BluTest
GLOBAL MICROBIOLOGY EXPERTISE

To Whom it May Concern

This work was contracted by BluTest Laboratories Ltd on behalf of DuoTech Ltd, 11-12 Queen's Square, Bristol, BS1 4NT for their Duomax product at the concentrations and under the conditions stated in the report for efficacy testing against Duck Hepatitis B virus.

Yours sincerely

Dr Chris Woodall
CEO
BluTest Laboratories Ltd
Glasgow, UK
5 July 2010

Robertson Incubator, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU
Tel: +44 (0)141 330 7340 Fax: +44 (0)141 330 7342 Email: info@blutest.com Website: www.blutest.com
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96

Microbac®

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

Volume _____

FINAL REPORT

VIRUCIDAL EFFECTIVENESS TEST
Duck Hepatitis B Virus
(Surrogate for Human Hepatitis B virus)

Test Agent
DuoMax

Test Organism
Duck Hepatitis B Virus; HepadnaVirus Testing, Inc.

Data Requirements
EPA Guidelines 810.2100 (g)

Author
S. Steve Zhou, Ph.D.

Study Completion Date
06/25/2010

Performing Laboratory
MICROBIOTEST
105 Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
739-101

Protocol Identification Number
BLU.1.12.02.09

Sponsor
BluTest Laboratories Ltd.
Robertson Incubator
4th Floor, Robertson Building
University of Glasgow
57 Dumbarton Road
Glasgow, G11 6 NU, United Kingdom

STATEMENT OF NO DATA CONFIDENTIALITY

Title: Virucidal Effectiveness Test – Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus)

Performed by: MICROBIOTEST
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 § 10(d)(1)(A), (B) or (C).

Company Agent _____ Title _____

Signature _____ Date _____

COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR § 160 with the following exceptions:

- Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test agent resides with the sponsor of the study.

The following technical personnel participated in this study:

Helen Christina, Zheng Chen

Study Director: MICROBIOTEST



S. Steve Zhou, Ph.D. — 06/25/2010 — Date

Submitted by:

_____ Name — _____ Title

_____ Signature — _____ Date

Sponsor: BluTest Laboratories Ltd.

_____ Name — _____ Title

_____ Signature — _____ Date

QUALITY ASSURANCE UNIT STATEMENT

Title: Virucidal Effectiveness Test - Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus)

The Quality Assurance Unit of MICROBIOTEST has inspected the Project Number 739-101 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	05/21/10	06/21/10	06/21/10
In Process	05/21/10	06/21/10	06/21/10
Final Report	06/21/10	06/21/10	06/21/10

Nathan S. Jones

Nathan Jones,
Manager, Quality Assurance Unit

06/25/10
Date

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TEST SUMMARY

TITLE: Virucidal Effectiveness 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 AGENT SUPPLIED BY THE SPONSOR OF THE STUDY:

DuoMax, received at MICROBIOTEST 05/17/10, and assigned DS No. A272

SPONSOR:

BluTest Laboratories Ltd.
Robertson Incubator
4th Floor, Robertson Building
University of Glasgow
57 Dumbarton Road
Glasgow, G11 6 NU, United Kingdom

TEST CONDITIONS

Challenge virus:

Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus); HepadnaVirus Testing, Inc.

Host:

Primary Duck Hepatocytes, Metzer Farms (duckling source)

Active ingredient(s):

Undisclosed

Neutralizer:

Fetal Bovine Serum + 1% Polysorbate 80 + 1% HEPES

Test agent preparation:

Ready to use (pre-diluted by the sponsor)

Contact time:

5 minutes

Contact temperature:

19C

Organic load:

Virus contained 100% duck serum

TEST CONDITIONS (continued)

Carrier inoculation and dry time:

2 x 2 inch area of glass carrier inoculated with 0.4 mL of virus and dried for 30 minutes

Test agent application:

2 mL of the test agent was added to the dried carriers

Media and reagents:

L-15 complete
Fetal Bovine Serum + 1% Polysorbate 80 + 1% Hepes
Phosphate Buffered Saline (PBS)
PBS + 0.5% Fetal Bovine Serum
Anti-DHBV envelope IgG MAb
Goat anti-mouse FITC-conjugated antibody
Blocker
4CN
Triethanolamine buffer
0.2M EGTA
1M $CaCl_2$
Collagenase
1M Hepes
80% Acetone
Hydrogen peroxide

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164. Testing was laboratory initiated on 05/21/10 and was concluded on 06/04/10. The study director signed the protocol on 05/21/10. 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 agent 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% fluorescent focus forming unit dose per mL ($FFFUD_{50}$/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 titer 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

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

RESULTS

Data are presented in Tables 1 – 4.

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

Log_{10} Reduction = $FFFUD_{50}$/mL (Plate Recovery Control) - $FFFUD_{50}$/mL (Test)

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

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

Key (for all tables):

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*	DuoMax
10^{-2}	0/4
10^{-3}	0/4
10^{-4}	0/4
10^{-5}	0/4
10^{-6}	0/4
10^{-7}	0/4
Titer (Log_{10} $FFFUD_{50}$/mL)	≤ 1.50
Load (Log_{10} $FFFUD_{50}$) per carrier (0.4 mL challenge)	**≤ 1.10**
Log_{10} Reduction	**≥ 4.50**

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

RESULTS (continued)

Table 2
Neutralizer Effectiveness and Cytotoxicity Related Controls

Dilution*	DuoMax	
	Neutralizer Effectiveness 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 mock inoculum.

Table 3
Viability Control Results

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

Table 4
Virus Recovery Controls

Dilution*	Plate Recovery Control	Virus Stock Titer Control
10^{-2}	4/4	4/4
10^{-3}	4/4	4/4
10^{-4}	4/4	4/4
10^{-5}	4/4	4/4
10^{-6}	2/4	4/4
10^{-7}	0/4	1/4
Titer (Log_{10} $FFFUD_{50}$/mL)	6.00	6.75
Load (Log_{10} $FFFUD_{50}$) per carrier (0.4 mL challenge)	**5.60**	**Not Applicable**

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

CONCLUSIONS

According to the regulatory agencies, the test agent passes the Virucidal Effectiveness 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, DuoMax passed the Virucidal Effectiveness Test when Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus), containing at least 5% organic soil, was exposed to the test agent for 5 minutes at 19C. All of the controls met the criteria for a valid test. These conclusions are based on observed data.