

FINAL REPORT

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

Test Substance

Total-Shield Plus

Lot Numbers

H21500823

H20300823

G22100723

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

Cameron J. Wilde

Study Completion Date

12/03/20

Performing Laboratory

Microbac Laboratories, Inc.

105 Carpenter Drive

Sterling, VA 20164

Laboratory Project Identification Number

1056-104

Protocol Identification Number

MCG.1.07.30.20

Sponsor

McGowan Industries, Inc.

12285 World Trade Dr. STE O

San Diego, CA 92128

STATEMENT OF NO DATA CONFIDENTIALITY

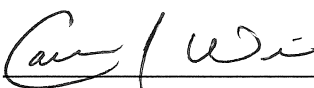
No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec.10(d)(1)(A), (B) or (C) and which pertains to a registered or previously registered pesticide is not entitled to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

Submitter signature: _____ Date: _____
Printed Name of Signer: _____
Printed Name of Company: _____

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: 12/03/2020
Typed Name: Cameron J. Wilde
Typed Name of Laboratory: Microbac Laboratories, Inc.

Sponsor Signature: _____ Date: _____
Printed Name: _____
Typed Name of Company: McGowan Industries, Inc.

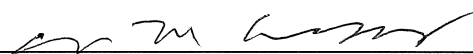
Submitter Signature: _____ Date: _____
Printed Name: _____
Typed Name of Company: McGowan Industries, Inc.

QUALITY ASSURANCE UNIT STATEMENT

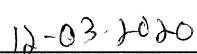
The Quality Assurance Unit of Microbac has inspected Project Number 1056-104 to be in compliance with current Good Laboratory Practice regulations, (40 CFR § 160).

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	09/30/20 10/01/20	10/01/20	10/01/20
In Process (Test)	09/30/20	10/01/20	10/01/20
Final Report	11/18/20	11/19/20	11/19/20



Jeanne M Anderegg for Danielle Downs, RQAP-GLP
Quality Assurance Specialist III



Date

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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.: 1056-104

Protocol No.: MCG.1.07.30.20

Test Method: ASTM International E1053-20 “Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces”

Sponsor: McGowan Industries, Inc.
12285 World Trade Dr. STE O
San Diego, CA 92128

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 Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus). This test was designed to simulate consumer use and was performed in conformance to EPA OCSP 810.2000 (2018) and 810.2200 (2018) Product Performance Test Guidelines.

Study Dates: Study Initiation: 09/30/20
Experimental Start: 09/30/20
Experimental End: 10/08/20
Study Completion: See page 1

TEST SUMMARY (continued)

Test Substance:	Total-Shield Plus	
	<ul style="list-style-type: none">• Lot No.: H21500823, received: 08/27/20, assigned DS No. K1186• Lot No.: H20300823, received: 08/27/20, assigned DS No. K1187• Lot No.: G22100723, received: 08/27/20, assigned DS No. K1188• Physical Description: Liquid• Storage Condition: Dark, Ambient Room Temperature• Active Ingredients: Phenol, Ethanol, 2-Phenylphenol• Dilution: Ready to use• Diluent: Not applicable	
Test Conditions:	Organic Soil Load:	5.0% Fetal Bovine Serum (FBS) in viral inoculum
	Contact Time:	5 minutes
	Contact Temperature:	20±1°C (Actual: 21°C)
	Contact Relative Humidity:	40%
Challenge Virus:	Sever Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)	
	<ul style="list-style-type: none">• Strain: USA-WA1/2020,• Source: BEI Resources, NR-52281	
Indicator Cells:	Vero E6 cells	
	<ul style="list-style-type: none">• Source: ATCC CRL-1586	
Incubation Time:	4-9 days	
Incubation Temperature:	36 ± 2°C with 5 ± 3% CO ₂	
Dilution Medium (DM):	Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS)	
Neutralizer:	MEM + 10% NCS + 0.5% Lecithin + 0.5% Polysorbate-80	
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:	Cameron J. Wilde Senior Scientist I (Study Director) Brandon G. Narvaez Associate Scientist II Callie Tang Associate Scientist I	

TEST PROCEDURES

Indicator Cells:

Vero E6 cells were obtained from ATCC and maintained in cell culture at $36 \pm 2^{\circ}\text{C}$ with $5 \pm 3\%$ 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 1.5×10^5 cells/mL at 1 mL per well.

Virus Inoculum:

Frozen viral stock was thawed on the day of the test. Stock virus contained an organic load of 5.0% FBS.

Test Substance:

The test substance was received ready to use.

Test Carriers:

Glass Petri dishes were heat sterilized in a hot air oven at 180°C for two hours followed by cooling and storing them at room temperature until use. Using a micropipette, a 0.4 mL aliquot of virus or dilution media (DM) was inoculated onto a glass carrier and immediately spread uniformly over the bottom using a sterile cell scraper. The inoculation was repeated for the remaining carriers. All carriers were dried for 30 minutes at 21°C and 40-45% RH. One carrier using virus was prepared for each batch of test substance. One carrier was prepared using DM for each batch of test substance for the Neutralizer Effectiveness/Viral Interference and Cytotoxicity controls. One carrier was prepared using virus for the Plate Recovery Control.

Test Substance Application and Exposure Conditions:

The test products were provided with spray bottles by the sponsor. Each spray bottle was not shaken prior to testing and was sprayed into a waste container to charge the spray bottle. A mock spray was performed prior to testing to determine the average volume of spray produced and the volume of neutralizer required for each batch for both test and controls. An average volume of 2.1 mL was produced during the mock sprays for H21500823, H20300823, and G22100723. The test substance was then sprayed onto the virus carriers in a horizontal position until thoroughly wet from a distance of 6". The treated carriers were then held for the contact time of 5 minutes at 21°C with 40% RH.

TEST PROCEDURES (continued)

Recovery of Samples:

After the contact time, the test substance was neutralized with 2.1 mL (average volume produced for each lot during mock sprays) of neutralizer. 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. An aliquot of the PNS was ten-fold serially diluted in DM.

Infectivity Assay:

Selected dilutions of the sample were inoculated onto the plates at 1.0 mL per well, 4 wells per dilution, and incubated at $36 \pm 2^{\circ}\text{C}$ with $5 \pm 3\%$ CO_2 . After 8 days, the plates were removed from incubation, scored, and recorded for test-substance specific cytotoxic effects and/or virus-specific cytopathic effect (CPE).

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

The control was performed 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 DM was used in lieu of virus inoculum to inoculate the carrier. 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 ten-fold serially diluted and 100 μL of virus stock (containing 1202 TCID_{50} units) was added individually to selected dilutions and held for at least the contact time. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

Cytotoxicity Control (CT):

This control was performed 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 however, no virus was added to the selected dilutions inoculated onto indicator cells plates and incubated in an identical manner as the test samples.

Plate Recovery Control (PRC):

This control was performed 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 2.1 mL of DM was used to treat the dried virus inoculum during test substance application. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

TEST PROCEDURES (continued)

Cell Viability Control (CVC):

This control was performed to demonstrate that the indicator host cells remained viable and to confirm the sterility of the media employed throughout the incubation period. Indicator cell plates were aspirated, and 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 to demonstrate that the titer of the stock virus was appropriate for use and that the viral infectivity assay was performed appropriately. An aliquot of the virus inoculum used in the study was ten-fold serially diluted in DM. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

PROTOCOL CHANGES

Protocol amendments:

No protocol amendments occurred during this study.

Protocol deviations:

No protocol deviations occurred during this study.

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, from 09/30/20 to 10/08/20. The study director signed the protocol on 09/30/20. The study completion date is the date the study director signed the final report. The individual test dates are as follows:

- Testing started at 3:16 pm on 09/30/20 and ended at 7:15 pm on 10/08/20

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 $\geq 4.8 \text{ Log}_{10} \text{ TCID}_{50}$ units.
- 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 CVC did not exhibit CPE.

CALCULATIONS

Titer Calculation:

The 50% Tissue Culture Infectious Dose per mL (TCID₅₀/mL) was determined using the Spearman-Kärber method using the following formula:

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

where: m = the logarithm of the dilution at which half 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
Σp_i = the sum of p_i (starting with the highest dilution producing 100% infection)

The values were converted to TCID₅₀/mL using a sample inoculum of 1.0 mL.

Viral Load Calculation:

Load (Log₁₀ TCID₅₀) per sample = Titer (Log₁₀ TCID₅₀/mL) + Log₁₀ [volume per sample (mL)]

Viral Reduction Calculation:

Log₁₀ Reduction = Initial Viral Load (Log₁₀ TCID₅₀*) – Output Viral Load (Log₁₀ TCID₅₀*)

* per assayed volume and per carrier

RESULTS

Results are presented in Tables 1 – 7.

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

RESULTS (continued)

Table 1
Plate Recovery Control (PRC)

Dilution*	PRC
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
Load (Log ₁₀ TCID ₅₀)**	6.37

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

**Per carrier (0.42 mL of Undilute [10⁰])

Table 2
Test Substance

Dilution*	Total-Shield Plus		
	Lot No. H21500823	Lot No. H20300823	Lot No. G22100723
10 ⁻²	T/4	T/4	T/4
10 ⁻³	T/4	T/4	T/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)	≤3.50	≤3.50	≤3.50
Load (Log ₁₀ TCID ₅₀)**	≤3.12	≤3.12	≤3.12
Log ₁₀ Reduction***	≥3.25	≥3.25	≥3.25

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

**Per carrier (0.42 mL of Undilute [10⁰])

***Per assayed volume and per carrier

RESULTS (continued)

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

Dilution*	Total-Shield Plus	
	Lot No. H21500823	
	NE/VI	CT
10 ⁻²	T/4	T/4
10 ⁻³	T/4	T/4
10 ⁻⁴	4/4	0/4

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

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

Dilution*	Total-Shield Plus	
	Lot No. H20300823	
	NE/VI	CT
10 ⁻²	T/4	T/4
10 ⁻³	T/4	T/4
10 ⁻⁴	4/4	0/4

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

Table 5
Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls

Dilution*	Total-Shield Plus	
	Lot No. G22100723	
	NE/VI	CT
10 ⁻²	T/4	T/4
10 ⁻³	T/4	T/4
10 ⁻⁴	4/4	0/4

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

RESULTS (continued)

Table 6
Cell Viability Control (CVC)

CVC
0/4
Cells were viable; media was sterile

Table 7
Virus Stock Titer Control (VST)

Dilution*	VST
10^{-4}	4/4
10^{-5}	4/4
10^{-6}	4/4
10^{-7}	2/4
10^{-8}	0/4
10^{-9}	0/4
Titer ($\text{Log}_{10} \text{TCID}_{50}/\text{mL}$)	7.00

*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 $\geq 3 \text{ Log}_{10}$ reduction on each test carrier in the presence or absence of cytotoxicity. If cytotoxicity is present, the virus control titer should be sufficient to demonstrate a $\geq 3 \text{ Log}_{10}$ reduction in viral titer on each test carrier beyond the level of cytotoxicity.

CONCLUSIONS

When tested as described, Total-Shield Plus, Lot Nos. H21500823, H20300823, and G22100723 passed the Virucidal Hard-Surface Efficacy Test when Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus) containing 5.0% FBS organic load was exposed to the test substance for 5 minutes at 21°C and 40% RH.

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

APPENDIX I



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

**McGowan Industries, Inc.
12285 World Trade Dr. STE O
San Diego, CA 92128**

July 30, 2020

Microbac Protocol: MCG.1.07.30.20

Microbac Project: 1056-104

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

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 OCSP 810.2000 (2018) and 810.2200 (2018) Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSP 810.2000 (2018), 810.2100 (2018), and 810.2200 (2018), Health Canada "Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs" (2020), and Health Canada "Guidance Document – Disinfectant Drugs" (2020) as applicable. 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".

TESTING CONDITIONS:

Virus will be dried on sterile glass Petri dishes at ambient temperature. One test substance (liquid), three batches (lots), will be tested in compliance with the EPA Lower Certified Limit Policy in 810.2000 at one exposure (contact) time at 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), Strain: USA-WA1/2020, Source: BEI Resources, NR-52281

Note: 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.

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:

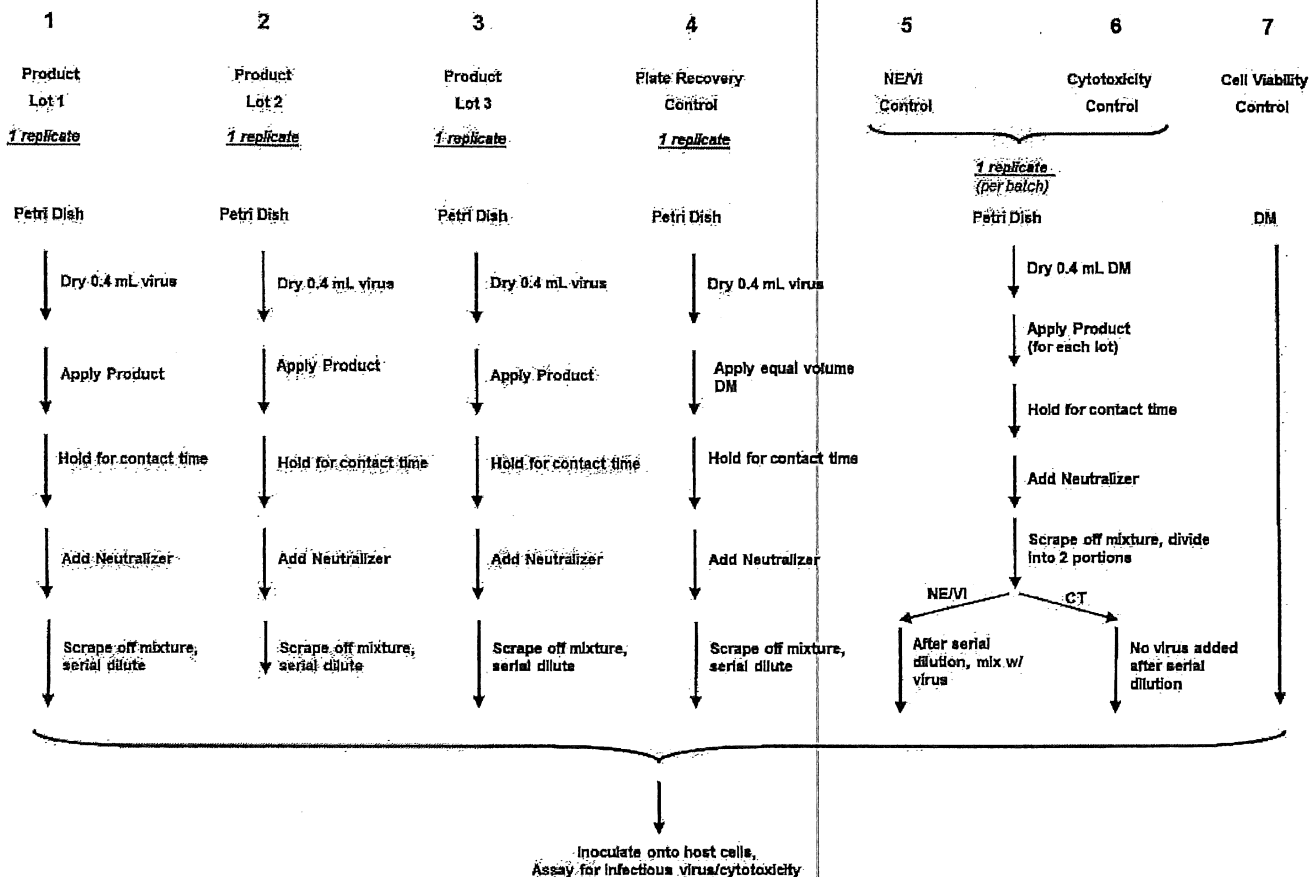
All Petri dishes, dilution tube racks, and host-containing apparatus will be appropriately labeled with the following information: virus, host, and test substance and/or project number.

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

NEVI: 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 NEVI and CT controls will be performed at one replicate per lot.

[Handwritten signature]

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 Log₁₀ 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 Log₁₀ 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 gel filtration columns (e.g., Sephacryl or Sephadex 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 $36\pm 2^{\circ}\text{C}$ with $5\pm 3\%$ 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 (TCID₅₀/mL) will be determined using the method of Spearman-Kärber (Kärber G., Arch. Exp. Pathol. Pharmacol. 1931, 162: 480-483). The TCID₅₀/carrier, i.e., the viral load per carrier, will be calculated as follows:

The Virus Load (TCID₅₀/carrier) will be calculated in the following manner:

Virus Load (Log₁₀ TCID₅₀) = Virus Titer (Log₁₀ TCID₅₀/mL) + Log₁₀ [Volume per sample (mL)]

The Log₁₀ Reduction Factor (LRF) will be calculated in the following manner:

Log₁₀ Reduction Factor = Initial viral load (Log₁₀ TCID₅₀, per assayed volume and per carrier) – Output viral load (Log₁₀ TCID₅₀, 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 log₁₀ 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 log₁₀ TCID₅₀ 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 $\geq 3 \log_{10}$ 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 $\geq 3 \log_{10}$ 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, Frequent Questions for the 2018 Series 810-Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines, August 2019.
5. Government of Canada, Health Canada, Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs, April 2020.
6. Government of Canada, Health Canada, Guidance Document – Disinfectant Drugs, April 2020.
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.

MISCELLANEOUS INFORMATION:

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

A. Test substance information:

Test substance name	Total-shield Plus		
EPA Reg. No. (if applicable)	U9658-3-96853		
Test substance batch No.	Lot 1 (H21500823)	Lot 2 (H20300823)	Lot 3 (G22100723)
Manufacture Date	Aug. 15, 2020	Aug. 3, 2020	July 21, 2020
Expiration Date	Aug. 15, 2022	Aug. 3, 2022	July 21, 2022
Active ingredient(s)	Phenol, Ethanol, 2-Phenylphenol		
Test substance storage conditions	<input checked="" type="checkbox"/> Ambient <input type="checkbox"/> Refrigerated <input type="checkbox"/> Other: _____		
Level of active ingredients in testing	<input checked="" type="checkbox"/> Lower Certified Limit (LCL) <input type="checkbox"/> At or below nominal		
SDS provided	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	C of A provided	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Dilution	<input checked="" type="checkbox"/> Ready to use <input type="checkbox"/> _____ (_____ parts test substance + _____ parts diluent)		
Diluent	<input checked="" type="checkbox"/> Not applicable <input type="checkbox"/> 400 ppm ± 2.9% AOAC hard water <input type="checkbox"/> 375 ppm OECD hard water (acceptable range: 338-394 ppm) <input type="checkbox"/> 200 ppm unsoftened tap water (acceptable range: 180-210 ppm) <input type="checkbox"/> Other: _____		
Contact time	5 minutes		
Contact temperature	<input checked="" type="checkbox"/> Room Temperature (20±1°C) <input type="checkbox"/> Other _____		
Organic Load	5.0% serum in viral inoculum.		

DAW

A. Test substance information (continued):

Test substance (spray) preparation	<input type="checkbox"/> Shake sprayer _____ times and spray into waste container to prime before use <input checked="" type="checkbox"/> Do not shake sprayer; spray into waste container to prime before use <input type="checkbox"/> Other: _____ <input type="checkbox"/> Not applicable
Test substance application	<input type="checkbox"/> Apply directly to dried virus via pipetting <input checked="" type="checkbox"/> Spray from <u>12</u> inches using <u>3</u> sprays or until thoroughly wet <input type="checkbox"/> Spray from _____ inches for _____ seconds or until thoroughly wet <input type="checkbox"/> Other: _____
Study conduct	<input checked="" type="checkbox"/> GLP <input type="checkbox"/> Non-GLP
Report submission	<input checked="" type="checkbox"/> EPA <input type="checkbox"/> Health Canada <input type="checkbox"/> Other: _____

PROTOCOL APPROVAL BY SPONSOR:

Sponsor Signature: _____

Date: 8/24/20

Printed Name: _____

Peteina C. Konikus

PROTOCOL APPROVAL BY STUDY DIRECTOR (Microbac):

Study Director Signature: _____

Date: 09/30/20

Printed Name: _____


Cameron J. Wilde

Don

Date Issued: 09/30/20 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 1056-104													
STUDY TITLE: VIRUCIDAL HARD-SURFACE EFFICACY TEST – Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)	STUDY DIRECTOR: Cameron Wilde  <div style="display: flex; justify-content: space-between;"> Signature Date 09/30/2020 </div>												
TEST MATERIAL(S): Total-Shield Plus	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="text-align: left;">BATCH (LOT) NO.</th> <th style="text-align: left;">DATE RECEIVED:</th> <th style="text-align: left;">DS NO.</th> </tr> <tr> <td>H21500823</td> <td>08/27/20</td> <td>K1186</td> </tr> <tr> <td>H20300823</td> <td>08/27/20</td> <td>K1187</td> </tr> <tr> <td>G22100723</td> <td>08/27/20</td> <td>K1188</td> </tr> </table>	BATCH (LOT) NO.	DATE RECEIVED:	DS NO.	H21500823	08/27/20	K1186	H20300823	08/27/20	K1187	G22100723	08/27/20	K1188
BATCH (LOT) NO.	DATE RECEIVED:	DS NO.											
H21500823	08/27/20	K1186											
H20300823	08/27/20	K1187											
G22100723	08/27/20	K1188											
PERFORMING DEPARTMENT(S): Virology and Toxicology	STORAGE CONDITIONS: Location: L2 <input checked="" type="checkbox"/> Dark <input checked="" type="checkbox"/> Ambient Room Temperature <input type="checkbox"/> Desiccator <input type="checkbox"/> Freezer <input type="checkbox"/> Refrigerator <input type="checkbox"/> Other:												
PROTECTIVE PRECAUTION REQUIRED: MSDS <input checked="" type="checkbox"/> Yes / <input type="checkbox"/> No													
PHYSICAL DESCRIPTION: <input type="checkbox"/> Solid <input checked="" type="checkbox"/> Liquid <input type="checkbox"/> Aerosol <input type="checkbox"/> Other:													
PURPOSE: See attached protocol. AUTHORIZATION: See client signature.													
PROPOSED EXPERIMENTAL START DATE: 09/30/20 TERMINATION DATE: 10/09/20													
CONDUCT OF STUDY: <input type="checkbox"/> FDA <input checked="" type="checkbox"/> EPA <input type="checkbox"/> R&D <input checked="" type="checkbox"/> GLP <input type="checkbox"/> GCP <input type="checkbox"/> Other:													
SPONSOR: McGowan Industries, Inc. 12285 World Trade Dr. STE O San Diego, CA 92128	CONTACT PERSON: Petrina Kohlrus Petrina@mcgowaninc.net												
TEST CONDITIONS: <div style="margin-bottom: 10px;"> Challenge organism: Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), Strain: USA-WA1/2020, Source: BEI Resources, NR-52281 </div> <div style="margin-bottom: 10px;"> Host cell line: Vero E6, Source: ATCC CRL-1586 </div> <div style="margin-bottom: 10px;"> Organic load: 5.0% FBS in virus inoculum </div> <div style="margin-bottom: 10px;"> Dilution medium: Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS) </div> <div style="margin-bottom: 10px;"> Active ingredient(s): Phenol, Ethanol, 2-Phenylphenol </div> <div style="margin-bottom: 10px;"> Dilution: Ready-to-use </div> <div style="margin-bottom: 10px;"> Neutralizer: MEM + 10% NCS + 0.5% Polysorbate-80 + 0.5% Lecithin </div> <div style="margin-bottom: 10px;"> Contact time(s): 5 minutes </div> <div style="margin-bottom: 10px;"> Contact temperature: Room Temperature (20±1°C) </div> <div style="margin-bottom: 10px;"> Incubation time: 4 – 9 days </div> <div style="margin-bottom: 10px;"> Incubation temperature: 36±2°C with 5±3% CO₂ </div> <div> Test product application: Spray from 6 inches using 3 sprays or until thoroughly wet. </div>													

APPENDIX II

**CERTIFICATE OF ANALYSIS
GERM PATROL LLC**

Product Name	NoviGuard™/ GermPatrol™ Total Shield Plus™/
Manufacturer's Name	Germ Patrol USA Corp
Batch Number	3 Test Batches A, B, and C
Sample size	16 ounces
Number of Samples	54 Bottles
ID Number Test Batch 1	H21500823
ID Number Test Batch 2	H20300823
ID Number Test Batch 3	G22100723
Address	180 Monohurst Rd
City, State, Zip	Franklin, NJ 07416
Product to be supplied for testing	<p>Viral testing: 500mL's per batch (3 batches at LCL)</p> <p>Bacterial testing: 2.5 liters per batch (3 batches at LCL for Quote #2, 3 batches at LCL for Quote #4, and 2 batches at Nominal value for Quote #3)</p>
Product Description	Multi-purpose Germicidal Spray
Signature of Person Responsible for Preparation	
Date Manufactured and expired	1-8/15/2020(23), 2-8/3/2020(23), 3-7/21/2020(23)

The 3 Batch samples were created for **McGowan Industries, Inc.**
2219 Margaret Dr.,Newport Beach, CA 92663 c: 949-677-4981.

All samples were Manufactured by Germ Patrol USA Corp. The samples consisted of there test batches comprised of 54 bottles total separated into 3 batches identified as

Batch A, B and C all to be used for testing purposes against the coronavirus known as Covid-19. These samples were created on August 19, 2020.

Test Batch A	Test Batch B	Test Batch C
272 oz	272 oz	187 oz
20 bottles	20 bottles	14 bottles
(three cases)	(three cases)	(two cases)

Boiling Point	148°F	Specific Gravity	0.83
Solubility in Water	TOTAL	Vapor Pressure (mm Hg)	135 @ 75°F
Vapor Density	Denser than air	Reactivity in Water	NONE
Appearance	Nearly colorless,	Melting Point	Below 32°F
ph. 7-8	Pounds per gallon or Bulk Density 8.6Lbs per Gal.	Alcohol: Odor Odor Threshold: No Data Available	Freezing Point 173°F (- 114°C)

Flash Point - °F 48° F	Method Used TCC	LEL: 6% UEL: 36%	Auto-Ignition Temp: 725°F
Stability: Stable <input checked="" type="checkbox"/> Unstable___	Empty drums should be completely drained, washed out and returned with bung in place.		
Extinguisher Media	Use water spray to cool fire and exposed surfaces and to protect personnel. Carbon dioxide also effective.		
Unusual Fire and Explosion hazards	NONE		
Lower Flammable Upper Flammable	Limit 3.3% Limit 19.0%		
Vapor Pressure Relative Vapor Density	44.6 mm Hg at 20°C (68°F) 20°C 1.59		

The Samples provided were created for Germ Shield Plus to be tested against the coronavirus known as Covid-19. Germ Patrol USA is a registered EPA manufacturing company in the state of New Jersey. All samples created conform to good manufacturing practices.