

Volume _____

FINAL REPORT

AOAC SPORICIDAL TEST
Using *Clostridium sporogenes* and Porcelain Penicylinders

Data Requirements
EPA Guidelines 810.2100 (b)

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Study Completion Date
October 4, 2002

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

Laboratory Project Identification Number
479-108

Submitted to: ENVIROFOAM TECHNOLOGIES, INC.
2903 Wall Triana Hwy., Suite 5B
Huntsville, AL 35824

STATEMENT OF NO DATA CONFIDENTIALITY

Title: AOAC Sporicidal Test Using *Clostridium sporogenes* and Porcelain Penicylinders

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 _____

_____ 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:

Angela L. Hollingsworth, Diane M. LeClercq, Felicia L. Sellers, Samina S. Raja, Temi Odebunmi, Hamid Bashir, Jessica D. Bocek

Study Director: MICROBIOTEST, INC.

_____	_____
Angela L. Hollingsworth	Date

Submitted by:

_____	_____
Name	Title
_____	_____
Signature	Date

Sponsor: ENVIROFOAM TECHNOLOGIES, INC.

_____	_____
Name	Title
_____	_____
Signature	Date

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: AOAC Sporicidal Test Using *Clostridium sporogenes* and Porcelain Penicylinders

The Quality Assurance Unit of MICROBIOTEST has inspected the Project Number 479-108 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	07/12/02	07/12/02	10/03/02
In Process	07/12/02 07/30/02	07/12/02 07/30/02	10/03/02 10/03/02
Final Report	10/03/02 10/04/02	10/04/02	10/04/02

Nathan S. Jones
Quality Assurance Unit

Date

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TEST SUMMARY

TITLE: AOAC Sporicidal Test Using *Clostridium sporogenes* and Porcelain Penicylinders

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. Easy Decon™ 4013 (Penetrator III), Lot No. 02-303, received at MICROBIOTEST on 06/29/02, and assigned DS No. 5830.
2. Easy Decon™ 4013 (Penetrator III), Lot No. 02-304, received at MICROBIOTEST on 06/29/02, and assigned DS No. 5831.
3. Easy Decon™ (Penetrator III RTM), Lot No. 02-116, which was at least 60 days old when tested, received at MICROBIOTEST on 07/19/02, and assigned DS No. 5860.
4. UHP (for use with all three lots of Easy Decon™), Lot No. 0705-72-300, received at MICROBIOTEST on 06/11/02 and 07/19/02, and assigned DS No. 5796.
5. Activator (for use with all three lots of Easy Decon™), Lot No. A013728301, received at MICROBIOTEST on 06/29/02 and 07/19/02, and assigned DS No. 5832.

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MICROBIOTEST, INC.

TEST CONDITIONS

Challenge microorganism:

Clostridium sporogenes, ATCC 3584

Active ingredient in test product:

Quaternary ammonium compounds, hydrogen peroxide

Neutralizer used:

Fluid thioglycollate medium containing 7.0% Polysorbate 80, 1.0% lecithin, 0.3% thioglycolic acid and catalase (FTM+)

Contact time:

4 hours

Contact temperature:

20±1C

Test material preparation:

85% by weight of the liquid Penetrator III or Penetrator III RTM test material was mixed with 8.2% by weight of UHP powder, followed by 6.8% by weight of Activator powder and mixed vigorously for at least two minutes.

Media and reagents:

Fluid Thioglycollate Medium containing 7.0% Polysorbate 80, 1.0% lecithin, 0.3% thioglycolic acid and catalase (FTM+)

Modified Fluid Thioglycollate Medium

HCl solution, 2.5N

Trypticase Soya Agar

Phosphate Buffered Saline (PBS)

PBS containing 1% Polysorbate 80

Lethen Broth (LB)

Gram stain reagents

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, INC., 105B Carpenter Drive, Sterling, VA 20164, from 07/12/02 to 08/05/02 and from 07/30/02 to 08/23/02. The study director signed the protocol 07/11/02. 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 - 2. The challenge microorganism was confirmed by Gram stain and colony morphology to be consistent with *C. sporogenes*. The sterility controls exhibited no growth. The neutralizer effectiveness and viability controls exhibited growth. An average of 13 colony-forming units (CFU) of *C. sporogenes* was added to the Neutralizer Effectiveness Controls for test date 07/12/02 and 14 CFU for test date 07/30/02. **The average carrier count was 2.0×10^5 CFU/carrier for test date 07/12/02 and 2.3×10^5 CFU/carrier for test date 07/30/02.** The pH for Lot No. 02-303 was 9.8, for Lot No. 02-304 was 9.8, and for Lot No. 02-116 was 9.6.

Table 1

Test Results

Results Expressed as No. of Positives (growth)/Total No. of Replicates

Microorganism	FTM+	LB
<i>C. sporogenes</i>	0/60	1/60
<i>B. subtilis</i>	0/60	1/60

RESULTS (continued)

Table 2

HCI Control Results

Results Expressed as Growth (+), No Growth (-) for The Subcultured MFTM Tubes

Microorganism	Contact Time (minutes)			
	2	5	10	20
<i>C. sporogenes</i>	+	+	+	-
<i>B. subtilis</i>	+	+	+	-

CONCLUSIONS

When tested as described, Easy Decon™ 4013 did pass the AOAC Sporicidal Test when *C. sporogenes* and *B. subtilis* spores dried on porcelain penicylinders were exposed to the test agent for four hours at 20±1C. All of the controls met the criteria established for a valid test. These conclusions are based on observed data.