

MICROBIOTEST

The Microbiology and Virology Laboratory

Volume _____

FINAL REPORT

VIRUCIDAL EFFICACY TEST USING SWINE INFLUENZA A VIRUS (H1N1)

Test Agent

Anolyte

Lot Numbers

Oct 4, 2009

Sept 25, 2009

Data Requirements

EPA Guidelines 810.2100 (g)

Author

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Study Completion Date

10/30/09

Performing Laboratory

MICROBIOTEST

105 Carpenter Drive

Sterling, Virginia 20164

Laboratory Project Identification Number

668-104

Protocol Identification Number

668.1.10.01.09

SPONSOR

Envirocleanse LLC

14019 SW Frwy. Ste 301-387

Sugar Land, TX 77478

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STATEMENT OF NO DATA CONFIDENTIALITY

Title: Virucidal Efficacy Test Using Swine Influenza A Virus (H1N1)

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 _____

_____ 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, Justin Zamorski

Study Director: MICROBIOTEST



S. Steve Zhou, Ph.D.

10/30/2009

Date

Submitted by:

Name

Title

Signature

Date

Sponsor:

Envirocleanse LLC

Name

Title

Signature

Date

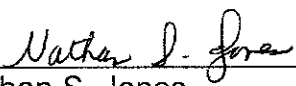
QUALITY ASSURANCE UNIT STATEMENT

Title of Study: Virucidal Efficacy Test Using Swine Influenza A Virus (H1N1)

The Quality Assurance Unit of MICROBIOTEST has inspected the Project Number 668-104 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	10/16/09	10/29/09	10/29/09
In Process	10/16/09	10/29/09	10/29/09
Final Report	10/29/09	10/29/09	10/29/09



Nathan S. Jones
Quality Assurance Unit

10/30/09
Date

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TEST SUMMARY

TITLE: Virucidal Efficacy Test Using Swine Influenza A Virus (H1N1)

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

TEST MATERIALS:

1. Anolyte; Lot No. Oct 4, 2009, received at MICROBIOTEST 10/09/09, and assigned DS No. 10402
2. Anolyte; Lot No. Sep 25, 2009, received at MICROBIOTEST 10/09/09, and assigned DS No. 10403

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TEST CONDITIONS

Challenge virus:

Swine Influenza A Virus (H1N1), A/Swine/1976/31; ATCC VR-99

Host:

MDCK cells, ATCC CCL-34

Active ingredient:

Hypochlorous acid

Neutralizer used:

Minimal Essential Medium (MEM) + 1% Fetal Bovine Serum (FBS) + 1%
HEPES + 0.01 mol/L $\text{Na}_2\text{S}_2\text{O}_3$

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 test agent was added to inoculated carriers.

Contact time:

10 minutes

Contact temperature:

21C

Organic load:

Serum was added to the virus to achieve an organic load of $\geq 5\%$

TEST CONDITIONS (continued)

Media and reagents:

MEM + 1 µg/mL Trypsin
MEM + 1% Fetal Bovine Serum + 1% HEPES + 0.01 mol/L Na₂S₂O₃
Phosphate Buffered Saline
Fetal Bovine Serum

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 10/16/09 and was concluded on 10/21/09. The study director signed the protocol on 10/15/09. 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 (TCID₅₀/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_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 TCID₅₀/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:

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

Viral Load ($\text{Log}_{10} \text{TCID}_{50}$) per carrier was determined in the following manner:

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

Key (for all tables):

X/y = X wells out of y wells inoculated exhibited positive viral Cytopathic Effect (CPE)

0/y = 0 out of y wells inoculated exhibited positive viral CPE; no cytotoxicity or bacterial contamination was observed in any of the wells inoculated

RESULTS (continued)**Table 1
Test Agent Results**

Dilution*	Analyte	
	Lot No: Oct 4, 2009	Lot No: Sep 25, 2009
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 (Log₁₀ TCID₅₀) per carrier (0.4mL challenge)	≤ 1.10	≤ 1.10
Log₁₀ Reduction	≥ 4.25	≥ 4.25

* Dilution refers to fold of dilution from virus inoculum.

**Table 2
Neutralizer Effectiveness and Cytotoxicity Related Controls**

Dilution*	Analyte Lot No: Oct 4, 2009	
	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 fold of dilution from mock inoculum.

RESULTS (continued)

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^{-3}	4/4	Not Determined
10^{-4}	4/4	4/4
10^{-5}	4/4	4/4
10^{-6}	1/4	2/4
10^{-7}	0/4	0/4
10^{-8}	0/4	0/4
10^{-9}	Not Determined	0/4
Titer (Log_{10} TCID ₅₀ /mL)	5.75	6.00
Load (Log_{10} TCID₅₀) per carrier (0.4mL challenge)	5.35	NA

* Dilution refers to fold of dilution from virus inoculum.
NA = not applicable

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, Anolyte passed the Virucidal Efficacy Test when Swine Influenza A Virus (H1N1), containing at least 5% organic soil, was exposed to the test agent for 10 minutes at 21C. All of the controls met the criteria for a valid test. These conclusions are based on observed data.