

FINAL REPORT
VIRUCIDAL EFFECTIVENESS TEST
Influenza virus

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

Data Requirements
Research & Development

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Laboratory Project Identification Number
514-103

Submitted to: North American Herb and Spice
1646 Addington Drive
Prescott, AZ 86301

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: Virucidal Effectiveness Test – Influenza virus

The Quality Assurance Unit of MICROBIOTEST, INC. has inspected the final report for Project Number 514-103 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	03/23/04 03/24/04	03/24/04	03/25/04

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, Samina S. Raja

Study Director: MICROBIOTEST, INC.

M. Khalid Ijaz, DVM, Ph.D.

Date

MICROBIOTEST, INC.

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TEST SUMMARY

TITLE: Virucidal Effectiveness Test – 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
1646 Addington Drive
Prescott, AZ 86301

TEST CONDITIONS

Challenge virus:

Influenza virus A2 / Japan / 305 / 57 (H2N2), 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:

N/A

Exposure time:

2, 5, 10, 15, and 20 minutes

Exposure temperature:

Ambient room temperature (20C)

Diluent:

Earle's Balanced Salt Solution (EBSS)

Dilution:

1% v/v (Oreganol P73 Extra Strength Formula) and 1% w/v (Oregacyn)

Organic load:

Viral stock contained at least 5% organic load

TEST CONDITIONS (continued)

Media and reagents:

Earle's Balanced Salt Solution (EBSS)
Phosphate Buffered Saline (PBS)
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 02/19/04 to 03/09/04. The study director signed the protocol 01/14/04. 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 - 10. A titration was performed to determine the titer of the viral stock. The 50% embryo lethal dose / embryo infectious dose per mL (ELD/EID₅₀/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. Log₁₀ 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 – Pre-Testing Data 2/19/04

Toxicity				
Oregacyn		Oreganol P73 Extra Strength Formula		
2.5% 2 replicates	0.25% 2 replicates	25% 1 replicate	2.5% 2 replicate	0.25% 2 replicates
DD	DD	0	00	00

Table 2 – Pre-Testing Data 2/25/04

Toxicity	
Oregacyn	Oreganol P73 Extra Strength Formula
1% 2 replicates	1% 2 replicates
00	00

Key: D = Dead embryo discarded following candling after one day of incubation
0 = No toxicity observed

RESULTS (continued)

Table 3 - Test Results

Dilution	Influenza A2 virus titer (ELD/EID ₅₀)/mL				
	Oreganol P73 Extra Strength Formula				
	Contact Time				
	2 minutes	5 minutes	10 minutes	15 minutes	20 minutes
10 ⁻²	++++	++++	++++	++++	++++
10 ⁻³	++++	++++	++++	-+++	++-+
10 ⁻⁴	+ - ++	++++	++++	+ - ++	+++ -
10 ⁻⁵	++ - +	+ - + -	++ - -	- + - -	- - - -
10 ⁻⁶	+ - - +	- + - -	- - - -	- - - -	- - - -
10 ⁻⁷	- - - -	- - - -	- - - -	- - - -	- - - -
(ELD/EID ₅₀)/mL	10 ^{5.56}	10 ^{5.23}	10 ^{5.00}	10 ^{4.33}	10 ^{4.17}

Key: + = Influenza A2 virus detected; hemagglutination observed

- = Influenza A2 virus not detected; no hemagglutination observed

RESULTS (continued)

Table 4 - Test Results

Dilution	Influenza A2 virus titer (ELD/EID ₅₀)/mL				
	Oregacyn				
	Contact Time				
	2 minutes	5 minutes	10 minutes	15 minutes	20 minutes
10 ⁻²	++++	++++	++++	++++	++++
10 ⁻³	++++	++++	++++	++++	++++
10 ⁻⁴	++++	++++	++-+	+ - + +	- + - -
10 ⁻⁵	++++	+ - + +	+ - - +	+ - + -	-----
10 ⁻⁶	+ - - +	+ - + -	+ - + -	-----	-----
10 ⁻⁷	-----	-----	-----	-----	-----
(ELD/EID ₅₀)/mL	10 ^{6.00}	10 ^{5.77}	10 ^{5.25}	10 ^{4.77}	10 ^{3.67}

Table 5 - Toxicity Control

	Toxicity Control	
	Oreganol P73 Extra Strength Formula	Oregacyn
10 ⁻¹	0 0 0 0	0 0 0 0
10 ⁻²	0 0 0 0	0 0 0 0
10 ⁻³	0 0 0 0	0 0 0 0

Key: + = Influenza A2 virus detected; hemagglutination observed
 - = Influenza A2 virus not detected; no hemagglutination observed
 0 = No toxicity observed

RESULTS (continued)

Table 6 – Input Virus Control and Virus Stock Titer

Dilution	Influenza A2 virus titer (ELD/EID ₅₀)/mL	
	Input Virus Control Contact Time: 20 minutes	Virus Stock Titer
10 ⁻¹	PNS	ND
10 ⁻²	+ D + +	
10 ⁻³	+ + + +	
10 ⁻⁴	+ + + +	D + + +
10 ⁻⁵	+ + + +	D + + +
10 ⁻⁶	+ + + +	+ + + +
10 ⁻⁷	+ - - +	+ + + +
10 ⁻²	ND	+ - - +
(ELD/EID ₅₀)/mL	≥10 ^{7.00}	≥10 ^{8.00}

Table 7 – Negative Control 2/19/04, 2/25/04, 2/26/04

- - - -

Table 8 – Uninoculated Control 2/19/04, 2/25/04, 2/26/04

- - - -

Key: + = Influenza A2 virus detected; hemagglutination observed
 - = Influenza A2 virus not detected; no hemagglutination observed
 D = Dead embryo discarded following candling after one day of incubation
 ND = not determined
 PNS = post neutralized sample

RESULTS (continued)Table 9 – Log₁₀ Reduction

Test Agent	Contact Time	Test Results (ELD/EID ₅₀)/mL	Input Virus Control (ELD/EID ₅₀)/mL	log ₁₀ Reduction
Oreganol P73 Extra Strength Formula	2 minutes	10 ^{5.56}	≥10 ^{7.00}	≥1.44
	5 minutes	10 ^{5.23}		≥1.77
	10 minutes	10 ^{5.00}		≥2.00
	15 minutes	10 ^{4.33}		≥2.67
	20 minutes	10 ^{4.17}		≥2.83

Table 10 – Log₁₀ Reduction

Test Agent	Contact Time	Test Results (ELD/EID ₅₀)/mL	Input Virus Control (ELD/EID ₅₀)/mL	log ₁₀ Reduction
Oregacyn	2 minutes	10 ^{6.00}	≥10 ^{7.00}	≥1.00
	5 minutes	10 ^{5.77}		≥1.23
	10 minutes	10 ^{5.25}		≥1.75
	15 minutes	10 ^{4.77}		≥2.23
	20 minutes	10 ^{3.67}		≥3.33

CONCLUSIONS

When tested as described, Oreganol P73 Extra Strength Formula and Oregacyn at final testing concentrations of 1%, inactivated virus in direct proportion to exposure time when Influenza A2 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.