



## FINAL STUDY REPORT

### STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces  
Utilizing Duck Hepatitis B Virus

### PRODUCT IDENTITY

ACCEL TB  
Lot 2-3646-REG-US and Lot 3-3647-REG-US

### DATA REQUIREMENT

Environmental Protection Agency Federal Register: August 25, 2000  
(Volume 65, Number 166)

### AUTHOR

Karen M. Ramm, B.A.  
Study Director

### STUDY COMPLETION DATE

April 26, 2005

### PERFORMING LABORATORY

ATS Labs  
1285 Corporate Center Drive, Suite 110  
Eagan, MN 55121

### SPONSOR

Virox Technologies  
2815 Bristol Circle, Unit 4  
Oakville, Ontario L6H6X5

### SPONSOR REPRESENTATIVE

Scientific & Regulatory Consultants, Inc.  
102 1/2 South Chauncey Street  
Columbia City, IN 46725-2306

### PROJECT NUMBER

A02454

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**STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS**

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

Company: Virox Technologies

Company Agent: Sally Hayes

Agent for Virox Technologies

Title

*Sally Hayes*  
Signature

Date: 04/27/05

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**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.

The procedures not performed by or under the direction of ATS Labs are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the test substance(s) and the harvesting of the primary hepatocyte cells.

Submitter: Sally Hayes  
Sally Hayes, Agent for Virox Technologies

Date: 04/27/05

Sponsor: Rhonda Jones  
Rhonda Jones, Agent for Virox Technologies

Date: 4-27-05

Study Director: Karen M. Ramm  
Karen M. Ramm, B.A.

Date: 4/26/05

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### QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. This study was inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director	Management
Critical Phase	November 22, 2004	November 22, 2004	April 26, 2005
Draft Report	December 10, 2004	December 10, 2004	
Final Report	April 26, 2005	April 26, 2005	

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor:

Rachelle L. Ewenson

Date:

04/26/05



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### STUDY PERSONNEL

STUDY DIRECTOR: Karen M. Ramm, B.A.

Professional personnel involved:

Kelleen Gutzmann, M.S. - Research Scientist II  
Katherine A. Paulson, M.L.T. - Research Assistant II

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## STUDY REPORT

### GENERAL STUDY INFORMATION

**Study Title:** Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus

**Project Number:** A02454

**Protocol Number:** SRC27022304.DHBV.1

**Sponsor:** Virox Technologies  
2815 Bristol Circle, Unit 4  
Oakville, Ontario L6H6X5

**Sponsor Representative:** Scientific & Regulatory Consultants, Inc.  
102 1/2 South Chauncey Street  
Columbia City, IN 46725-2306

**Test Facility:** ATS Labs  
1285 Corporate Center Drive, Suite 110  
Eagan, MN 55121

### TEST SUBSTANCE IDENTITY

**Test Substance Name:** ACCEL TB

**Lot/Batch(s):** Lot 2-3646-REG-US and Lot 3-3647-REG-US

#### **Test Substance Characterization**

Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The characterization of the BTC 835 validation control substance is the responsibility of the manufacturer. A certified copy of the Certificate of Analysis supplied by the manufacturer is maintained in the study file.

### STUDY DATES

**Date Sample Received:** March 11, 2004  
**Study Initiation Date:** September 29, 2004  
**Experimental Start Date:** November 22, 2004  
**Experimental End Date:** December 3, 2004  
**Study Completion Date:** April 26, 2005

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## OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a disinfectant against duck Hepatitis B virus according to test criteria and methods approved by the U.S. Environmental Protection Agency (EPA) for registration of a product as virucide. This study followed the guidelines set forth in the Environmental Protection Agency Federal Register: August 25, 2000 (Volume 65, Number 166).

Human Hepatitis B virus (HBV), a member of the Hepadnaviridae Family of enveloped DNA-containing viruses, presents a serious public safety concern. However, at present, there is no reliable *in vitro* infectivity assay for these viruses and the most reliable *in vivo* system uses primates (chimpanzees). Duck Hepatitis B virus, also a member of Hepadnaviridae Family, serves as a valuable model virus for human Hepatitis B virus, since these viruses share many similar characteristics.

## SUMMARY OF RESULTS

Test Substance:	ACCEL TB, Lot 2-3646-REG-US and Lot 3-3647-REG-US
Dilution:	Ready to use (RTU)
Virus:	Duck Hepatitis B virus
Exposure Time:	One minute
Exposure Temperature:	20±1°C
Organic Soil Load:	100% Duck serum
Efficacy Result:	Two lots of ACCEL TB met the test criteria specified in the study protocol. The results indicate <b>complete inactivation</b> of duck Hepatitis B virus under these test conditions.

## TEST SYSTEM

### 1. Virus

The DHBV16 strain of the duck Hepatitis B virus (DHBV) used in this assay was obtained from Hepadnavirus Testing, Palo Alto, CA and consists of duck Hepatitis B virus serum obtained from congenitally infected ducklings. The virus was stored at ≤ -70°C until the day of use. On the day of use, three aliquots of stock virus (Lot D2-H) were removed, thawed, combined and refrigerated until use in the assay. The stock virus cultures contained 100% duck serum as the organic soil load. The stock virus tested demonstrated fluorescence typical of DHBV on primary duck hepatocytes.

### 2. Indicator Cell Cultures

Cultures of primary duck hepatocytes were prepared utilizing ducklings received from Metzger Farms. Upon receipt, the ducklings were screened for the presence of the test virus via a polymerase chain reaction (PCR) specific for DHBV at ViroMed Laboratories, Inc. Only ducklings verified to be free of the test virus and less than seven days old were utilized in this assay. An *in situ* perfusion of the duck liver utilizing S-MEM containing collagenase was performed by an outside vendor. The hepatocytes were then seeded into sterile six well disposable tissue culture labware by personnel from ATS Labs. The cultures were maintained and used at the appropriate density and incubated at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub>.

All cell culture documentation will be retained for the cell cultures used in this assay with respect to source, summary of the perfusion process, seeding densities, and the general condition of the cells.

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### 3. Test Medium

Test medium used in this study was Leibovitz L-15 medium supplemented with 0.1% glucose, 10 $\mu$ M dexamethasone, 10 $\mu$ g/mL insulin, 20 mM HEPES, 10  $\mu$ g/mL gentamicin, and 100 units/mL penicillin.

The following table lists the test and control groups, the dilutions assayed, and the numbers of cultures used. See text for a more detailed explanation.

<b>SAMPLES TESTED FOR THE PRESENCE OF VIRUS</b>			
<b>Test or Control Group</b>	<b>Dilutions Assayed Per Carrier (log<sub>10</sub>)</b>	<b>Cultures per Dilution</b>	<b>Total Cultures Inoculated</b>
Negative Controls	N/A	4	2-4/group
Input Virus Control	-4,-5,-6,-7	4	16
Dried Virus Control (performed in duplicate)	-4,-5,-6,-7	4	32
Test Sample - Lot #1 (performed in duplicate)	-2,-3,-4	4	24
Test Sample - Lot #2 (performed in duplicate)	-2,-3,-4	4	24
Cytotoxicity Control - Lot #1	-2,-3	2	4
Cytotoxicity Control - Lot #2	-2,-3	2	4
Neutralization Control - Lot #1	-2,-3	2	4
Neutralization Control - Lot #2	-2,-3	2	4
175 ppm BTC 835 Validation Control (performed in duplicate)	-2,-3,-4	4	24
Cytotoxicity Control - 175 ppm BTC 835	-2,-3	2	4
Neutralization Control - 175 ppm BTC 835	-2,-3	2	4
350 ppm BTC 835 Validation Control (performed in duplicate)	-2,-3,-4	4	24
Cytotoxicity Control - 350 ppm BTC 835	-2,-3	2	4
Neutralization Control - 350 ppm BTC 835	-2,-3	2	4

## TEST METHOD

### 1. Preparation of Test Substance

Two lots of ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US) were used as received from the Sponsor. The test substance was in solution as determined by visual observation.

### 2. Preparation of the BTC Validation Control

BTC 835 (EPA Registration # 1839-32) received from Stepan Company, Northfield, IL was utilized as the validation control substance. The manufacture date is August 28, 2003. The expiration date is August 28, 2005. A 175 parts per million (ppm) and a 350 ppm concentration of the control substances were made in sterile deionized water.

According to the certificate of analysis supplied with the control substance, the stock BTC 835 is a 50.21% concentration (502,100 ppm). From this stock solution, a 1:100 dilution of the stock BTC 835 was made by adding 1.0 mL of BTC 835 to a 100 mL volumetric flask and adding sterile deionized water to the 100 mL mark, which equals a 5021 ppm concentration. The 350 ppm concentration of BTC 835 used in the study was made by adding 1.0 mL of the 5021 ppm concentration of BTC 835 to 13.35 mL sterile deionized water. The 175 ppm concentration was prepared by adding 4.0 mL of the 350 ppm concentration of BTC 835 to 4.0 mL sterile deionized water. Both concentrations of the control were in solution as determined by visual observation and used on the day of preparation.

### 3. Preparation of Virus Films

Films of virus were prepared at staggered intervals by spreading 0.2 mL of virus inoculum uniformly over the bottoms of ten separate 100 X 15mm sterile glass petri dishes. The virus films were air-dried at 20.0°C in a relative humidity of 35% for 30 minutes. The virus films were dry as determined by visual observation.

### 4. Sephadex Gel Filtration

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 test substance by filtration through Sephadex gel. Columns of Sephadex LH-20-100 were equilibrated with phosphate buffered saline containing 1% albumin, centrifuged for three minutes to clear the void volume, loaded with 2.0 mL of virus-test substance mixture and immediately passed through the column utilizing the syringe plunger.

### 5. Input Virus Control

On the day of test, 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.

### 6. Treatment of Virus Films with Test Substance

For each lot of test substance, separate dried virus films were exposed to 2.0 mL of the use dilution for one minute at 20.0°C. Following the exposure time, the plates were scraped with a cell scraper to resuspend the contents of the plate and the virus-test substance mixture was immediately passed through a Sephadex column utilizing the syringe plunger in order to detoxify the mixture. The filtrate ( $10^{-1}$  dilution) was then titered by serial dilution and assayed for infectivity.

7. Treatment of Virus Control Films

Two virus films were prepared as previously described. The control films were individually exposed to 2.0 mL of test medium for the same amount of time as the test film was exposed to the test substance. The virus films were individually scraped with a plastic cell scraper and passed through individual Sephadex columns in the same manner as the test virus. The filtrates were then titered by 10-fold serial dilution and assayed for infectivity.

8. BTC 835 Validation Control Substance

Two dried virus films were exposed to 2.0 mL of each concentration of the BTC 835 control substance (175 ppm and 350 ppm) for 10 minutes at 20.0°C. Following the exposure time, each plate was individually scraped with a cell scraper to resuspend the contents and the virus-control substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrates were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

9. Cytotoxicity Assay

A 2.0 mL aliquot of the use dilution of the test substance and each concentration of the BTC 835 control substance were filtered through individual Sephadex columns utilizing the syringe plunger. The filtrates were titered by 10-fold serial dilution and assayed for cytotoxicity. Cytotoxicity of the duck hepatocytes was scored at the same time as virus-test substance and virus control cultures.

10. Assay of Non-Virucidal Level of Test Substance

Each dilution of the Sephadex-filtered test substance (cytotoxicity control dilutions) and each concentration of the BTC 835 control substance were mixed with an aliquot of low titer stock virus, and the resulting mixtures of dilutions were assayed for infectivity in order 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 the reduction in infectivity by the test substance.

11. Infectivity Assays

Primary duck hepatocytes were used as the indicator cell line in the infectivity assays. Cells contained in cell culture labware were inoculated in quadruplicate with 1.0 mL of the dilutions prepared from the virus control, test, and validation control substances. The cytotoxicity and neutralization control dilutions were inoculated in duplicate. A 2.0 mL aliquot of test medium was added to each test and control well in addition to the inoculum. Uninfected indicator cell cultures (negative cell controls) were inoculated with test medium alone. The inoculum was allowed to adsorb overnight at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub>. Following the adsorption period, a 3.0 mL aliquot of test medium was added to each cell culture well. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub> for ten days. The test medium was aspirated from each test and control well and replaced with 4.0 mL of fresh medium as needed throughout the incubation period. On the final day of incubation, the cultures were observed microscopically for test substance cytotoxicity and the cells were then fixed with ethanol. An indirect immunofluorescence assay was then performed using a monoclonal antibody specific for the envelope protein of the DHBV.

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### **STUDY ACCEPTANCE CRITERIA**

A valid test requires 1) that at least 4 log<sub>10</sub> of infectivity be recovered from the dried virus control films; 2) that when cytotoxicity is evident, at least a 3-log reduction in viral titer is demonstrated beyond the cytotoxic level; and 3) that the cell controls be negative for infectivity. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions.

### **PROTOCOL CHANGES**

#### **Protocol Amendments:**

The following protocol amendments occurred during the course of this study:

- 1) To aid in increasing the number of hepatocytes recovered during the perfusion of the duck livers, the protocol is amended to change the base medium utilized during the perfusion from Williams' Medium E to S-MEM.
- 2) Per Sponsor request, the protocol is amended to reflect the Sponsor's new address. The current address is 2815 Bristol Circle, Unit 4, Oakville, Ontario L6H6X5

#### **Protocol Deviations:**

The following protocol deviations occurred during the course of this study:

- 1) The harvest of the primary hepatocyte report provided by the supplier of the hepatocytes does not specify where the perfusions were performed, a deviation of the protocol. Every effort was made to have the supplier correct the report however the supplier was unresponsive to all efforts made. In the opinion of the Study Director, this protocol deviation does not affect the integrity of the assay as the location of the perfusions is not pertinent to the outcome of the assay.
- 2) The perfusion procedure raw data was not delivered to the testing facility to be archived with the study. Every effort was made by the testing facility to obtain the raw data however the supplier was unresponsive to all efforts made. A final report authored by the supplier outlining the procedure for harvesting the primary hepatocytes was received by the testing facility. In the opinion of the Study Director, this protocol deviation does not affect the integrity of the assay as the hepatocytes infected with the test virus performed as expected, all test controls were valid.

### **DATA ANALYSIS**

#### **Calculations**

Viral and cytotoxicity titers are expressed as -log<sub>10</sub> of the 50 percent titration endpoint for infectivity (TCID<sub>50</sub>) or cytotoxicity (TCD<sub>50</sub>), respectively, as calculated by the method of Spearman Karber.

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

#### **Statistical Analysis**

The log<sub>10</sub> reduction in infectivity was calculated using the revised EPA approved method for calculating the Most Probable Number (MPN) as obtained from the EPA on January 4, 2001.

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## **STUDY RETENTION**

### **Record Retention**

All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. These original data include, but are not limited to, the following:

1. Certified copy of final study report.
2. Original signed protocol.
3. Any protocol amendments/deviation notifications.
4. All handwritten raw data for control and test substances including, but not limited to notebooks, data forms and calculations.
5. All measured data used in formulating the final report.
6. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in 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 per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test material.

## **REFERENCES**

1. Annual Book of ASTM Standards on Water and Environmental Microbiology, 2000, E1053-97, Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces.
2. U.S. Environmental Protection Agency Pesticide Assessment Guidelines, Subdivision G: Product Performance, 91-2(f), November 1982.
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, DIS/TSS-7, November 12, 1981.
4. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Schmidt, N.J. and Emmons, R.W. editors. Sixth edition, 1989. p. 18-20.
5. 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.
6. Environmental Protection Agency Federal Register: August 25, 2000 (Volume 65, Number 166).
7. Statistical Analysis of Hepatitis B Carrier Test Data (Revised 1-01) Template for Calculating the Log Reduction (LR) and Associated Standard Error (SE). M. Hamilton, Center for Biofilm Engineering, Montana State University. January 9, 2001, Published January 4, 2001

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## **STUDY RESULTS**

Results of the test with two lots of ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US) exposed to duck Hepatitis B virus for one minute are shown in Tables 1 and 2. The input titer (not dried) of the virus was  $5.5 \log_{10}$ . The titer of the virus control was  $4.75 \log_{10}$  for Replicate #1 and  $4.5 \log_{10}$  for Replicate #2. The MPN of the two virus control replicates is 36164 and 23979, respectively. Following exposure, test virus infectivity was not detected in either replicate of either lot of the virus-test substance mixture at any dilution tested ( $\leq 1.5 \log_{10}$ ). The MPN for the test replicates is  $\leq 23.979$ . Test substance cytotoxicity was not observed at any dilution tested ( $\leq 1.5 \log_{10}$ ). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at  $\leq 1.5 \log_{10}$ .

Utilizing the statistical program provided by the EPA, the log reduction in viral titer is  $\geq 3.09$  and the standard error of the log reduction is 0.09.

Results of the test with two concentrations of BTC 835 are shown in Tables 3 and 4. Following the 10 minute exposure time, test virus infectivity was detected in the virus/BTC 835 mixture of the 175 ppm concentration at  $\geq 4.5 \log_{10}$  for Replicate #1 and at  $4.0 \log_{10}$  for Replicate #2. Following the ten minute exposure time, test virus infectivity was detected in the 350 ppm concentration of the virus/BTC 835 mixture at  $3.25 \log_{10}$  for Replicate #1 and at  $3.5 \log_{10}$  for Replicate #2. Control substance cytotoxicity was not observed at any dilution tested ( $\leq 1.5 \log_{10}$ ). The neutralization control (non-virucidal level of the test substance) indicates that the BTC 835 control substance was neutralized at  $\leq 1.5 \log_{10}$ .

## **STUDY CONCLUSION**

**Under the conditions of this investigation, in the presence of a 100% duck serum soil load, ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US), ready to use, demonstrated complete inactivation of duck Hepatitis B virus following a one minute exposure time at 20.0°C, as required by the U.S. EPA for virucidal label claims.**

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

**The use of the ATS Labs name, logo or any other representation of ATS Labs, other than distribution of this report in its entirety, without the written approval of ATS Labs is prohibited. In addition, ATS Labs may not be referred to in any form of promotional materials, press releases, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the express written permission of ATS Labs.**

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**TABLE 1: Virus Control and Test Substance Assay Results**

**Effects of ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US) Following a One Minute Exposure to DHBV Dried on an Inanimate Surface**

Dilution	Input Virus Control	Dried Virus Control		DHBV + Lot 2-3646-REG-US		DHBV + Lot 3-3647-REG-US	
		Replicate #1	Replicate #2	Replicate #1	Replicate #2	Replicate #1	Replicate #2
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 <sup>-2</sup>	NT	NT	NT	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 <sup>-3</sup>	NT	NT	NT	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 <sup>-4</sup>	++++	++++	++++	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 <sup>-5</sup>	++++	0+00	0 0 0 0	NT	NT	NT	NT
10 <sup>-6</sup>	0 0 0 0	0 0 0 0	0 0 0 0	NT	NT	NT	NT
10 <sup>-7</sup>	0 0 0 0	0 0 0 0	0 0 0 0	NT	NT	NT	NT
TCID <sub>50</sub> /1.0 mL	10 <sup>5.5</sup>	10 <sup>4.75</sup>	10 <sup>4.5</sup>	≤10 <sup>1.5</sup>	≤10 <sup>1.5</sup>	≤10 <sup>1.5</sup>	≤10 <sup>1.5</sup>
MPN	NA	36164	23979	≤23.979	≤23.979	≤23.979	≤23.979
Log <sub>10</sub> MPN	NA	4.55828	4.37983	≤1.37983	≤1.37983	≤1.37983	≤1.37983
MPN Log Reduction	NA	NA		≥3.09			

**TABLE 2: Test Substance Cytotoxicity and Neutralization Control Results**

Dilution	Cytotoxicity Control Lot 2-3646-REG-US	Cytotoxicity Control Lot 3-3647-REG-US	Neutralization Control Lot 2-3646-REG-US	Neutralization Control Lot 3-3647-REG-US
Cell Control	0 0	0 0	0 0	0 0
10 <sup>-2</sup>	0 0	0 0	++	++
10 <sup>-3</sup>	0 0	0 0	++	C +
TCD <sub>50</sub> /1.0 mL	≤10 <sup>1.5</sup>	≤10 <sup>1.5</sup>	See below	See below

(+) = Positive for the presence of test virus

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

(C) = Bacterial contamination present, unable to determine CPE. The replicate cell culture well was not used for calculation purposes.

(NT) = Not tested

(NA) = Not applicable

(MPN) = Most Probable Number

The results of the neutralization control indicate that both lots of the test substance were neutralized at the dilution equivalent to ≤1.5 log<sub>10</sub> TCID<sub>50</sub>/1.0 mL as compared to the treated test samples.

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**TABLE 3: BTC 835 Assay Results****Effects of BTC 835 Following a Ten Minute Exposure to DHBV Dried on an Inanimate Surface**

Dilution	DHBV+ 175 ppm BTC 835		DHBV+ 350 ppm BTC 835	
	Replicate #1	Replicate #2	Replicate #1	Replicate #2
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 <sup>-2</sup>	++++	CC++	++C+	++++
10 <sup>-3</sup>	++++	++++	+++0	++++
10 <sup>-4</sup>	++++	+0+0	0000	0000
TCID <sub>50</sub> /1.0 mL	≥10 <sup>4.5</sup>	10 <sup>4.0</sup>	10 <sup>3.25</sup>	10 <sup>3.5</sup>

**TABLE 4: BTC 835 Cytotoxicity and Neutralization Control Results**

Dilution	Cytotoxicity Control	Cytotoxicity Control	Neutralization Control	Neutralization Control
	175 ppm BTC 835	350 ppm BTC 835	175 ppm BTC 835	350 ppm BTC 835
Cell Control	0 0	0 0	0 0	0 0
10 <sup>-2</sup>	0 0	0 0	++	++
10 <sup>-3</sup>	0 0	0 0	++	+C
TCD <sub>50</sub> /1.0 mL	≤10 <sup>1.5</sup>	≤10 <sup>1.5</sup>	See below	See below

(+) = Positive for the presence of test virus

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

(C) = Bacterial contamination present, unable to determine CPE. The replicate cell culture well was not used for calculation purposes.

Results of the neutralization control indicate that both concentrations of the BTC 835 control substance were neutralized at the dilution equivalent to ≤1.5 log<sub>10</sub> TCID<sub>50</sub>/1.0 mL as compared to the treated BTC 835 samples.