

Volume \_\_\_\_\_

**FINAL REPORT**  
**VIRUCIDAL EFFICACY TEST**  
**Using Human Immunodeficiency virus Type 1**

**TEST AGENT**  
**Easy Decon 200-531X**

**Data Requirements**  
**EPA Guidelines 810.2100 (g)**

**Author**  
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**Study Completion Date**  
**05/13/08**

**Performing Laboratory**  
**MICROBIOTEST**  
**105 Carpenter Drive**  
**Sterling, Virginia 20164**

**Laboratory Project Identification Number**  
**634-107**

**Submitted to: EFT Holdings, Inc.**  
**1012 Oster Drive, Suite A**  
**Huntsville, AL 35816**

## STATEMENT OF NO DATA CONFIDENTIALITY

Title: Virucidal Efficacy Test Using Human Immunodeficiency virus Type 1

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 \_\_\_\_\_ Title \_\_\_\_\_

\_\_\_\_\_  
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:

Lauren A. Blaszak

Study Director: MICROBIOTEST

\_\_\_\_\_  
S. Steve Zhou, Ph.D.

\_\_\_\_\_  
Date

Submitted by:

\_\_\_\_\_  
Name

\_\_\_\_\_  
Title

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

Sponsor: EFT Holdings, Inc.

\_\_\_\_\_  
Name

\_\_\_\_\_  
Title

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

**MICROBIOTEST**

### QUALITY ASSURANCE UNIT STATEMENT

Title of Study: Virucidal Efficacy Test Using Human Immunodeficiency virus Type 1

The Quality Assurance Unit of MICROBIOTEST has inspected the Project Number 634-107 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	04/09/08	05/05/08	05/09/08
In Process	04/09/08	04/09/08	05/09/08
Final Report	05/05/08	05/05/08	05/09/08

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

\_\_\_\_\_  
Date

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## TEST SUMMARY

**TITLE:** Virucidal Efficacy Test Using Human Immunodeficiency virus Type 1

**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. Easy Decon 200-531X components: Penetrator, Fortifier, Booster; Lot No. T-1003, received at MICROBIOTEST 04/03/08, and assigned DS No. 9302.
2. Easy Decon 200-531X components: Penetrator, Fortifier, Booster; Lot No. T-1004, received at MICROBIOTEST 04/03/08, and assigned DS No. 9303.

**SPONSOR:** EFT Holdings, Inc.  
1012 Oster Drive, Suite A  
Huntsville, AL 35816

## TEST CONDITIONS

Challenge virus:

Human Immunodeficiency virus Type 1, Zeptomatrix Corporation

Host:

C8166 cells, University of Pennsylvania

Active ingredient in test product:

Hydrogen Peroxide/Benzyl-C12-C16 Alkyl Di-Methyl Chlorides

Neutralizer used:

Fetal bovine serum (FBS) + 0.3% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> + 1% catalase

Carrier Inoculation and Dry Time:

2 x 2 inch area of glass carrier inoculated with 0.2 mL of virus and dried for 30 minutes.

Test Agent Application:

Carriers were thoroughly sprayed until wet from a distance of 6 inches.

Test Agent Preparation:

1. Added entire contents of bottle (Penetrator, Part 1 pre-measured), to the flask.
2. Added entire contents of bottle (Fortifier, Part 2 pre-measured), to the flask.
3. Added entire contents of bottle (Booster, Part 3 pre-measured), to the flask.
4. Mixed the contents ensuring all contents were thoroughly blended.
5. Used within 8 hours of mixing.

Contact time:

10 minutes

Contact temperature:

23 - 24C

Organic load:

Viral stock contained ≥ 5% organic load.

Media and reagents:

Fetal bovine serum + 0.3% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> + 1% catalase  
RPMI + 10% Fetal bovine serum  
Sephacryl columns

## STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164. Testing was initiated on 04/09/08 and was concluded on 04/21/08. The study director signed the protocol on 04/09/08. 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 (TCID<sub>50</sub>/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<sub>k</sub> = the logarithm of the smallest dosage which induces infection in all cultures
- d = the logarithm of the dilution factor
- p<sub>i</sub> = the proportion of positive results at dilution i

The values were converted to TCID<sub>50</sub>/ml using a sample inoculum of 0.05 ml.

### **CALCULATION OF TITER (continued)**

When a sample contains a low concentration of virus there is a discrete probability that if only a fraction of the sample is tested for virus, that fraction will test negative due to random distribution of virus throughout the total sample. The probability,  $p$ , that the sample analyzed does not contain infectious virus is expressed by:  $p = [(V-v)/V]^y$ , where  $V$  is the total volume of the container,  $v$  is the volume of the fraction being tested, and  $y$  is the absolute number of infectious viruses randomly distributed in the sample. If  $V$  is sufficiently large relative to  $v$ , the Poisson distribution can approximate  $p$ :

$$P = e^{-cv} \quad \text{or} \quad c = -[\ln(P)] / v$$

Where  $c$  is the concentration of infectious virus and  $v$  is the total sample volume. The amount of virus which would have to be present in the total sample in order to achieve a positive result with 95% confidence ( $p = 0.05$ ) is calculated as

$$c = -[\ln(0.05)] / v = 3 / v$$

If all  $n$  dishes are negative, the virus titer after the process is considered to be less than or equal to this value. The total volume of sample assayed is  $v = v'nd$ , where  $v'$  is the test volume in a dish,  $n$  is the number of dishes per sample, and  $d$  is the sample dilution.

### **RESULTS**

Data is presented in Tables 1 – 5.

The  $\log_{10}$  Reduction Factor was calculated in the following manner:

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

**RESULTS (continued)**

**Table 1  
 Titer Results  
 Test Agent**

Dilution	Easy Decon 200-531X	
	Lot No. T-1003	Lot No. T-1004
10 <sup>-2</sup>	C/8	C/8
10 <sup>-3</sup>	0/8	0/8
10 <sup>-4</sup>	0/8	0/8
10 <sup>-5</sup>	0/8	0/8
10 <sup>-6</sup>	0/8	0/8
10 <sup>-7</sup>	0/8	0/8
TCID <sub>50</sub> /mL	≤ 10 <sup>3.83</sup>	≤ 10 <sup>3.83</sup>

**Table 2  
 Titer Results**

**Neutralizer Effectiveness and Cytotoxicity Related Controls**

Dilution	Easy Decon 200-531X Lot No. T-1003	
	Neutralizer Effectiveness Control	Cytotoxicity Control
10 <sup>-2</sup>	C/8	C/8
10 <sup>-3</sup>	8/8	0/8
10 <sup>-4</sup>	8/8	0/8

**Table 3  
 Viability Control Results**

Cell Viability Control
0/8; cells were viable, media was sterile

Key: x/y = x wells out of y wells inoculated showed positive viral cytopathic effect (CPE)  
 0 = 0 wells out of y wells inoculated showed positive viral cytopathic effect (CPE); no  
 cytotoxicity observed in any inoculated wells.  
 C/y = Cytotoxicity observed in y number of wells

## RESULTS (continued)

**Table 4**  
**Titer Results**

Dilution	Plate Recovery Control	Column Titer Control
$10^{-2}$	8/8	8/8
$10^{-3}$	8/8	8/8
$10^{-4}$	8/8	8/8
$10^{-5}$	5/8	5/8
$10^{-6}$	4/8	3/8
$10^{-7}$	0/8	2/8
TCID <sub>50</sub> /mL	$10^{6.93}$	$10^{7.05}$

Key: x/y = x wells out of y wells inoculated showed positive viral cytopathic effect (CPE)  
 0 = 0 wells out of y wells inoculated showed positive viral cytopathic effect (CPE); no cytotoxicity observed in any inoculated wells.  
 C/y = Cytotoxicity observed in y number of wells

**Table 5**  
**Log<sub>10</sub> Reduction**  
**Easy Decon 200-531X**

Sample	Initial Titer Log <sub>10</sub> TCID <sub>50</sub> /mL	Output Titer Log <sub>10</sub> TCID <sub>50</sub> /mL	Log <sub>10</sub> Reduction
Lot No. T-1003	$10^{6.93}$	$\leq 10^{3.83}$	$\geq 3.10$
Lot No. T-1004	$10^{6.93}$	$\leq 10^{3.83}$	$\geq 3.10$

## 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, Easy Decon 200-531X (Lot Nos. T-1003 and T-1004) passed the Virucidal Efficacy Test when Human Immunodeficiency virus Type 1, containing at least 5% organic soil, was exposed to the test agent for 10 minutes at 23 - 24C. All of the controls met the criteria for a valid test. These conclusions are based on observed data.