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FINAL REPORT
Virucidal Efficacy Test
Avian Influenza virus

Test Agent:
Sniper

Data Requirements
EPA Guidelines 810.2100 (g)

Author
Peggy R. Cherwoo

Study Completion Date
August 8, 2006

Testing Laboratory
Accugen Laboratories, Inc.
50 West 75th Street
Willowbrook, IL 60527

Laboratory Project Identification Number
786-80501

Submitted to: Global Environmental Restoration
PO Box 248
Carencro, Lousiana 70520

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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:

Peggy R. Cherwoo, Patrick M. Zoder, Tracey Kelly, Lauren A. Blaszak

Study Director:

_____ _____
Tehseen Naqvi Date

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: Virucidal Efficacy Test – Avian Influenza Virus

The Quality Assurance Unit has inspected the Project Number 786-80501 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	07/20/06	08/04/06	08/08/06
In Process	07/20/06	07/20/06	08/08/06
Final Report	08/04/06	08/04/06	08/08/06

A.Raza M.D.; Dcpath, MPH
Director Quality Assurance Unit

TEST SUMMARY

TITLE: Virucidal Efficacy Test – Avian Influenza Virus

STUDY DESIGN: This study was performed according to the signed protocol and project sheets issued by the Study Director

See Project Sheets (Appendix I)
See signed protocol (Appendix II)

TEST MATERIALS SUPPLIED BY THE SPONSOR OF THE STUDY:

1. Sniper, Lot No.101, received at Accugen 07/05/06, and assigned DS No.80501.
2. Sniper, Lot No.102, received at Accugen 07/05/06, and assigned DS No. 80502

SPONSOR: **Global Environmental Restoration**
PO Box 248
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TEST CONDITIONS

Challenge virus:
Avian Influenza virus, Type A, (H9N2), Turkey/Wis/66; SPAFAS

Host:
Embryonated chicken eggs; BE eggs

Organic load:
Viral stock contained ≥5% organic load

Active ingredient in test product:
Chlorine dioxide

Neutralizer used:
Fetal bovine serum + 0.3% $Na_2S_2O_3$

Contact time:
10 minutes

Contact temperature:
Ambient temperature (22C)

Dilution:
Ready to use

Carrier Inoculation:
Test carriers were inoculated with 0.2mL viral stock and dried for 35 minutes at 22C.

Media and reagents:
FBS + 0.3% $Na_2S_2O_3$
Earle's balanced salt solution (EBSS)
Phosphate buffered saline
Chicken red blood cells

STUDY DATES

The laboratory phase of this test was performed from 07/20/06 to 07/27/06. The study director signed the protocol 07/18/06. 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 Accugen and the sponsor will be stored in the archives

RESULTS

Results are presented in Tables 1 – 4. The 50% embryo infectious/lethal dose per mL (EID/ELD_{50}/mL) was determined from the test and relevant control data using the method of Reed and Muench, Am. J. of Hyg. 1938, 27:493. The host viability control demonstrated media sterility and with one exception embryo viability (one of four embryos was non-viable upon candling at the conclusion of incubation). Virus was not detected in the host viability control. All controls including neutralizer effectiveness, toxicity, and recovery titer met the criteria required for a valid test. The test agent was effectively neutralized. The neutralized test agent mixture did not render the host system resistant to viral infection. Infectious virus was not detected in the host system after exposure to the test agent as described.

The log_{10} reduction (LR) was calculated in the following manner:

Log_{10} reduction = Infectious virus titer recovered from plate recovery control – Infectious virus titer recovered from test.

RESULTS (continued)

Table 1
Test Results

Dilution	Sniper	
	Lot No. 101	Lot No. 102
10^{-2}	0 0* 0 0	0 0 0 0
10^{-3}	0 0 0* 0*	0 0 0 0
10^{-4}	0 0 0 0*	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0
$EID/ELD_{50}/mL$	$\leq 10^{1.50}$	$\leq 10^{1.50}$

Table 2
Neutralizer Effectiveness and Cytotoxicity Related Controls

Dilution	Sniper Lot No. 101		
	Neutralizer Effectiveness Control	Toxicity Control	Toxicity-related Viral Interference Control
10^{-2}	0* + + 0*	0 0 0 0	+ + + +
10^{-3}	+ + + +	0 0 0 0	+ + + +
10^{-4}	+ + + +	0 0 0 0	+ + + +

Table 3
Control results

Dilution	Avian Influenza Virus Plate Recovery Control
10^{-2}	+ + + +
10^{-3}	+ + + +
10^{-4}	+ + + +
10^{-5}	+ + + +
10^{-6}	+ + + +
10^{-7}	+ + + +
$EID/ELD_{50}/mL$	$\geq 10^{7.50}$

Host Viability Control
0 0 0* 0

Key: + = Avian Influenza virus infected embryos were detected, hemagglutination observed.
0 = Avian Influenza virus infected embryos not detected, no hemagglutination observed; no toxicity.
0* = Avian influenza virus infected embryos not detected, no hemagglutination observed.Non-viable embryo.

RESULTS (continued)

Table 4
Log_{10} Reduction

Sniper	
Lot No. 101	Lot No. 102
≥6.00	≥6.00

CONCLUSIONS

When tested as described, Sniper passed the Virucidal Efficacy Test when Avian Influenza virus, containing a 5% organic load, was exposed to the test agent for 10 minutes at 22C. All of the controls met the criteria for a valid test. These conclusions are based on observed data.