FINAL REPORT

Virucidal Effectiveness Test
Canine parvovirus
Using unglazed, clay tiles

Test Agent System:
Dry Steam Vapor System, 2400 Series,
TANCS® Equipped

Data Requirements
EPA Guidelines 810.2100 (g)

Author
Lauren A. Blaszak

Study Completion Date
pending

Performing Laboratory
MICROBIOTEST
105 Carpenter Drive
Sterling, Virginia 20164

MICROBIOTEST Project Identification Number
567-105

Submitted to: Advanced Vapor Technologies, LLC
7719 230th Street, SW
Edmonds, WA 98026
STATEMENT OF NO DATA CONFIDENTIALITY

Title: Virucidal Effectiveness Test – Canine parvovirus - Using unglazed, clay tiles

Performed by: MICROBIOTEST Sponsor: Advanced Vapor Technologies, LLC
105 Carpenter Drive 7719 230th Street, SW
Sterling, Virginia 20164 Edmonds, WA 98026

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 ______________________________ _______________
Name ______________________________ Title ______________________________

Signature ______________________________ Date ______________________________

MICROBIOTEST
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

__________________________________________  ___________
Lauren A. Blaszak                         Date

Submitted by:  

__________________________________________  ___________
Name                          Title

__________________________________________  ___________
Signature                      Date

Sponsor: ADVANCED VAPOR TECHNOLOGIES, LLC

__________________________________________  ___________
Name                          Title

__________________________________________  ___________
Signature                      Date


**QUALITY ASSURANCE UNIT STATEMENT**

Title of Study: Virucidal Effectiveness Test – Canine parvovirus - Using unglazed, clay tiles

The Quality Assurance Unit of MICROBIOTEST has inspected the Project Number 567-105 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.

<table>
<thead>
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<th>PHASE INSPECTED</th>
<th>DATE OF INSPECTION</th>
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<th>DATE REPORTED TO MANAGEMENT</th>
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<tr>
<td>Protocol</td>
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<td>Final Report</td>
<td>12/15/06</td>
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Nathan S. Jones, RQAP–GLP  
Manager, Quality Assurance Unit  
Date
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TEST SUMMARY

TITLE: Virucidal Effectiveness Test – Canine parvovirus – Using unglazed, clay tiles

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:

Dry Steam Vapor System, 2400 series, TANCS® equipped, Serial No. 5505053936, received at MICROBIOTEST 09/28/06, and assigned DS No. 8432.

Note: The Dry Steam Vapor System equipment was operated by a representative of the sponsor.

Note: the 6cm x 6cm x 1.2cm tiles were received by the sponsor on 11/16/06.

SPONSOR: Advanced Vapor Technologies, LLC
7719 230th Street, SW
Edmonds, WA  98026
TEST CONDITIONS

Challenge virus:

Canine parvovirus (NIKE/CPV-2b), American BioResearch Laboratories

Host:

CrFK cells, American BioResearch Laboratories

Organic load:

Viral stock contained $\geq$5% organic load

Active ingredient in test product:

Dry steam vapor generated from tap water

Contact time:

The Dry Steam Vapor System was operated by a representative of the sponsor to treat the test tiles for a contact time of 7 seconds. The nozzle brush was held over the tile and gently moved in a back and forth motion.

Contact temperature:

Ambient temperature (22C)

Dilution:

Ready to use

Tile Inoculation:

A marked 4 × 4 centimeter area of the smooth surface of unglazed clay test tiles (approximately 6 × 6 × 1.2 centimeters) was inoculated with 0.2mL viral stock and dried for 20 minutes at 22C.

Media and reagents:

- RPMI 1640 containing 5% Newborn calf serum
- Phosphate Buffered saline (PBS)
- 80% acetone
- PBS containing 0.5% fetal bovine serum
- CPV direct FA conjugate
- Tap water
STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164, from 11/29/06 to 12/11/06. The study director signed the protocol 11/29/06. 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.

RESULTS

Results are presented in Tables 1 – 4. The 50% fluorescent focus forming unit dose per mL (FFFD_{50}/mL) was determined from the test and relevant control data using the method of Reed and Muench, Am. J. of Hyg. 1938, 27:493. The cell viability control demonstrated cell viability and media sterility. Virus was not detected in the cell viability control. All controls including cytotoxicity and tile recovery titer met the criteria required for a valid test. Infectious virus was not detected in the host system after exposure to the test agent as described.

The log_{10} reduction (LR) was calculated in the following manner:

\[ \text{Log}_{10} \text{ reduction} = \frac{\text{Infectious virus titer recovered from tile recovery control} - \text{Infectious virus titer recovered from test}}{\text{Infectious virus titer recovered from test}} \]
# RESULTS (continued)

**Table 1**

Test Results

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Dry Steam Vapor System, 2400 Series, TANCS® equipped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TILE 1</td>
</tr>
<tr>
<td>10^{-2}</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>10^{-3}</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>10^{-4}</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>10^{-5}</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>10^{-6}</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>10^{-7}</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>FFFUD_{50/mL}</td>
<td>≤10^{1.50}</td>
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</table>

**Table 2**

Cytotoxicity Control

<table>
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<th>Dilution</th>
<th>Dry Steam Vapor System, 2400 Series, TANCS® equipped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytotoxicity Control</td>
</tr>
<tr>
<td>10^{-2}</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>10^{-3}</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>10^{-4}</td>
<td>0 0 0 0</td>
</tr>
</tbody>
</table>

**Table 3**

Control results

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Canine parvovirus Tile Recovery Control</th>
<th>Cell Viability Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^{-2}</td>
<td>+ + + +</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>10^{-3}</td>
<td>+ + + +</td>
<td></td>
</tr>
<tr>
<td>10^{-4}</td>
<td>+ + + +</td>
<td></td>
</tr>
<tr>
<td>10^{-5}</td>
<td>+ 0 + 0</td>
<td></td>
</tr>
<tr>
<td>10^{-6}</td>
<td>0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>10^{-7}</td>
<td>0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>FFFUD_{50/mL}</td>
<td>10^{5.00}</td>
<td></td>
</tr>
</tbody>
</table>

Key: + = Canine parvovirus infected cells were detected, fluorescence observed  
0 = Canine parvovirus infected cells not detected, no fluorescence observed; no cytotoxicity observed
RESULTS (continued)

Table 4
Log_{10} Reduction

<table>
<thead>
<tr>
<th>Dry Steam Vapor System, 2400 series, TANCS® equipped</th>
<th>TILE 1</th>
<th>TILE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TILE 1</td>
<td>≥3.50</td>
<td></td>
</tr>
<tr>
<td>TILE 2</td>
<td>≥3.50</td>
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CONCLUSIONS

When tested as described, Dry Steam Vapor system, 2400 series, TANCS® equipped, passed the Virucidal Effectiveness Test when Canine parvovirus, containing at least 5% organic load, was exposed to the test agent for 7 seconds at 22°C. All of the controls met the criteria for a valid test. These conclusions are based on observed data.