ams Labs. - SOP TMCV-005 – Testing of Virucidal Activity of Disinfectants by Suspension Test Method.

ASTM E1052-97 Standard Test Method to Assess the Activity of Microbiocides against Viruses in Suspension

MATERIALS AND METHODS

A. VIRUS STRAIN

The test virus used in this study was Mumps virus (ATCC VR-1379) which was obtained from the ATCC. The virus was stored in liquid nitrogen prior to use.

B. CELL SUBSTRATE

The host cells used in this study were Vero (ATCC CCL-81) cells, which were obtained from Adelaide University. The host cells were stored in liquid nitrogen prior to use. Cells were thawed and sub-cultured in EMEM cell growth medium.

C. TEST PRODUCT

Germagic Disinfectant (Thyme) Lot: DB17-148-04, Exp: 28/03/2022, with the active ingredients of Thyme Oil BP – 1.49% w/w. Certificate of Analysis was released as "Germagic".

D. REAGENTS AND SUPPLIERS

1. Eagles MEM (EMEM), supplied by Lonza (Lot # 0000736166 / Exp: 03/08/2020)
2. L-glutamine, supplied by Gibco (Lot # 1952633 Exp: 30/01/2020)
3. Foetal Bovine Serum (FBS), supplied by Gibco (Lot # NZ01117-1, Exp: 11/2022)
4. Trypsin/Versene-EDTA, supplied by Gibco (Lot # 1997839 Exp: 30/11/2019)
5. Flat-bottom microtiter plates, supplied by Crown Scientific
6. Hepes Buffer supplied by Gibco (Lot # 1952649 Exp: 30/10/2020)
7. Antibiotic-Antimycotic by Gibco (Lot: 2041557 Exp: 30/10/2019)
8. Phosphate Buffer Solution (PBS), supplied by Eurofins l ams Labs (Exp: 09/08/2019)

9. Non-Essential Amino Acid by Gibco ((Lot # 1964490 Exp: 30/10/2019)

E. TEST CONDITION

Test Commencement Date	11/07/2019
Test Virus	Mumps virus ATCC VR-1379
Test Disinfectant	Germagic Disinfectant (Thyme) DB17-148-04 (Exp: 28/03/2022)
Contact time and test temperature	10 minutes and room temperature
Test Condition	Neat

F. PREPARATION OF CELL SUBSTRATE

1. All work was carried out in a class 2 biosafety cabinet.
2. Growth media was prepared by combining the following reagents in EMEM in their specified proportions; FBS (5%), L-glutamine (1%), NEAA (1%), Antibiotic-Antimycotic (1%) and Hepes Buffer (1%).
3. A 25 cm^2 flask was prepared containing a monolayer of Vero (ATCC CCL-81).
4. Once the monolayer reached confluency the growth media was discarded and the cell monolayer washed twice with 2 mL PBS, with the remaining PBS being discarded.
5. A volume of 0.5 mL Trypsin was then added to the flask, which was subsequently incubated at 37°C ± 2°C for approximately 5 minutes until cells were visibly lifting from the flask. Progress was checked using an inverted microscope.
6. When all the cells were detached, 5 mL of growth media was added and the flask was shaken gently to resuspend the cells.
7. A volume of 1 mL of the cell suspension was removed and diluted in 10 mL of fresh growth media. Using a multichannel pipette, 100 μL of this cell suspension was dispensed into each well of a 96 well microtiter plate.
8. The plates were incubated in the CO_2 incubator with an atmosphere of 5% CO_2 in air at a temperature of 37°C ± 2°C for 18-24 hours.

G. PREPARATION OF VIRUS/DISINFECTANT TEST

1. A vial of Mumps virus was removed from liquid nitrogen storage and thawed in a 37°C ±1°C water bath for approximately 5 minutes. A volume of 0.2 mL of virus suspension was transferred into 2mL of sample.
2. After 10 minutes contact time; 0.2 mL of the disinfectant-virus mixture was then transferred to maintenance media and further diluted. Dilutions of the disinfectant-virus mixture were plated out onto the cell monolayer. The surviving virus particles were then assayed by serial dilution with quadruplicate.

H. PREPARATION OF POSITIVE VIRUS CONTROL

The positive control was prepared by transferring 0.2mL of virus into 2ml of EMEM maintenance media. The suspension was serially diluted and assayed for infectivity with quadruplicate.

I. PREPARATION OF CYTOTOXICITY CONTROL

An aliquot of 0.2 mL of maintenance media was added to 2mL of disinfectant and subjected to the same treatment as test sample. At the end of the contact time, 0.2 ml of the liquid was added to 1.8mL maintenance media containing 50% FBS (10^{-1}) and serially diluted up to 10^{-3} dilutions. Infectivity was assayed by plating out an aliquot of 100 μL of each dilution into quadruplicate wells.

J. PREPARATION OF NEUTRALISATION CONTROL

Dilutions of neutralised sample filtrate from (10^{-2} to 10^{-3}) of the Cytotoxicity control (I) were spiked with 0.1 mL of low titre of virus suspension to determine the dilution (s) at which the disinfectant virucidal activity is completely neutralised. A volume of 0.1 mL of spiked neutralisation dilutions were plated out in quadruplicate along with the test and virus control plate.

K. METHOD FOR VIRAL ASSAY

1. Maintenance medium was prepared by combining the following reagents in EMEM in their specified proportions; FBS (2%), L-glutamine (1%), NEAA (1%), Antibiotic-Antimycotic (1%) and Hepes Buffer (1%).
2. The virus was diluted in series from 10^{-2} to 10^{-6}.

L. INOCULATION OF THE TEST SAMPLES AND CONTROLS

1. Commencing with the highest dilution of the test samples, 100µl from each dilution was dispensed into quadruplicate wells.
2. When all wells were filled, the plate was incubated at 37°C ± 1°C in a humidified atmosphere of 5% - 7% CO_2.

M. READING VIRUS TITRATION RESULTS

1. The test plate was periodically examined over seven days for presence / absence of cytopathic effect (CPE) and cytotoxicity.
2. The positive wells and negative wells at each dilution were recorded on the Virus Titration Worksheet

N. CALCULATION OF VIRUS TITRE

The Reed & Muench LD50 Method was used for determining the virus titre endpoint.

RESULTS

The titre of the test Mumps virus control was 4.80 log_{10}. There was no virus infectivity observed in the disinfectant-virus test mixture at any dilution assayed after the 10 minutes contact time.

There was cytotoxicity observed at 10^{-1} dilution tested (1.5 log_{10}). The neutralisation control shows no viral inhibition at 10^{-2} dilution, which means the sample was neutralised at 1.50 log_{10}. All cell substrate controls were normal.

Considering the cytotoxicity and neutralisation test results, the sample Germagic has shown significant virucidal efficacy against Mumps virus by achieving 3.3 log reduction in virus concentration after 10 minutes exposure period at room temperature.

TABLE 1: Virus Control Results

Virus Dilution	Number Inoculated	Virus Control	Cytotoxicity	Neutralisation	Germagic
10^{-1}	4	N/A	C	C	C
10^{-2}	4	$4^+/4$	$0^+/4$	$4^+/4$	$0^+/4$
10^{-3}	4	$4^+/4$	$0^+/4$	$4^+/4$	$0^+/4$
10^{-4}	4	$2^+/4$	N/A	N/A	$0^+/4$
10^{-5}	4	$2^+/4$	N/A	N/A	$0^+/4$
10^{-6}	4	$1^+/4$	N/A	N/A	N/A
Total host	N/A	**20**	**8**	**8**	**16**
Log_{10}	-	**4.8**	**1.5**	**1.5**	**1.5**
Log_{10} Reduction of Virus after Treatment				**3.3**	

Calculated virus titre = $10^{4.8}TCID_{50/0.1ml}$ (4.8 log_{10})

Note: Presence of virus in each response is recorded as "+"
Absence of virus in each response is recorded as "0"
Cytotoxic response is recorded as "C"

CONCLUSION:

The sample "Germagic Disinfectant (Thyme)" has demonstrated significant virucidal efficacy against Mumps virus (ATCC VR-1379), after 10 minutes contact time at room temperature. The sample has achieved a 3.3 log reduction in virus titre.

DATA STORAGE

The raw data and a copy of this report will be archived in the records section at Eurofins I ams Laboratories for a minimum period of 5 years.

SUMMARY

This study was designed to evaluate the virucidal properties of “Germagic Disinfectant (Thyme)” by a quantitative suspension method based upon ASTM E1052-97. In this test the solution reduced the Rubella virus titre by 3.0 logs after the 10 minute exposure period at room temperature.

INTRODUCTION

A study was required by Felton Grimwade & Bosisto’s to evaluate a disinfectant product for virucidal properties against Rubella virus. A contact time of 10 minutes was designated by the sponsor.

OBJECTIVE

This study was conducted to determine the virucidal activity of “Germagic Disinfectant (Thyme)” against Rubella virus using accepted criteria for making virucidal claims.

REFERENCES

Therapeutic Goods (Standard for Disinfectants and Sanitary Products) (TGO104) Order 2019.

Eurofins I ams Labs. - SOP TMCV-005 – Testing of Virucidal Activity of Disinfectants by Suspension Test Method.

ASTM E1052-97 Standard Test Method to Assess the Activity of Microbiocides against Viruses in Suspension

MATERIALS AND METHODS

A. VIRUS STRAIN

The test virus used in this study was Rubella virus (ATCC VR-1359) which was obtained from the ATCC. The virus was stored in liquid nitrogen prior to use.

B. CELL SUBSTRATE

The host cells used in this study were LLC-MK2 (ATCC CCL-7) cells, which were obtained from In Vitro Technologies. The host cells were stored in liquid nitrogen prior to use. Cells were thawed and sub-cultured in EMEM cell growth medium.

C. TEST PRODUCT

Germagic Disinfectant (Thyme) Lot: DB17-148-04, Exp: 28/03/2022, with the active ingredients of Thyme Oil BP – 1.49% w/w. Certificate of Analysis was released as “Germagic”.

D. REAGENTS AND SUPPLIERS

1. Eagles MEM (EMEM), supplied by Lonza (Lot # 0000736166 / Exp: 03/08/2020)
2. L-glutamine, supplied by Gibco (Lot # 1952633 Exp: 30/01/2020)
3. Foetal Bovine Serum (FBS), supplied by Gibco (Lot # NZ01117-1, Exp: 11/2022)
4. Trypsin/Versene-EDTA, supplied by Gibco (Lot # 1997839 Exp: 30/11/2019)
5. Flat-bottom microtiter plates, supplied by Crown Scientific
6. Hepes Buffer supplied by Gibco (Lot # 1952649 Exp: 30/01/2020)
7. Antibiotic-Antimycotic by Gibco (Lot: 2041557 Exp: 30/10/2019
8. Phosphate Buffer Solution (PBS), supplied by Eurofins I ams Labs (Exp: 09/08/2019)

9. Non-Essential Amino Acid by Gibco (Lot # 1964490 Exp: 30/10/2019)

E. TEST CONDITION

Test Commencement Date	30/07/2019
Test Virus	Rubella virus ATCC VR-1359
Test Disinfectant	Germagic Disinfectant (Thyme) DB17-148-04 (Exp: 28/03/2022)
Contact time and test temperature	10 minutes and room temperature
Test Condition	Neat

F. PREPARATION OF CELL SUBSTRATE

1. All work was carried out in a class 2 biosafety cabinet.
2. Growth media was prepared by combining the following reagents in EMEM in their specified proportions; FBS (5%), L-glutamine (1%), NEAA (1%), Antibiotic-Antimycotic (1%) and Hepes Buffer (1%).
3. A 25 cm^2 flask was prepared containing a monolayer of LLC-MK2 (ATCC CCL-7).
4. Once the monolayer reached confluency the growth media was discarded and the cell monolayer washed twice with 2 mL PBS, with the remaining PBS being discarded.
5. A volume of 0.5 mL Trypsin was then added to the flask, which was subsequently incubated at 37°C ± 2°C for approximately 5 minutes until cells were visibly lifting from the flask. Progress was checked using an inverted microscope.
6. When all the cells were detached, 5 mL of growth media was added and the flask was shaken gently to resuspend the cells.
7. A volume of 1 mL of the cell suspension was removed and diluted in 10 ml of fresh growth media. Using a multichannel pipette, 100 μL of this cell suspension was dispensed into each well of a 96 well microtiter plate.
8. The plates were incubated in the CO_2 incubator with an atmosphere of 5% CO_2 in air at a temperature of 37°C ± 2°C for 18-24 hours.

G. PREPARATION OF VIRUS/DISINFECTANT TEST

1. A vial of Rubella virus was removed from liquid nitrogen storage and thawed in a 37°C ±1°C water bath for approximately 5 minutes. A volume of 0.2 mL of virus suspension was transferred into 2 mL of sample.
2. After 10 minutes contact time; 0.2 mL of the disinfectant-virus mixture was then transferred to maintenance media and further diluted. Dilutions of the disinfectant-virus mixture were plated out onto the cell monolayer. The surviving virus particles were then assayed by serial dilution with quadruplicate.

H. PREPARATION OF POSITIVE VIRUS CONTROL

The positive control was prepared by transferring 0.2 mL of virus into 2 mL of EMEM maintenance media. The suspension was serially diluted and assayed for infectivity with quadruplicate.

I. PREPARATION OF CYTOTOXICITY CONTROL

An aliquot of 0.2 mL of maintenance media was added to 2 mL of disinfectant and subjected to the same treatment as test sample. At the end of the contact time, 0.2 mL of the liquid was added to 1.8 mL maintenance media containing 50% FBS (10^{-1}) and serially diluted up to 10^{-3} dilutions. Infectivity was assayed by plating out an aliquot of 100 μL of each dilution into quadruplicate wells.

J. PREPARATION OF NEUTRALISATION CONTROL

Dilutions of neutralised sample filtrate from (10^{-2} to 10^{-3}) of the Cytotoxicity control (I) were spiked with 0.1 mL of low titre of virus suspension to determine the dilution (s) at which the disinfectant virucidal activity is completely neutralised. A volume of 0.1 mL of spiked neutralisation dilutions were plated out in quadruplicate along with the test and virus control plate.

K. METHOD FOR VIRAL ASSAY

1. Maintenance medium was prepared by combining the following reagents in EMEM in their specified proportions; FBS (2%), L-glutamine (1%), NEAA (1%), Antibiotic-Antimycotic (1%) and Hepes Buffer (1%).
2. The virus was diluted in series from 10^{-2} to 10^{-7}.

L. INOCULATION OF THE TEST SAMPLES AND CONTROLS

1. Commencing with the highest dilution of the test samples, 100 µl from each dilution was dispensed into quadruplicate wells.
2. When all wells were filled, the plate was incubated at 37°C ± 2°C in a humidified atmosphere of 5% - 7% CO_2.

M. READING VIRUS TITRATION RESULTS

1. The test plate was periodically examined over seven days for presence / absence of cytopathic effect (CPE) and cytotoxicity.
2. The positive wells and negative wells at each dilution were recorded on the Virus Titration Worksheet

N. CALCULATION OF VIRUS TITRE

The Reed & Muench LD50 Method was used for determining the virus titre endpoint.

RESULTS

The titre of the test Rubella virus control was 5.5 $\log_{10}$. There was no virus infectivity observed in the disinfectant-virus test mixture at any dilution assayed after the 10 minutes contact time.

There was cytotoxicity observed at 10^{-2} dilution tested (2.5 $\log_{10}$). The neutralisation control shows no viral inhibition at 10^{-3} dilution, which means the sample was neutralised at 2.50 $\log_{10}$. All cell substrate controls were normal.

Considering the cytotoxicity and neutralisation test results, the sample “Germagic Disinfectant (Thyme)” has shown significant virucidal efficacy against Rubella virus by achieving 3.0 log reduction in virus concentration after 10 minutes exposure period at room temperature.

TABLE 1: Virus Control Results

Virus Dilution	Number Inoculated	Virus Control	Cytotoxicity	Neutralisation	Germagic
10^{-1}	4	$4^{+}/4$	C	C	C
10^{-2}	4	$4^{+}/4$	C/4	C/4	C/4
10^{-3}	4	$4^{+}/4$	$0^{+}/4$	$4^{+}/4$	$0^{+}/4$
10^{-4}	4	$4^{+}/4$	N/A	N/A	$0^{+}/4$
10^{-5}	4	$3^{+}/4$	N/A	N/A	$0^{+}/4$
10^{-6}	4	$2^{+}/4$	N/A	N/A	$0^{+}/4$
10^{-7}	4	$1^{+}/4$	N/A	N/A	N/A
Total host	N/A	**28**	**8**	**8**	**20**
Log_{10}	-	**5.5**	**2.5**	**2.5**	**2.5**
Log_{10} Reduction of Virus after Treatment				**3.0**	

Calculated virus titre = $10^{5.5}TCID_{50/0.1ml}$ (5.5 log_{10})

Note: Presence of virus in each response is recorded as "+"
Absence of virus in each response is recorded as "0"
Cytotoxic response is recorded as "C"

CONCLUSION:

The sample "Germagic Disinfectant (Thyme)" has demonstrated significant virucidal efficacy against Rubella virus (ATCC VR-1359), after 10 minutes contact time at room temperature. The sample has achieved a 3.0 log reduction in virus titre.

DATA STORAGE

The raw data and a copy of this report will be archived in the records section at Eurofins I ams Laboratories for a minimum period of 5 years.