

**FINAL REPORT**  
**VIRUCIDAL EFFECTIVENESS TEST**  
**Avian Influenza virus**

**Test Agents: Oreganol P73 Extra Strength Formula  
and  
Oregacyn**

**Data Requirements**  
**Research & Development**

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**Laboratory Project Identification Number**  
**514-106**

**Submitted to: NORTH AMERICAN HERB AND SPICE**  
**1455 Lakeside Dr.**  
**Waukegan, IL 60085**

## QUALITY ASSURANCE UNIT STATEMENT

Title of Study: Virucidal Effectiveness Test – Avian Influenza virus

The Quality Assurance Unit of MICROBIOTEST, INC. has inspected the final report for Project Number 514-106 for accuracy in data transcription.

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>
Final Report	05/03/04	05/03/04	05/04/04

\_\_\_\_\_  
Nathan S. Jones, RQAP-GLP  
Quality Assurance Unit

\_\_\_\_\_  
Date

## COMPLIANCE STATEMENT

This study was conducted under my direction according to the protocol and extant SOPs.

The following technical personnel participated in this study:

Zheng Chen, Nausheen Javed, Samina S. Raja

Study Director: MICROBIOTEST, INC.

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M. Khalid Ijaz, DVM, Ph.D.

\_\_\_\_\_  
Date

**MICROBIOTEST, INC.**

## TABLE OF CONTENTS

FINAL REPORT - COVER PAGE .....	1
QUALITY ASSURANCE UNIT STATEMENT .....	2
COMPLIANCE STATEMENT .....	2
TABLE OF CONTENTS .....	3
TEST SUMMARY .....	4
TEST CONDITIONS.....	5-6
STUDY DATES AND FACILITIES.....	6
RECORDS TO BE MAINTAINED.....	6
RESULTS.....	7-10
CONCLUSIONS.....	10
APPENDIX I .....	
APPENDIX II.....	

## TEST SUMMARY

**TITLE:** Virucidal Effectiveness 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. Oreganol P73 Extra Strength Formula, received at MICROBIOTEST, INC. on 06/23/03 and assigned DS No. 6287.
2. Oregacyn, Lot No. 111102, received at MICROBIOTEST, INC. on 06/23/03 and assigned DS No. 6288.

**SPONSOR:** NORTH AMERICAN HERB AND SPICE  
1455 Lakeside Dr.  
Waukegan, IL 60085

## TEST CONDITIONS

Challenge virus:

Avian Influenza virus type A, Turkey / Wis / 66 strain (H9N2),  
Charles River Laboratories

Host:

Embryonated chicken eggs, B&E Eggs

Active ingredient in test product:

Wild Oregano Oil & Olive Oil Base (Oreganol P73 Extra Strength Formula)  
Wild Oregano, Wild Cumin, Wild Sage, Wild Cinnamon (Oregacyn)

Neutralizer:

Not applicable

Exposure time:

2, 5, 10, 15, and 20 minutes

Exposure temperature:

Ambient room temperature (22C)

Diluent:

Earle's Balanced Salt Solution

Dilution:

25% v/v (Oreganol P73 Extra Strength Formula) and 25% w/v (Oregacyn)

Organic load:

Viral stock contained at least 5% organic load

## **TEST CONDITIONS (continued)**

### Media and reagents:

Earle's Balanced Salt Solution  
Phosphate Buffered Saline  
Chicken red blood cells

### Test Agent Application:

For both test agents tested at the appropriate concentration: 0.5mL of viral inoculum was added to 4.5mL of diluted test agent and held for the specified contact times. At the completion of the 2, 5, 10, 15, and 20 minute contact times, the virus-test agent mixture was serially diluted tenfold in EBSS. 0.2mL of selected dilutions was inoculated into embryonated chicken eggs.

## **STUDY DATES AND FACILITIES**

The laboratory phase of this test was performed at MICROBIOTEST, INC., 105B Carpenter Drive, Sterling, VA 20164, from 04/07/04 to 04/21/04. The study director signed the protocol 04/06/04. 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, INC., 105B Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

## RESULTS

Results are presented in Tables 1 - 6. A titration was performed to determine the titer of the viral stock. The 50% embryo lethal dose / embryo infectious dose per mL (ELD/EID<sub>50</sub>/mL) was determined from the virus stock, test, and relevant control data using the method of Reed and Muench, 1938. The negative control demonstrated host viability and media sterility. Virus was not recovered in the negative control or the uninoculated control. Log<sub>10</sub> reduction was calculated using the following equation:

$$\text{Log}_{10} (\text{Input Virus Control}) - \text{Log}_{10} (\text{Test Results}) = \text{Log}_{10} \text{Reduction}$$

Table 1 - Test Results

Dilution	Avian Influenza type A virus titer (ELD/EID <sub>50</sub> /mL)				
	Oreganol P73 Extra Strength Formula				
	Contact Time				
	2 minutes	5 minutes	10 minutes	15 minutes	20 minutes
10 <sup>-2</sup>	++++	++++	++++	++++	++++
10 <sup>-3</sup>	++++	++++	++++	+++ -	+ - - +
10 <sup>-4</sup>	++++	++++	+++ -	- + - +	+ - - +
10 <sup>-5</sup>	- + - +	+++ -	- + + -	- + - -	+ - + -
10 <sup>-6</sup>	+ - - +	+ - + -	+ - + -	- - - +	- - - -
10 <sup>-7</sup>	+ + - -	- - - -	- - - -	- - - -	- - - -
ELD/EID <sub>50</sub> /mL	≥10 <sup>6.00</sup>	10 <sup>5.75</sup>	10 <sup>5.25</sup>	10 <sup>4.22</sup>	10 <sup>4.00</sup>

Key: + = Avian Influenza type A virus detected; hemagglutination observed  
 - = Avian Influenza type A virus not detected; no hemagglutination observed

**RESULTS (continued)**

Table 2 - Test Results

Dilution	Avian Influenza type A virus titer (ELD/EID <sub>50</sub> /mL)				
	Oregacyn				
	Contact Time				
	2 minutes	5 minutes	10 minutes	15 minutes	20 minutes
10 <sup>-2</sup>	++++	++++	++++	++++	++-+
10 <sup>-3</sup>	++++	++++	++++	+--	+--+
10 <sup>-4</sup>	++++	+++-	-+-+	--+-	-+-+
10 <sup>-5</sup>	-+++	-+--	-+--	+---	----
10 <sup>-6</sup>	++--	----	----	--+-	----
10 <sup>-7</sup>	----	D---	----	----	----
ELD/EID <sub>50</sub> /mL	10 <sup>5.77</sup>	10 <sup>4.50</sup>	10 <sup>4.23</sup>	10 <sup>3.63</sup>	10 <sup>3.56</sup>

Table 3 - Toxicity Control

	Toxicity Control	
	Oreganol P73 Extra Strength Formula	Oregacyn
10 <sup>-2</sup>	----	-D--
10 <sup>-3</sup>	----	----
10 <sup>-4</sup>	----	----

Key: + = Avian Influenza type A virus detected; hemagglutination observed  
 - = Avian Influenza type A virus not detected; no hemagglutination observed  
 D = Dead embryo discarded following candling after one day of incubation



**RESULTS (continued)**

Table 4 – Input Virus Control and Virus Stock Titer

Dilution	Avian Influenza type A virus titer (ELD/EID <sub>50</sub> /mL)	
	Input Virus Control Contact Time: 20 minutes	Virus Stock Titer
10 <sup>-1</sup>	PNS	++++
10 <sup>-2</sup>	++++	++++
10 <sup>-3</sup>	++++	++++
10 <sup>-4</sup>	++++	++++
10 <sup>-5</sup>	++++	++++
10 <sup>-6</sup>	++++	++++
10 <sup>-7</sup>	- + - -	+ - - +
10 <sup>-8</sup>	ND	+ - - -
ELD/EID <sub>50</sub> /mL	≥10 <sup>6.67</sup>	≥10 <sup>7.23</sup>

Key: + = Avian Influenza type A virus detected; hemagglutination observed  
 - = Avian Influenza type A virus not detected; no hemagglutination observed  
 ND = Not determined  
 PNS = Post neutralized sample

**RESULTS (continued)**Table 5 – Log<sub>10</sub> Reduction

Test Agent	Contact Time	Test Results (ELD/EID <sub>50</sub> )/mL	Input Virus Control (ELD/EID <sub>50</sub> )/mL	log <sub>10</sub> Reduction
Oregonol P73 Extra Strength Formula	2 minutes	≥10 <sup>6.00</sup>	≥10 <sup>6.67</sup>	<, >, or = 0.67
	5 minutes	10 <sup>5.75</sup>		≥0.92
	10 minutes	10 <sup>5.25</sup>		≥1.42
	15 minutes	10 <sup>4.22</sup>		≥2.45
	20 minutes	10 <sup>4.00</sup>		≥2.67

Table 6 – Log<sub>10</sub> Reduction

Test Agent	Contact Time	Test Results (ELD/EID <sub>50</sub> )/mL	Input Virus Control (ELD/EID <sub>50</sub> )/mL	log <sub>10</sub> Reduction
Oregoncyn	2 minutes	10 <sup>5.77</sup>	≥10 <sup>6.67</sup>	≥0.90
	5 minutes	10 <sup>4.50</sup>		≥2.17
	10 minutes	10 <sup>4.23</sup>		≥2.44
	15 minutes	10 <sup>3.63</sup>		≥3.04
	20 minutes	10 <sup>3.56</sup>		≥3.11

**CONCLUSIONS**

When tested as described, Oregonol P73 Extra Strength Formula and Oregoncyn at final testing concentrations of 25% inactivated virus in direct proportion to exposure time when Avian Influenza virus was exposed to each test agent for 2, 5, 10, 15, and 20 minutes at ambient room temperature. All of the controls met the criteria established for a valid test. These conclusions are based on observed data.