MRID 46498201

TRADE SECRET

MICROBIOTEST, INC

The Microbiology and Virology Laboratory

Volume _____

FINAL REPORT

H-26819 and H-26820: USE DILUTION TEST SUPPLEMENTAL MICROORGANISM (*Staphylococcus aureus*, MRSA, ATCC 33592)

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

Author
Angela L. Hollingsworth

Study Completion Date
March 7, 2005

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

Laboratory Project Identification Number
473-108

Sponsor Study Number
DCSE-2005-002

Study Sponsor:
E. I. DUPONT DE NEMOURS AND COMPANY
DUPONT CHEMICAL SOLUTIONS ENTERPRISE
P.O. Box 80023
Wilmington, DE 19880-0023

105B Carpenter Drive, Sterling, VA 20164 • 703-925-0100 • Fax: 703-925-9366
www.microbiotest.com

STATEMENT OF NO DATA CONFIDENTIALITY

Title: H-26819 and H-26820: Use Dilution Test – Supplemental Microorganism (*S. aureus*, MRSA, ATCC 33592)

Performed by: MICROBIOTEST, INC.
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: E. I. DUPONT DE NEMOURS AND COMPANY

Company Agent _____ March 10, 2005
Date

We have submitted this material to the United States Environmental Protection Agency specifically under the provisions contained in FIFRA as amended and, hereby, consent to use and disclosure of this material by EPA according to FIFRA. Notwithstanding the wording of our marking TRADE SECRET, this marking, by itself, conveys no supplemental claims of confidentiality under FIFRA Sections 10(a) or 10 (b). In submitting this material to EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection or right involving this material that would have been claimed by the company if this material had not been submitted to the EPA, nor do we waive any protection or right provided under FIFRA Section 10(g).

COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR § 160 with the following exceptions:

- Information on the identity, strength, purity, stability, uniformity, and chemical composition of the test agent resides with the sponsor of the study.

The following technical personnel participated in this study:

Felicia L. Sellers, Rita M. Peralta, Temitope O. Odebunmi, Elin V. Hughes

Study Director: MICROBIOTEST, INC.

T. Odebunmi — 3/7/05
Temitope O. Odebunmi — Date

Submitted by: E. I. DUPONT DE NEMOURS AND COMPANY

Nancy B. Lomax — Registration Manager
Name — Title

Nancy B. Lomax — 3/10/05
Signature — Date

Sponsor: E. I. DUPONT DE NEMOURS AND COMPANY

Nancy B. Lomax — Registration Manager
Name — Title

Nancy B. Lomax — 3/10/05
Signature — Date

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: H-26819 and H-26820: Use Dilution Test – Supplemental Microorganism (*S. aureus*, MRSA, ATCC 33592)

The Quality Assurance Unit of MICROBIOTEST has inspected Project Number 473-108 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.

PHASE INSPECTED	DATE OF INSPECTION	DATE REPORTED TO STUDY DIRECTOR	DATE REPORTED TO MANAGEMENT
Protocol	01/25/05	01/25/05	01/25/05
In Process	01/25/05	01/25/05	01/25/05
Final Report	02/01/05	02/01/05	02/02/05

Nathan S. Jones, RQAP-GLP
Quality Assurance Unit

03/07/05
Date

TABLE OF CONTENTS

TEST SUMMARY

TITLE: H-26819 and H-26820: Use Dilution Test – Supplemental Microorganism (*S. aureus*, MRSA, ATCC 33592)

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:

Substance Tested: Virkon® S, EPA Reg. #71654-6

Synonyms/Codes: H-26819 and H-26820

ID Number: H-26819, Lot No. 5471, manufactured on Jan. 26, 2003, received at MICROBIOTEST, INC. 01/18/05, and assigned DS No. 7217.

H-26820, Lot No. 19861, manufactured on Aug. 20, 2004, received at MICROBIOTEST, INC. 01/18/05, and assigned DS No. 7218.

Composition:

ACTIVE INGREDIENTS	
Potassium peroxymonosulfate	(21.41%)
Sodium chloride	(1.50%)
OTHER INGREDIENTS	(77.09%)

Known Impurities: None

Physical Characteristics: Free flowing powder

TEST SUMMARY (continued)

Sponsor: E. I. DUPONT DE NEMOURS AND COMPANY
DuPont Chemical Solutions Enterprise
P.O. Box 80023
Wilmington, DE 19880-0023

Study Initiated: 01/24/05
Study Completed: 03/07/05

TEST CONDITIONS

Challenge microorganism (adjusted to yield carrier counts between $1.0 \times 10^4 - 2.0 \times 10^6$ colony forming units /carrier):

Staphylococcus aureus (MRSA), ATCC 33592

Active ingredients in test product:

Potassium Peroxymonosulfate
Sodium chloride

Neutralizer used:

Letheen Broth containing 0.1% $Na_2S_2O_3$

Contact time:

10 Minutes

Contact temperature:

Room temperature (19C)

Diluent(s):

400ppm hard water

Dilution(s):

1% (1g test agent + 99mL diluent)

Serum:

Heat-inactivated horse serum was added to the inoculum for a final concentration of 5% organic load.

Carrier type:

Stainless steel penicylinder

TEST CONDITIONS (continued)

Media and reagents:

Nutrient Agar
Nutrient Broth
Asparagine solution, 0.1%
Sodium hydroxide solution, 1N
Letheen Broth containing 0.1% $Na_2S_2O_3$
Phosphate Buffered Saline
Phosphate Buffered Saline containing 1% Polysorbate 80
Heat-inactivated horse serum
Tryptic Soy Agar
Gram stain reagents
400ppm hard water
Mueller Hinton Agar
Oxacillan antibiotic disk

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, INC., 105 Carpenter Drive, Sterling, VA 20164, and from 01/25/05 to 01/28/05. The study director signed the protocol on 01/24/05. 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., 105 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 Gram stain and colony morphology to be consistent with *S. aureus* (MRSA). The sterility control exhibited no growth. The viability and neutralizer effectiveness controls exhibited growth. An average of 88 colony-forming units (CFU)/tube of *S. aureus* (MRSA) was added to the neutralizer effectiveness control. All bacteriostasis streaks were negative for growth. *S. aureus* (MRSA) was confirmed as resistant to Oxacillin as a zone of inhibition measuring 0mm was observed using an Oxacillin-impregnated disk. *S. aureus* is resistant to Oxacillin when a zone of inhibition of ≤10mm is observed.

Table 1

Test Results

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

Microorganism	H-26819 Lot No. 5471	H-26820 Lot No. 19861
S.aureus (MRSA)	0/10	0/10

Table 2

Carrier Counts

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

Microorganism	Avg. CFU/carrier
S.aureus (MRSA)	1.0×10^5

CONCLUSION

When tested as described, Virkon® S (H-26819 and H-26820) passed the Use Dilution Test when *S. aureus* (MRSA), containing a 5% organic load, was exposed to the test agent for 10 minutes at 19C. All of the controls met the criteria established for a valid test. These conclusions are based on observed data.

APPENDIX I
PROJECT SHEET(S)

Date Issued: 01/23/05 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 473-109*

STUDY TITLE: Use Dilution Test Supplemental Microorganism

STUDY DIRECTOR: Temitope O. Odebunmi
T. Odebunmi 1-24-05
Signature Date

TEST MATERIAL(S):	**LOT NO.**	**DATE RECEIVED:**	**DS NO.**
Virkon® S	H26819 Lot 5471	01/18/05	7217
Virkon® S	H26820 Lot 19861	01/18/05	7218

PERFORMING DEPARTMENT(S): Applied Microbiology Laboratory

STORAGE CONDITIONS: Location: D2
■ 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: 01/25/05 **TERMINATION DATE:** 01/28/05

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■GLP ☐ GCP ■ Other: CAL DPR

SPONSOR: E.I. DUPONT DENEMOURS & CO.
DuPont Chemical Solutions Ent.
P.O. Box 80023
Wilmington, DE 19880

CONTACT PERSON:
Dr. Carl W. Erkenbrecher
Telephone No. (302) 695-8652
FAX No. (302) 695-8680

TEST CONDITIONS:

Challenge organism(s):	*Staphylococcus aureus* (MRSA), ATCC 33592
Active ingredient(s):	Potassium Peroxymonosulfate
Neutralizer(s):	Letheen broth containing 0.1% $Na_2S_2O_3$
Contact Time(s):	10 minutes
Contact Temperature(s):	Room temperature
Diluent(s):	400ppm hard water
Dilution(s):	1% (1g test material + 99mL diluent)
Serum:	■ Yes / ☐No (Heat-inactivated horse serum to achieve 5% organic load)
Incubation Time(s):	48±2hours, 24±2 hours (for streaks)
Comments:	NA

#wrong #TCO 1/24/05

APPENDIX II
SIGNED PROTOCOL

MICROBIOTEST PROTOCOL

USE DILUTION TEST
SUPPLEMENTAL MICROORGANISM

Prepared for
E.I. DuPont deNemours & Co.
DuPont Chemical Solutions Ent.
P.O. Box 80023
Wilmington, DE 19880

December 29, 2004

OBJECTIVE:

This test is designed to substantiate disinfectant effectiveness claims for a product to be registered with the US Environmental Protection Agency for use in health-care facilities. It measures the potential of the test agent to disinfect hard surfaces contaminated with bacteria. 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 microorganism per lot of test agent will be evaluated using two lots of the test agent. Methicillin resistant *Staphylococcus aureus* (MRSA) 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 of the study: *Staphylococcus aureus* (MRSA), ATCC 33592

2. Media and reagents:
   a. Nutrient Broth (NB).
   b. Recovery broth containing neutralizers (RB+).
   c. Recovery broth.
   d. Nutrient Agar (NA).
   e. Phosphate Buffered Saline (PBS).
   f. PBS containing 1% Polysorbate 80 (PBS+).
   g. Heat-inactivated horse serum.

3. Laboratory equipment and supplies including polished stainless steel penicylinders

TEST SYSTEM IDENTIFICATION:

All Petri dishes and dilution tube racks will be labeled with microorganism, test agent and project number.

EXPERIMENTAL DESIGN:

A. Inocula preparation:

Bacteria from stock cultures will be transferred into NB and incubated. Daily transfers will be made for at least three consecutive days (but no more than 30 days). Tubes of 10-mL NB will be inoculated with one loopful of inoculum per tube and incubated. After 48-54 hours, cultures will be used for contaminating the carriers.

For each microorganism, the NB cultures will be pooled into a sterile flask and adjusted if necessary to yield carrier counts of 1 x 10^4 to 2 x 10^6 colony-forming units (CFU). If requested by the sponsor, horse serum will be added to the cultures to achieve an organic load of 5%.

Each 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.

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, cooled and stored at room temperature until use.

The asparagine solution will be decanted and the carriers will be covered with the prepared inoculum (20 carriers per tube of 20-mL inocula) for 15 min at ambient temperature; then the broth will be removed and the carriers will be 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 throughout the test. One contaminated carrier will be added to each tube; the tube swirled to mix; and the carrier allowed remaining 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 for 48±2 hours at 37±2C 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 and toxicity:

A test tube containing ten mL of one of the test agent lots 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 and fewer than 100 CFU of the challenge microorganism will be added to the tube. The CFU added to the tube will be confirmed.

All tubes and plates will be incubated with the test.

3. Carrier counts:

The average CFU per carrier will be determined using three carriers. Dried carriers will be placed individually into tubes containing 10 mL PBS with 1% Polysorbate 80. The tubes will be subjected to ultrasound in a cleaning (not cavitating) sonicator. Serial ten-fold dilutions of each suspension will be performed in PBS blanks. Duplicate one-mL aliquots from selected dilutions will be plated in Nutrient Agar pour plates. All plates will be incubated with the test and the average CFU/carrier determined.

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. Bacteriostasis control:

If, after two days incubation, no growth is observed in any tube, all tubes will be streaked onto agar plate and incubated for 24±2 hours at 37±2C. No growth on these plates will eliminate bacteriostasis as the cause for lack of growth in the test tubes.

6. Antibiotic Resistance:

A bead from the previously frozen stock culture will be inoculated into 10mL of NB. The culture will be incubated for 24±2 hours at 37±2C. After the initial incubation, an individual Mueller Hinton Agar (MHA) will be streaked with the test microorganism in a crosshatch pattern. After crosshatching, an appropriate antibiotic disk will be added to the center of the plate. The plate will be incubated for 24±2 hours at 37±2C. Upon the completion of incubation, the plate will be observed and the zone of inhibition will be measured and documented. The inhibition zone is defined as the area immediately surrounding the antibiotic disk. Using the Zone Diameter Interpretive Standards provided from the manufacturer of the antibiotic disks, the zone of inhibition will be determined to be Resistant, Intermediate, or Susceptible. The final determination will be reported in the final report.

7. Confirmation of challenge microorganisms:

All of the viability controls and at all of the test tubes showing growth will be streaked onto agar plates and incubated for 24±2 hours at 37±2C. Gram stains will be performed from these streaks in order to confirm growth of the challenge microorganisms.

PRODUCT EVALUATION CRITERIA:

According to EPA, the compound passes the test if visible growth is observed in none 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.

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 are in the range of 1×10^4 to 2×10^6 CFU/carrier.
- The neutralizer is effective and non toxic

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.
- The control results.

RECORDS TO BE MAINTAINED:

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

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

The proposed experimental start and termination dates; additional information about the test agent; challenge microorganism used; media and reagent identification; and the type of neutralizers employed in the test will be addressed in a project sheet issued separately. The date the study director signs project sheet number one will be the initiation date. All project sheets will be forwarded to the study sponsor.

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

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned before initiation of the test. Resumes for technical personnel are maintained and are available on request. This study will be conducted at MICROBIOTEST, INC., 105B Carpenter Drive, Sterling, VA 20164.

MISCELLANEOUS INFORMATION:

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

A. Name and address: E.I. DuPont deNemours & Co.
DuPont Chemical Solutions Ent.
P.O. Box 80023
Wilmington, DE 19880

B. Test agent: Virkon S
Active ingredient: Potassium Peroxymonosulfates
Lot 1: H 26819 Lot 5471
Lot 2: H 26820 Lot 19861

Dilution to be tested: 1/90
Diluent: 400 ppm hard water (AOAC hard water has 2.9% deviation)
Exposure time: 10 minutes
Exposure temperature: ____ ±2C or ☒ Room temperature

C. Organic load – serum added to achieve 5% in the inoculum: ☒ yes ☐ no.

D. Precautions/storage conditions: refer to MSDS or certificate of analysis
☒ provided ☐ not provided

E. Additional information: _____

REPORT HANDLING:

The sponsor intends to submit this information to (CHOOSE ONE ONLY):

☒ EPA ☐ FDA ☐ Health Canada ☒ CAL DPR
☐ ARTG ☐ non GLP ☐ other _____

PROTOCOL APPROVAL:

Sponsor: Carl W. Eisenbacher Date: 1-14-05