MICROBIOTEST, INC
The Microbiology and Virology Laboratory

Volume

FINAL REPORT

AOAC USE DILUTION TEST
Using *Aspergillus niger*

Test Agent: Easy Decon™ 200-5000
Test Agent: Easy Decon™ 200-8000

Data Requirements
EPA Guidelines 810.2100 (c), (d), (e)

Author
Donna B. Suchmann

Study Completion Date
April 7, 2004

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

Laboratory Project Identification Number
479-111

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

STATEMENT OF NO DATA CONFIDENTIALITY

Title: AOAC Use Dilution Test Using *Aspergillus niger*

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:

Felicia L. Sellers, Angela L. Turner, Camila Buendia

Study Director: MICROBIOTEST, INC.

Felicia L. Sellers — Date: 4/7/04

Submitted by:

Name — Title

Signature — Date

Sponsor: ENVIROFOAM TECHNOLOGIES, INC.

Name — Title

Signature — Date

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: AOAC Use Dilution Test Using *Aspergillus niger*

The Quality Assurance Unit of MICROBIOTEST has inspected Project Number 479-111 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	03/04/04	03/04/04	04/01/04
In Process	03/04/04	03/04/04	04/01/04
Final Report	04/01/04	04/01/04	04/05/04

Nathan S. Jones

Nathan S. Jones, RQAP–GLP
Quality Assurance Unit

04/07/04
Date

TABLE OF CONTENTS

TEST SUMMARY

TITLE: AOAC Use Dilution Test Using *Aspergillus niger*

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 DF 200 Binary Blend Penetrator 5000, Lot No. 03141, received at MICROBIOTEST, INC. 02/19/04, and assigned DS No. 6600.

2. DF 200 Binary Blend Penetrator 8000, Lot No. 04-31, received at MICROBIOTEST, INC. 02/06/04, and assigned DS No. 6588.

3. DF 200 Binary Blend Fortifier, Lot No. 243072006, received at MICROBIOTEST, INC. on 02/06/04.

4. DF 200 Binary Blend Booster, Lot No. 42018, received at MICROBIOTEST, INC. on 02/06/04.

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

TEST CONDITIONS

Challenge microorganism:

Aspergillus niger, ATCC 16404

Active ingredient in test product:

Veriquat, B1216, H_2O_2

Neutralizer used:

Sabouraud Dextrose Broth containing 0.25% Yeast Extract, 0.1% Sodium Thioglycollate, 0.6% Sodium Thiosulfate , 0.5% Polysorbate 80, and 0.7% Lecithin

Contact time:

60 Minutes

Contact temperature:

20±2C

Test agent preparation:

For each lot of Penetrator, 49% by weight of Penetrator was combined with 49% by weight Fortifier and mixed for 1-2 minutes. Then, 2% by weight Booster was added and mixed for 2 minutes.

Media and reagents:

Sabouraud Dextrose Agar (SDA)
Sterile saline solution
Phosphate Buffered Saline
Asparagine solution, 0.1%
Sodium hydroxide solution, 1N

TEST CONDITIONS (continued)

Media and reagents: (continued)

Sabouraud Dextrose Broth containing 0.25% Yeast Extract, 0.1% Sodium Thioglycollate, 0.6% Sodium Thiosulfate , 0.5% Polysorbate 80, and 0.7% Lecithin
Phosphate Buffered Saline containing 1% Polysorbate 80

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, INC., 105B Carpenter Drive, Sterling, VA 20164, from 03/04/04 to 03/19/04. The study director signed the protocol 03/04/04. 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 and 2. The challenge microorganism was confirmed by wet mount to be consistent with *A. niger*. The sterility control exhibited no growth. The viability and neutralizer effectiveness controls exhibited growth. An average of 88 colony-forming units (CFU) of *A. niger* were added to the neutralizer effectiveness controls. Due to the turbid nature of the neutralizer, test and control tubes were streaked onto SDA for growth observations. Test tube streaks exhibited no growth, negating fungistasis. Pre-test inoculum counts were 2.3×10^8 CFU/mL.

RESULTS (continued)

Table 1

Test Results

Results Expressed as Number of Tubes Exhibiting Growth Total Number of Tubes

Microorganism	Easy Decon™ 200-5000	Easy Decon™ 200-8000
A. niger	0/10	0/10

Table 2

Carrier Counts

Results Expressed as Average Colony Forming Units (CFU) per Carrier

Microorganism	Avg. CFU/carrier
A. niger	3.4×10^6

CONCLUSION

When tested as described, Easy Decon™ 200-5000 and Easy Decon™ 200-8000 passed the AOAC Use Dilution Test when *A. niger* was exposed to the test agent for 60 minutes at 20±2C. All of the controls met the criteria established 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: 03/03/04 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No: 479-111

STUDY TITLE: AOAC Use Dilution Test Using *Aspergillus niger*

STUDY DIRECTOR: Felicia L. Sellers
Felicia Sellers 3/4/04
Signature Date

TEST MATERIAL (S):	**IDENTIFICATION NO.**	**DATE RECEIVED:**	**DS NO.**
EasyDECON™ 200-5000	03141*	02/19/04	6600
EasyDECON™ 200-8000	04-31*	02/06/04	6588

PERFORMING DEPARTMENT (S): Applied Microbiology Laboratory

STORAGE CONDITIONS: Location: E4
■ Dark ■ Ambient Room Temperature
☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:

PROTECTIVE PRECAUTION REQUIRED: MSDS ■ Yes ☐ No

PHYSICAL DESCRIPTION: ☐ Solid ■ Liquid ☐ Aerosol ☐ Other:

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 03/04/04 **TERMINATION DATE:** 03/19/04

CONDUCT OF STUDY: ☐ FDA ☐ EPA ■ R&D ■ GLP ☐ GCP ■ Other (Internal use of sponsor)

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

CONTACT PERSON: Robert H. Comstock
Telephone No. 256-774-8417
FAX No. 256-461-7806

TEST CONDITIONS:

Challenge organism(s): *Aspergillus niger*, ATCC 16404

Active ingredient(s): Veriquat, B1216, H202

Neutralizer(s): Sabouraud Dextrose Broth containing 0.25% Yeast Extract, 0.1% Sodium Thioglycollate, 0.6% Sodium Thiosulfate , 0.5% Polysorbate 80, and 0.7% Lecithin (SDB++)

Contact Time(s): 60 Minutes Contact Temperature(s): 20±2C

Diluent(s): NA Dilution(s): RTU

Serum: ☐ Yes / ■ No

Incubation Time(s): At least ten days (Test and Control tubes)
3-5 days (control plates, streaks and/or fungistasis)

Incubation Temperature(s): 25-30C

Comments: To prepare activated Easy Decon™ the following will be performed. For each lot of Penetrator*, a solution containing 49% (wt) Penetrator will be combined with 49% (wt) Fortifier. This will be mixed for 1-2 minutes until homogeneous. Then, 2% (wt) Booster will be added, and mixed for 2 minutes. The mixture will be used within the first two hours of preparation.

Date Issued: 03/03/04 Project Sheet No. 2 Page No. 1 Laboratory Project Identification No: 479-111			
STUDY TITLE: AOAC Use Dilution Test Using *Aspergillus niger*	**STUDY DIRECTOR:** Felicia L. Sellers Signature 3/4/04 Date		
TEST MATERIAL (S):	**IDENTIFICATION NO.**	**DATE RECEIVED:**	**DS NO.**
EasyDECON™ 200-5000	03141*	02/19/04	6600
EasyDECON™ 200-8000	04-31*	02/06/04	6588
PERFORMING DEPARTMENT (S): Applied Microbiology Laboratory	**STORAGE CONDITIONS:** Location: E4 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
SPONSOR: ENVIROFOAM TECHNOLOGIES, INC. 2903 Wall Triana Hwy. Suite 5B Huntsville, AL 35824	**CONTACT PERSON:** Robert H. Comstock Telephone No. 256-774-8417 FAX No. 256-461-7806		

EXPLANATION:

This project sheet was issued to document the following:

Protocol Amendment(s):

1 The name of the test material on Page 8 of the protocol (Miscellaneous Information), correspondence received with the test material and the bottles themselves are inconsistent. Per the sponsor the activated test agent will be referred to as Easy Decon™ 200-5000 (Lot No. 03141 and Easy Decon™ 200-8000 (Lot No. 04-31)

2. The two lot numbers 03141 and 04-31 refer to the penetrator solutions (DF 200 Binary Blend Penetrator 5000 and DF 200 Binary Blend Penetrator 8000 respectively). In addition one lot of the fortifier solution (DF 200 Binary Blend Fortifier) lot number 243072006 and booster solution (DF 200 Binary Blend Booster) was received on 02/06/04 and will be used to prepare the activated test material. To prepare activated Easy Decon™ the following will be performed. For each lot of Penetrator*, a solution containing 49% (wt) Penetrator will be combined with 49% (wt) Fortifier. This will be mixed for 1-2 minutes until homogeneous. Then, 2% (wt) Booster will be added, and mixed for 2 minutes. The mixture will be used within the first two hours of preparation.

Date Issued: 04/04/04 Project Sheet No. 3 Page No. 1 Laboratory Project Identification No. 479-111

STUDY TITLE: AOAC Use Dilution Test Using *Aspergillus niger*	**STUDY DIRECTOR:** Felicia L. Sellers Signature 4/7/04 Date		
TEST MATERIAL (S):	**IDENTIFICATION NO.**	**DATE RECEIVED:**	**DS NO.**
EasyDECON™ 200-5000	03141	02/19/04	6600
EasyDECON™ 200-8000	04-31	02/06/04	6588
PERFORMING DEPARTMENT (S): Applied Microbiology Laboratory	**STORAGE CONDITIONS:** Location: E4 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
SPONSOR: ENVIROFOAM TECHNOLOGIES, INC. 2903 Wall Triana Hwy. Suite 5B Huntsville, AL 35824	**CONTACT PERSON:** Robert H. Comstock Telephone No. 256-774-8417 FAX No. 256-461-7806		

EXPLANATION:

This project sheet was issued to document the following:

Protocol Deviation (s):

1. Page 3 of the protocol (Inoculum preparation) requires use of NGA for transfers and density determination of the conidial suspension. SDA was used. This deviation had no impact on the study since all controls met the criteria for a valid test.

2. Page 3 of the protocol requires incubation of transfers at 25 – 30C. On 02/08/04 – 02/10/04 MBT had a temperature recording of 24C. This deviation had no impact on the study since all controls met the criteria for a valid test.

3. Page 6 of the protocol requires that colony morphology be documented. Colony morphology was not documented. This deviation had no impact on the study since the cell morphology was consistent with *Aspergillus niger*, ATCC 16404.

Protocol Amendment(s):

3. Page 6 of the protocol requires carrier counts of at least 1 X 10^6CFU/carrier for the test results to be acceptable for evaluation. The test results will be acceptable for evaluation if the inoculum counts are at least 1 X 10^6CFU/mL and the carrier count controls are at least 1 X 10^4CFU/carrier. This amendment serves to correct the test acceptance criteria.

APPENDIX II
SIGNED PROTOCOL

MICROBIOTEST PROTOCOL

AOAC USE DILUTION TEST

USING *Aspergillus niger*

Prepared for
ENVIROFOAM TECHNOLOGIES, INC.
2903 Wall Triana Hwy.
Suite 5B
Huntsville, AL 35824

January 15, 2004

MICROBIOTEST Protocol: 479.1.01.15.04

MICROBIOTEST Project Number: 479-111

OBJECTIVE:

This test is designed to substantiate fungicidal effectiveness claims for a product to be registered with the Environmental Protection Agency. It measures the potential of the test agent to disinfect hard surfaces contaminated with fungus. The test follows *Official Methods of Analysis*, Sixteenth edition, 1995, AOAC; is required by EPA DIS/TSS 1 & 2.

TESTING CONDITIONS:

A total of ten replicates per lot of test agent will be evaluated using two lots. *Aspergillus niger* cultures dried on stainless steel penicylinders will be exposed to the test agent at the temperature and for the time stipulated by the sponsor. The carriers will be removed from the test agent, neutralized and cultured.

MATERIALS

A. Test agents supplied by the sponsor: 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 by specified by the sponsor prior to the initiation of testing.

The sponsor assures MICROBIOTEST, INC. 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 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, INC., including, but not limited to:

1 Challenge microorganism, required by the sponsor: *Aspergillus niger*, ATCC 16404.

2. Media and reagents:
   a. Neopeptone Glucose Agar (NGA)
   b. Sabouraud Dextrose Agar plates (SDA)
   b. Sabouraud Dextrose Broth (SDB)
   c. Asparagine solution, 0.1%
   d. Sodium hydroxide solution, 1N (NaOH)
   e. Recovery broth with neutralizers – SDB containing neutralizers
   f. PBS with 1% Polysorbate 80 (PBS+)
   g. Sterile saline (SS)
   h. Heat-inactivated horse serum (if required)

3. Laboratory equipment and supplies including polished stainless steel penicylinders

EXPERIMENTAL DESIGN:

A. Inoculum preparation:

The fungus will be inoculated from the stock culture onto NGA plates and incubated at 25 - 30C for ≥10, but 15≤ days or until sporulation occurs. When the cultures appear to be mature, the mycelial mats will be removed from the surface of at least five plates and macerated with SS in a sterile glass tissue grinder. The suspension will be filtered through sterile glass wool to remove the hyphae.

The density of the conidial suspension will be determined by serially diluting the prepared culture in SS. Aliquots from selected dilution will be plated on duplicate NGA plates. The plates will be incubated for 3-5 days at 25-30C. The suspension will be stored at 2 - 10C for ≤ 4 weeks before use.

If requested by the sponsor, horse serum will be added to the cultures to achieve an organic load of 5%. NO

The inoculum will be agitated on a Vortex-type mixer for 3-4 seconds, then allowed to sit for ten minutes and decanted into a sterile flask.

Twenty-mL aliquots will be transferred into 25x150 mm sterile tubes, with mixing of the inoculum between transfers.

B. Carrier preparation:

The carriers will be soaked overnight in 1N NaOH, rinsed with tap water until a neutral pH is reached, then rinsed twice with deionized water.

Cleaned carriers will be placed in multiples of 10 into sterile tubes, covered with 0.1% asparagine solution, steam-sterilized for 20 min at 121C, cooled and stored at room temperature until use.

The carriers will be placed into the broth and remain in contact with the inoculum (20 carriers per tube of 20-mL inoculum) for 15 min at ambient temperature; then they will be removed from the broth and placed into sterile, Petri dishes matted with filter paper, and dried at 37±2C for 20 - 40 min.

C Test agent preparation:

The test agent will be prepared according to the sponsor's specifications and dispensed in 10-mL aliquots into sterile test tubes. The tubes will be placed in a water bath and allowed to come to test temperature for at least ten minutes before testing.

D. Test:

Tubes containing the test agent will be maintained at testing temperature ±2C throughout the test. One contaminated carrier will be added to each tube; the tube swirled to mix; and the carrier allowed to remain in contact with the test agent for a time specified by the sponsor of the study. After the contact time, the carriers will be removed, transferred to recovery broth with neutralizers and the tubes will be thoroughly shaken. All tubes will be incubated at least ten days at 25 - 30C and the results recorded as visible growth or no visible growth.

E. Controls:

1 Sterility controls:

One tube of recovery broth with neutralizers containing a single sterile carrier will be incubated with the test.

2. Neutralizer effectiveness:

One tube containing ten mL of the test agent will be allowed to equilibrate to testing temperature for at least 10 min. A single sterile carrier will be added to the tube and held for the same time as the test carriers.

After the contact time, the carrier will be added to a tube containing recovery broth with neutralizers. Fewer than 100 CFU of the challenge microorganism will be added to the tube. The CFU added to the tube will be confirmed on duplicate SDA plates. 60 MIN.

The tube will be incubated with the test. All plates will be incubated for 3-5 days at 25-30C.

3. Carrier counts:

The average CFU per carrier will be determined using three carriers. Inoculated carriers will be placed individually into tubes containing 10 mL PBS+. The tubes will be subjected to ultrasound for 5 min in a cleaning (not cavitating) sonicator. Serial ten-fold dilutions of each suspension will be performed in PBS blanks. Duplicate aliquots from selected dilutions will be plated on SDA. All plates will be incubated for 3-5 days at 25-30C. The colonies will be enumerated and CFU/carrier will be calculated.

4. Viability controls:

Two inoculated carriers will be inoculated into tubes of recovery broth with neutralizers and incubated with the test to serve as comparison for the test cultures.

5. Observations of growth/Confirmation /Fungistasis controls:

Due to the turbid nature of the recovery broth with neutralizers, all test replicates and control tubes (viability, neutralizer effectiveness, and sterility) will be streaked onto SDA plates and incubated for 3-5 days at 25-30C.

All streaks will be observed for growth or no growth. Absence of growth on all of the test replicates streaks will negate fungistasis as the cause for lack of growth.

All of the viability controls and any of the test replicates showing growth will be confirmed through wet mount identification and the morphology will be documented.

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 carrier counts should be at least 1×10^6 CFU/carrier.

PRODUCT EVALUATION CRITERIA:

According to EPA, the compound passes the test if no visible growth is observed in any of the subculture broths (0/10) for any lot of test agent and the controls meet their stipulated criteria. There is no statistical method proposed for this protocol.

DATA PRESENTATION:

The final report will include the following information:

- The number of positive carriers per microorganism per lot.
- The average colony-forming units per carrier.

STUDY DATES:

The anticipated date of study initiation (date when the study director signs the protocol) is upon receipt of test agent and letter of authorization with a purchase order number and a signed protocol. The date the study director signs the final report is the study completion date.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes for the technical personnel are maintained and are available on request. This study will be conducted in the Applied Microbiology Laboratory at MICROBIOTEST, INC., 105B Carpenter Drive, Sterling, Virginia 20164.

RECORDS TO BE MAINTAINED:

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

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

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

REPORT FORMAT:

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

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

MISCELLANEOUS INFORMATION:

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

A. Name and address: ENVIROFOAM TECHNOLOGIES, INC.
2903 Wall Triana Hwy.
Suite 5B
Huntsville, AL 35824

B. Test agent: EASY DECON(TM) 200-8000 & 200-5000

1st Lot No: 04-31 2nd Lot No: 03-141

Active ingredients: VERIDIAN B1216, H_2O_2

Dilution to be tested: NONE Diluent: _____

Contact time: 60 MIN

Contact temperature: 20±2C

C Organic load Serum added to inoculum to achieve 5% load. Yes ☐ No ■

D. Precautions/storage conditions – see 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

PROTOCOL APPROVAL:

Sponsor: _____ Date: 2/18/04