

**MICROBIOTEST, INC**  
*The Microbiology and  
Virology Laboratory*

## **FINAL REPORT**

# **VIRUCIDAL EFFECTIVENESS TEST Canine parvovirus**

**BioSurf**

Data Requirements  
EPA Guideline 810.2100 (g)

Author  
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Study Completion Date  
04/12/01

Performing Laboratory  
MicroBioTest, Inc.  
105B Carpenter Drive  
Sterling, Virginia 20164

Laboratory Project Identification Number  
455-105

**Submitted to: Micrylium  
4590 Dufferin Street  
Toronto, Ontario M3H 5S5  
Canada**



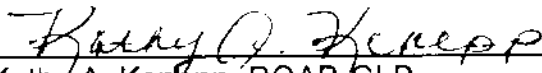
**QUALITY ASSURANCE UNIT STATEMENT**

TITLE OF STUDY: Virucidal Effectiveness Test – Canine parvovirus

The Quality Assurance Unit of MicroBioTest, Inc. has inspected the Project Number 455-105 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	03/02/01	03/02/01	03/20/01
In-Process	03/02/01	03/02/01	03/20/01
Final Report	03/26/01	03/27/01	04/09/01

  
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 Kathy A. Kerepp, RQAP-GLP  
 Manager, Quality Assurance Unit

04/11/01  
 Date

## TEST SUMMARY

**TITLE:** Virucidal Effectiveness Test – Canine parvovirus

**STUDY DESIGN:** See Project Sheets (Appendix I)  
See signed protocol (Appendix II)

**TEST MATERIALS:**

1. BioSurf, Lot No. 044, received at MicroBioTest, Inc. on 09/29/00 and assigned DS No. 4995.
2. BioSurf, Lot No. 068, received at MicroBioTest, Inc. on 11/27/00 and assigned DS No. 5047.

**SPONSOR:** Micrylium  
4590 Dufferin Street  
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## TEST CONDITIONS

**Virus tested:**

Canine parvovirus, CPV-265 (James A. Baker Institute for Animal Health Cornell University) – frozen viral stocks were thawed on the day of the test.

**Host cells:**

NLFK, (James A. Baker Institute for Animal Health, Cornell University)

**Active ingredient:**

Ethanol/chlorhexidine

**Neutralizers used:**

Fetal bovine serum  
Sephacryl columns S-1000

**Contact time:**

50 seconds

**Contact temperature:**

20±2C

**Dilution:**

Ready-to-use spray

**Application:**

Spray for 15 seconds at 6 inches from carrier

**Organic load:**

Virus replicated in cells grown in 5% or greater fetal bovine serum.

**Media and reagents:**

Liebovitz-15 + McCoy's complete tissue culture medium  
Phosphate Buffered Saline (PBS)  
Fetal bovine serum  
PBS + 0.5% fetal bovine serum  
Earle's balanced salt solution  
Sephacryl S-1000

**Media and reagents (continued):**

Sterile deionized water  
 Tissue culture grade alcohol  
 Anti-CPV direct conjugate

**STUDY DATES AND FACILITIES**

The laboratory phase of this test was performed at MicroBioTest, Inc., 105B Carpenter Drive, Sterling, Virginia 20164, from 03/02/01 to 03/11/01. The study director signed the protocol 03/01/01. The study completion date is the date the study director signed the final report.

Any 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, Inc. and the sponsor will be stored in the archives at MicroBioTest, Inc., 105B Carpenter Drive, Sterling, Virginia 20164.

**RESULTS**

Results and data are presented in Tables 1 – 3. For this test, 0.2 mL of inoculum was applied to each carrier and allowed to dry for 30 minutes at 20C. All controls, including neutralizer effectiveness, cytotoxicity, plate recovery, column titer, virus stock titer, cell viability and sterility, met the established criteria for a valid test. The fluorescent focus forming unit dose 50% per mL FFFUD<sub>50</sub>/mL was determined from the virus stock, test, column titer and plate recovery data using the method of Reed and Muench, 1938.

Table 1

Test Results for BioSurf  
 Canine parvovirus

Dilution	Lot No. 044	Lot No. 068
10 <sup>-1</sup>	PNS	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>	ND	ND
FFFUD <sub>50</sub> /mL	≤10 <sup>1.50</sup>	≤10 <sup>1.50</sup>

Key: -- = No FF observed  
 PNS = Post neutralized sample  
 ND = Not determined

**RESULTS (continued)**

Table 2

## Control Results for Canine parvovirus

Dilution	Neutralizer Effectiveness	Cytotoxicity
$10^{-1}$	PNS	PNS
$10^{-2}$	++++	0000
$10^{-3}$	++++	0000
$10^{-4}$	++++	0000

Table 3

## Additional Control Results for Canine parvovirus

Dilution	Plate recovery	Column Titer Control	Virus Stock Titer
$10^{-1}$	PNS	PNS	++++
$10^{-2}$	++++	++++	++++
$10^{-3}$	++++	++++	++++
$10^{-4}$	++++	++++	++++
$10^{-5}$	++++	++++	++++
$10^{-6}$	+--+	+++-	++++
$10^{-7}$	----	----	+++-
$10^{-8}$	ND	ND	----
FFFUD <sub>50</sub> /mL	$10^{6.00}$	$10^{6.33}$	$10^{7.33}$

Key: PNS = Post neutralized sample  
 ND = Not determined  
 + = FFFU observed  
 - = No FFFU observed  
 0 = No cytotoxicity observed

**CONCLUSIONS**

When tested as described, BioSurf, exposed to the challenge virus for 50 seconds, at 20±2C, proved to be an effective virucidal agent against Canine parvovirus. The neutralizer was shown to be effective, and the controls demonstrated good infectivity of the challenge virus. These conclusions are based on observed data.