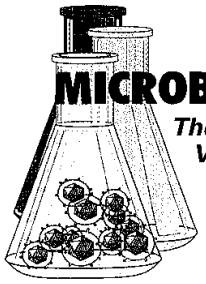

MICROBIOTEST, INC
The Microbiology and Virology Laboratory

Volume _____

FINAL REPORT

VIRUCIDAL EFFECTIVENESS TEST
Coronavirus

TEST AGENT: Accelerated Hydrogen Peroxide

Data Requirements
EPA Guidelines 810.2100 (g)

Author
M. Khalid Ijaz, DVM, Ph.D.

Study Completion Date
07/02/03

Performing Laboratory
MICROBIOTEST, INC.
105B Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
506-101

Sponsor: **VIROX TECHNOLOGIES**
6705 Millcreek Dr., Unit 4
Mississauga, Ontario
L5N 5M4

STATEMENT OF NO DATA CONFIDENTIALITY

Title: Virucidal Effectiveness Test - Coronavirus

Performed by: MICROBIOTEST, INC.
105B 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: _____ _____
Signature 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:

Zheng Chen, Tien V. Mai

Study Director: MICROBIOTEST, INC.

M. Khalid Ijaz, DVM, Ph.D. — Date: 07/02/03

Submitted by:

Name — Title

Signature — Date

Sponsor: VIROX TECHNOLOGIES

Navid Omidbakhsh — Director of Research & Development

Name — Title

Signature — Date: 07/03/03

QUALITY ASSURANCE UNIT STATEMENT

Title of study: Virucidal Effectiveness Test - Coronavirus

The Quality Assurance Unit of MICROBIOTEST, INC. has inspected the Project Number 506-101 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	05/29/03	06/10/03	06/30/03
In process	05/29/03	06/10/03	06/30/03
Final report	06/30/03	06/30/03	07/02/03

Nathan S. Jones 07/02/03

Nathan S. Jones
Quality Assurance Unit

Date

TABLE OF CONTENTS

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:

1. Accelerated Hydrogen Peroxide, Lot No. 12783, received at MICROBIOTEST on 04/16/03, and assigned DS No. 6208.

2. Accelerated Hydrogen Peroxide, Lot No. 12784, received at MICROBIOTEST on 04/16/03, and assigned DS No. 6209.

SPONSOR:

VIROX TECHNOLOGIES
6705 Millcreek Dr., Unit 4
Mississauga, Ontario
L5N 5M4

TEST CONDITIONS

Challenge virus:

Human Coronavirus, ATCC VR-740

Host:

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

Active ingredient in test product:

Hydrogen peroxide

Neutralizer used:

Fetal bovine serum (FBS)

Contact time:

5 minutes

Contact temperature:

Room Temperature (24C)

Carrier Inoculation:

0.2mL of stock virus was used to inoculate carriers. Carriers were dried for 40 minutes

Dilution:

Ready to use

Organic load:

Viral stock contained at least 5% organic load

TEST CONDITIONS (continued)

Media and reagents:

Earle's Balanced Salt Solution (EBSS)
Eagle's Minimum Essential Medium containing 10% fetal bovine serum (CCM)
Sephacryl S-1000
Phosphate Buffered Saline containing 0.5% FBS
Fetal bovine serum (FBS)

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, INC., 105B Carpenter Drive, Sterling, VA 20164, from 05/29/03 to 06/10/03. The study director signed the protocol 05/29/03. 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 - 3. A titration was performed to determine the titer of the viral stock. The 50% cell cutlure infectious does per mL ($CCID_{50}$/mL) was determined by the method of Reed and Muench, 1938. The cell viability control demonstrated MRC-5 cell viability and media sterility. Virus was not detected in the cell viability control.

RESULTS (continued)

Table 1

Test Results

Dilutions (log_{10})	Accelerated Hydrogen Peroxide, Lot No. 12783	Accelerated Hydrogen Peroxide, Lot No. 12784
-2	- - - -	- - - -
-3	- - - -	- - - -
-4	- - - -	- - - -
-5	- - - -	- - - -
-6	- - - -	- - - -
-7	- - - -	- - - -
$CCID_{50}$/mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$

Table 2

Neutralizer Effectiveness and Cytotoxicity Controls

Dilutions (log_{10})	Accelerated Hydrogen Peroxide, Lot No. 12783	
	Neutralizer Effectiveness Control	Cytotoxicity Control
-2	+ + + +	0 0 0 0
-3	+ + + +	0 0 0 0
-4	+ + + +	0 0 0 0

Key: + = Cytopathic effects observed
- = No cytopathic effects observed
0 = No cytotoxicity observed

RESULTS (continued)

Table 3

Plate Recovery Control, Column Titer Control, and Virus Stock Titer

Dilutions ($\log_{10}$)	Plate Recovery Control	Column Titer Control	Virus Stock Titer
-1	PNS	PNS	+ + + +
-2	+ + + +	+ + + +	+ + + +
-3	+ + + +	+ + + +	+ + + +
-4	+ + + +	+ + + +	+ + + +
-5	+ - + +	+ - + +	+ + + +
-6	- + - -	+ - - +	+ - + +
-7	- - - -	- - - -	- - - -
-8	ND	ND	- - - -
$CCID_{50}$/mL	$10^{5.50}$	$10^{5.77}$	$10^{6.33}$

Key: PNS = Post-neutralized sample
+ = Cytopathic effects observed
- = No cytopathic effects observed
ND = Not determined

CONCLUSIONS

When tested as described, Accelerated Hydrogen Peroxide passed the Virucidal Effectiveness Test when Coronavirus, containing at least 5% organic load, was exposed to the test material for 5 minutes at room temperature (24C). All of the controls met the criteria for a valid test. These conclusions are based on observed data.

APPENDIX I
PROJECT SHEET(S)

MicroBioTest, Inc., 105B Carpenter Dr., Sterling, Virginia 20164

Date Issued: 05/29/03 Project Sheet No. **1** Page No. **1** Laboratory Project Identification No. **506-101**			
STUDY TITLE: Virucidal Effectiveness Test Coronavirus	**STUDY DIRECTOR:** M. Khalid Ijaz DVM, Ph.D. Signature		Date 05/29/03
TEST MATERIAL(S): Accelerated Hydrogen Peroxide	**LOT NO.** 12783 12784	**DATE RECEIVED:** 04/16/03 04/16/03	**DS NO.** 6208 6209
PERFORMING DEPARTMENT(S): Cell Culture, Mycobacteriology, and Virology Laboratory	**STORAGE CONDITIONS:** Location: A1 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
PROTECTIVE PRECAUTION REQUIRED: MSDS ■ Yes / ☐No			
PHYSICAL DESCRIPTION: ☐ Solid ■Liquid ☐ Aerosol ■ Other: Spray bottle			
PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.			
PROPOSED EXPERIMENTAL START DATE: 05/29/~~00~~03 **TERMINATION DATE:** 06/16/~~00~~03			
CONDUCT OF STUDY: ☐ FDA ☑ EPA ☐ R&D ☐GLP ☐ GCP ■ Other: Health Canada ~~Regulatory~~			
SPONSOR: Virox Technologies 6705 Millcreek Drive, unit 4 Mississauga, Ontario L5N 5M4	**CONTACT PERSON:** Navid Omidbakhsh Telephone No. 905-813-0110 x 114 FAX No. 905-815~~0200~~30220		

TEST CONDITIONS:

Challenge organism(s): Human coronavirus (ATCC VR-740)

Host(s): MRC-5 cells, (Diagnostic Hybrids, Inc., Athens, OH)

Active ingredient(s): Hydrogen peroxide **Columns used:** ■ Yes/ ☐ No

Neutralizer(s): Fetal bovine serum (FBS)

Contact Time(s): 5 minutes **Contact Temperature(s):** Room Temperature

Diluent(s): N/A **Dilution(s):** Ready to use

Serum: ■ Yes / ☐ No (virus already contains at least 5% organic load)

Incubation Time(s): 10 – 14 days **Incubation Temperature(s):** 33±1C in 5±1 CO_2

Comment(s), Test Application: 2 mL of the test product will be applied to the tested carrier

MicroBioTest, Inc., 105B Carpenter Dr., Sterling, Virginia 20164

Date Issued: 07/01/03 Project Sheet No. **2** Page No. **1** Laboratory Project Identification No. **506-101**			
STUDY TITLE: Virucidal Effectiveness Test Coronavirus	**STUDY DIRECTOR:** M. Khalid Ijaz DVM, Ph.D. Signature		Date
TEST MATERIAL(S): Accelerated Hydrogen Peroxide	**LOT NO.** 12783 12784	**DATE RECEIVED:** 04/16/03 04/16/03	**DS NO.** 6208 6209
PERFORMING DEPARTMENT(S): Cell Culture, Mycobacteriology, and Virology Laboratory	**STORAGE CONDITIONS:** Location: A1 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ☐GLP ☐ GCP ■ Other: Health Canada Regulatory			
SPONSOR: Virox Technologies 6705 Millcreek Drive, unit 4 Mississauga, Ontario L5N 5M4	**CONTACT PERSON:** Navid Omidbakhsh Telephone No. 905-813-0110 x 114 FAX No. 905-8150200		

EXPLANATION:

This project sheet was issued to document the following:

1. The Media and reagent section of the protocol lists PBS as a media/reagent used for this study. PBS alone is not needed in any part of the test and therefore will not be used. In addition, CCM is referred to as "Eagle's minimum essential media containing 10% fetal bovine serum." This will be equivalent to "Minimum essential media eagle's containing 10% fetal bovine serum." This amendment serves to clarify the usage of PBS as well as redefine CCM for this study.
2. The Cell culture section of the protocol indicates 35±2C as the incubation/adsorption conditions used for this study. To optimize the conductivity of viral replication of the virus-host system, host-containing plates used for this study will be adsorbed and incubated at 33±1C in 5±1% CO_2. This amendment serves to adjust the incubation conditions used for this study.

APPENDIX II
SIGNED PROTOCOL

MICROBIOTEST PROTOCOL

VIRUCIDAL EFFECTIVENESS TEST

Coronavirus

Prepared for:
Virox Technologies
6705 Millcreek Drive, Unit 4
Mississauga, Ontario L5N 5M4

April 11, 2003

MICROBIOTEST PROTOCOL: VIR.1.04.11.03

MICRBIOTEST Project Number: 506-10'

OBJECTIVE:

This test is designed to substantiate virucidal effectiveness claims for a product to be labeled as a virucide. It determines the potential of the test agent to disinfect hard surfaces contaminated with viruses. The test is designed to simulate consumer use and conforms to EPA Guidelines DIS/TSS-7, November 1981, and follows the procedure outlined in the American Society for Test Materials (ASTM) test method designated E 1053-97.

TESTING CONDITIONS:

Virus will be dried on a sterile glass Petri dish at ambient temperature. Two lots of disinfectant will be used to treat the dried virus according to the label claims. After a defined exposure period, the test agent-virus mixture will be scraped from the surface, collected, neutralized and assayed for the presence of infectious virions.

MATERIALS:

A. Test, control and reference substances will be supplied by the sponsor of the study (see last page).

The test agent will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test agent such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures MICROBIOTEST testing facility management that the test agent has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MICROBIOTEST will retain all unused test agents for a period of at least three months after completion of the test, then discard them in a manner that meets the approval of the safety officer.

B. Materials supplied by MICROBIOTEST, including, but not limited to:

1. Challenge virus (requested by the sponsor of the study): Human Coronavirus (ATCC VR-740)

2. Host cell line: MRC-5 cells (Diagnostic Hybrids, Inc., Athens, OH)

3. Laboratory equipment and supplies.

4. Media and reagents:
   a. Eagle's minimum essential medium containing 10% fetal bovine serum (CCM)
   b. Phosphate buffered saline (PBS).
   c. Earle's balanced salt solution (EBSS)
   d. Neutralizer(s)
   e. Sephacryl columns (if necessary)
   f. PBS containing 0.5% fetal bovine serum

TEST SYSTEM IDENTIFICATION:

All Petri dishes, dilution tube racks, and host-containing apparatus will be labeled with the following information: virus, host, test agent and project number.

EXPERIMENTAL DESIGN:

All of the procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at MICROBIOTEST. SOPs and Logs are referred to in the raw data and are required as part of GLP regulations.

A. Inoculum preparation:

Viral stocks are purchased from American Type Culture Collection (ATCC) or other reputable sources that identify them by scientifically accepted methods and are propagated at MICROBIOTEST. Records are maintained that demonstrate the origin of the virus. The virus stocks will be stored at an ultra-low temperature.

Frozen viral stocks will be thawed on the day of the test (fresh stock cultures may be used at the discretion of the Study Director).

B. Carrier preparation:

An aliquot of 0.2 mL of stock virus will be spread, with the cell scraper, over an area of approximately 4 in^2 that has been marked on the underside of pre-sterilized Petri dishes. Then the virus will be allowed to dry for 30 to 60 minutes at room temperature. The drying time and temperature will be recorded. One carrier will be prepared for each lot and the Plate recovery control, additionally; one plate will be prepared for the Neutralizer effectiveness control using EBSS.

C. Test agent preparation:

The agent will be prepared according to the sponsor's directions or proposed label claims.

E. Test:

At least 10^4 infectious units of the virus inoculum will be spread over the surface of one sterile glass Petri dishes with a cell scraper and allowed to dry for 30 to 60 min at room temperature. After the inoculum has dried, 2.0 mL of the test agent will be added. The plates will remain at the temperature and for the time specified by the sponsor.

After the contact period, the test agent will be neutralized with 2.0 mL of appropriate neutralizer and the mixture will be scraped from the surface of the dish with a cell scraper. This will be considered approximately one log_{10} dilution.

If columns are required for proper neutralization, 0.5 mL of each sample will be loaded into separate pre-spun Sephacryl columns. The columns will be spun for 4 minutes at 1000 rpm. The samples will be aseptically removed from the columns and dispensed into dilution tubes containing EBSS. The final dilution will be 1:10. Ten-fold serial dilutions will be performed. If columns are not used, serial tenfold dilutions of neutralized virus will be prepared in EBSS.

For spray type agents, the agent will be used as per sponsor's instructions, the volume dispensed will be measured and an equal volume of neutralizer will be used. Following spraying and contact time, the procedure for processing the samples will be the same as described earlier (see above).

F. Cell culture:

The residual infectious virus in both test and controls will be detected by cytopathic effect. Selected dilutions of the neutralized inoculum/test agent mixture will be added to cultured host cells, and incubated at 35±2C for 90-120 minutes for viral adsorption. Post-adsorption, the sample containing EBSS will be aspirated, plates washed once with EBSS and refed with CCM. The cultures will be incubated at 35±2C for 10-14 days. Post-incubation the cytopathic effects (CPE) will be scored by examining both test and controls. The observations will be recorded.

G. Controls:

1. Neutralizer effectiveness: (NE)

This control will determine if any residual active ingredient is present after neutralization.

One lot of the test agent will be used for the neutralizer effectiveness control. This control will be processed exactly as the test procedure but instead of viral inoculum, EBSS will be added. Post-test, and neutralization, a 1.0-mL sample will be divided into two portions, [one for cytotoxicity control (see below) and one for neutralizer effectiveness] and loaded onto pre-spun columns if required.

If columns are used, each sample will be passed through an individual column. If columns are not used, the neutralizer effectiveness sample will be ten-fold serially diluted in EBSS and virus (100 µL of stock) will be added to each dilution and incubated for a period equivalent to the contact time. Then these samples will be used to inoculate host (eggs) as described for the test procedure.

2. Cytotoxicity: (CT)

The cytotoxicity sample, acquired from the neutralizer effectiveness control, will be ten-fold serially diluted in EBSS, having no virus added. Select dilutions will be inoculated and incubated in the same manner as the rest of the test and controls. These effects are distinct from cytopathic effects due to virus infection, which will be evident in the stock titer and plate recovery cultures.

3. Plate recovery: (PRC)

The virus inoculum will be spread over the surface of a sterile glass Petri dish and left to dry at ambient temperature for 30 to 60 minutes. A volume of EBSS equivalent to that of the test agent will be added to the dried virus. Post-contact time, virus will be subjected to the identical neutralization procedure as the test agent. This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

When samples are required to pass through the Sephacryl columns, a column titer control (CTC) will also be performed (by assaying a portion of PRC before passing through the columns) to determine any affect on infectious virus titer while passing the columns (see below).

The results from this control are compared with the test results to confirm recovery of at least four log of infectious virus in this control following drying and neutralization. Its titer is used to compare with the titers of the test results to reach the acceptable test criteria (see below).

4. Column titer control:

This control will be performed only if Sephacryl columns are used. It is performed to determine any affect on infectious virus titer while passing the columns.

The sample for this control will be acquired from a portion of the Plate recovery control, prior to passing through the columns.

This sample is directly ten-fold serially diluted in EBSS, then processed in the same manner as the rest of the test and controls.

5. Virus stock titer: (VST)

In order to determine the original virus titer, an aliquot of the virus inoculum will be tenfold serially diluted in EBSS, and assayed (see above) as described for the test and other controls.

6. Cell viability control: (CVC)

Four wells will be inoculated with EBSS during viral adsorption and CCM during the incubation phase of the study. This control will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will also confirm the sterility of the media employed throughout the assay period.

H. Calculation:

The $CCID_{50}$/mL will be determined from the virus stock, test and plate recovery data using the method of Reed and Muench, Am. J. of Hyg. 1938, 27:493. The test results shall be reported as the reduction of the virus titer due to treatment with test material expressed as log_{10}.

PRODUCT EVALUATION CRITERIA:

According to the regulatory agencies, the test agent passes the test if there is complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a three-log reduction in titer must be demonstrated beyond the cytotoxic level.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The infectious virus recovered from the PRC control must be $\geq$ 4-$\log_{10}$.
- Viral-induced toxicity must be distinguishable from test agent induced cytotoxic effects.

DATA PRESENTATION:

The final report will include the following information in tabular form (if appropriate) for both the test and control cultures:

- Virus stock titer
- Test results
- Plate recovery
- Neutralizer system employed and effectiveness data
- Cytotoxicity results
- The volume of virus used, drying time and ambient temperature of the test
- Host recovery system

REPORT FORMAT:

MICROBIOTEST employs a standard report format for each test design. Each final report provides the following information:

- Sponsor identification
- Test agent identification
- Type of assay and project number
- Dates of study initiation and completion
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria
- Signed Quality Assurance and Compliance Statements

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at MICROBIOTEST, INC., 105B Carpenter Drive, Sterling, Virginia 20164. Chemical analyses, if required, will be performed at an analytical laboratory to be identified in the final report.

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, INC., 105B Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test agent; challenge virus and host cell line monolayers used; and the type of neutralizers to be employed in the test will be addressed in a project sheet issued separately for each study. The study sponsor should sign all project sheets.

MICROBIOTEST PROTOCOL: Virucidal Effectiveness Test - Coronavirus

MISCELLANEOUS INFORMATION:

The following information is to be completed by the sponsor prior to initiation of the study:

A. Name and address: Virox Technologies
6705 Millcreek Drive, Unit 4
Mississauga, Ontario L5N 5M4

B. Test Agent: Accelerated Hydrogen Peroxide

Lot No. 1: 12784 Lot No. 2: 12783

Active ingredient: Hydrogen Peroxide

Dilution to be tested: Ready to use Diluent: —

Spray time: N/A Spray distance: N/A

Exposure time: 5 min Exposure temperature: RT ±2C

C. All virucidal studies are conducted in the presence of at least 5% organic load.

D. Additional instructions: _____

E. Precautions/storage conditions: refer to MSDS or certificate of analysis
☒ provided ☐ not provided

REPORT HANDLING:

The sponsor intends to submit this information to: ☐ the EPA ☐ the FDA
☐ CAL DPR ☐ ARTG ☐ non GLP ☐ other ✓

HEALTH CANADA
Regulatory Affairs
TPP

PROTOCOL APPROVAL:

Sponsor: [redacted] Date: 04/14/03

Protocol: VIR.1.04.11.03

MICROBIOTEST, INC.

NS - 04/14/03