FINAL REPORT

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

Test Substance
TMXPAE

Lot Numbers
TMXPAE-200326-1
TMXPAE-200326-2
TMXPAE-200326-3

Test Organism
Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2)(COVID-19 Virus), Strain: USA-WA1/2020 Source: BEI Resources, NR-52281

Test Guidelines
EPA (2018) Guidelines 810.2000 and 810.2200 (G)

Author
Cory Chiossone

Study Completion Date
08/24/20

Performing Laboratory
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Laboratory Project Identification Number
596-102

Protocol Identification Number
596.1.04.13.20

Sponsor
Laboratoire M2
4005-A, Rue de la Garlock
Sherbrooke (Quèbec) J1L 1W9
Canada

STATEMENT OF NO DATA CONFIDENTIALITY

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec.10(d)(1)(A), (B) or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Submitter signature: Denise Burnside Date: 08/24/2020
Typed Name of Signer: Denise Burnside
Typed Name of Company: Agent for Laboratoire M2

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The following is a detailed description of all differences between the practices used in the study and those required by 40 CFR part § 160:

- Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study.

Study Director Signature: _____ Date: 08/24/2020
Typed Name: Cory Chiossone
Typed Name of Laboratory: Microbac Laboratories, Inc.

Sponsor Signature: Denise Burnside Date: 08/24/2020
Typed Name of Signer: Denise Burnside
Typed Name of Company: Agent for Laboratoire M2

Submitter Signature: Denise Burnside Date: 08/24/2020
Typed Name of Signer: Denise Burnside
Typed Name of Company: Agent for Laboratoire M2

QUALITY ASSURANCE UNIT STATEMENT

The Quality Assurance Unit of Microbac has inspected Project Number 596-102 to be in compliance with current Good Laboratory Practice regulations, (40 CFR § 160).

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below.

Phase Inspected	Date of Inspection	Date Reported to Study Director	Date Reported to Management
Protocol	06/02/20 06/04/20	06/04/20	06/04/20
In Process (Carrier Preparation)	06/02/20	06/04/20	06/04/20
Draft Final Report	07/07/20 07/08/20	07/14/20	07/14/20
Final Report	08/24/20	08/24/20	08/24/20

Danielle Downs 08/24/2020

Danielle Downs, RQAP-GLP
Quality Assurance Specialist III

Date

TABLE OF CONTENTS

TEST SUBSTANCE CHARACTERIZATION

Test Substance characterization as to the identity, strength, purity, solubility and composition, as applicable, according to 40 CFR, Part 160, Subpart F [160.105] was documented prior to its use in the study. The Test Substance Certificate of Analysis Reports, provided by the sponsor, are found in Appendix II.

TEST SUMMARY

Study Title:	VIRUCIDAL HARD-SURFACE EFFICACY TEST – Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2)(COVID-19 Virus)
Project No.:	596-102
Protocol No.:	596.1.04.13.20
Test Method:	ASTM International E1053-20 "Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces"
Sponsor:	Laboratoire M2 4005-A, Rue de la Garlock Sherbrooke (Quèbec) J1L 1W9 Canada
Testing Facility:	Microbac Laboratories, Inc. 105 Carpenter Drive Sterling, VA 20164
Study Objective:	This test was performed in order to substantiate virucidal efficacy claims for a test substance to be labeled as a virucide by determining the potential of the test substance to disinfect hard surfaces contaminated with SARS-CoV-2. This test was designed to simulate consumer use and was performed in conformance to EPA OCSPP 810.2000 (2018) and 810.2200 (2018) Product Performance Test Guidelines, Frequent Questions for the 2018 Series 810 – Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines (2018), as well as the Health Canada's Disinfectant Drugs (April 2020) and Safety and Efficacy Requirements for Surface Disinfectant Drugs (April 2020).
Study Dates:	Study Initiation: 06/02/20 Experimental Start: 06/02/20 Experimental End: 06/11/20 Study Completion: See page 1

TEST SUMMARY (continued)

Test Substance: TMXPAE

- Lot No.: TMXPAE-200326-1, Received: 04/01/20, DS No. K346
- Lot No.: TMXPAE-200326-2, Received: 04/01/20, DS No. K347
- Lot No.: TMXPAE-200326-3, Received: 04/01/20, DS No. K348
- Physical Description: Liquid
- Storage Condition: Ambient Room Temperature
- Active Ingredients: 0.212% Thymol (TMXPAE-200326-1), 0.213% Thymol (TMXPAE-200326-2), 0.208% Thymol (TMXPAE-200326-3)
- Dilution: 1:1.03 (1 part test substance + 0.03 parts diluent) (TMXPAE-200326-1 and TMXPAE-200326-2) Ready-to-use (TMXPAE-200326-3)
- Diluent: Sterile DI Water

Test Conditions: Organic Soil Load: 5.0% Newborn Calf Serum (NCS) in viral inoculum
Contact Time: 55 Seconds
Contact Temperature: Room temperature (20±1°C) (Actual: 21°C)
Contact Relative Humidity: 34-35% RH
Note: Test conditions apply to all test and control carriers.

Challenge Virus: Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2)(SARS-Related Coronavirus 2) (COVID-19 Virus)

- Strain: USA-WA1/2020
- Source: BEI Resources, NR-52281

Strain USA-WA1/2020 was isolated from an oropharyngeal swab from a patient with a respiratory illness who had recently returned from travel to the affected region of China and developed clinical disease (COVID-19) in January 2020 in Washington, USA (https://www.beiresources.org/Catalog/animalviruses/NR-52281.aspx).

Indicator Cells: Vero E6 cells

- Source: ATCC CRL-1586

Incubation Time: 4 – 9 days (Actual: 9 days)

Incubation Temperature: 36 ± 2°C with 5 ± 3% CO_2

Dilution Medium (DM): Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS)

TEST SUMMARY (continued)

Neutralizer:	MEM + 10% NCS +0.5% Polysorbate-80 + 0.5% Lecithin + 3% HEPES + 0.025N NaOH
Test substance diluent:	Sterile DI Water
Study Design:	This study was performed according to the signed protocol and project sheet(s) issued by the Study Director (see Appendix I).
Study Personnel:	Cory Chiossone — Lab Manager Alivia Rinaldi — Associate Scientist I Elizabeth Franco — Associate Scientist III Cameron Wilde — Senior Scientist I

TEST PROCEDURES

Indicator Cells:

Vero E6 cells were obtained from ATCC and maintained in cell culture at 36 ± 2°C with 5 ± 3% CO_2 prior to seeding. The indicator cell plates were prepared the day prior to inoculation with test sample. The cells were seeded in 24-well plates at a density of 1.0 x 10^5 cells/mL at 1 mL per well.

Virus Inoculum:

The stock virus was propagated in Vero E6, the cell supernatant was clarified, aliquoted, and stored at -60 to -90°C. Frozen viral stock was thawed on the day of the test.

Challenge Virus:

Original stock virus contained 5.0% Newborn Calf Serum.

Test Substance:

Test substance was received ready to use (Lot No. TMXPAE-200326-3) or was diluted 1 part test substance + 0.03 parts diluent (175 mL of test substance + 5.25 mL Sterile DI Water) (Lot Nos. TMXPAE-200326-1 and TMXPAE-200326-2). Test substance was stored at ambient temperature and required no equilibration. Test substance was used within 3 hours of preparation.

Test Carriers:

100mm diameter glass carriers were sterilized on 06/01/20 for 2 hours before being inoculated with 0.4 mL of virus inoculum. The inoculum was spread with a cell scraper over the bottom of the carriers. The virus was dried for 30 minutes at 21°C with 34-36% Relative Humidity (RH) until

TEST PROCEDURES (continued)

they were visually dry. One carrier was be prepared for each lot of the test substance using virus. One carrier was prepared for the plate recovery control using virus. Additionally, one carrier was prepared for each lot of test substance for the neutralizer effectiveness/viral interference and cytotoxicity controls using dilution media in lieu of virus as the inoculum.

Test Substance Application and Exposure Conditions:

The test substance, at its use dilution, was dispensed into Microbac provided spray bottles and inverted 3 times. A mock spray action was performed by applying each batch of test substance, as specified by the Sponsor, onto at least two blank Petri dishes. The volume dispensed onto each dish was measured and averaged for each batch. The average spray volume per batch was 2.0 mL and this average spray volume for each batch was used for the neutralizer volume for all applicable runs. The average volume of the mock spray for the three lots was used as the volume of dilution media added to the plate recovery control.

One carrier per test substance batch was evaluated. Using 4 sprays from the spray bottles the product was applied to the test carriers from a distance of 6-8 inches. The dried virus film was completely covered by the test substance. After the product was applied a stopwatch was started and the test carriers were held for the contact time of 55 seconds at 21°C with 34-35% RH in a horizontal position.

Recovery of Samples:

After the contact time, the test substance was neutralized with 2.0 mL of neutralizer, as determined by the mock spray volume. The mixture was scraped from the surface of the carrier with a cell scraper. This post-neutralized sample (PNS) was considered the 10^{-1} dilution. A 0.5 mL aliquot of the PNS was ten-fold serially diluted in 4.5 mL aliquots of DM.

Selected dilutions were inoculated onto 24-well host cell plates at 1.0 mL per well, 4 wells per dilution, and adsorbed for 24 hours 49 minutes at 36 ± 2°C with 5 ± 3% CO_2 (actual: 35.91 ± 0.37°C with 5% CO_2). After adsorption, the media was aspirated; the cells were refed with 1.0 mL of fresh DM and returned to incubation at 36 ± 2°C with 5 ± 3% CO_2 (Actual: 36.26 ± 0.28°C with 5% CO_2) for an additional 8 days.

Infectivity Assay:

The residual infectious virus in both test and controls was detected by viral-induced cytopathic effect (CPE). CPE is defined as cell rounding and sloughing off of the cell monolayer. After 9 days of incubation at 36 ± 2°C with 5 ± 3% CO_2 (actual: 36.22 ± 0.31°C with 5% CO_2), the plates were removed, scored, and recorded for test-substance specific cytotoxic effects and/or virus-specific cytopathic effect (CPE).

TEST PROCEDURES (continued)

Neutralizer Effectiveness and Viral Interference Control (NE/VI):

The control was performed concurrently with the test to assess whether residual active ingredient was present after neutralization (Neutralizer Effectiveness) or if the neutralized test substance interferes with virus infectivity (Viral Interference). The NE/VI was prepared identically to the test sample except 0.4 mL of DM was used in lieu of virus inoculum to inoculate the carrier. 4 sprays of the test substance were applied to the carriers and held for the contact time (55 seconds). 2.0 mL of neutralizer was then added. After test substance application and neutralization, the PNS was divided into two portions, one for NE/VI and one for Cytotoxicity (see below). For the NE/VI, a 0.5 mL aliquot of the PNS was 10-fold serially diluted into 4.5 mL of DM and 100 µL of virus stock (containing 1413 $TCID_{50}$ units) was added individually to 4.5 mL of selected dilutions and held for at least the contact time (actual: 55 seconds). This post-neutralized sample (PNS) was considered the 10^{-1} dilution. Selected dilutions were inoculated onto indicator cell plates at 1.0 mL per well, 4 wells per dilution and incubated in an identical manner as the test samples.

Cytotoxicity Control (CT):

This control was performed concurrently with the test to assess the cytotoxic effects of the test substance on indicator cells. The CT (obtained from the NE/VI) was prepared identically to the NE/VI except no virus was added to the selected dilutions inoculated onto indicator cells plates and incubated in an identical manner as the test sample. This control was performed for each lot of test substance at one replicate per lot. A 0.5 mL aliquot of the PNS was ten-fold serially diluted in 4.5 mL aliquots of DM. Selected dilutions were inoculated onto indicator cell plates at 1.0 mL per well, 4 wells per dilution and incubated in an identical manner as the test samples.

Plate Recovery Control (PRC):

This control was performed concurrently with the test with a single inoculated carrier to establish the input viral load to compare with the test substance results to evaluate the viral reduction by the test substance. The PRC was prepared identically to the test sample except 2.0 mL DM was used in lieu of test substance to treat the dried virus inoculum. The average volume of the mock spray for the three lots was used as the volume of dilution media added to the plate recovery control. After treatment the carriers were held for the 55 second contact time used during test substance application. 2.0 mL of neutralizer was added after the contact time. This post-neutralized sample (PNS) was considered the 10^{-1} dilution. A 0.5 mL aliquot of the PNS was ten-fold serially diluted in 4.5 mL aliquots of DM. Selected dilutions were inoculated onto indicator cell plates at 1.0 mL per well, 4 wells per dilution and incubated in an identical manner as the test samples.

TEST PROCEDURES (continued)

Cell Viability Control (CVC):

This control was performed concurrently with the test to demonstrate that the indicator host cells remained viable and to confirm the sterility of the media employed throughout the incubation period. 1.0 mL of DM was added to 4 wells of indicator cells and incubated in an identical manner as the test samples.

Virus Stock Titer Control (VST):

This control was performed concurrently with the test to demonstrate that the titer of the stock virus was appropriate for use and that the viral infectivity assay was performed appropriately. A 0.5 mL aliquot of the virus inoculum used in the study was ten-fold serially diluted in 4.5 mL aliquots of DM. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples. The host cell plates were inoculated at 1.0 mL per well, 4 wells per dilution.

PROTOCOL CHANGES

Protocol Amendments:

1. Protocol page 15 states that the organic load will be Fetal Bovine Serum. The organic load will actually be Newborn Calf Serum. This amendment serves to correct the organic load source on protocol page 15.

2. Protocol References section, reference 4, is listed as:

   U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2000, 810.2100, and 810.2200, August 2019.

   The reference should be instead:

   U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Frequent Questions for the 2018 Series 810-Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines, August 2019.

   This amendment serves to correct reference 4 in the References section of the protocol.

3. The References section of the Protocol give References 5 and 6 as:

   5. Health Canada, January 2014. Guidance Document – Disinfectant Drugs.

   6. Health Canada, January 2014. Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.

   These references should be:

   5. Health Canada, April 2020. Guidance Document – Disinfectant Drugs.

PROTOCOL CHANGES (continued)

6. Health Canada, April 2020. Guidance Document – Safety and Efficacy Requirements for Surface Disinfectant Drugs.

This amendment serves to update references 5 and 6 in the References section of the Protocol.

4. The records to be maintained section of the protocol states that all protocol amendments and deviations will be sent to Sponsor for approval and signature. It should state that "all protocol amendments and deviations related to test procedure will be sent to Sponsor for approval and signature". This amendment serves to clarify the protocol regarding this statement.

5. The test substance preparation section states "The prepared test substance, if not within the stipulated test temperature range, will be pre-equilibrated to the test temperature prior to use in the study as applicable". To clarify, it should state, "If the test temperature and the test substance (or prepared test substance, as applicable) temperature are ambient, no pre-equilibration is required. If either temperature is non-ambient, the test substance (or prepared test substance, as applicable) to be used in testing must be pre-equilibrated to the test temperature prior to use in the study". This amendment serves to clarify the test substance preparation section.

Protocol Deviations:

No protocol deviations occurred during this study.

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed in the BSL-3 Laboratory at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, from 06/02/20 to 06/11/20. The study director signed the protocol on 06/02/20. The study completion date is the date the study director signed the final report. The individual test dates are as follows:

- Testing started at 3:28 pm on 06/02/20 and ended at 3:30 pm on 06/11/20.

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test substance records, the final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

TEST ACCEPTANCE CRITERIA

The test was considered acceptable for test substance evaluation due to the criteria below being satisfied:

- The infectious virus recovered from the PRC was ≥ 4.8 Log_{10} $TCID_{50}$ units per carrier.
- Viral-induced CPE was distinguishable from test substance induced cytotoxicity (if any).
- Virus was recovered from dilutions of the NE/VI control not exhibiting cytotoxicity.
- The Cell Viability Control (assay negative control) did not exhibit virus.

CALCULATIONS

Calculation of virus added per NE/VI dilution:

$$D = C * (A / B)$$

where: A = Units of virus per mL in the stock virus (in natural number, not logarithm number – based on the certified titer)
B = The fold of dilution (in natural number, not logarithm number)
C = Volume of the diluted virus added per NE/VI dilution
D = Units of virus per NE/VI dilution

Titer Calculation:

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 of 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
$\sum p_i$ = the 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.

Viral Load Calculation:

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

CALCULATIONS (continued)

Viral Reduction Calculation:

Log_{10} Reduction = Initial Viral Load (Log_{10} $TCID_{50}$*) – Output Viral Load (Log_{10} $TCID_{50}$*)

* per assayed volume and per carrier

RESULTS

Results are presented in Tables 1 – 5.

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 cytopathic effect

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

Table 1
Plate Recovery Control (PRC)

Dilution*	PRC
10^{-3}	4/4
10^{-4}	4/4
10^{-5}	4/4
10^{-6}	3/4
10^{-7}	0/4
10^{-8}	0/4
Titer (Log_{10} $TCID_{50}$/mL)	6.25
Load (Log_{10} $TCID_{50}$)**	5.85

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

RESULTS (continued)

Table 2
Test Substance

Dilution	TMXPAE		
	Lot No. TMXPAE-200326-1	Lot No. TMXPAE-200326-2	Lot No. TMXPAE-200326-3
10^{-2}	T/4	T/4	T/4
10^{-3}	0/4	0/4	0/4
10^{-4}	0/4	0/4	0/4
10^{-5}	0/4	0/4	0/4
10^{-6}	0/4	0/4	0/4
10^{-7}	0/4	0/4	0/4
Titer (Log_{10} $TCID_{50}$/mL)	≤ 2.50	≤ 2.50	≤ 2.50
Load (Log_{10} $TCID_{50}$)**	≤ 2.10	≤ 2.10	≤ 2.10
Log_{10} Reduction***	≥ 3.75	≥ 3.75	≥ 3.75

*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

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

Dilution*	TMXPAE					
	Lot No. TMXPAE-200326-1		Lot No. TMXPAE-200326-2		Lot No. TMXPAE-200326-3	
	NE/VI	CT	NE/VI	CT	NE/VI	CT
10^{-2}	T/4	T/4	T/4	T/4	T/4	T/4
10^{-3}	4/4	0/4	4/4	0/4	4/4	0/4
10^{-4}	4/4	0/4	4/4	0/4	4/4	0/4

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

RESULTS (continued)

Table 4
Cell Viability Control (CVC)

CVC
0/4
Cells were viable; media was sterile

Table 5
Virus Stock Titer Control (VST)

Dilution*	VST
10^{-3}	4/4
10^{-4}	4/4
10^{-5}	4/4
10^{-6}	4/4
10^{-7}	1/4
10^{-8}	0/4
Titer (Log_{10} $TCID_{50}$/mL)	6.75

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

TEST SUBSTANCE EVALUATION CRITERIA

According to the US Environmental Protection Agency, the test substance passes the test if the following criteria are met:

- The test substance must demonstrate a ≥ 3 Log_{10} reduction on each test carrier in the presence or absence of cytotoxicity, taking into account the level of neutralization. If cytotoxicity is present, the virus control titer should be increased, if necessary, to demonstrate a ≥ 3 log10 reduction in viral titer on each surface beyond the cytotoxic and neutralization level.

CONCLUSIONS

When tested as described, TMXPAE, Lot Nos: TMXPAE-200326-1, TMXPAE-200326-2, TMXPAE-200326-3, passed the Virucidal Hard-Surface Efficacy Test when Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2)(COVID-19 Virus), containing 5.0% Newborn Calf Serum, was exposed to the test substance for 55 seconds at 21°C and 34-35% RH. All test lots demonstrated complete inactivation of SARS-CoV-2.

All controls met the criteria for a valid test. These conclusions are based on observed data.

REFERENCES

1. ASTM E1053-20, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020.

2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing, February 2018.

3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides, Guidance for Efficacy Testing, February 2018.

4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Frequent Questions for the 2018 Series 810-Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines, August 2019.

5. Health Canada, April 2020. Guidance Document – Disinfectant Drugs.

6. Health Canada, April 2020. Guidance Document – Safety and Efficacy Requirements for Surface Disinfectant Drugs.

7. U.S. Environmental Protection Agency, Office of Pesticide Programs Microbiology Laboratory SOP MB-30-02: Standard Operating Procedure for Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 21, 2019.

8. Association of Official Analytical Chemists (AOAC) International, Official Method 960.09: Germicidal and Detergent Sanitizing Action of Disinfectants. Official Methods of Analysis of the AOAC, 2013 edition.

9. Organisation for Economic Co-operation and Development (OECD) Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides Used on Hard Non-Porous Surfaces. Series on Testing Assessment No. 187 and Series on Biocides No. 6, June 21, 2013.

APPENDIX I

MICROBAC®

Microbac Protocol

VIRUCIDAL HARD-SURFACE EFFICACY TEST -

Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

Testing Facility
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
Laboratoire M2
4005-A, Rue de la Garlock
Sherbrooke (Québec) J1L 1W9
Canada

April 30, 2020

Microbac Protocol: 596.1.04.13.20

Microbac Project: 5910-102

OBJECTIVE:

This test is designed to substantiate virucidal effectiveness claims for a liquid or spray test substance to be labeled as a virucide. It determines the potential of the test substance to disinfect hard surfaces contaminated with the test virus. The test is designed to simulate consumer use and conforms to EPA OCSPP 810.2000 (2018) and 810.2200 (2018) Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2000 (2018), 810.2100 (2018), and 810.2200 (2018), as well as the Health Canada "Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs" (January 2014). This protocol follows the procedure outlined in the ASTM International test method designated E1053-20, "Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces". The study design also aligns with EPA guidance provided to Scientific & Regulatory Consultants, Inc. (Letter from K. Willis, EPA OPP AD Science Branch Chief, March 25, 2020).

TESTING CONDITIONS:

Virus will be dried on a suitable sterile hard surface at ambient temperature. Three lots of one test substance (liquid) will be tested in compliance with the EPA Lower Certified Limit Policy in 810.2000 at one contact time and one replicate (N=1). The test substance will be used to treat the dried virus on a glass Petri dish carrier. One carrier will be tested for each lot of test substance and the appropriate controls. After a defined exposure period as specified by the Sponsor, the test substance-virus mixture will be neutralized, scraped off from the surface, collected, and tested for the presence of infectious virions.

MATERIALS:

A. Test, control and reference substances will be supplied by the Sponsor of the study. Microbac will append the Sponsor-provided Certificate(s) of Analysis (CoA) to this study report, as per CFR 40.160.105:

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance shall be determined and shall be documented by the Sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented and retained by the Sponsor.

When relevant to the conduct of the study the solubility of each test, control, or reference substance shall be determined by the Sponsor before the experimental start date. The stability of the test, control, or reference substance shall be determined by the Sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis.

The test substance will be tested as supplied by the Sponsor unless directed otherwise. All operations performed on the test substance such as dilution or specialized storage conditions must be specified by the Sponsor before initiation of testing.

The Sponsor assures Microbac testing facility management that the test substance/formulation has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

Microbac will retain all unused test substances for a period of one year upon completion of the test, and then discard them in a manner that meets the approval of the safety officer or return them to the Sponsor. The test materials and the paper records will be retained in accordance with FIFRA. Microbac will contact the Study Sponsor to arrange for transfer of records when/if the test substance is returned to the Sponsor.

B. Materials supplied by Microbac, including, but not limited to:

1. Challenge virus (requested by the Sponsor of the study): Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus) (SARS-Related Coronavirus 2), Strain: USA-WA1/2020, Source: BEI Resources, NR-52281. Strain USA-WA1/2020 was isolated from an oropharyngeal swab from a patient with a respiratory illness who had recently returned from travel to the affected region of China and developed clinical disease (COVID-19) in January 2020 in Washington, USA (https://www.beiresources.org/Catalog/animalviruses/NR-52281.aspx).

2. Host cell line: Vero E6 cells, ATCC CRL-1586

3. Laboratory equipment and supplies.

4. Fetal Bovine Serum or another appropriate source of serum as the soil load used for testing with SARS-CoV-2 (if applicable) as requested by the Sponsor.

5. Media and reagents:

   Media and reagents relevant to the virus-host system and test substance being tested will be documented in the first project sheet and data pack.

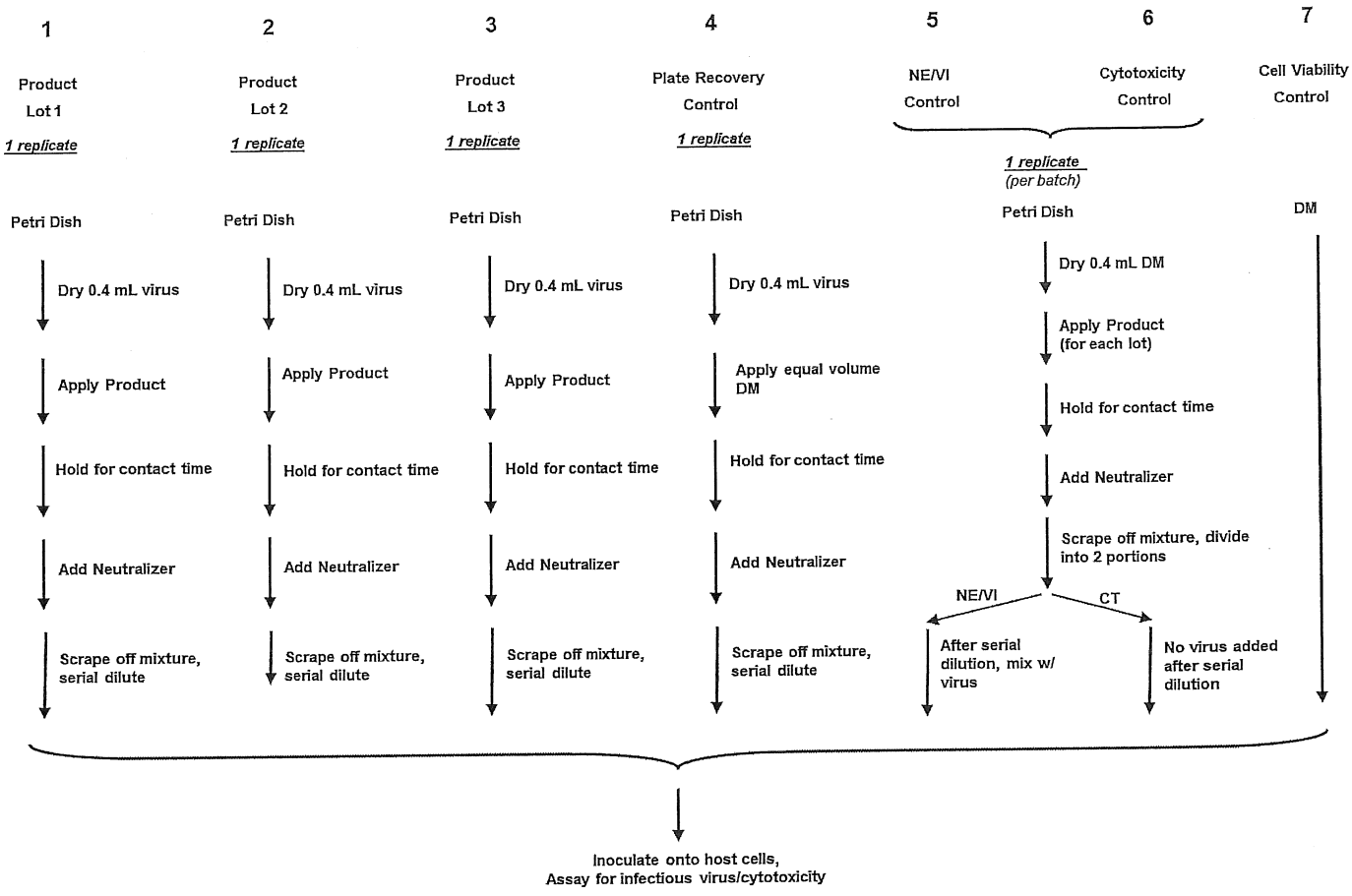
TEST SYSTEM IDENTIFICATION:

All Petri dishes, dilution tube racks, and host-containing apparatus will be appropriately labeled with the following information: virus, host, and test substance and/or project number.

EXPERIMENTAL DESIGN:

All of the procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at Microbac. SOPs and Logs are referred to in the raw data and are required as part of GLP regulations. The study flow diagram is shown in Figure 1, with details described in the following sections.

FIGURE 1



DM: Dilution Medium
NE/VI: Neutralizer Effectiveness/Viral Interference control CT: Cytotoxicity Control
Note: One test substance, three lots, will be tested at one exposure (contact) time and one replicate (N=1). The NE/VI and CT controls will be performed at one replicate per lot.

A. Inoculum preparation:

Viral stocks are purchased from reputable sources that identify them by scientifically accepted methods and may have been further propagated at Microbac. Records are maintained that demonstrate the origin of the virus. The virus stocks are stored at an ultra-low temperature.

Frozen viral stocks will be thawed on the day of the test. Serum will be added to the viral stock to achieve an organic load of 5.0% (if not already 5.0%), unless otherwise directed by the Sponsor and pre-agreed by Microbac. If the challenge virus culture is

standardized by concentration or dilution, or if a column is used, these manipulations must be documented and reported.

Note: A level of approximately 4.8 – 6.3 Log_{10} virus challenge per carrier (as indicated by the plate recovery control load) when there is no cytotoxicity associated with the test substance, or approximately 3.0 – 4.5 Log_{10} per carrier beyond the level of cytotoxicity when present, should be achieved whenever possible.

B. Carrier preparation:

For each lot of the test substance an aliquot of 0.4 mL of stock virus will be added, and spread with a cell scraper over the bottom of pre-sterilized glass Petri dishes (100mm diameter). This volume will remain consistent among all test and control runs. Carriers treated with virus will be dried at ambient temperature. The drying time, temperature, and relative humidity will be recorded and reported.

One carrier will be prepared for each lot of the test substance using virus. One carrier will be prepared for the plate recovery control using virus. Additionally, one carrier will be prepared for each lot of test substance for the neutralizer effectiveness/viral interference and cytotoxicity controls using dilution media in lieu of virus as the inoculum.

C. Test substance preparation:

Note: Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance/formulation resides with the Sponsor of the study.

The test substance will be prepared exactly according to the Sponsor's directions (if provided). If the Sponsor requests dilution of the test substance, the diluted test substance will be used for testing within three hours of preparation. The prepared test substance, if not within the stipulated test temperature range, will be pre-equilibrated to the test temperature prior to use in the study as applicable.

D. Test:

Three lots of the test substance (liquid) will be tested at one contact time and one replicate (N=1). Note: The temperature and relative humidity during the exposure period will be recorded and reported.

For direct liquid application test substance, for each run, after the inoculum has dried, 2.0 mL of the test substance will be added. After addition, a stopwatch will immediately be started to measure the contact time. The dried virus film must be completely covered by the test substance. The plates will remain at the temperature and for the time specified by the Sponsor. After the contact period, the test substance will be neutralized with 2.0 mL of appropriate neutralizer and the mixture will be scraped from the surface of the dish with a cell scraper. This post-neutralized sample (PNS) will be considered approximately a 10^{-1} dilution. The temperature and relative humidity during the exposure period will be recorded and reported.

For a spray application test substance, an aliquot of the test substance, ready-to-use, will be dispensed into a sterilized spray bottle, if not provided by the Sponsor. Unless otherwise directed by the Sponsor, the spray bottle will then be shaken 2 – 3 times to ensure homogeneity and sprayed to charge the spray bottle. A mock spray action will be performed by applying the test substance as the Sponsor directs onto at least two blank Petri dishes. Then the volume dispensed onto each dish will be measured and averaged. This averaged volume from the mock spray runs will be used for the neutralizer volume for all applicable runs and for the Plate recovery control runs. The test substance will be sprayed onto the virus carriers in a horizontal position until thoroughly wet from a distance of 6" – 8" or as directed by the Sponsor. Each carrier will be held in a horizontal position for the exposure time as specified by the Sponsor. After the contact period, the test substance will be neutralized with an appropriate neutralizer using the averaged volume from the mock spray runs. The neutralizer-test substance mixture will be scraped off from the surface of the dish with a cell scraper. This post-neutralized sample (PNS) will be considered approximately a 10^{-1} dilution. The temperature and relative humidity during the exposure period will be recorded and reported.

If Sephacryl columns are used to aid in the neutralization and to further reduce the cytotoxicity, each inoculum/test substance/neutralizer mixture sample will be loaded onto a pre-spun Sephacryl column. Following the passage through columns, the eluates will be aseptically collected and serially ten-fold diluted in DM. If columns are not used, serial ten-fold dilutions of the inoculum/test substance/neutralizer mixture will directly be prepared in DM.

E. Infectivity assay:

The residual infectious virus in all test and control samples will be detected by viral-induced cytopathic effect (CPE).

Selected dilutions of the neutralized inoculum/test substance mixture (test samples) and control samples will be added to cultured host cells (at least four wells per dilution, per reaction mixture) and incubated at 36±2°C with 5±3% CO_2 for total 4 – 9 days. The host cells may be washed twice with phosphate buffered saline prior to inoculation. The inoculated culture will be observed and refed with fresh media as necessary, during the incubation period. These activities, if applicable, will be recorded. The host cells will then be examined microscopically for presence of infectious virions. The resulting virus-specific CPE and test substance-specific cytotoxic effects will be scored by examining all test and control samples. These observations will be recorded.

F. Controls:

1. Plate recovery control (PRC):

This concurrent control will be performed with a single inoculated carrier, concurrently with the test substance runs. The temperature and relative humidity during the exposure period will be recorded and reported.

The virus inoculum will be spread over the surface of a sterile glass Petri dish and left to dry at ambient temperature. A volume of DM equivalent to that of the test substance will be added to the dried virus and the plate held for the Sponsor requested contact time at the requested exposure temperature. Post-contact time, virus will be subjected to the identical neutralization procedure as used for the test substance. Serial 10-fold dilutions of the samples will be prepared in DM and selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in Section E "Infectivity Assay". This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

To achieve a valid test, at least 4.8-Log_{10} of infectious virus per carrier must be recovered from this control following drying and neutralization. The titer from this control will be used to calculate the log_{10} reduction of the virus titer

post treatment with the test substance (see below).

2. Neutralizer effectiveness/Viral interference control (NE/VI):

This concurrent control will determine if residual active ingredient is present after neutralization and if the neutralized test substance interferes with the virus infection system. This control will be performed for each lot of test substance at one replicate per lot concurrently with testing. The temperature and relative humidity during the exposure period will be recorded and reported.

The test substance will be processed exactly as the test procedure but in lieu of virus inoculum, dried DM will be exposed to the test substance and assayed as previously described. Post-treatment and neutralization, the neutralized DM/test substance mixture will be divided into two portions, one for the cytotoxicity control and the other for the neutralizer effectiveness/viral interference control and processed as in the test.

If columns are used, each portion will be passed through individual columns and the eluate will be serially diluted ten-fold in DM. If columns are not used, each portion will be directly diluted using serial ten-fold dilutions in DM.

The neutralizer effectiveness/viral interference control sample will be diluted as follows: using dilution test tubes and appropriate pipette, an aliquot of the PNS will be used for making serial 10-fold dilutions in DM (for example, 0.5 mL sample + 4.5 mL DM). Following serial dilution, 0.1 mL of a low titered virus, containing approximately 1,000 – 5,000 infectious units of virus, will be added to 4.5 mL of each dilution and held for a period of no shorter than the contact time.

Selected dilutions of these samples will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section.

3. Cytotoxicity control (CT):

This concurrent control will be performed for each lot of test substance at one replicate per lot.

The cytotoxicity sample, acquired from the neutralizer effectiveness/viral interference control run, will be diluted and have no virus added. Selected dilutions will be inoculated onto host cells and incubated in the same manner as the rest of the test and control samples. Cytotoxicity will be scored at the same time as the test samples; cytotoxic effects are distinct from virus-induced cytopathic effects, which will be evident in the plate recovery control cultures.

4. Column titer control (to be performed only if a Sephacryl column is used):

This concurrent control will be performed to determine any affect the columns may have on infectious virus titer. It will be performed in a single run.

The sample for this control will be acquired from a portion of the PRC, prior to passing through the columns and will be serially diluted in DM, then processed in the same manner as the test.

5. Cell viability control:

This concurrent control will be performed in a single run. It will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the DM employed throughout the assay period. At least four wells of cells will receive only DM and will be incubated and processed with both test and other controls. This will serve as the negative control.

6. Virus Stock Titer control (VST)

This concurrent control will be performed in a single run. An aliquot of the virus used in the study will be directly serially diluted and inoculated onto the host cells to confirm the titer of the stock virus. This control will demonstrate that the titer of the stock virus is appropriate for use and that the viral infectivity assay is performed appropriately.

G. Calculation:

The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber (Kärber G., Arch. Exp. Pathol. Pharmakol. 1931, 162: 480-483). The $TCID_{50}$/carrier, i.e., the viral load per carrier, will be calculated as follows:

The Virus Load ($TCID_{50}$/carrier) will be calculated in the following manner:
Virus Load (Log_{10} $TCID_{50}$) = Virus Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [Volume per sample (mL)]

The Log_{10} Reduction Factor (LRF) will be 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)

These analyses will be described in detail in the final report. The test results will be reported as the log_{10} reduction of the virus titer per carrier and per volume post-treatment with the test substance.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The infectious virus recovered from the PRC control must be at least 4.8 log_{10} $TCID_{50}$ units per carrier.
- Viral-induced cytopathic effect must be distinguishable from test substance induced cytotoxic effects (if any).
- Virus must be recovered from the neutralizer effectiveness/viral interference control (not exhibiting cytotoxicity).
- The Cell Viability Control (assay negative control) must not exhibit virus.

TEST SUBSTANCE EVALUATION CRITERIA:

According to the US Environmental Protection Agency, the test substance passes the test if the following are met:

- The product must demonstrate a ≥ 3 log_{10} reduction on each surface in the presence or absence of cytotoxicity and taking into account the level of neutralization; and
- If 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 and neutralization level.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, Virginia 20164.

REGULATORY COMPLIANCE AND QUALITY ASSURANCE (GLP studies only):

This study will be performed in compliance with the US Environmental Protection Agency's Good Laboratory Practices (GLP) regulations, 40 CFR 160 (note: information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance/formula resides with the Sponsor of the study unless otherwise stated).

The Quality Assurance Unit of Microbac will inspect the conduct of the study for GLP compliance. The dates and a description of the phase(s) inspected, and the dates that findings are reported to management and the study director will be included in the final report.

PROTOCOL AMENDMENTS AND DEVIATIONS:

Any protocol amendment(s) and protocol deviation(s) identified will be reported in project sheet(s) and included in the final report.

REPORT FORMAT:

A draft report will be provided to the Sponsor for review prior to finalization. The report will contain all items required by EPA GLP (40 CFR Part 160.185), EPA 810.2000 (2018) and 810.2200 (2018) and be in compliance with EPA PR Notice 2011-3. Microbac employs a standard report format for each test design. Each final report will provide all the information in the citations above including (but not limited to):

- Sponsor identification
- Test substance identification
- Manufacture date for each product lot
- Type of assay and project number
- Study start and end time (clock time)
- Interpretation of results and conclusions

- Test results presented in tabular form
- Methods and evaluation criteria
- Description of protocol deviations and protocol amendments (if applicable)
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)
- Certificate of Analysis for each test lot (for GLP studies only; if provided by the Sponsor)
- List of personnel (and respective titles) involved in the study

RECORDS TO BE MAINTAINED:

For all GLP studies, the original signed final report or an electronic copy will be sent to the Sponsor. The original signed final report, or a copy thereof, will be maintained in the study file. If requested, a draft report will be provided to the Sponsor for review prior to finalization of the report.

All raw data, protocol, protocol modifications, test substance records, the final report (or copy thereof), and correspondence between Microbac and the Sponsor will be stored in the archives at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The Sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates, additional information about the test substance, challenge virus identity, host cell line monolayers, and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the study initiation date. All project sheets issued containing protocol amendments or deviations will be forwarded to the study Sponsor for approval and signature.

REFERENCES (if applicable)

1. ASTM E1053-20, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020.

2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing, February 2018.

3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides, Guidance for Efficacy Testing, February 2018.

4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2000, 810.2100, and 810.2200, August 2019.

5. Health Canada, January 2014. Guidance Document – Disinfectant Drugs.

6. Health Canada, January 2014. Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.

7. U.S. Environmental Protection Agency, Office of Pesticide Programs Microbiology Laboratory SOP MB-30-02: Standard Operating Procedure for Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 21, 2019.

8. Association of Official Analytical Chemists (AOAC) International, Official Method 960.09: Germicidal and Detergent Sanitizing Action of Disinfectants. Official Methods of Analysis of the AOAC, 2013 edition.

9. Organisation for Economic Co-operation and Development (OECD) Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides Used on Hard Non-Porous Surfaces. Series on Testing Assessment No. 187 and Series on Biocides No. 6, June 21, 2013.

MISCELLANEOUS INFORMATION:

The following information is to be completed by the Sponsor prior to initiation of the study (please check all applicable open boxes):

B. Test substance information:

Test substance name	TMXPAE		
EPA Reg. No.	87742-1	87742-1	87742-1
Test substance lot numbers	TMXPAE-200326-1	TMXPAE-200326-2	TMXPAE-200326-3
Manufacture Date	03/26/2020	03/26/2020	03/26/2020
Expiration Date	03/26/2021	03/26/2021	03/26/2021
Active ingredient(s)	Batch TMXPAE-200326-1: 0.212% Thymol Batch TMXPAE-200326-2: 0.213% Thymol Batch TMXPAE-200326-3: 0.208% Thymol		
Test substance storage conditions	■ Ambient □ Refrigerated □ Other:_____		
Level of active ingredients in testing	■ Lower Certified Limit (LCL) □ At or below nominal		
SDS provided	■ Yes □ No	C of A provided	■ Yes □ No
Dilution	■ Ready to use: See below* ■ See below* (See below* parts test substance + See below* parts diluent)		
Diluent	□ Not applicable □ 400 ppm ± 2.9% AOAC hard water □ 375 ppm OECD hard water (acceptable range: 338-394 ppm) □ 200 ppm unsoftened tap water (acceptable range: 180-210 ppm) ■ Other: sterile DI water		
Contact time	55 seconds		
Contact temperature	■ Room Temperature (20±1°C) □ Other :_____		
Organic Load	□ 5.0% serum in viral inoculum ■ Other: 5.0% FBS in viral inoculum		

*Dilutions for testing:
Batch TMXPAE-200326-1 - Dilute 1:1.03 (1 part test substance + 0.03 parts diluent)
Batch TMXPAE-200326-2 – Dilute 1:1.03 (1 part test substance + 0.03 parts diluent)
Batch TMXPAE-200326-3 - Ready to Use (no dilution)

Test substance information (continued):

Test substance preparation (spray)	□ Shake sprayer _____ times and spray into waste container to prime before use □ Do not shake sprayer; spray into waste container to prime before use ■ Other: Invert (do not shake) sprayer at least three times and spray into waste container to prime before use □ Not applicable
Test substance application	□ Apply directly to dried virus via pipetting ■ Spray from 6-8" inches using ≥ 4 sprays or until thoroughly wet □ Spray from _____ inches for _____ seconds or until thoroughly wet □ Other: _____
Study conduct	■ GLP □ Non-GLP
Report submission	■ EPA ■ Health Canada □ Other: _____

PROTOCOL APPROVAL BY SPONSOR:

Sponsor Signature: Denise Burnside Date: 05/08/2020

Printed Name: Denise Burnside, Agent for Laboratoire M2

PROTOCOL APPROVAL BY STUDY DIRECTOR (Microbac):

Study Director Signature: _____ Date: 06/02/2020

Printed Name: Cory Chiossone

Date Issued: 06/02/20	Project Sheet No. 1	Page No. 1	Laboratory Project Identification No. 596-102

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

STUDY DIRECTOR: Cory Chiossone

Signature 06/02/20 Date

TEST MATERIAL(S):	**LOT NO.**	**DATE RECEIVED:**	**DS NO.**
TMXPAE	TMXPAE-200326-1	04/01/20	K346
	TMXPAE-200326-2	04/01/20	K347
	TMXPAE-200326-3	04/01/20	K348

PERFORMING DEPARTMENT(S): Virology and Toxicology

STORAGE CONDITIONS: Location: F5
☐ Dark ■ Ambient Room Temperature
☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:

PROTECTIVE PRECAUTION REQUIRED: MSDS ■ Yes / ☐ No

PHYSICAL DESCRIPTION: ☐ Solid ■ Liquid ☐ Aerosol

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 06/02/20 **TERMINATION DATE:** 06/10/20

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: Health Canada

SPONSOR: Laboratoire M2
4005-A, Rue de la Garlock
Sherbrooke (Québec) J1L 1W9
Canada

CONTACT PERSON: Denise Burnside
dburnside@srcconsultants.com

TEST CONDITIONS:

Challenge organisms: Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), Strain USA-WA1/2020; BEI Resources, NR-52281

Host: Vero E6 cells, ATCC CRL-1586

Organic load: 5.0% Newborn Calf Serum in viral inoculum

Disinfectant application: Spray from 6-8 inches using ≥ 4 sprays until thoroughly wet.

Active Ingredient: 0.212% Thymol (K346), 0.213% Thymol (K347), 0.208% Thymol (K348)

Dilution medium: Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS)

Dilution: 1:1.03 (1 part test substance + 0.03 parts diluent) (K346 and K347), Ready to use (K348)

Diluent: Sterile DI Water

Neutralizer(s): MEM + 10% NCS + 0.5 % polysorbate-80 + 0.5% lecithin + 3% HEPES + 0.025N NaOH

Contact time: 55 seconds

Contact temperature: Ambient room temperature (20±1°C)

Incubation time(s): 4 – 9 days

Incubation temperature(s): 36±2C in 5±3% CO_2

PROTOCOL AMENDMENTS:

1. Protocol page 15 states that the organic load will be Fetal Bovine Serum. The organic load will actually be Newborn Calf Serum. This amendment serves to correct the organic load source on protocol page 15.

Date Issued: 07/27/20	Project Sheet No. 2	Page No. 1	Laboratory Project Identification No. 596-102

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

STUDY DIRECTOR: Cory Chiossone

Signature 07/30/20 Date

TEST MATERIAL(S): TMXPAE

LOT NO.	DATE RECEIVED:	DS NO.
TMXPAE-200326-1	04/01/20	K346
TMXPAE-200326-2	04/01/20	K347
TMXPAE-200326-3	04/01/20	K348

PERFORMING DEPARTMENT(S): Virology and Toxicology

STORAGE CONDITIONS: Location: F5
☐ Dark ■ Ambient Room Temperature
☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: Health Canada

SPONSOR: Laboratoire M2
4005-A, Rue de la Garlock
Sherbrooke (Québec) J1L 1W9
Canada

CONTACT PERSON: Denise Burnside
dburnside@srcconsultants.com

PROTOCOL AMENDMENTS:

2. Protocol References section, reference 4, is listed as:

   U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2000, 810.2100, and 810.2200, August 2019.

   The reference should be instead:

   U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Frequent Questions for the 2018 Series 810-Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines, August 2019.

   This amendment serves to correct reference 4 in the References section of the protocol.

3. The References section of the Protocol give References 5 and 6 as:

   5. Health Canada, January 2014. Guidance Document – Disinfectant Drugs.

   6. Health Canada, January 2014. Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.

   These references should be:

   5. Health Canada, April 2020. Guidance Document – Disinfectant Drugs.

   6. Health Canada, April 2020. Guidance Document – Safety and Efficacy Requirements for Surface Disinfectant Drugs.

   This amendment serves to update references 5 and 6 in the References section of the Protocol.

4. The records to be maintained section of the protocol states that all protocol amendments and deviations will be sent to Sponsor for approval and signature. It should state that "all protocol amendments and deviations related to test procedure will be sent to Sponsor for approval and signature". This amendment serves to clarify the protocol regarding this statement.

Amendment(s) to or deviation(s) from the protocol accepted by the sponsor:

Denise Burnside 07/30/2020
Signature Date

MBT Lab Form: 015k

Date Issued: 08/20/20 Project Sheet No. 3	Page No. 1 Laboratory Project Identification No. 596-102
STUDY TITLE: VIRUCIDAL HARD-SURFACE EFFICACY TEST – Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)	**STUDY DIRECTOR:** Cory Chiossone Signature 08/21/20 Date

TEST MATERIAL(S): TMXPAE	**LOT NO.**	**DATE RECEIVED:**	**DS NO.**
	TMXPAE-200326-1	04/01/20	K346
	TMXPAE-200326-2	04/01/20	K347
	TMXPAE-200326-3	04/01/20	K348

PERFORMING DEPARTMENT(S): Virology and Toxicology	**STORAGE CONDITIONS:** Location: F5 ☐ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: Health Canada

SPONSOR: Laboratoire M2 4005-A, Rue de la Garlock Sherbrooke (Québec) J1L 1W9 Canada	**CONTACT PERSON:** Rhonda Jones rjones@srcconsultants.com

PROTOCOL AMENDMENTS:

5. The test substance preparation section states "The prepared test substance, if not within the stipulated test temperature range, will be pre-equilibrated to the test temperature prior to use in the study as applicable". To clarify, it should state, "If the test temperature and the test substance (or prepared test substance, as applicable) temperature are ambient, no pre-equilibration is required. If either temperature is non-ambient, the test substance (or prepared test substance, as applicable) to be used in testing must be pre-equilibrated to the test temperature prior to use in the study". This amendment serves to clarify the test substance preparation section.

Amendment(s) to or deviation(s) from the protocol accepted by the sponsor:

Signature 8-21-20 Date

MBT Lab Form: 015k

APPENDIX II

APPENDIX B: CERTIFICATES OF ANALYSIS

Product Safety Labs

CERTIFICATE OF ANALYSIS

Product: TMXPAE

Lot #: TMXPAE-200326-1

PSL Reference No.: 200331-4D

Date of Analysis: April 2, 2020

Date of Manufacture: March 26, 2020

Result:

Thymol: 0.212%

Approval: _____ May 4, 2020
Lyla Ansah-Johnson, BS, MBA
Analytical Services
Product Safety Labs
Date

QA Release: _____ May 4, 2020
Quality Assurance
Product Safety Labs
Date

This material was analyzed in compliance with Good Laboratory Practice (40 CFR 160) standards. Data are reported in PSL GLP Study No. 52634

PRODUCT SAFETY LABS
2394 US Highway 130
Dayton, NJ 08810
USA

732-438-5100
psl@productsafetylabs.com
www.productsafetylabs.com

Product Safety Labs

CERTIFICATE OF ANALYSIS

Product: TMXPAE

Lot #: TMXPAE-200326-2

PSL Reference No.: 200331-5D

Date of Analysis: April 2, 2020

Date of Manufacture: March 26, 2020

Result:

Thymol: 0.213%

Approval: _____ May 4, 2020
Date

Lyla Ansah-Johnson, BS, MBA
Analytical Services
Product Safety Labs

QA Release: _____ May 4, 2020
Date

Quality Assurance
Product Safety Labs

This material was analyzed in compliance with Good Laboratory Practice (40 CFR 160) standards. Data are reported in PSL GLP Study No. 52634

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Dayton, NJ 08810
USA

732-438-5100
psl@productsafetylabs.com
www.productsafetylabs.com

Product Safety Labs

CERTIFICATE OF ANALYSIS

Product: TMXPAE

Lot #: TMXPAE-200326-3

PSL Reference No.: 200331-6D

Date of Analysis: April 2, 2020

Date of Manufacture: March 26, 2020

Result:

Thymol: 0.208%

Approval: _____ May 4, 2020
Date

Eyla Ansah-Johnson, BS, MBA
Analytical Services
Product Safety Labs

QA Release: _____ May 4, 2020
Date

Quality Assurance
Product Safety Labs

This material was analyzed in compliance with Good Laboratory Practice (40 CFR 160) standards. Data are reported in PSL GLP Study No. 52634

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