

Volume \_\_\_\_\_

**FINAL REPORT**

**INITIAL VIRUCIDAL EFFICACY TEST – Duck Hepatitis B Virus  
(Surrogate for Human Hepatitis B Virus)**

**Test Agent**

**SNIPER<sup>®</sup>**

**Lot Number**

**108-171-2**

**108-172-1**

**Test Organism**

**Duck Hepatitis B Virus, HepadnaVirus Testing**

**Test Guideline**

**EPA Guidelines 810.2200 (f)(2)**

**Author**

**S. Steve Zhou, Ph. D**

**Study Completion Date**

**11/27/2012**

**Performing Laboratory**

**MICROBIOTEST**

**A Division of Microbac Laboratories, Inc.**

**105 Carpenter Drive**

**Sterling, Virginia 20164**

**Laboratory Project Identification Number**

**813-105**

**Protocol Identification Number**

**813.2.11.01.12**

**Sponsor**

**GER, Inc.**

**P.O. Box 667**

**Carencro, LA 70507**

## STATEMENT OF NO DATA CONFIDENTIALITY

Title: INITIAL VIRUCIDAL EFFICACY TEST – Duck Hepatitis B Virus  
(Surrogate for Human Hepatitis B Virus)

Performed by: MICROBIOTEST  
A Division of Microbac Laboratories, Inc.  
105 Carpenter Drive  
Sterling, Virginia 20164

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA sec. 10(d)(1)(A), (B) or (C).

Submitter signature: \_\_\_\_\_ Date: \_\_\_\_\_

Typed Name of Signer: \_\_\_\_\_

Typed Name of Company: \_\_\_\_\_ GER Inc. \_\_\_\_\_

## 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 agent resides with the sponsor of the study.

Study Director signature: Salimatu Lukula Date: 11/27/2012  
Typed Name: Salimatu Lukula, M.S.  
Typed Name of Laboratory: MicroBioTest, a division of Microbac Laboratories, Inc.

Sponsor signature: \_\_\_\_\_ Date: \_\_\_\_\_  
Typed Name of Signer: \_\_\_\_\_  
Typed Name of Company: GER Inc.


Submitter signature: \_\_\_\_\_ Date: \_\_\_\_\_  
Typed Name of Signer: \_\_\_\_\_  
Typed Name of Company: GER Inc.

### QUALITY ASSURANCE UNIT STATEMENT

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

The Quality Assurance Unit of MICROBIOTEST has inspected Project Number 813-105 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.

<u>PHASE INSPECTED</u>	<u>DATE OF INSPECTION</u>	<u>DATE REPORTED TO STUDY DIRECTOR</u>	<u>DATE REPORTED TO MANAGEMENT</u>
Protocol	11/07/12	11/07/12	11/07/12
In Process (Test)	11/07/12	11/07/12	11/07/12
Final Report	11/21/12	11/21/12	11/21/12
	 _____ Jeanne M. Anderegg Manager, Quality Assurance Unit		<u>11-27-12</u> _____ Date

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## **TEST SUMMARY**

**TITLE:** INITIAL VIRUCIDAL 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).

### **TEST MATERIALS SUPPLIED BY THE SPONSOR OF THE STUDY:**

1. SNIPER®; Lot No. 108-171-2; received at MICROBIOTEST on 11/02/12; and assigned DS No. C849
2. SNIPER®; Lot No. 108-172-1; received at MICROBIOTEST on 11/02/12; and assigned DS No. C850

**SPONSOR:** GER, Inc.  
P.O. Box 667  
Carencro, LA 70507

## TEST CONDITIONS

Challenge virus:

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

Host:

Primary Duck Hepatocyte cells  
Duckling source: Metzger Farms

Active ingredient in test product:

Chlorine Dioxide ( $\text{ClO}_2$ )

Neutralizer:

Fetal Bovine Serum (FBS) + 0.5%  $\text{Na}_2\text{S}_2\text{O}_3$

Dilution medium/cell culture medium:

L-15 Complete

Contact time:

5 minutes and 10 minutes

Contact temperature and relative humidity:

Ambient Room Temperature (20C); at 21.3 - 21.4% RH

Carriers:

Glass petri dishes

Carrier inoculation/dry time:

2 x 2 inch area of glass carrier inoculated with 0.4 mL of virus and dried  
for 30 minutes at 19-20C and 20.6 - 21.3% RH

Organic load:

Virus stock contained 100% duck serum

### **TEST CONDITIONS (continued)**

Dilution:

Ready to use

Media and reagents:

L-15 Complete  
Fetal Bovine Serum + 0.5% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>  
Phosphate Buffered Saline  
Anti-DHBV envelope IgG MAb  
Goat anti-mouse FITC-conjugated antibody  
80% Acetone

### **STUDY DATES AND FACILITIES**

The laboratory phase of this test was performed at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164, from 11/06/12 to 11/19/12. The study director signed the protocol 11/06/12. On the day of test conduct on 11/06/12, the testing started at 1:00 pm and ended at 2:40 pm. The study completion date is the date the study director signed the final report.

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 material records, the final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 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<sub>50</sub>/mL) was determined using the Spearman-Kärber method using the following formula:

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

where:

- m = the logarithm of the titer relative to the test volume
- x<sub>k</sub> = the logarithm of the smallest dosage which induces infection in all cultures
- d = the logarithm of the dilution factor
- p<sub>i</sub> = the proportion of positive results at dilution i

The values were converted to TCID<sub>50</sub>/mL using a sample inoculum of 1.0mL.

## RESULTS

Data are presented in Tables 1 – 7.

The Log<sub>10</sub> Reduction Factor (LRF) was calculated in the following manner:

$$\text{Log}_{10} \text{ Reduction} = \text{Log}_{10} \text{ TCID}_{50} (\text{Plate Recovery Control}) - \text{Log}_{10} \text{ TCID}_{50} (\text{Test})$$

The Load (Log<sub>10</sub> TCID<sub>50</sub>) per carrier was calculated in the following manner:

$$\text{Load (Log}_{10} \text{ TCID}_{50}) = \text{Titer (Log}_{10} \text{ TCID}_{50}/\text{mL}) + \text{Log}_{10} [\text{volume per carrier (mL)}]$$

The Average Viral Log<sub>10</sub> Load from n replicates was determined as follows:

$$\text{Log}_{10} \left[ \frac{10^{(\text{Log}_{10} \text{Load}_1)} + 10^{(\text{Log}_{10} \text{Load}_2)} + \dots + 10^{(\text{Log}_{10} \text{Load}_n)}}{n} \right]$$

## RESULTS (continued)

### Key (for all tables):

C/y= Cytotoxicity observed in y wells inoculated

X/y = virus was detected by immunofluorescence observed in X wells out of y wells inoculated

0/y = no virus was detected by lack of immunofluorescence observed in y wells inoculated; no cytotoxicity observed

**Table 1**  
**Test Agent Results**

Dilution*	SNIPER® Lot No. 108-171-2			
	5 minutes		10 minutes	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
$10^{-2}$	C/4	C/4	C/4	C/4
$10^{-3}$	0/4	0/4	0/4	0/4
$10^{-4}$	0/4	0/4	0/4	0/4
$10^{-5}$	0/4	0/4	0/4	0/4
$10^{-6}$	0/4	0/4	0/4	0/4
$10^{-7}$	0/4	0/4	0/4	0/4
Titer ( $\text{Log}_{10}$ TCID <sub>50</sub> /mL)	≤ 2.50	≤ 2.50	≤ 2.50	≤ 2.50
<b>Load (<math>\text{Log}_{10}</math> TCID<sub>50</sub>) per carrier (0.4 mL challenge)</b>	<b>≤ 2.10</b>	<b>≤ 2.10</b>	<b>≤ 2.10</b>	<b>≤ 2.10</b>
<b><math>\text{Log}_{10}</math> Reduction</b>	<b>≥ 3.25</b>	<b>≥ 3.25</b>	<b>≥ 3.14</b>	<b>≥ 3.14</b>

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

## RESULTS (continued)

**Table 2**  
**Test Agent Results**

Dilution*	SNIPER® Lot No. 108-172-1			
	5 minutes		10 minutes	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
$10^{-2}$	C/4	C/4	C/4	C/4
$10^{-3}$	0/4	0/4	0/4	0/4
$10^{-4}$	0/4	0/4	0/4	0/4
$10^{-5}$	0/4	0/4	0/4	0/4
$10^{-6}$	0/4	0/4	0/4	0/4
$10^{-7}$	0/4	0/4	0/4	0/4
Titer ( $\text{Log}_{10}$ TCID <sub>50</sub> /mL)	≤ 2.50	≤ 2.50	≤ 2.50	≤ 2.50
<b>Load (<math>\text{Log}_{10}</math> TCID<sub>50</sub>) per carrier (0.4 mL challenge)</b>	<b>≤ 2.10</b>	<b>≤ 2.10</b>	<b>≤ 2.10</b>	<b>≤ 2.10</b>
<b>Log<sub>10</sub> Reduction</b>	<b>≥ 3.25</b>	<b>≥ 3.25</b>	<b>≥ 3.14</b>	<b>≥ 3.14</b>

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

**Table 3**  
**Neutralizer Effectiveness and Cytotoxicity Related Controls**

Dilution*	SNIPER® (Lot No.108-171-2)	
	Neutralizer Effectiveness Control	Cytotoxicity Control
$10^{-2}$	C/4	C/4
$10^{-3}$	4/4	0/4
$10^{-4}$	4/4	0/4

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

## RESULTS (continued)

**Table 4**  
**Neutralizer Effectiveness and Cytotoxicity Related Controls**

Dilution*	SNIPER® (Lot No.108-172-1)	
	Neutralizer Effectiveness Control	Cytotoxicity Control
10 <sup>-2</sup>	C/4	C/4
10 <sup>-3</sup>	4/4	0/4
10 <sup>-4</sup>	4/4	0/4

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

**Table 5**  
**Virus Recovery Controls**

Dilution*	Plate Recovery Control			
	5 minutes		10 minutes	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
10 <sup>-2</sup>	4/4	4/4	4/4	4/4
10 <sup>-3</sup>	4/4	4/4	4/4	4/4
10 <sup>-4</sup>	4/4	4/4	4/4	4/4
10 <sup>-5</sup>	3/4	4/4	2/4	3/4
10 <sup>-6</sup>	2/4	1/4	2/4	2/4
10 <sup>-7</sup>	0/4	0/4	0/4	0/4
Titer (Log <sub>10</sub> TCID <sub>50</sub> /mL)	5.75	5.75	5.50	5.75
<b>Load (Log<sub>10</sub> TCID<sub>50</sub>) per carrier (0.4 mL challenge)</b>	<b>5.35</b>	<b>5.35</b>	<b>5.10</b>	<b>5.35</b>
<b>Average Load (Log<sub>10</sub> TCID<sub>50</sub>) per carrier</b>	<b>5.35</b>		<b>5.24</b>	

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

## RESULTS (continued)

**Table 6**  
**Viability Control Results**

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

**Table 7**  
**Virus Recovery Controls**

Dilution*	Virus Titer Control
$10^{-3}$	4/4
$10^{-4}$	4/4
$10^{-5}$	4/4
$10^{-6}$	3/4
$10^{-7}$	1/4
$10^{-8}$	0/4
<b>Titer (<math>\text{Log}_{10} \text{TCID}_{50}/\text{mL}</math>)</b>	<b>6.50</b>

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

## CONCLUSIONS

According to the regulatory agencies, the test agent passes the Virucidal Efficacy Test if there is complete inactivation of the challenge virus at all dilutions. When cytotoxicity is evident, at least a three-log reduction in titer must be demonstrated beyond the cytotoxic level.

When tested as described, SNIPER® (Lot No. 108-171-2 and 108-172-1) passed the Virucidal Efficacy Test when Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus), containing  $\geq 5\%$  serum, was exposed to both lots of the test agent for 5 minutes and 10 minutes at 20C. All of the controls met the criteria for a valid test. These conclusions are based on observed data.

## APPENDIX



**MICROBIOTEST**

A Division of Microbac Laboratories, Inc.  
105-B Carpenter Drive  
Sterling, VA 20164

## **MICROBIOTEST PROTOCOL**

### **INITIAL VIRUCIDAL EFFICACY TEST**

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

Testing Facility  
**MICROBIOTEST**

A Division of Microbac Laboratories, Inc.  
105 Carpenter Drive  
Sterling, VA 20164

Prepared for  
**GER, Inc.**  
P.O. Box 667  
Carencro, LA 70507

**July 27, 2012**

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MICROBIOTEST Protocol: 813.2.11.01.12

MICROBIOTEST Project: 813-105

## OBJECTIVE:

This test is designed to substantiate virucidal effectiveness claims for a product to be labeled as a virucide. It determines the potential of the test agent to disinfect hard surfaces contaminated with Duck Hepatitis B Virus. The test is designed to simulate consumer use and conforms to EPA OCSP 810.2000 and 810.2200 Product Performance Test Guidelines, and follows the procedure outlined in the American Society for Test Materials (ASTM) test method designated E1053.

## TESTING CONDITIONS:

Virus will be dried on a suitable sterile hard surface at ambient temperature. Two lots of one type of test agent will be used to treat the dried viruses. After a defined exposure period as specified by the sponsor, the test agent-virus mixture will be scraped from the surface, collected, neutralized and tested for the presence of infectious virions. One concentration of the test agent will be evaluated at two exposure (contact) times. Two replicate runs will be performed for each test condition.

## MATERIALS:

- A. Test, control and reference substances will be supplied by the sponsor of the study (see last page).

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

The sponsor assures MICROBIOTEST testing facility management that the test agent has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MICROBIOTEST will retain all unused test agents for a period of at least three months after completion of the test, and then discard them in a manner that meets the approval of the safety officer.



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

1. Challenge virus requested by the sponsor of the study: Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus)
2. Host cell lines: primary duck hepatocytes
3. Laboratory equipment and supplies.
4. Media and reagents:

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

**TEST SYSTEM IDENTIFICATION:**

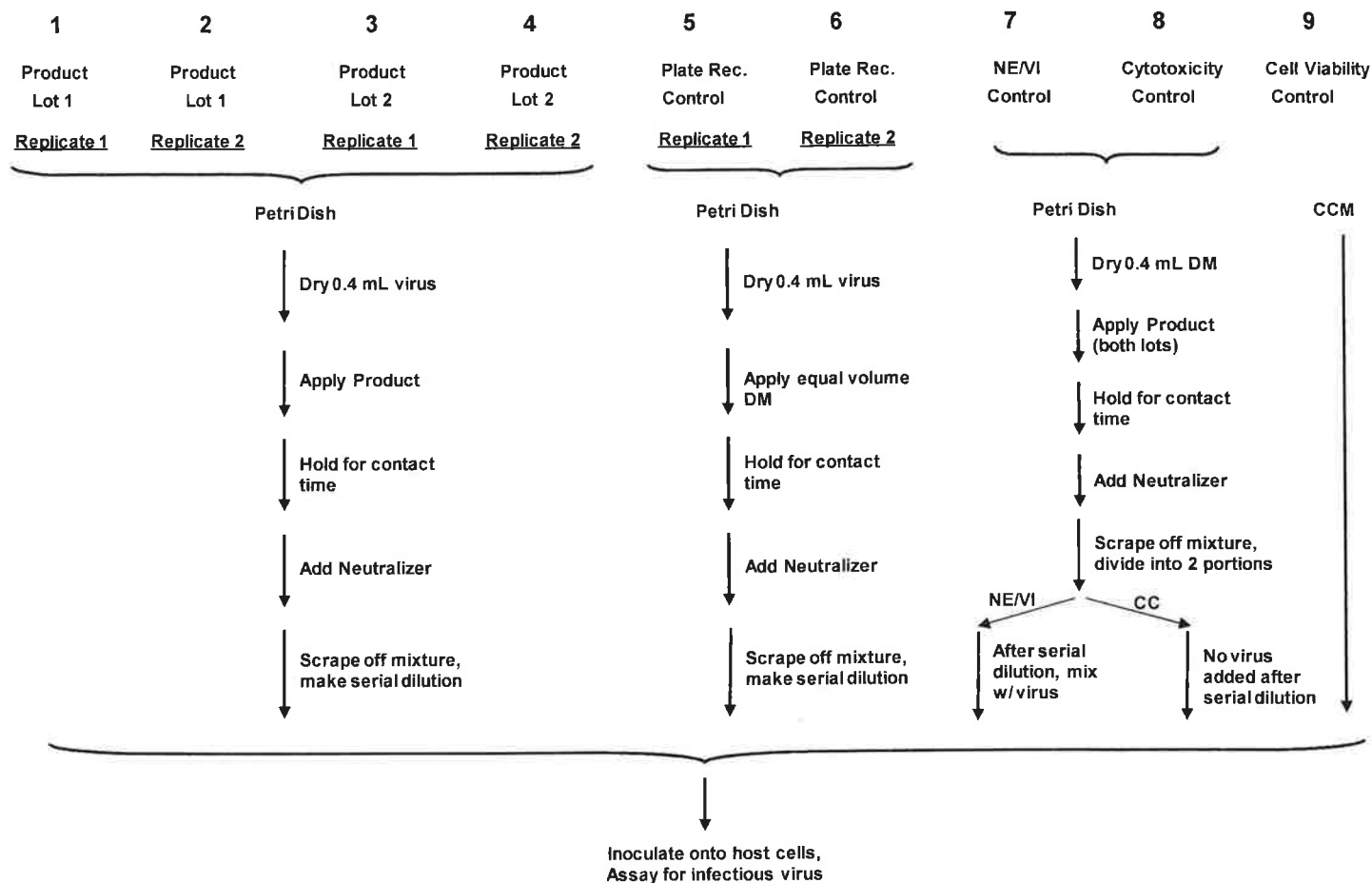
All Petri dishes, dilution tube racks, and host-containing apparatus will be appropriately labeled with the following information: virus, host, and test agent and/or project number.

**EXPERIMENTAL DESIGN:**

All of the procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at MICROBIOTEST. 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

CC: Cytotoxicity Control

*Note 1: The volume of the virus to be applied onto each carrier may be changed depending on the titer of the virus. This volume will remain consistent among all test and control runs.*

*Note 2: Two contact times will be tested for the Product and Plate Recovery Control. The NE/VI and CC controls will be tested on the longer contact time only as a worst case scenario.*

A. Inoculum preparation:

Viral stocks are purchased from reputable sources that identify them by scientifically accepted methods and may have been propagated at MICROBIOTEST. 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 (fresh stock cultures may be used at the discretion of the Study Director). Serum will be added to viral stock to achieve an organic load of 5% (if not already 5% or above) unless otherwise directed by the Sponsor.

B. Carrier preparation:

For each lot of the test agent, an aliquot of 0.4 mL of stock virus will be spread over an area of approximately 4 in<sup>2</sup> that has been marked on the underside of pre-sterilized glass Petri dishes. Note: The volume of the test virus to be applied onto each carrier may be changed at the discretion of the Study Director. 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.

Eight carriers (two carriers per lot per contact time) will be prepared for the test agent using virus. Four carriers will be prepared for the plate recovery control using virus (two carriers per contact time). Additionally, two carriers (one carrier per lot, longer contact time only) will be prepared for the neutralizer effectiveness/viral interference and cytotoxicity controls using dilution medium (DM) in lieu of virus.

C. Test agent preparation:

The test agent will be prepared exactly according to the sponsor's directions.

D. Test:

Two lots of the test agent will be tested at two contact times. Two replicates will be tested for each lot and each contact time of the test agent.

For direct liquid application products, for each replicate 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 agents, the agent will be used as the sponsor directs, the volume dispensed will be measured and an equal volume of neutralizer will be used. Following the contact time, the procedure for processing the samples will be the same as described earlier.

If Sephadex columns are used to aid in the neutralization and to further reduce the cytotoxicity, each inoculum/test agent/neutralizer mixture sample will be loaded onto a pre-spun Sephadex 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 agent/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 agent mixtures will be inoculated onto Primary duck hepatocytes (four wells per dilution per reaction mixture) and incubated at  $36\pm 2^{\circ}\text{C}$  in  $5\pm 1\%$   $\text{CO}_2$  for 20-30 hours for viral adsorption. After adsorption, the monolayer will be refed with CCM and returned to the above listed incubation conditions for an additional 9-13 days. During the incubation phase the media may be replaced with fresh CCM every 2-4 days to maintain the cells. After incubation the infectious DHBV will be assayed by immunofluorescence assay according to MICROBIOTEST SOP M1006.VI.013 (current version).

F. Controls:

1. Plate recovery control:

This control will be performed in duplicate runs on each of the two contact times. 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 agent will be added to the dried virus. Post-contact time, virus will be subjected to the identical neutralization procedure as the test agent. 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 4.0-log 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:

This control will determine if residual active ingredient is present after neutralization and if the neutralized test agent interferes with the virus infection system. This control will be performed on both lots of the product at one replicate per lot using the longer contact time only as a worst-case scenario.

The test agent will be processed exactly as the test procedure but in lieu of virus inoculum, dried DM will be exposed to the test agent and assayed as previously described. Post-treatment and neutralization, the neutralized DM/test agent 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, the neutralizer effectiveness sample (0.5 mL) will be directly diluted using serial ten-fold dilutions in DM.

Following serial dilution of the reaction mixture in DM, virus (100 µL of a low titer virus inoculum) will be added to each dilution and held for a period

equivalent or greater than the contact time. Then the selected dilutions will be used to inoculate host cells as described for the test procedure.

3. Cytotoxicity control:

This control will be performed on both lots of the product at one replicate per lot using the longer contact time only 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.

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 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)

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 infectious dose per mL (TCID<sub>50</sub>/mL) will be determined using the method of Spearman-Kärber, or another appropriate method. The test results will be reported as the reduction of the virus titer due to treatment with test agent expressed as log<sub>10</sub>.

**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 MICROBIOTEST, 105 Carpenter Drive, Sterling, Virginia 20164.

**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  $\geq 4.0\text{-log}_{10}$ .
- Viral-induced cytopathic effect must be distinguishable from test agent 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.

**PRODUCT EVALUATION CRITERIA:**

According to the regulatory agencies, the test agent passes the test if there is complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a three-log reduction in titer must be demonstrated beyond the cytotoxic level.

## **REPORT FORMAT:**

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

- Sponsor identification
- Test agent 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)

## **RECORDS TO BE MAINTAINED:**

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 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 agent; 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.



**MISCELLANEOUS INFORMATION:**

The following information is to be completed by the sponsor prior to initiation of the study:

- A. Name and address: GER, Inc.  
P.O. Box 667  
Carencro, LA 70507
- B. Test Agent Name: SNIPER<sup>®</sup>  
Active Ingredient: Chlorine Dioxide (ClO<sub>2</sub>)  
Lot 1 number: 108-172-1; ≥60 day aged: ☐ yes ☒ no  
(Manufacture or expiration date: 10-30-12)  
Lot 2 number: 108-171-2; ≥60 day aged: ☒ yes ☐ no  
(Manufacture or expiration date: 8/02/11)
- C. Testing conditions:
- Dilution to be tested: ☒ Ready to use; or  
☐ \_\_\_\_\_ (\_\_\_\_\_ part test agent + \_\_\_\_\_ parts diluent)
- Diluent: ☒ not applicable (ready to use)  
☐ Other: \_\_\_\_\_
- Exposure (Contact) time 1: ☐ 5 minutes  
Exposure (Contact) time 2: ☐ 10 minutes  
Exposure temperature: ☒ Room temperature (20±1C)
- Spray application: ☐ not applicable  
☐ \_\_\_\_\_ number of sprays  
☒ until thoroughly wet
- Spraying distance: ☐ not applicable  
☒ 6 – 8 inches  
☐ Other: \_\_\_\_\_
- D. Organic load in virus inoculum: ☒ ≥ 5% serum; or ☐ \_\_\_\_\_
- E. Precautions/storage conditions: MSDS and/or C of A provided: ☒ yes ☐ no

F. Note: prior to dispensing, the bottles of test agent should be gently shaken 2-3 times.  
*Continued on next page.*

**MISCELLANEOUS INFORMATION: (continued)**

**REPORT HANDLING:**

The sponsor intends to submit this information to:

☒ US EPA      ☐ US FDA      ☐ Health Canada      ☐ CAL DPR  
☐ ARTG      ☐ other: Internal Purposes

**STUDY CONDUCT:**   ☐ GLP   ☐ non-GLP

**PROTOCOL APPROVAL BY SPONSOR:**

Sponsor Signature: Alan Buel Campbell      Date: 11/11/12

Sponsor Printed Name: Alan Buel Campbell

**PROTOCOL APPROVAL BY STUDY DIRECTOR (MICROBIOTEST):**

Study Director Signature: Salimatu Lukula      Date: 11/16/2012

**Salimatu Lukula**

Study Director Printed Name: \_\_\_\_\_

Date Issued: 11/06/12 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 813-105			
<b>STUDY TITLE:</b> INITIAL VIRUCIDAL EFFICACY TEST – Duck Hepatitis B Virus (surrogate for Human Hepatitis B Virus)		<b>STUDY DIRECTOR:</b> Salimatu Lukula, M.S. <i>Salimatu Lukula</i> 11/06/2012 Signature Date	
<b>TEST AGENT (S):</b> SNIPER®	<b>LOT NO.:</b> 108-171-2 108-172-1	<b>DATE RECEIVED:</b> 11/02/12 11/02/12	<b>DS NO.:</b> C849 C850
<b>PERFORMING DEPARTMENT(S):</b> Virology	<b>STORAGE CONDITION:</b> Location: C5 <input checked="" type="checkbox"/> Dark <input checked="" type="checkbox"/> Ambient Room Temperature <input type="checkbox"/> Desiccator <input type="checkbox"/> Freezer <input type="checkbox"/> Refrigerator		
<b>PROTECTIVE PRECAUTION REQUIRED:</b> MSDS <input checked="" type="checkbox"/> Yes / <input type="checkbox"/> No			
<b>PHYSICAL DESCRIPTION:</b> <input type="checkbox"/> Solid <input checked="" type="checkbox"/> Liquid <input type="checkbox"/> Aerosol <input type="checkbox"/> Other:			
<b>PURPOSE:</b> See attached protocol. <b>AUTHORIZATION:</b> See client signature.			
<b>PROPOSED EXPERIMENTAL START DATE:</b> 11/06/12 <b>TERMINATION DATE:</b> 11/30/12			
<b>CONDUCT OF STUDY:</b> <input type="checkbox"/> FDA <input checked="" type="checkbox"/> EPA <input type="checkbox"/> R&D <input checked="" type="checkbox"/> GLP <input type="checkbox"/> GCP <input type="checkbox"/> Other:			
<b>SPONSOR:</b> GER, Inc. P.O. Box 667 Carencro, LA 70507		<b>CONTACT PERSON:</b> Alan Bud Campbell Telephone No. 337-235-4710 Email: alanbud@environmentrestoration.com	
<b>TEST CONDITIONS:</b>			
Challenge organism:	Duck Hepatitis B Virus (DHBV), HepadnaVirus Testing		
Host cell line:	Primary Duck Hepatocyte cells, Metzger Farm (duckling source)		
Active ingredient(s):	Chlorine Dioxide (ClO <sub>2</sub> )		
Dilution(s):	Ready to Use		
Neutralizer:	Fetal Bovine Serum + 0.5% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>		
Dilution Medium:	L-15 complete		
Organic load:	Virus contains 100% duck serum		
Exposure time:	5 minutes and 10 minutes		
Exposure temperature:	Room temperature (20±1C)		
Incubation time:	10 - 14 days	Incubation temperature: 36±2C and 5±1%CO <sub>2</sub>	
Comments:	Prior to dispensing, the bottles of test agent will be gently shaken 2-3 times. Carriers will be sprayed from a distance of 6-8 inches until thoroughly wet.		

Date Issued: 11/27/12 Project Sheet No. 2 Page No. 1 Laboratory Project Identification No. 813-105

**STUDY TITLE:** INITIAL VIRUCIDAL EFFICACY TEST – Duck Hepatitis B Virus (surrogate for Human Hepatitis B Virus)**STUDY DIRECTOR:** Salimatu Lukula, M.S.

*Salimatu Lukula* 11/27/12  
 Signature Date

**TEST AGENT (S):**

SNIPER®

**LOT NO.:**

108-171-2

108-172-1

**DATE RECEIVED:**

11/02/12

11/02/12

**DS NO.:**

C849

C850

**PERFORMING DEPARTMENT(S):**

Virology and Molecular Biology

**STORAGE CONDITION:** Location: C5
☒ Dark   ☒ Ambient Room Temperature

☐ Desiccator   ☐ Freezer   ☐ Refrigerator
**CONDUCT OF STUDY:** ☐ FDA ☒ EPA ☐ R&D ☒ GLP ☐ GCP ☐ Other:**SPONSOR:** GER, Inc

P.O. Box 667

Carencro, LA 70507

**CONTACT PERSON:** Alan Bud Campbell

Telephone No. 337-235-4710

Email: alanbud@environmentrestoration.com

## Protocol Amendments:

1. Protocol, page 4 - figure 1 states CCM as the reagent for CVC. The correct statement is DM. This amendment serves to correct the typographical error on page 4.
2. Protocol, page 6, section E – The protocol states that “the monolayer will be refed with CCM...”. The correct statement is “the monolayer will be refed with DM....”. This amendment serves to correct the typographical error on page 6.
3. Protocol, Page 12- The study conduct is not specified. The conduct of the study is GLP. This amendment serves to clarify the study conduct.
4. Protocol, Page 11 – The exposure times are listed, but not checked. Per sponsor, the exposure times are 5 and 10 minutes. This amendment serves to clarify the exposure times.