

FINAL REPORT
VIRUCIDAL EFFECTIVENESS TEST
Coronavirus

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

Data Requirements
Research Development

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Performing Laboratory
MICROBIOTEST, INC.
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Laboratory Project Identification Number
514-101

Submitted to: North American Herb and Spice
9812 East Karen Drive
Scottsdale, AZ 85260

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: Virucidal Effectiveness Test – Coronavirus

The Quality Assurance Unit of MICROBIOTEST has inspected the final report for Project Number 514-101 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	08/11/03	08/11/03	08/14/03

Nathan S. Jones
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.

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 – Coronavirus

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 on 06/23/03 and assigned DS No. 6287.
2. Oregacyn, Lot No. 111102, received at MICROBIOTEST on 06/23/03 and assigned DS No. 6288.

SPONSOR: North American Herb and Spice
9812 East Karen Drive
Scottsdale, AZ 85260

TEST CONDITIONS

Challenge virus:

Human Coronavirus (HCoV), ATCC VR-740

Host:

MRC-5 cells, Diagnostic Hybrids, Inc., Athens, OH

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 (21C)

Diluent:

Earle's Balanced Salt Solution (EBSS)

Dilution:

1:1000 (Oreganol P73 Extra Strength Formula) and 1:10000 (Oregacyn)

Organic load:

Viral stock contained at least 5% fetal bovine serum

TEST CONDITIONS (continued)

Media and reagents:

Minimum Essential Medium Eagle containing 10% fetal bovine serum (CCM)
Phosphate Buffered Saline (PBS)
Earle's Balanced Salt Solution (EBSS)

Test Agent Application:

For both test agents tested at the appropriate concentration: 1mL of viral inoculum was added to 9mL 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. Selected dilutions were inoculated into 4 wells of MRC-5 monolayers.

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, INC., 105B Carpenter Drive, Sterling, VA 20164, from 07/17/03 to 07/29/03. The study director signed the protocol 07/17/03. 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 – 7. A titration was performed to determine the titer of the viral stock. The 50% cell culture infectious unit does per mL (CCID₅₀/mL) was determined from the test and pertinent control data using the method of Reed and Muench, Am. J. of Hyg. 1938, 27:493. The cell viability control demonstrated MRC-5 cell viability and media sterility. Virus was not recovered in the cell viability control.

Table 1 – Cytotoxicity 7/11/03

Cytotoxicity		
Oreganol P73 Extra Strength Formula (10%)	Oregacyn (5%)	Oregacyn (1%)
CCCC	CCCC	CCCC

Table 2– Cytotoxicity 7/14/03

Cytotoxicity			
Dilution (log ₁₀)	Oreganol P73 Extra Strength Formula (10%)	Oregacyn (5%)	Oregacyn (1%)
-1	CCCC	CCCC	CCCC
-2	0000	CCCC	0000
-3	0000	0000	0000

Table 3– Cytotoxicity 7/16/03

Cytotoxicity		
Dilution (log ₁₀)	Oreganol P73 Extra Strength Formula (10%)	Oregacyn (1%)
-2	0000*	0000

*20% cytotoxicity observed

Key: C = cytotoxicity observed

0 = no cytotoxicity observed

RESULTS (continued)

Table 4 - Test Results

Dilutions (log ₁₀)	Human Coronavirus titer (CCID ₅₀ /mL)				
	Oreganol P73 Extra Strength Formula				
	CONTACT TIME				
	2min	5 min	10 min	15 min	20 min
-1	++++	++++	++++	++++	++++
-2	++++	+++ -	++ - +	+ - - -	- - - -
-3	- + - +	+ - - -	- - - -	- - - -	- - - -
-4	- - - -	- - - -	- - - -	- - - -	- - - -
-5	- - - -	- - - -	- - - -	- - - -	- - - -
-6	- - - -	- - - -	- - - -	- - - -	- - - -
CCID ₅₀ /mL	10 ^{3.00}	10 ^{2.50}	10 ^{2.33}	10 ^{1.67}	10 ^{1.50}

Key: + = HCoV infected cells detected by cytopathic effects observed
 - = no HCoV infected cells detected by lack of cytopathic effects observed

RESULTS (continued)

Table 5 - Test Results

Dilutions (log ₁₀)	Human Coronavirus titer (CCID ₅₀ /mL)				
	Oregacyn Lot No. 111102				
	CONTACT TIME				
	2min	5min	10 min	15 min	20 min
-1	++++	++++	++++	+ - + +	----
-2	++ - +	- + - -	----	----	----
-3	----	----	----	----	----
-4	----	----	----	----	----
-5	----	----	----	----	----
-6	----	----	----	----	----
CCID ₅₀ /mL	10 ^{2.33}	10 ^{1.67}	10 ^{1.50}	10 ^{1.33}	≤10 ^{1.50}

Table 6 - Cytotoxicity Control

Dilutions (log ₁₀)	Cytotoxicity Control	
	Oreganol P73 Extra Strength Formula	Oregacyn Lot No. 111102
-1	0 0 0 0	0 0 0 0
-2	0 0 0 0	0 0 0 0
-3	0 0 0 0	0 0 0 0

Key: + = HCoV infected cells detected by cytopathic effects observed
 - = no HCoV infected cells detected by lack of cytopathic effects
 observed
 0 = no cytotoxicity observed

RESULTS (continued)

Table 7 – Input Virus Control and Virus Stock Titer

Dilutions (log ₁₀)	Human Coronavirus titer (CCID ₅₀ /mL)	
	Input Virus Control	Virus Stock Titer
-1	PNS	+ + + +
-2	+ + + +	+ + + +
-3	+ + + +	+ + + +
-4	+ + + +	+ + + +
-5	+ - + -	+ + + +
-6	+ + - -	+ + - -
-7	- - - -	- - + -
-8	ND	- - - -
CCID ₅₀ /mL	10 ^{5.50}	10 ^{6.23}

Key: + = HCoV infected cells detected by cytopathic effects observed
 - = HCoV cells not detected by a lack of cytopathic effects observed
 ND = not determined
 PNS = post-neutralized sample

CONCLUSIONS

When tested as described, Oreganol P73 Extra Strength Formula and Oregacyn at final testing concentrations of 0.1% and 0.01% respectively, inactivated HCoV in direct proportion to exposure time when HCoV 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.