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Volume _____

FINAL REPORT

VIRUCIDAL HARD-SURFACE EFFICACY TEST – Duck Hepatitis B Virus (surrogate for Human Hepatitis B Virus)

Test Substance
Envirocleanse A

Lot Numbers
110218
101918

Test Organism
Duck Hepatitis B Virus, Strain: Grimaud, Hepadnavirus Testing

Test Guidelines
EPA Guideline 810.2000
EPA Guideline 810.2200 (G)

Author
Zheng Chen, M.S.

Study Completion Date
12/04/18

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

Laboratory Project Identification Number
668-118

Protocol Identification Number
668.2.10.25.18

Sponsor
Envirocleanse LLC
12621 W. Airport Blvd., Ste. 200
Sugar Land, TX 77478

STATEMENT OF NO DATA CONFIDENTIALITY

Title: VIRUCIDAL HARD-SURFACE EFFICACY TEST – Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus)

Performed by: Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

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: _____

Typed Name of Signer: _____

Typed Name of Company: Envirocleanse LLC

COMPLIANCE STATEMENT

The following is a detailed description of all differences between the practices used in the study and those required by 40 CFR 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: 12/04/2018

Typed Name: Zheng Chen, M.S

Typed Name of Laboratory: Microbac Laboratories, Inc.

Sponsor signature: _____ Date: _____

Typed Name of Signer: Scott G. Mack

Typed Name of Company: Envirocleanse LLC

Submitter signature: _____ Date: _____

Typed Name of Signer: _____

Typed Name of Company: Envirocleanse LLC

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: VIRUCIDAL HARD-SURFACE EFFICACY TEST – Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus)

The Quality Assurance Unit of Microbac has inspected Project Number 668-118 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	11/08/18	11/08/18	11/08/18
In Process (Test)	11/08/18	11/08/18	11/08/18
Final Report	11/29/18	11/29/18	11/29/18

Jeanne M. Anderegg, RQAP-GLP
Quality Assurance Manager

12-04-2018
Date

TABLE OF CONTENTS

TEST SUMMARY

Title: VIRUCIDAL HARD-SURFACE EFFICACY TEST – Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus)

Study design: This study was performed according to the signed protocol and project sheet(s) issued by the Study Director (See Appendix I).

Test substances supplied by the sponsor of the study:

1. Envirocleanse A; Lot No. 110218, received at Microbac on 11/06/18 and assigned DS No. I641.

2. Envirocleanse A; Lot No. 101918, received at Microbac on 11/06/18 and assigned DS No. I642.

Sponsor: Envirocleanse LLC
12621 W. Airport Blvd., Ste. 200
Sugar Land, TX 77478

TEST CONDITIONS

Challenge virus:

Duck Hepatitis B Virus, Strain: Grimaud, Hepadnavirus Testing

Host:

Primary Duck Hepatocyte cells, Metzer Farms (duckling source)

Active ingredients:

Hypochlorous Acid (HOCl)

Neutralizer:

L-15 Complete + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 0.5% $Na_2S_2O_3$

Dilution medium:

L-15 Complete

Dilution:

Ready to use

Contact times:

2 minutes
10 minutes

Contact temperature and relative humidity (RH):

Room Temperature 20±1°C (Actual: 21°C); 31 - 33% RH

Carrier inoculation and dry time:

Glass carriers were inoculated with 0.4 mL of virus in a 4 in^2 area and dried for 40 minutes at 20-21°C and 31 - 32% RH

Organic load:

≥5% serum in virus inoculum (100% Duck Serum)

TEST CONDITIONS (continued)

Test substance application:

Each carrier was sprayed 3 times from a distance of 6”- 8” until thoroughly wet.

Media and reagents:

L-15 Complete + 10% FBS + 0.5% Polysorbate 80 + 0.5% $Na_2S_2O_3$
L-15 Complete
Phosphate Buffered Saline
JP1.7C12.2H10 (DHBsAg)
FITC conjugated goat anti-mouse antibody
80% Acetone

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, from 11/08/18 to 11/21/18. The study director signed the protocol on 11/08/18. The study completion date is the date the study director signed the final report. The individual test date was:

- Testing started at 1:30 pm on 11/08/18 and ended at 11:30 am on 11/21/18

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.

CALCULATION OF TITER

The 50% Tissue Culture Infectious Dose per mL ($TCID_{50}$/mL) was determined using the Spearman-Karber method using the following formula:

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

where:

m = the logarithm of the dilution at which half of the wells are infected relative to the test volume
x_k = the logarithm of the smallest dosage which induces infection in all cultures
d = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i
$\sum p_i$ = the sum of p_i (starting with the highest dilution producing 100% infection)

The values were converted to $TCID_{50}$/mL using a sample inoculum of 1.0 mL.

RESULTS

Results are presented in Tables 1– 9.

The Log_{10} Reduction Factor was calculated in the following manner:

Log_{10} Reduction Factor = Log_{10} $TCID_{50}$ (Plate Recovery Control) - Log_{10} $TCID_{50}$ (Test)

The Load (Log_{10} $TCID_{50}$) per carrier was calculated in the following manner:

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

Key (for all tables):

T/y = Cytotoxicity observed in y wells inoculated; viral immunofluorescence could not be determined
X/y = Virus was detected by immunofluorescence observed in X wells out of y wells inoculated
0/y = 0 out of y wells inoculated exhibited positive viral immunofluorescence; no cytotoxicity or bacterial contamination was observed in any of the wells inoculated

RESULTS (continued)

Table 1
Test Substance

Dilution*	Envirocleanse A, 10 minutes, Lot No. 110218	
	Replicate 1	Replicate 2
10^{-2}	0/4	0/4
10^{-3}	0/4	0/4
10^{-4}	0/4	0/4
10^{-5}	0/4	0/4
10^{-6}	0/4	0/4
10^{-7}	0/4	0/4
Titer (Log_{10} $TCID_{50}$/mL)	≤1.50	≤1.50
Load (Log_{10} $TCID_{50}$) per carrier (0.4 mL volume of Undilute)	≤1.10	≤1.10
Log_{10} Reduction	≥3.89	≥3.89

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

Table 2
Test Substance

Dilution*	Envirocleanse A, 10 minutes, Lot No. 101918	
	Replicate 1	Replicate 2
10^{-2}	0/4	0/4
10^{-3}	0/4	0/4
10^{-4}	0/4	0/4
10^{-5}	0/4	0/4
10^{-6}	0/4	0/4
10^{-7}	0/4	0/4
Titer (Log_{10} $TCID_{50}$/mL)	≤1.50	≤1.50
Load (Log_{10} $TCID_{50}$) per carrier (0.4 mL volume of Undilute)	≤1.10	≤1.10
Log_{10} Reduction	≥3.89	≥3.89

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

RESULTS (continued)

Table 3
Test Substance

Dilution*	Envirocleanse A, 2 minutes, Lot No. 110218	
	Replicate 1	Replicate 2
10^{-2}	2/4	0/4
10^{-3}	0/4	0/4
10^{-4}	0/4	0/4
10^{-5}	0/4	0/4
10^{-6}	0/4	0/4
10^{-7}	0/4	0/4
Titer (Log_{10} $TCID_{50}$/mL)	2.00	≤1.50
Load (Log_{10} $TCID_{50}$) per carrier (0.4 mL volume of Undilute)	1.60	≤1.10
Log_{10} Reduction	3.39	≥3.89

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

Table 4
Test Substance

Dilution*	Envirocleanse A, 2 minutes, Lot No. 101918	
	Replicate 1	Replicate 2
10^{-2}	0/4	2/4
10^{-3}	0/4	0/4
10^{-4}	0/4	0/4
10^{-5}	0/4	0/4
10^{-6}	0/4	0/4
10^{-7}	0/4	0/4
Titer (Log_{10} $TCID_{50}$/mL)	≤1.50	2.00
Load (Log_{10} $TCID_{50}$) per carrier (0.4 mL volume of Undilute)	≤1.10	1.60
Log_{10} Reduction	≥3.89	3.39

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

RESULTS (continued)

Table 5
Neutralizer Effectiveness/Viral Interference and Cytotoxicity Controls

Dilution*	Envirocleanse A, Lot No. 110218	
	Neutralizer Effectiveness/ Viral Interference Control	Cytotoxicity Control
10^{-2}	4/4	0/4
10^{-3}	4/4	0/4
10^{-4}	4/4	0/4

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

Table 6
Neutralizer Effectiveness/Viral Interference and Cytotoxicity Controls

Dilution*	Envirocleanse A, Lot No. 101918	
	Neutralizer Effectiveness/ Viral Interference Control	Cytotoxicity Control
10^{-2}	4/4	0/4
10^{-3}	4/4	0/4
10^{-4}	4/4	0/4

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

Table 7
Plate Recovery Control

Dilution*	Plate Recovery Control	
	Replicate 1	Replicate 2
10^{-3}	4/4	4/4
10^{-4}	4/4	4/4
10^{-5}	3/4	2/4
10^{-6}	1/4	1/4
10^{-7}	0/4	0/4
10^{-8}	0/4	0/4
Titer (Log_{10} $TCID_{50}$/mL)	5.50	5.25
Load (Log_{10} $TCID_{50}$) per carrier (0.4 mL volume of Undilute)	5.10	4.85
Average Load (Log_{10} $TCID_{50}$) per carrier	4.99	

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

RESULTS (continued)

Table 8
Virus Stock Titer Control

Dilution*	Virus Stock Titer Control
10^{-4}	4/4
10^{-5}	4/4
10^{-6}	2/4
10^{-7}	2/4
10^{-8}	0/4
10^{-9}	0/4
Titer (Log_{10} $TCID_{50}$/mL)	6.50

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

Table 9
Viability Control Results

Cell Viability Control
0/4
Cells were viable; media was sterile

CONCLUSIONS

According to the US Environmental Protection Agency, the test substance passes the Virucidal Hard-Surface Efficacy Test if there is a ≥ 3 log10 reduction on each surface in the presence or absence of cytotoxicity.

When tested as described, Envirocleanse A, Lot Nos. 110218 and 101918, passed the Virucidal Hard-Surface Efficacy Test when Duck Hepatitis B Virus, containing 100% serum, was exposed to the test substance for 2 minutes and for 10 minutes at 21°C and 31 - 33% relative humidity. All of the controls met the criteria for a valid test. These conclusions are based on observed data.

APPENDIX I

MICROBAC®

Microbac Protocol

VIRUCIDAL HARD-SURFACE EFFICACY TEST -

Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus)

Testing Facility
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA20164

Prepared for
Envirocleanse LLC
12621 W. Airport Blvd., Ste. 200
Sugar Land, TX 77478

October 25, 2018

Microbac Protocol: 668.2.10.25.18

Microbac Project: 668-118

OBJECTIVE:

This test is designed to substantiate virucidal effectiveness claims for a test substance to be labeled as a virucide. It determines the potential of the test substance to disinfect hard surfaces contaminated with the test virus. The test is designed to simulate consumer use and conforms to EPA OCSPP 810.2000 (2018) and 810.2200 (2018) Product Performance Test Guidelines, and follows the procedure outlined in the ASTM International test method designated E1053-11, "Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces".

TESTING CONDITIONS:

Virus will be dried on a suitable sterile hard surface at ambient temperature. One test substance (liquid), two batches (lots), will be tested at two contact times and two replicates (N=2). The test substance will be used to treat the dried virus on a glass Petri dish carrier. 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 (see last page). 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 for each batch 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 of each batch.

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 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 to 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): Duck Hepatitis B Virus (DHBV), Strain: Grimaud, HepadnaVirus Testing

2. Host cell line: Primary duck hepatocytes, Metzer Farms

3. Laboratory equipment and supplies.

4. Media and reagents:

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

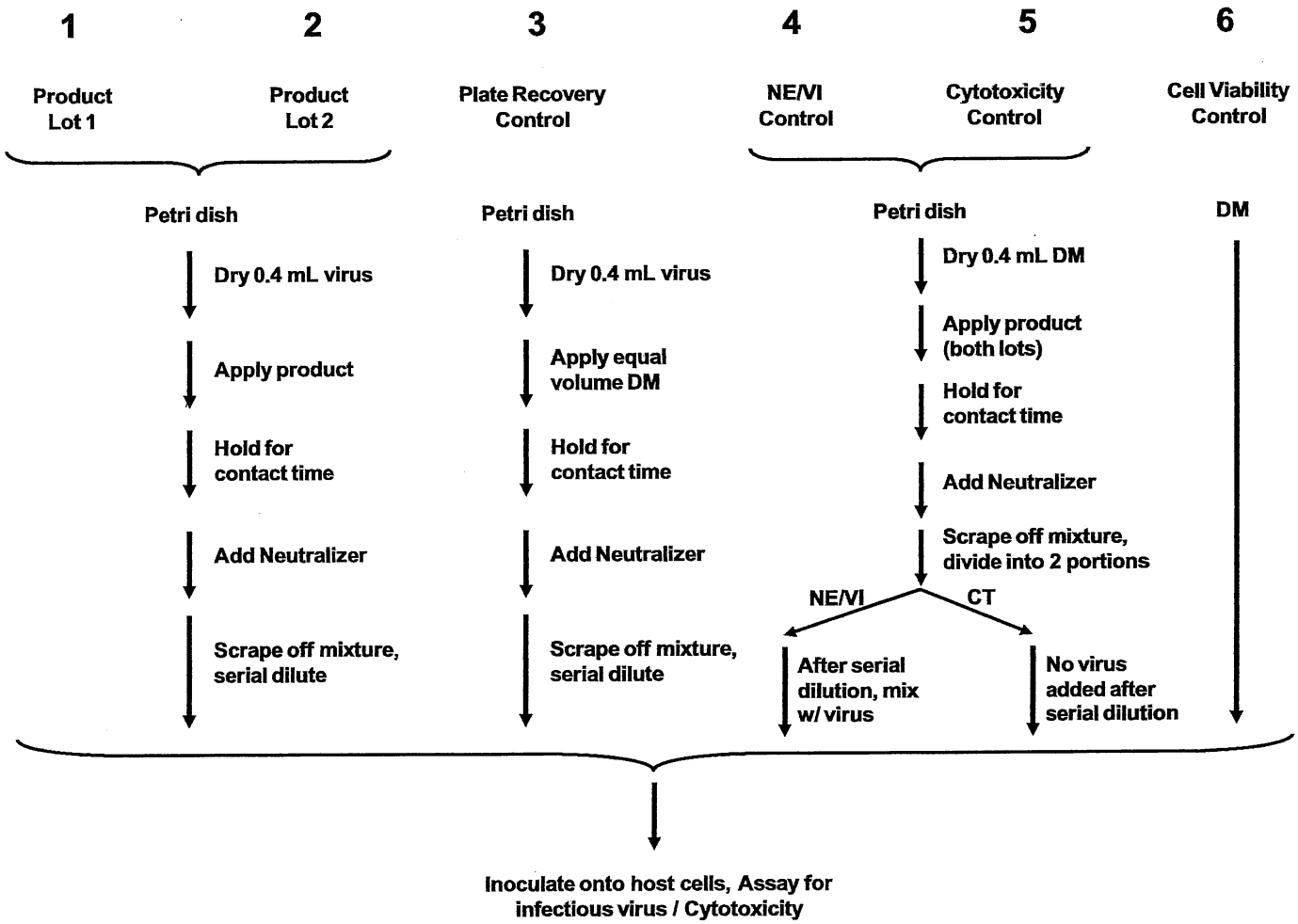
TEST SYSTEM IDENTIFICATION:

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

EXPERIMENTAL DESIGN:

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

FIGURE 1



DM: Dilution Medium
NE/VI: Neutralizer Effectiveness/Viral Interference control
CT: Cytotoxicity Control

*Note: Two lots of the test substance (liquid) will be tested at **two contact times** and two replicates (N=2).*

A. Inoculum preparation:

Viral stocks are purchased from reputable sources that identify them by scientifically accepted methods and may have been 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. The serum content of the virus stock is 100% duck serum. No additional serum (such as fetal bovine serum) will be added to the virus stock.

Note: a level of approximately 4.8 – 6.8 Log_{10} virus challenge (as indicated by the plate recovery control load) when there is no cytotoxicity associated with the test substance, or approximately 3.0 – 5.0 Log_{10} beyond the level of cytotoxicity when present, should be achieved whenever possible.

B. Carrier preparation:

For each lot and each contact time of the test substance, an aliquot of 0.4 mL of stock virus will be spread over an area of approximately 4 in^2 that has been marked on the underside of pre-sterilized glass Petri dishes. This volume will remain consistent among all test and control runs. Then the virus will be allowed to dry at ambient temperature. The drying time and temperature will be recorded.

Two carriers will be prepared for each lot and each contact time of the test substance using virus. Two carriers 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 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 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:

Two lots of the test substance (liquid) will be tested at two contact times and two replicates (N=2).

For direct liquid application test substance, for each run, after the inoculum has dried, 2.0 mL of the test agent will be added. The dried virus film must be completely covered by the test agent. The plates will remain at the temperature and for the time specified by the sponsor. After the contact period, the test agent 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 will be considered approximately a 10^{-1} dilution.

For spray type test substance, an aliquot of the test substance, ready-to-use, will be dispensed into a sterilized spray bottle. 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 for all applicable runs and for the Plate recovery control runs. Then the test substance will be sprayed onto the virus carriers in a horizontal position until thoroughly wet from a distance of 6" – 8". 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; and the 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.

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

E. Infectivity assay:

The residual infectious virus in both test and controls will be detected by immunofluorescent staining targeting the S envelop protein of DHBV (DHBsAg).

Selected dilutions of the neutralized inoculum/test substance mixtures will be inoculated onto Primary duck hepatocytes (four wells per dilution per reaction mixture) and incubated at 36±2°C in 5±3% CO_2 overnight (approximately 20-30 hours) for viral adsorption. After adsorption, the monolayer will be refed with media and returned to the above listed incubation conditions for a total of 10-14 days. During the incubation phase the media may be replaced with fresh media every 2-4 days to maintain the cells. After incubation the infectious DHBV will be assayed by immunofluorescence assay according to Microbac SOP M1006.VI.013 (current version).

F. Controls:

1. Plate recovery control (PRC):

This control will be performed in duplicate runs, concurrently with the test substance runs, using the longer contact time as a worst-case scenario.

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. Post-contact time, virus will be subjected to the identical neutralization procedure as the test substance. This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

The results from this control will be compared with the test results to confirm recovery of at least 4.8-Log_{10} of infectious virus in this control following drying and neutralization. Its titer will be used to compare with the titers of the test results to reach the acceptable test criteria (see below).

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

This 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 both lots of test substance at one replicate, using the longer contact time as a worst-case scenario.

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 cytotoxicity control and the other for neutralizer effectiveness/viral interference control and processed as 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. Then these samples will be used to inoculate host cells as described for the test procedure.

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 the "Infectivity Assay" section.

3. Cytotoxicity control (CT):

This control will be performed for both lots of test substance at one replicate, using the longer contact time as a worst-case scenario.

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

G. Calculation:

The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber (Kärber G., Arch. Exp. Pathol. Pharmakol. 1931, 162: 480-483) or other appropriate methods such as Reed and Muench (Am. J. of Hyg. 1938, 27:493). In the case where a sample contains no detectable virus, a statistical analysis may be performed based on Poisson distribution (International Conference on Harmonization, Topic Q5A, 1999: 24-25) to determine the theoretical maximum possible titer for that sample. These analyses will be described in detail in the final report. The test results will be reported as reduction of the virus titer post treatment with the test article expressed as log_{10}.

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

The Log_{10} Reduction Factor (LRF) will be calculated in the following manner:
Log_{10} Reduction Factor = Initial viral load (Log_{10} $TCID_{50}$) – Output viral load (Log_{10} $TCID_{50}$)

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

TEST SUBSTANCE EVALUATION CRITERIA:

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

- The product must demonstrate a ≥ 3 log_{10} reduction on each surface in the presence or absence of cytotoxicity; and
- If cytotoxicity is present, the virus control titer should be increased if necessary to demonstrate a ≥ 3 log_{10} reduction in viral titer on each surface beyond the cytotoxic 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, 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 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 of the inspections and the dates that findings are reported to the study management and 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:

Microbac employs a standard report format for each test design. Each final report will provide at least the following information:

- Sponsor identification
- Test substance identification
- Type of assay and project number
- Dates of study initiation and completion
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)
- Certificate of Analysis, if provided by the Sponsor (for GLP studies only)

RECORDS TO BE MAINTAINED:

For all GLP studies, the original signed final report will be sent to the Sponsor.

All raw data, protocol, protocol modifications, test substance records, copy of final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac, 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 and host cell line monolayers used 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 initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

HEP B

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. Name and address: Envirocleanse LLC
12621 W. Airport Blvd., Ste. 200
Sugar Land, TX 77478

B. Test substance information:

Test substance name	ANOLITE	
Batch (Lot) No.	Batch (Lot) 1	Batch (Lot) 2
	110218	10/9/18
Active ingredient(s)	HYPOCHLOROUS ACID	HYPOCHLOROUS ACID
Manufacture Date	11-2-18	10-19-18
Expiration Date	1-2-19	12-19-18
Lower Certified Limit (LCL)?	☒ Yes ☐ No ☐ N/A	☒ Yes ☐ No ☐ N/A
Dilution	■ Ready to use; or ☐ Dilute _____ part(s) concentrate + _____part(s) diluent	
Diluent	■ Not applicable ☐ _____ ppm ±2.9% AOAC hard water ☐ Other _____	
Spray application	☒ Spray until thoroughly wet; from a distance of 6 – 8 inches	
Contact temperature	Room Temperature (20±1°C)	
Contact time #1	☒ 2 minute(s) 0 seconds	
Contact time #2	☒ 10 minute(s) 0 seconds	
Organic Load	DHBV inoculum contains 100% duck serum	

Continued on next page

MISCELLANEOUS INFORMATION (Continued):

C. Precautions/storage conditions: MSDS and/or CofA provided: ☑ Yes ☐ No

(Note: CofA, if provided, will be included in the final report.) DARK AMBIENT ROOM TEMPERATURE

REPORT HANDLING:

The sponsor intends to submit this information to: ☑ US EPA ☐ Other: _____

STUDY CONDUCT: GLP

PROTOCOL APPROVAL BY SPONSOR:

Sponsor Signature: _____ Date: 11-5-18

Printed Name: _____

PROTOCOL APPROVAL BY STUDY DIRECTOR (Microbac):

Study Director Signature: _____ Date: 11/08/2018

Printed Name: Zheng Chen

Date Issued: 11/08/18 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 668-118

STUDY TITLE: VIRUCIDAL HARD-SURFACE EFFICACY TEST – Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus)	**STUDY DIRECTOR:** Zheng Chen, M.S. Signature 11/08/18 Date		
TEST MATERIAL(S): Envirocleanse A	**BATCH (LOT)** 110218 101918	**DATE RECEIVED:** 11/06/18 11/06/18	**DS NO.** I641 I642
PERFORMING DEPARTMENT(S): Virology and Toxicology	**STORAGE CONDITIONS:** Location: J6 ■ Dark ■Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐Refrigerator ☐ Other:		
PROTECTIVE PRECAUTION REQUIRED: MSDS ■ Yes / ☐ No			
PHYSICAL DESCRIPTION: ☐ Solid ■Liquid ☐ Aerosol ☐ Other:			
PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.			
PROPOSED EXPERIMENTAL START DATE: 11/08/18 **TERMINATION DATE:** 11/21/18			
CONDUCT OF STUDY: ☐ FDA ■ EPA ☐R&D ■ GLP ☐ GCP ☐ Other:			
SPONSOR: Envirocleanse LLC 12621 W Airport Blvd., Ste. 200 Sugar Land, TX 77478	**CONTACT PERSON:** Scott Mack Email: smack@eco-enviro.com		

TEST CONDITIONS:

Challenge organism: Duck Hepatitis B Virus (DHBV), Strain: Grimaud, HepadnaVirus Testing, Inc.

Host cell line: Primary Duck Hepatocytes, Metzer Farms

Organic load: 100% duck serum in viral inoculum

Dilution medium: L-15 Complete

Active ingredient(s): Hypochlorous Acid (HOCl)

Neutralizer: L-15 Complete + 10% Fetal Bovine Serum + 0.5% $Na_2S_2O_3$ + 0.5% Polysorbate-80

Dilution: Ready to use

Contact time(s): 2 minutes
10 minutes

Contact temperature: Room Temperature (20±1°C)

Incubation time: 10-14 days

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

Note: Spray until thoroughly wet from a distance of 6 – 8 inches

PROTOCOL AMENDMENT(S):

1. On page 13, Miscellaneous Information, section B of the protocol, the test substance name is listed as "Anolite". Per the sponsor, this should be "Envirocleanse A". This amendment serves to clarify this section of the protocol.

2. On page 13, Miscellaneous Information, section B of the protocol, there are write overs for the manufacture date and expiration date for batch 1. These should be "11-2-18" and "1-2-19" respectively. This amendment serves to clarify this section of the protocol.

APPENDIX II

CERTIFICATE OF ANALYSIS

Product: Envirocleanse A

Active Ingredient: Hypochlorous acid (CAS No. 7790-92-3)

Lot: 101918

Date of Production: 10/19/2018

Analysis: Total Chlorine

Date of Analysis: 11/06/2018

Method: Iodometric Determination using Sodium Thiosulfate (HACH Method 8209 by Digital Titrator)

Result:

% Total Chlorine	% HOCl
0.0431	0.0319

EMSL Analytical, Inc.
5950 Fairbanks N. Houston Rd, Houston, TX 77040
Phone: (713) 686-3635 Fax: (713) 686-3645 Web: www.emsl.com

CERTIFICATE OF ANALYSIS

Product: Envirocleanse A

Active Ingredient: Hypochlorous acid (CAS No. 7790-92-3)

Lot: 110218

Date of Production: 11/02/2018

Analysis: Total Chlorine

Date of Analysis: 11/06/2018

Method: Iodometric Determination using Sodium Thiosulfate
(HACH Method 8209 by Digital Titrator)

Result:

% Total Chlorine	% HOCl
0.0460	0.0341

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