FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: SARS-Related Coronavirus 2

PRODUCT IDENTITY

Envirocleanse-A
Lot 11/30/20 1, Lot 11/30/20 2 and Lot 11/30/20 3

TEST GUIDELINE

OCSPP 810.2200

PROTOCOL NUMBER

ECL01102120.SARS2.2

AUTHOR

Laura Schueller, B.S.
Study Director

STUDY COMPLETION DATE

January 19, 2021

PERFORMING LABORATORY

Analytical Lab Group-Midwest
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Envirocleanse, LLC
22762 Westheimer Pkwy, Suite 515
Katy, TX 77450

PROJECT NUMBER

A31676

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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).

Company: Envirocleanse, LLC

Company Agent: _____

_____
Title

_____ Date:_____
Signature

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160 with the following exceptions:

The following studies were not performed following GLP regulations: characterization and stability of the compounds.

Submitter:_____ Date:_____

Sponsor:_____ Date:_____

Study Director:_____ Date: 1/19/2021
Laura Schueller, B.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to Management and the Study Director.

Phase Inspected	Date of Phase Inspection	Date Reported to Study Director	Date Reported to Management
Critical Phase Audit: Preparation of Test Substance	January 5, 2021	January 5, 2021	January 6, 2021
Final Report	January 18, 2021	January 18, 2021	January 19, 2021

Quality Assurance Specialist: Cody Ann Date: 1/19/21

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STUDY PERSONNEL

STUDY DIRECTOR: Laura Schueller, B.S.

Professional Personnel Involved:

Amy Backler, M.S.	- Manager, Study Director Operations
Erica Flinn, B.A.	- Manager, Core Services Laboratory Operations
Ryan Williams, B.S.	- Manager, Chemistry Program
Kayla Helmberger, B.S.	- Manager, Chemistry Laboratory
Miranda Peskar, B.S.	- Virology Laboratory Supervisor
Kasey Thompson, B.S.	- Virologist
Maria Cullen, B.A.	- Associate Virologist
Tanner Straus, B.S.	- Associate Virologist
Vanessa Bell, B.S.	- Chemist

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Project Number: A31676

Protocol Number: ECL01102120.SARS2.2

Sponsor: Envirocleanse, LLC
22762 Westheimer Pkwy, Suite 515
Katy, TX 77450

Testing Facility: Analytical Lab Group-Midwest
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name: Envirocleanse-A

Lot/Batch(s): Lot 11/30/20 1, Lot 11/30/20 2 and Lot 11/30/20 3

Manufacture Date: Lot 11/30/20 1 – November 30, 2020
Lot 11/30/20 2 – November 30, 2020
Lot 11/30/20 3 – November 30, 2020

Test Substance Characterization
Test substance characterization as to identity, strength, purity, stability and uniformity, as applicable, was document prior to its use in the study, however, not in accordance to 40 CFR, Part 160, Subpart F [160.105]. The analysis of free available chlorine may be found in Appendix I.

STUDY DATES

Date Sample Received: December 10, 2020
Study Initiation Date: January 4, 2021
Experimental Start Date: January 5, 2021 (Start time: 2:24 p.m.)
Experimental End Date: January 12, 2021 (End time: 2:10 p.m.)
Study Completion Date: January 19, 2021

OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure was to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to the U.S. Environmental Protection Agency (EPA) and Health Canada.

SUMMARY OF RESULTS

Test Substance:	Envirocleanse-A, Lot 11/30/20 1, Lot 11/30/20 2 and Lot 11/30/20 3
Dilutions:	330 ppm, defined as 116.2 mL test substance + 33.8 mL sterile deionized water (Lot 11/30/20 1), applied as a trigger spray 330 ppm, defined as 119.3 mL test substance + 30.7 mL sterile deionized water (Lot 11/30/20 2), applied as a trigger spray 330 ppm, defined as 115.4 mL test substance + 34.6 mL sterile deionized water (Lot 11/30/20 3), applied as a trigger spray
Virus:	SARS-Related Coronavirus 2, BEI Resources NR-52281, Strain Isolate USA-WA1/2020
Exposure Time:	1 minute
Exposure Temperature:	Room temperature (19.25°C)
Exposure Humidity:	20.61%
Organic Soil Load:	5% fetal bovine serum
Efficacy Result:	Three lots of Envirocleanse-A (Lot 11/30/20 1, Lot 11/30/20 2 and Lot 11/30/20 3) met the performance requirements specified in the study protocol. The results indicate **a ≥3 $\log_{10}$ reduction in titer** of SARS-Related Coronavirus 2 under these test conditions as required by the U.S. EPA and Health Canada.

TEST SYSTEM

1. Virus

The Isolate USA-WA1/2020 strain of SARS-Related Coronavirus 2 used for this study was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH (BEI Resources NR-52281). The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at ≤-70°C until the day of use. On the day of use, an aliquot of stock virus (Lot SARS2-9) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Coronavirus on Vero E6 cells.

2. Indicator Cell Cultures

Cultures of Vero E6 cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CRL-1586). The cells were propagated by Analytical Lab Group-Midwest personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO_2. On the day of testing, the cells were observed as having proper cell integrity and confluency, and therefore, were acceptable for use in this study.

All cell culture documentation was retained for the cell cultures used in the assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

3. Test Medium

The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-inactivated fetal bovine serum (FBS), 10 μg/mL gentamicin, 100 units/mL penicillin, 2.5 μg/mL amphotericin B, 2.0 mM L-glutamine, 0.1 mM NEAA and 1.0 mM sodium pyruvate.

TEST METHOD

1. Preparation of Test Substance

Three lots of Envirocleanse-A (Lot 11/30/20 1, Lot 11/30/20 2 and Lot 11/30/20 3), were tested at 330 ppm, defined as 116.2 mL test substance + 33.8 mL sterile deionized water (116.20 mL product + 33.80 mL water) for Lot 11/30/20 1, defined as 119.3 mL test substance + 30.7 mL sterile deionized water (119.30 mL product + 30.70 mL water) for Lot 11/30/20 2, and defined 115.4 mL test substance + 34.6 mL sterile deionized water (115.40 mL product + 34.60 mL water) for Lot 11/30/20 3, applied as a trigger spray, as requested by the Sponsor. The test substance was prepared in Analytical Lab Group-Midwest trigger spray bottles and appeared homogeneous as determined by visual observation. The use dilution of the test substance was used on the day of preparation and was at the exposure temperature prior to use.

2. Preparation of Virus Films
Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of four separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 19.25°C in a relative humidity of 20.61% until visibly dry (20 minutes).

3. Preparation of Sephadex Gel Filtration Columns
To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel. On the day of testing, Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

4. Input Virus Control (TABLE 1)
On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

5. Treatment of Virus Films with the Test Substance (TABLE 2)
For each lot of test substance, one dried virus film was individually exposed for 1 minute at room temperature (19.25°C) and 20.61% relative humidity to the amount of spray released under use conditions. The carriers were sprayed using 3 sprays, until thoroughly wet, at a distance of 6 to 8 inches, and held covered for the exposure time. The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

6. Treatment of Dried Virus Control Film (TABLE 1)
One virus film was prepared as previously described (paragraph 2). The virus control film was exposed to 2.00 mL of test medium in lieu of the test substance and held covered for 1 minute at room temperature (19.25°C) and 20.61% relative humidity. Just prior to the end of the exposure time, the virus control was scraped with a cell scraper and at the end of the exposure time the virus mixture was immediately passed through a Sephadex column in the same manner as the test virus (paragraph 5). The filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

7. Cytotoxicity Controls (TABLE 3)
Each lot of the test substance was sprayed as previously described (paragraph 5) onto separate sterile petri dishes and held covered for the 1 minute exposure time at room temperature (19.25°C) and 20.61% relative humidity. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper and at the end of the exposure time the contents were immediately passed through a Sephadex column utilizing a syringe plunger. The filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for cytotoxicity. Cytotoxicity of the Vero E6 cell cultures was scored at the same time as the virus-test substance and virus control cultures.

8. Assay of Non-Virucidal Level of Test Substance (Neutralization Control) (TABLE 4)
Each dilution of the neutralized test substance (cytotoxicity control dilutions) was challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, was retained. Dilutions that showed virucidal activity were not considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures was inoculated with a 100 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus (approximately 1000 infectious units) was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates.

9. Infectivity Assays
The Vero E6 cell line, which exhibits cytopathic effect (CPE) in the presence of SARS-Related Coronavirus 2, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C (37.0°C) in a humidified atmosphere of 5-7% CO_2 (6.0% CO_2) in sterile disposable cell culture labware. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.

10. Statistical Methods: Not applicable

PLANNED PROTOCOL CHANGES

Protocol Amendments:
No protocol amendments were required for this study.

Planned Protocol Deviations:
No planned protocol deviations occurred during this study.

DATA ANALYSIS

Calculation of Titers

Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier) :

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

Log_{10} of Y = the $TCID_{50}$/carrier (Example: $10^{5.50}$ or 5.50 Log_{10})

*$TCID_{50}$ value calculated based on the volume inoculated per well

Calculation of Log Reduction

The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control Log_{10} $TCID_{50}$ – Test Substance Log_{10} $TCID_{50}$ = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

Calculation of Infectious Units

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus:$10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

STUDY ACCEPTANCE CRITERIA

U.S. EPA and Health Canada Submission

A valid test requires 1) that at least 4.8 log_{10} of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 log_{10} reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 log_{10} reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at Analytical Lab Group-Midwest following the record retention policy outlined in the internal SOP ALS-0032. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of the final study report.
7. Study-specific SOP deviations made during the study.

Test Substance Retention

The test substance will be discarded following study completion. It is the responsibility of the Sponsor to retain a sample of the test substance.

REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-12 (Reapproved 2017).
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, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.

STUDY RESULTS

Results of tests with three lots of Envirocleanse-A (Lot 11/30/20 1, Lot 11/30/20 2 and Lot 11/30/20 3), diluted to 330 ppm, defined as 116.2 mL test substance + 33.8 mL sterile deionized water (Lot 11/30/20 1), defined as 119.3 mL test substance + 30.7 mL sterile deionized water (Lot 11/30/20 2), and defined as 115.4 mL test substance + 34.6 mL sterile deionized water (Lot 11/30/20 3), applied as a trigger spray, exposed to SARS-Related Coronavirus 2 in the presence of a 5% fetal bovine serum organic soil load at room temperature (19.25°C) and 20.61% relative humidity for 1 minute are shown in Tables 1-4. All cell controls were negative for test virus infectivity.

The titer of the input virus control was 6.50 $\log_{10}$/100 µL. The titer of the dried virus control was 5.50 $\log_{10}$/100 µL (5.80 $\log_{10}$/carrier). Following exposure, test virus infectivity was not detected in the virus-test substance mixture in any lot at any dilution tested [≤0.50 $\log_{10}$/100 µL (≤0.80 $\log_{10}$/carrier)]. Test substance cytotoxicity was not observed in any lot at any dilution tested (≤0.50 $\log_{10}$/100 µL). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤0.50 $\log_{10}$/100 µL for all lots.

Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer, per volume inoculated per well and per carrier was ≥5.00 $\log_{10}$ for all lots.

STUDY CONCLUSION

Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, Envirocleanse-A, diluted to 330 ppm, defined as 116.2 mL test substance + 33.8 mL sterile deionized water (Lot 11/30/20 1), defined as 119.3 mL test substance + 30.7 mL sterile deionized water (Lot 11/30/20 2), and defined as 115.4 mL test substance + 34.6 mL sterile deionized water (Lot 11/30/20 3), applied as a trigger spray, demonstrated a ≥3 log_{10} reduction in titer of SARS-Related Coronavirus 2 following a 1 minute exposure time at room temperature (19.25°C) and 20.61% relative humidity as required by the U.S. EPA and Health Canada.

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.

TABLE 1: Virus Control Results

Input Virus Control and Dried Virus Control Following a 1 Minute Exposure Time

Dilution	Input Virus Control	Dried Virus Control
Cell Control	0 0	0 0 0 0
10^{-1}	+ +	+ + + +
10^{-2}	+ +	+ + + +
10^{-3}	+ +	+ + + +
10^{-4}	+ +	+ + + +
10^{-5}	+ +	+ + + +
10^{-6}	+ +	0 0 0 0
10^{-7}	0 0	NT
$TCID_{50}$/100 µL	$10^{6.50}$	$10^{5.50}$
$TCID_{50}$/carrier	NA	$10^{5.80}$

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(NA) = Not applicable
(NT) = Not tested

TABLE 2: Test Results

Effects of Envirocleanse-A Following a 1 Minute Exposure to SARS-Related Coronavirus 2 Dried on an Inanimate Surface

Dilution	SARS-Related Coronavirus 2 + Lot 11/30/20 1	SARS-Related Coronavirus 2 + Lot 11/30/20 2	SARS-Related Coronavirus 2 + Lot 11/30/20 3
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$\leq 10^{0.50}$
$TCID_{50}$/carrier	$\leq 10^{0.80}$	$\leq 10^{0.80}$	$\leq 10^{0.80}$

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present

TABLE 3: Cytotoxicity Control Results

Cytotoxicity of Envirocleanse-A on Vero E6 Cell Cultures

Dilution	Cytotoxicity Control Lot 11/30/20 1	Cytotoxicity Control Lot 11/30/20 2	Cytotoxicity Control Lot 11/30/20 3
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
TCD_{50}/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$\leq 10^{0.50}$

(0) = No test virus recovered and/or no cytotoxicity present

TABLE 4: Neutralization Control Results

Non-Virucidal Level of the Test Substance (Neutralization Control)

Dilution	Test Virus + Cytotoxicity Control Lot 11/30/20 1	Test Virus + Cytotoxicity Control Lot 11/30/20 2	Test Virus + Cytotoxicity Control Lot 11/30/20 3
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	+ + + +	+ + + +
10^{-2}	+ + + +	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +	+ + + +
10^{-6}	+ + + +	+ + + +	+ + + +

(+) = Positive for the presence of test virus after low titer stock virus added (neutralization control)
(0) = No test virus recovered and/or no cytotoxicity present

Results of the non-virucidal level control indicate that the test substance was neutralized at a $TCID_{50}$/100 µL of ≤0.50 log_{10} for all lots.

APPENDIX I: Free Available Chlorine Analysis of Envirocleanse-A

Envirocleanse-A Lot	Replicate	ppm Active	% Difference	Average ppm Active
11/30/20 1	1	425	0	426 ppm
	2	426		
11/30/20 2	1	415	0	415 ppm
	2	414		
11/30/20 3	1	429	0	429 ppm
	2	429		

The above three lots of Envirocleanse-A (Lot 11/30/20 1, Lot 11/30/20 2, and Lot 11/30/20 3) were diluted to 330 ppm for use in testing

(For Laboratory Use Only)
Analytical Lab Group-Midwest Project # A31676 1-4-21
Test Substance Tracking # TS121020.ECL01 SMW 12-10-20

PROTOCOL

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: SARS-Related Coronavirus 2

PROTOCOL NUMBER

ECL01102120.SARS2.2

SPONSOR

Envirocleanse, LLC
22762 Westheimer Pkwy, Suite 515
Katy, TX 77450

PERFORMING LABORATORY

Analytical Lab Group-Midwest
1285 Corporate Center Drive, Suite 110
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DATE

October 21, 2020

EXACT COPY
INITIALS LAJ DATE 1/18/21

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

PURPOSE

The purpose of this study is to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

TEST SUBSTANCE CHARACTERIZATION

According to 40 CFR, Part 160, Subpart F [160.105] test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Analytical Lab Group-Midwest. Analytical Lab Group-Midwest will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once Analytical Lab Group-Midwest receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is December 14, 2020. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of January 11, 2021. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Analytical Lab Group-Midwest.

If a test must be repeated, or a portion of it, because of failure by Analytical Lab Group-Midwest to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of Analytical Lab Group-Midwest nor any of its employees are to be used in advertising or other promotion without written consent from Analytical Lab Group-Midwest.

The Sponsor is responsible for any rejection of the final report by the regulatory agency of its submission concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Analytical Lab Group-Midwest final report and notify Analytical Lab Group-Midwest of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Analytical Lab Group-Midwest will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Regulatory agencies require that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. Each agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The Vero E6 cell line, which supports the growth of the SARS-Related Coronavirus 2, will be used in this study. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A film of virus, dried on a glass surface, is exposed to the test substance for a specified exposure time. At the end of the exposure time, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

STUDY DESIGN

Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

The inoculated carriers are exposed to the test substance for the Sponsor specified exposure time. At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is inoculated into indicator cell cultures. The resuspended virus control film and each batch of test substance alone will be treated in exactly the same manner. For analysis of infectivity, cultures will be held for the appropriate incubation period at the end of which time cultures will be scored for the presence of the test virus. Cultures will be monitored at that time for cell viability. Uninfected indicator cell cultures will be carried in parallel and similarly monitored. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. In addition to the above titrations for infectivity and cytotoxicity, the residual virucidal activity of the test substance after neutralization will be determined by adding a low titer of stock virus to each dilution of the test substance (cytotoxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

VIRUS

The Isolate USA-WA1/2020 strain of SARS-Related Coronavirus 2 to be used for this study was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH (BEI Resources NR-52281). Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at ≤ -70°C until the day of use. Alternate methods of viral propagation may be utilized based on the growth requirements of the virus. The propagation method will be specified in the raw data and in the report. On the day of use the appropriate number of aliquots are removed, thawed, combined (if applicable) and maintained at a refrigerated temperature until used in the assay. **Note:** If the Sponsor requests an organic soil load challenge, fetal bovine serum (FBS) or the requested organic soil will be incorporated into the stock virus aliquot. The stock virus aliquot will be adjusted to yield the percent organic soil load requested.

INDICATOR CELL CULTURES

Cultures of Vero E6 cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CRL-1586). The cells are propagated by Analytical Lab Group-Midwest personnel. The cells are seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO_2. The confluency of the cells will be appropriate for the test virus. Vero E6 cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the final report.

All cell culture documentation is retained for the cell cultures used in this assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

TEST MEDIUM

The test medium used for this assay is Minimum Essential Medium (MEM) supplemented with 0-10% (v/v) heat inactivated fetal bovine serum. The medium may also be supplemented with one or more of the following: 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 1.0-2.0 mM L-glutamine, and 0.5 – 5 µg/mL trypsin. The composition of the test medium may be altered based on the virus and/or cells. The composition of the medium will be specified in the raw data and in the report.

PREPARATION OF TEST SUBSTANCE

The dilution of test substance(s) will be used as recommended by the Sponsor. The product will be pre-equilibrated to the desired test temperature if applicable.

PREPARATION OF VIRUS FILMS

Films of virus will be prepared by spreading 200 µL of virus inoculum uniformly over the bottom of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥20 minutes). A calibrated timer will be used for timing the drying. The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

One dried virus film per batch of test substance will be assayed unless otherwise requested.

TEST METHOD

Preparation of Sephadex Gel Filtration Columns

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

Input Virus Control

On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

Treatment of Virus Films with the Test Substance

For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time(s) and temperature. A calibrated timer will be used for timing the exposure. The actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate (10^{-1} dilution) is then titered by serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

Treatment of Dried Virus Control Film

The appropriate number of virus films are prepared as described previously for each exposure time assayed. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure and the actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the virus films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate (10^{-1} dilution) is then titered by serial dilution and assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns.

Cytotoxicity Control
A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger and the filtrate is diluted serially in medium and inoculated into cell cultures for assay of cytotoxicity concurrently with the virus control and test substance-treated virus samples. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns.

Assay of Non-Virucidal Level of Test Substance (Neutralization Control)
Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures will be inoculated with a 100 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates.

Infectivity Assays
The Vero E6 cell line, which exhibits cytopathic effect (CPE) in the presence of SARS-Related Coronavirus 2, will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. The input virus control will be inoculated in duplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware for approximately seven days. Periodically throughout the incubation time the cultures may be microscopically observed for the absence or presence of CPE, cytotoxicity and for viability. The observations will be recorded on the raw data worksheets; only the results from the final observations will be reported. The infectious units of the low titer stock virus will be calculated and included in the final report.

DATA ANALYSIS

Calculation of Titers
Viral and cytotoxicity titers will be expressed as -$\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$\text{- Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier) :

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$\log_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.50}$ or 5.50 $\log_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

Calculation of Log Reduction
The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $\log_{10}$ $TCID_{50}$ – Test Substance $\log_{10}$ $TCID_{50}$ = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

Calculation of Infectious Units

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus:$10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

The specialized virucidal testing section of Analytical Lab Group-Midwest maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: N/A

STUDY ACCEPTANCE CRITERIA

Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

U.S. EPA and Health Canada Submission

A valid test requires 1) that at least 4.8 log_{10} of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 log_{10} reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 log_{10} reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

If the test substance fails to meet the test acceptance criteria and the dried virus control fails to meet the control acceptance criteria, the study is considered valid and no repeat testing is necessary, unless requested by the Sponsor.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number.

For any studies with presence of contamination in subculture media, a control failure, system failure, technician error, etc. the Repeat Testing Policy from the Series 810 Guidelines FAQ document will be followed.

FINAL REPORT
The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, a conclusion as it relates to the purpose of the test and all other items required by 40 CFR Part 160.185. A draft report may be requested by the Sponsor. The final report will be prepared once the Sponsor has reviewed the draft report and notified the Study Director to complete the study.

PROTOCOL CHANGES
If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

TEST SUBSTANCE RETENTION
Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be **discarded** following study completion unless otherwise requested.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at Analytical Lab Group-Midwest following the record retention policy outlined in the internal SOP ALS-0032. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of the final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at Analytical Lab Group-Midwest. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study, which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

PROPOSED STATISTICAL METHODS: N/A

REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-12 (Reapproved 2017).
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, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April, 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April, 2020. Guidance Document - Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.

STUDY INFORMATION

(All blank sections are verified by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)

Test Substance (Name and Lot/Batch Number exactly as it should appear on final report)

Test Substance Name	Lot/Batch Number
Envirocleanse-A ①	~~#1~~ ① 11/30/20 1
Envirocleanse-A ①	~~#2~~ ① 11/30/20 2
Envirocleanse-A ①	~~#3~~ ① 11/30/20 3

Testing at the lower certified limit (LCL) for the hardest-to-kill virus on your label is required for registration.

Product Description

☐ Quaternary ammonia ☐ Peracetic acid ☐ Sodium hypochlorite
☐ Iodophor ☐ Peroxide ☑ Other Hypochlorous acid

Approximate Test Substance Active Concentration (upon submission to Analytical Lab Group-Midwest):

② ~~500 ppm if fail,~~ 338 ppm if pass first test

(This value is used for neutralization planning only. This value is not intended to represent characterization values.)

Storage Conditions

☑ Room Temperature
☐ 2-8°C
☐ Other _____

Hazards

② ☑ None known: Use Standard Precautions
☐ Material Safety Data Sheet, Attached for each product
☐ As Follows: _____

Product Preparation

② ~~☑~~ No dilution required, Use as received (RTU) ~ do not use over the recommended FAC ppm

② ☒ *Dilution(s) to be tested:

330-338 ppm ② defined as ② see attachment + ② see attachment amount. If above directed FAC ppm, dilute with deionized water until FAC ppm levels are satisfactory.
(example: 1 oz/gallon) (amount of test substance) (amount of diluent)

☐ AOAC Synthetic Hard Water: 400 ppm (360-420 ppm)
☐ Un-softened Tap Water: 200 ppm (180-210 ppm)
☐ OECD Hard Water: 375 ppm (338-394 ppm)
☒ Other Deionized Water ②

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Virus: SARS-Related Coronavirus 2

Exposure Time: ② ~~10 minutes if fail the first test,~~ 1 minute if pass first test

Exposure Temperature: ☑ Room temperature (to be based on regulatory agency of submission)
☐ Other: _____ °C (please specify range)

Directions for application of aerosol/spray products:

☐ Spray instructions are not applicable.

Trigger spray application:

☑ Spray carriers using 3 sprays, or until thoroughly wet, at a distance of 6 to 8 inches.
☐ Spray carriers using _____ sprays at a distance of _____ to ____ inches/cm. (circle one)

Aerosol spray application:

☐ Spray carriers for _____ seconds, or until thoroughly wet, at a distance _____ to ____ inches/cm.

Organic Soil Load

☐ 1% fetal bovine serum
☑ 5% fetal bovine serum
☐ Other _____

① Per email dated ~~12/14/20~~ LAS 12/14/20 WE LAS 12/14/20
② Per email dated 12/10/20
12/22/20.
LAS 1/4/21

Number of Carriers to be Tested

☑ One (typical for U.S. EPA / Health Canada submission)
☐ Other: _____

SPRAY BOTTLES USED IN TESTING (section only applicable for spray products)

To ensure expected levels of product are delivered, it is recommended that the Sponsor provide the spray bottles used in testing. Please indicate the desired source of the sprayer bottles used in testing:

(1) ☑ Sprayer(s) and bottle(s) are provided by the Sponsor
(1) ☒ General purpose spray bottle(s) are to be provided by Analytical Lab Group-Midwest
☐ The spray nozzle(s) are provided by the Sponsor and general purpose bottle(s) will be provided by Analytical Lab Group-Midwest

REGULATORY AGENCY(S) THAT MAY REVIEW DATA

☑ U.S. EPA
☑ Health Canada
☐ Not applicable - For internal/other use only (Efficacy result will be based on U.S. EPA requirements)

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP or Development Study)

PROTOCOL MODIFICATIONS

☐ Approved without modification
☑ Approved with modification
Reference 8 is updated to: Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - (1) ☒ Yes ☒ No (1)

TEST SUBSTANCE SHIPMENT STATUS

(This section is for informational purposes only.)
☐ Test Substance is already present at Analytical Lab Group-Midwest.
☑ Test Substance has been or will be shipped to Analytical Lab Group-Midwest.
Date of expected receipt at Analytical Lab Group-Midwest: December 23rd

TESTING FACILITY MANAGEMENT VERIFICATION OF 40 CFR PART 160 SUBPART B (160.31(D))

Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing: ☑ **Yes** ☐ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** (1) ☒ **No***

Stability testing of the formulation has been or will be completed prior to or concurrent with efficacy testing:
☑ **Yes** ☐ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☒ **No*** (1)

**If testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.*

(1) Per email dated 12/22/20. VM 1/4/21

PROPRIETARY INFORMATION

APPROVAL SIGNATURES

SPONSOR:

NAME: Joey Waid TITLE: Sales Manager

SIGNATURE: DATE: 11/6/20

PHONE: (281) 201 - 4544 EMAIL: J.Waid@eco-enviro.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ❑ See Attached

Analytical Lab Group-Midwest:

NAME: Laura Schueller
Study Director

SIGNATURE: DATE: 1/4/2021
Study Director

Attachment to Protocol: ECL01102120.SARS2.2

LRS 1/4/21

Chemistry Verifications

- **Protocol #:** ECL01102120.SARS2.2
- **Method #:** ACM-0007
- **Test Substance Name:** Envirocleanse-A Lot #: 11/30/20 1, 11/30/20 2, and 11/30/20 3
- **Tracking #:** TS121020.ECL01
- **Active Ingredient:** Free Available Chlorine (FAC)
- **Approximate Expected Concentration of Test Substance:** ≤338 ppm, with an acceptable range of 300-338 ppm, targeting 330-338 ppm
- **# of lots of test substance**: 3 lots
- **# of Replicates:** 2 reps
- **Specification:** %Difference ≤ 10%
- **Chemistry Testing, Test as:** Ready to Use
- **Viral Testing, Test as:** Test substance will be diluted to target 330-338 ppm, with an acceptable range of 300-338 ppm, after chemistry testing has determined the FAC level. The test substance will be diluted using $C_1V_1=C_2V_2$, using 150 mL as V_2. Example calculation is as follows: (titrated FAC ppm value) (X) = (330ppm) (150 mL).