FINAL STUDY REPORT

STUDY TITLE

AOAC Use-Dilution Method

Test Organism:

Clostridium difficile (ATCC 9689)

PRODUCT IDENTITY

MDF-500 Part A & B
Lot 1: AZB30/BZB30 and Lot 2: AXA-02/BXB-02

DATA REQUIREMENTS

U.S. EPA 40 CFR Part 158
"Data Requirements for Registration"
Pesticide Assessment Guidelines - Subdivision G, 91-2 (i)

AUTHOR

Amy S. Jeske, B.S.
Study Director

STUDY COMPLETION DATE

April 12, 2007

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Modec, Inc.
4725 Oakland Street
Denver, CO 80239

SPONSOR REPRESENTATIVE

Ag-Chem Consulting
12208 Quinque Lane
Clifton, VA 20124

PROJECT NUMBER

A04824

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B), or (C).

Company: Modec, Inc.

Company Agent: James Tellman

CFO

Title

Signature

Date: 4/12/07

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160.

The studies not performed by or under the direction of ATS Labs are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the compound(s).

Submitter: _____ Date: 4/12/07

Sponsor: _____ JAMES TELLMAN Date: 4/12/07

Study Director: _____ Amy S. Jeske, B.S. Date: 4/12/07

QUALITY ASSURANCE UNIT SUMMARY

Study: AOAC Use-Dilution Method

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. These studies have been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director	Management
Critical Phase	April 5, 2007	April 5, 2007	April 12, 2007
Final Report	April 10, 2007	April 11, 2007	

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: Brenda S. Date: 4/12/07

TABLE OF CONTENTS

STUDY PERSONNEL

STUDY DIRECTOR: Amy S. Jeske, B.S.

Professional personnel involved:

David Rottjakob, M.T.	- Director, Microbiology Services
Scott R. Steinagel, B.S.	- Microbiology Laboratory Supervisor
Becky Lien, B.A.	- Research Scientist I
Adam W. Pitt, B.S.	- Research Assistant II
Peter Toll, B.S.	- Research Assistant I
Lisa Slusser, B.S.	- Research Assistant I
Katherine C. Sager, B.S.	- Research Assistant I

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Use-Dilution Method

Project Number: A04824

Protocol Number: MOD02031607.UD

Sponsor:
Modec, Inc.
4725 Oakland Street
Denver, CO 80239

Sponsor Representative:
Ag-Chem Consulting
12208 Quinque Lane
Clifton, VA 20124

Test Facility:
ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name: MDF-500 Part A & B

Lot/Batch(s): Lot 1: AZB30/BZB30 and Lot 2: AXA-02/BXB-02

Test Substance Characterization
Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor.

STUDY DATES

Date Sample Received: February 12, 2007
Study Initiation Date: March 23, 2007
Experimental Start Date: April 5, 2007
Experimental End Date: April 9, 2007
Study Completion Date: April 12, 2007

OBJECTIVE

The objective of this study was to determine the efficacy of the Sponsor's product following the AOAC Use-Dilution Method in compliance with the U.S. Environmental Protection Agency requirements set forth in the Pesticide Assessment Guidelines.

SUMMARY OF RESULTS

Test Substance:	MDF-500 Part A & B (Lot 1: AZB30/BZB30 and Lot 2: AXA-02/BXB-02)
Dilution:	Combine equal parts of Part A with Part B and mix well
Test Organism:	*Clostridium difficile* (ATCC 9689)
Exposure Time:	Ten minutes
Exposure Temperature:	20±1°C
Organic Soil Load:	5% fetal bovine serum
Efficacy Result:	MDF-500 Part A & B demonstrated efficacy of two lots against *Clostridium difficile*, and therefore, meets the requirements set forth by the U.S. EPA for disinfectant label claims following a ten minute exposure period.

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium
Clostridium difficile	9689	Fluid Thioglycollate Medium and Tryptic Soy Agar with 5% Sheep Blood (BAP)

The microorganism used in this study was obtained from the American Type Culture Collection, Manassas, Virginia.

Recovery Media

Neutralizing Subculture Medium:	Primary: Fluid Thioglycollate Medium with 0.14% Lecithin, 1.0% Tween 80 and 0.01% Catalase (See Deviation 1) Secondary: Fluid Thioglycollate Medium
Agar Plate Medium:	Tryptic Soy Agar with 5% Sheep Blood (BAP)

Reagents

Organic Soil Load Description:	5% fetal bovine serum (FBS)

Carriers

Stainless steel penicylinders were pre-soaked overnight in 1.0 N NaOH, washed in water until rinse water was neutral to phenolphthalein, and autoclaved in 0.1% asparagine.

TEST METHOD

Preparation of Test Substance

The test substance was prepared by mixing 60.0 mL of Part A with 60.0 mL of Part B for each lot. The test substance was homogenous as determined by visual observation and was used within three hours of preparation.

Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20±1°C water bath and allowed to equilibrate for ≥10 minutes.

Preparation of Test Organism

All test organism preparation and incubation was performed in an anaerobic chamber. From a frozen stock an initial tube of Fluid Thioglycollate Medium was inoculated and incubated for three days at 35-37°C under anaerobic conditions. From this initial broth suspension, one loopful (0.01 mL) was transferred to a second tube of Fluid Thioglycollate Medium and incubated for two days at 35-37°C under anaerobic conditions. Following this incubation, 0.10 mL aliquots of the second broth culture were transferred and spread onto BAP plates. The plates were incubated for ≈15 hours at 35-37°C under anaerobic conditions (See Deviation 2).

After incubation, the organism growth was harvested by removing the growth from the plates using a sterile loop. The organism growth was suspended in Fluid Thioglycollate medium. The harvested test culture was lighter than a 0.5 McFarland standard prior to carrier inoculation.

Addition of Organic Soil Load

A 1.30 mL aliquot of FBS was added to 24.7 mL of harvested organism suspension to yield a 5% fetal bovine serum soil load.

Contamination of Carriers

Sterile penicylinders were immersed for 15 minutes in a harvested suspension of the test organism, at a ratio of 1 carrier per 1.0 mL broth. The penicylinders were then dried on filter paper in a sterile petri dish at 35-37°C for 40 minutes in the anaerobic chamber at a 47.8% relative humidity.

Exposure Conditions

This portion of the study was performed outside of the anaerobic chamber. Carriers were used in testing immediately following removal from the anaerobic chamber. For each prepared test substance, 10 contaminated and dried carriers were individually transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the prepared test substance at the requested dilution and exposed for ten minutes at 20±1°C.

Test System Recovery

Following exposure, each exposed carrier was then transferred by hook needle at identical staggered intervals to 10 mL of Fluid Thioglycollate Medium with 0.14% Lecithin, 1.0% Tween 80 and 0.01% Catalase. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Fluid Thioglycollate Medium ≥30 minutes after subculture of the first carrier.

Incubation and Observation

The neutralized subculture tubes and plates were incubated for 4 days at 35-37°C in an anaerobic chamber. Following incubation, the subcultures were visually examined for the presence or absence of visible growth.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" was performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control
The serum used for soil load was cultured, incubated, and visually examined for growth. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control
A representative uninoculated carrier was added to the subculture medium. The subculture medium containing the carrier was incubated and examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control
A representative sample of uninoculated neutralizing subculture medium was incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control
A representative inoculated carrier was added to the subculture medium. The subculture medium containing the carrier was incubated and visually examined for growth. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control
The neutralization of the test substance was confirmed by exposing sterile carriers (representing not less than 10% of the total number of test carriers) to the test substance and transferring them to primary subculture tubes containing 10 mL of neutralizing subculture medium. Carriers were then transferred from primary subculture tubes into individual secondary subculture tubes ≥30 minutes following the primary transfer. The subculture tubes containing the exposed carriers were inoculated with ≤100 CFU of the test organism (the test organism dilutions used for inoculating the subculture tubes were used immediately following dilution), incubated under test conditions and visually examined for the presence of growth. This control was performed with multiple replicates using different dilutions of the test organism. A standardized spread plate procedure using a 0.10 mL aliquot of the test organism dilution was run concurrently in order to enumerate the number of CFU actually added. The control result was reported using data from the most appropriate dilution.

The acceptance criterion for this study control is growth after inoculation with ≤100 CFU.

Carrier Population Control
Inoculated carriers were added at a ratio of 1 carrier to 10 mL neutralizing broth and vortex mixed. Appropriate serial ten-fold dilutions were prepared and 0.10 mL aliquots were spread plated on agar plate medium, and incubated. Following incubation, the resulting colonies were enumerated and the CFU/carrier calculated. The acceptance criterion for this study control is a minimum of 1.0×10^4 CFU/carrier.

Verification of Spores Present in the Initial Suspension Control
A 3.0 mL aliquot of the prepared test organism suspension was heat shocked at 50±2°C for 10 minutes. Aliquots of 1.00 mL and 200 µL of the heat shocked prepared test organism suspension were spread plated in duplicate using standard microbiological techniques on *Clostridium difficile* Selective Agar. Following incubation, the organism plates were observed to enumerate the concentration of spores present in the prepared test organism suspension used for inoculation of the test carriers. This control was run for informational purposes only, there is no acceptance criterion.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The EPA efficacy performance requirements for label claims state that the disinfectant must kill the microorganism on 10 out of the 10 inoculated carriers.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

PROTOCOL CHANGES

Protocol Amendments:
No protocol amendments were required for this study.

Protocol Deviations:
1. The protocol calls for the use of Fluid Thioglycollate Medium with 0.14% Lecithin, 1.0% Tween 80 and 0.05% Catalase for the primary neutralizer. Inadvertently, Fluid Thioglycollate Medium with 0.14% Lecithin, 1.0% Tween 80 and 0.01% Catalase was used. This deviation did not negatively impact the study outcome as the combination of primary and secondary subculture media demonstrated effective neutralization in the neutralization confirmation control, therefore all results are valid.

2. The protocol states that the inoculated blood agar plates will be incubated for 20±4 hours prior to harvesting. Inadvertently, the inoculated blood agar plates were removed from the incubator following ≈ 15 hours of incubation. While this is less than called for in the protocol, the carrier quantitation control results met the acceptance criteria and the verification of spores present in the initial suspension control indicated that no spores were found in the initial suspension. Since this test was designed to use the vegetative form of *Clostridium diffiicile* this deviation is acceptable.

DATA ANALYSIS

Calculations

Carrier Population Control Calculation:

$$\text{CFU/carrier} = \frac{(\text{average number colonies/plate @ dilution}) \times (\text{dilution factor}) \times (\text{volume neutralizer})}{(\text{number of carriers tested}) \times (\text{volume plated})}$$

The carrier population was calculated and reported using data from the most appropriate dilution(s).

Statistical Analysis
None used.

STUDY RETENTION

Record Retention

All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. The original data includes, but is not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Test Substance Retention

The test substance will be discarded following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test material.

REFERENCES

1. Association of Official Analytical Chemists (AOAC), 1990. Use-Dilution Tests, p. 135-137. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.

2. Association of Official Analytical Chemists (AOAC), 1990. Germicidal and Detergent Sanitizing Action of Disinfectants, p. 139 [Preparation of Synthetic Hard Water]. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.

3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Efficacy Data Requirements, Disinfectants for Use on Hard Surfaces, DIS/TSS-1.

4. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1979. Efficacy Data Requirements, Supplemental Recommendations, DIS/TSS-2.

5. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Subseries 91A: Public Health Uses. *In* Pesticide Assessment Guidelines – Subdivision G (Product Performance).

RESULTS

For Control and Neutralization Results, see Tables 1-4.

All data measurements/controls including the culture purity, viability, organic soil load sterility, neutralizing subculture medium sterility, carrier sterility, neutralization confirmation, and carrier population were within acceptance criteria.

For Test Results, see Table 5.

ANALYSIS

MDF-500 Part A & B (Lot 1: AZB30/BZB30 and Lot 2: AXA-02/BXB-02), prepared by mixing parts A & B together equally, demonstrated no growth of *Clostridium difficile* (ATCC 9689) in any of the 10 primary subculture tubes and no growth in any of the 10 secondary subculture tubes following a ten minute exposure period in the presence of a 5% fetal bovine serum organic soil load.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum organic soil load, MDF-500 Part A & B (Lot 1: AZB30/BZB30 and Lot 2: AXA-02/BXB-02), prepared by mixing parts A & B together equally, demonstrated efficacy against *Clostridium difficile* as required by the U.S. EPA for disinfectant label claims following a ten minute exposure period.

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.

TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control	Results
	***Clostridium difficile* (ATCC 9689)**
Purity Control	Pure
Viability Control	Growth
Organic Soil Sterility Control	No Growth
Neutralizing Subculture Medium Sterility Control – Primary (Fluid Thioglycollate Medium with 0.14% Lecithin, 1.0% Tween 80 and 0.01% Catalase)	No Growth
Neutralizing Subculture Medium Sterility Control – Secondary (Fluid Thioglycollate Medium)	No Growth
Carrier Sterility Control	No Growth

TABLE 2: CARRIER POPULATION CONTROL RESULTS

Test Organism	Date Performed	Result
Clostridium difficile (ATCC 9689)	4/5/07	8.6×10^6 CFU/carrier

CFU = Colony Forming Unit

TABLE 3: VERIFICATION OF SPORES PRESENT IN INITIAL SUSPENSION

Test Organism	Date Performed	CFU/plate		Average Result	
		200 µL	1.0 mL	200 µL	1.0 mL
Clostridium difficile (ATCC 9689)	4/5/07	0, 0	0, 0	<1 CFU/200 µL	<1 CFU/1.0 mL

CFU = Colony Forming Unit

TABLE 4: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Date Performed	Average Inoculum (CFU/mL)	Number of Subculture Tubes	
				Tested	Positive
MDF-500 Part A & B, Lot 1: AZB30/BZB30	*Clostridium difficile* (ATCC 9689)	4/5/07	40	1	1
MDF-500 Part A & B, Lot 2: AXA-02/BXB-02				1	1

CFU = Colony Forming Unit

The neutralization controls demonstrated growth, eliminating bacteriostasis as a cause of lack of growth in the test system.

TABLE 5: TEST RESULTS

Test Substance	Test Organism	Date Performed	Sample Dilution	Number of Carriers	
				Exposed	Showing Growth**
MDF-500 Part A & B, Lot 1: AZB30/BZB30	*Clostridium difficile* (ATCC 9689)	4/5/07	Combine equal parts A & B	1°=10 2°=10	1°=0 2°=0
MDF-500 Part A & B, Lot 2: AXA-02/BXB-02				1°=10 2°=10	1°=0 2°=0

** Number of carriers showing growth of the test organism.
1° Primary Subculture
2° Secondary Subculture

(For Laboratory Use Only)
ATS Labs Project # A 0 4 8 2 4
3/27/07

ATS LABS

PROTOCOL

AOAC Use-Dilution Method

Test Organism:

Clostridium difficile (ATCC 9689)

PROTOCOL NUMBER

MOD02031607.UD

PREPARED FOR

Modec, Inc.
4725 Oakland Street
Denver, CO 80239

SPONSOR REPRESENTATIVE

Ag-Chem Consulting
12208 Quinque Lane
Clifton, VA 20124

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PREPARED BY

David Rottjakob, M.T.
Director, Microbiology Services

DATE

March 16, 2007

EXACT COPY
INITIALS AES DATE 4/12/07

1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

AOAC Use-Dilution Method

SPONSOR: Modec, Inc.
4725 Oakland Street
Denver, CO 80239

SPONSOR REPRESENTATIVE: Ag-Chem Consulting
12206 Quinque Lane
Clifton, VA 20124

TEST FACILITY: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE
The purpose of this study is to determine the efficacy of the sponsor's product following the AOAC Use Dilution Method in compliance with the U.S. Environmental Protection Agency requirements set forth in the Pesticide Assessment Guidelines.

SUBSTANCE CHARACTERIZATION
Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The test substance shall be characterized by the Sponsor prior to the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is April 2, 2007. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of April 30, 2007. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

If a test must be repeated, or a portion of it, due to failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of ATS Labs or any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

The Sponsor is responsible for any rejection of the final report by the United States FDA or EPA concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM
The U.S. Environmental Protection Agency requires that a specific bacterial claim for a test substance intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed bacteria. This is accomplished in the laboratory by treating the target bacteria with the disinfectant (test substance) under conditions which simulate as closely as possible the actual conditions under which the test substance is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting data. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A film of bacterial cells dried on a surface of stainless steel carriers is exposed to the test substance for a specified contact time. After exposure, the carriers are transferred to vessels containing neutralizing subculture media and assayed for survivors. Appropriate viability, carrier population and neutralization controls are performed. The current version of Standard Operating Procedure CGT-4400 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Clostridium difficile	9689	Fluid Thioglycollate Medium and Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, anaerobic

The test organism to be used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Carriers

Carriers will be screened according to AOAC Official Method of Analysis and any carrier positive for growth will be discarded. Only penicylinders showing no growth may be used. Stainless steel penicylinders will be pre-soaked overnight in 1.0N NaOH, washed in water until neutral and autoclaved in 0.1% asparagine.

Preparation of Test Organism

All test organism incubation will be performed in an anaerobic chamber. From a frozen stock an initial tube of Fluid Thioglycollate Medium will be inoculated and incubated for 2-5 days at 35-37°C under anaerobic conditions. This culture is termed the "initial broth suspension." From this initial broth suspension, one loopful (0.01 mL) will be transferred to a second tube of Fluid Thioglycollate Medium and incubated for 2 days at 35-37°C under anaerobic conditions. Following this incubation, 0.1 mL aliquots of the second broth culture will be transferred and spread onto BAP plates. The plates will be incubated for 20±4 hours at 35-37°C under anaerobic conditions.

After incubation, the organism growth will be harvested by removing the organism growth using a sterile loop or swab and adding it to a tube of Fluid Thioglycollate Medium. This test culture suspension will be adjusted to approximately match a 0.5 McFarland standard prior to carrier inoculation. This procedure will be performed in an anaerobic chamber.

An organic soil load may be added to the test culture per Sponsor's request.

Contamination of Carriers

The penicylinders will be transferred to the culture and immersed for 15 minutes in the prepared suspension at a ratio of 1 carrier per 1.0 mL culture. The inoculated carriers will be dried on filter paper in a sterile petri dish at 35-37°C for 40 minutes in the anaerobic chamber. The drying conditions (temperature and humidity) will be appropriate for the test organism. The actual drying conditions will be clearly documented.

Preparation of Test Substance

The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the diluted test substance(s) shall be used within three hours of preparation. Ten (10) mL of the test substance at its use-dilution will be aliquoted into the required number of sterile 25 x 150 mm tubes. The tubes will be placed into a waterbath at the specified exposure temperature, and allow to equilibrate for ≥10 minutes prior to testing.

Exposure Conditions

Each contaminated and dried carrier will be placed into a separate tube containing 10 mL of the test substance at its use-dilution for the desired exposure time and temperature. This portion of the testing may be performed outside of the anaerobic chamber. If testing is performed outside of the anaerobic chamber the carriers will be used in testing immediately following removal from the anaerobic chamber.

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Test System Recovery
Following the Sponsor specified exposure period each medicated carrier will be transferred by hook needle at staggered intervals to 10 mL of neutralizing broth. If necessary, carriers will be transferred into individual secondary subculture tubes containing 10 mL neutralizing broth ≥30 minutes after subculture of first carrier. This portion of the procedure may be performed outside of the anaerobic chamber.

Incubation and Observation
All subculture tubes and plates will be incubated 2-5 days at 35-37°C. Plates will be incubated under anaerobic conditions (or other appropriate time/temperatures).

Following incubation, the subculture tubes will be visually examined for growth. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination.

Representative subculture tubes showing growth will be subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control
The serum used for soil load will be cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control
A representative uninoculated carrier will be added to the neutralizing subculture medium. The subculture medium containing the carrier will be incubated and examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control
A representative sample of uninoculated neutralizing subculture medium will be incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control
A representative inoculated carrier will be added to the subculture medium. The subculture medium containing the carrier will be incubated and visually examined for growth. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control
The neutralization of the test substance will be confirmed by exposing sterile carriers (representing not less than 10% of the total number of test carriers) to the test substance and transferring them to primary subcultures containing 10 mL of neutralizing subculture medium. If performed in the test procedure, carriers will then be transferred from primary subcultures into individual secondary subcultures ≥30 minutes following the primary transfer. The subcultures containing the exposed carriers will be inoculated with ≤100 colony forming units (CFU) of each test organism, incubated under test conditions and visually examined for the presence of growth. This control will be performed with multiple replicates using different dilutions of the test organism. **NOTE: The organism dilutions shall be used immediately after they have been diluted.** A standardized spread plate procedure will be run concurrently in order to enumerate the number of CFU actually added. **A 0.1 mL aliquot of the appropriate dilutions will be plated in duplicate. The control result will be reported using data from the most appropriate dilution.**

The acceptance criterion for this study control is growth following inoculation with ≤100 CFU.

OR:

Ten percent of the subcultures containing carriers showing no growth will be inoculated with ≤100 CFU of each test organism and incubated. This control will be performed with multiple replicates representing different dilutions of the test organism. A standardized spread plate procedure will be run concurrently in order to enumerate the number of CFU actually added. The control result will be reported using data from the most appropriate dilution.
The acceptance criterion for this study control is growth following inoculation with ≤100 CFU.

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Carrier Population Control
Inoculated carriers will be added at a ratio of 1 carrier to 10 mL neutralizing broth and vortex mixed. Appropriate serial ten-fold dilutions will be prepared and 0.1 mL aliquots will be plated on agar plate medium, and incubated. Following incubation, the resulting colonies will be enumerated and the CFU/carrier calculated. The acceptance criterion for this study control is a minimum of 1.0×10^4 CFU/carrier.

Verification of Spores Present in the Initial Suspension Control
Three (3.0) mL of the prepared test organism suspension will be heat shocked at 80 ± 2°C for 20 minutes. One (1.0) mL and 200 µL samples of the heat shocked prepared test organism suspension will be spread plated in duplicate using standard microbiological techniques. Following incubation, the organism plates will be observed to enumerate the concentration of spores present in the prepared test organism suspension used for inoculation of the test carriers. This control is run for informational purposes only, there is no acceptance criterion. *Clostridium difficile* Selective Agar will be used for this control parameter.

SD listed 10⁴ incorrect temperature ASJ 3/23/07

+ vortex time listed. It should be 10 minutes ASJ 3/23/07

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM
ATS Labs maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including bacterial strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subculture tubes, etc. during the course of the test. Test subculture tubes are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The EPA efficacy performance requirements for label claims state that the disinfectant must kill the microorganism on 10 out of the 10 inoculated carriers.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

REPORT
The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the bacterial strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

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PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

PRODUCT DISPOSITION

It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. Association of Official Analytical Chemists (AOAC), 1990. Use-Dilution Tests, p. 135-137. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.
2. Association of Official Analytical Chemists (AOAC), 1990. Germicidal and Detergent Sanitizing Action of Disinfectants, p. 139 [Preparation of Synthetic Hard Water]. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Efficacy Data Requirements, Disinfectants for Use on Hard Surfaces, DIS/TSS-1.
4. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1979. Efficacy Data Requirements, Supplemental Recommendations, DIS/TSS-2.
5. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Subseries 91A: Public Health Uses. *In* Pesticide Assessment Guidelines – Subdivision G (Product Performance).

DATA ANALYSIS

Calculations

Carrier Population Control Calculation:

$$\text{CFU/carrier} = \frac{(\text{average number colonies/plate @ dilution}) \times (\text{dilution factor}) \times (\text{volume neutralizer})}{(\text{number of carriers tested}) \times (\text{volume plated})}$$

The carrier population is calculated and reported using data from the most appropriate dilution(s).

Statistical Analysis
None used.

STUDY INFORMATION
(All sections must be completed prior to submitting protocol)

Sponsor (Date/Initial): 21 MAR 2007

Test Substance (Name & Batch Numbers, *including ≥60 day old batch* - exactly as it should appear on final report):
MDF-500 Part A & B (Lot 1: AZR30/BZR30 & Lot 2: AXA-02/BXB-02)

Specify ≥60 day old batch: N/A

Expiration Date: 2-2008

Product Description:
- ☑ Quaternary ammonia
- ☐ Iodophor
- ☐ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☐ Other

Test Substance Active Concentration (upon submission to ATS Labs): 3.6% Part A & 7.95% Part B

Neutralization/Subculture Broth:
- ☑ Primary – Fluid Thioglycollate Medium + 0.14% Lecithin, 1.0% Tween 80 and 0.05% Catalase
  Secondary – Fluid Thioglycollate Medium
- ☐ ATS Labs' Discretion. The Sponsor authorizes additional fees for specific neutralization media (if necessary).

Storage Conditions:
- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other:

Hazards:
- ☑ None known: Use Standard Precautions
- ☐ Material Safety Data Sheet, Attached for each product
- ☐ As Follows:

Product Preparation
- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilutions/Concentrations to be tested: Mix Parts A & B together equally (1 part A & 1 part B)
  - ☐ Deionized Water (Filter Sterilized)
  - ☐ Tap Water (Filter Sterilized)
  - ☐ AOAC Synthetic Hard Water: _____ PPM
  - ☐ Other

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Organism: *Clostridium difficile* (ATCC 9689)

Carrier Number: 10 per batch

Exposure Time: 10 Minutes

Exposure Temperature: 20 ± 1 °C

Organic Soil Load:
- ☑ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
- ☐ No Organic Soil Load Required
- ☐ Other:

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TEST SUBSTANCE SHIPMENT STATUS

☑ Has been used in one or more previous studies at ATS Labs.
☐ Has been shipped to ATS Labs (but has not been used in a previous study).
Date shipped to ATS Labs:_____ Sent via overnight delivery? ☐ Yes ☐ No
☐ Will be shipped to ATS Labs.
Date of expected receipt at ATS Labs:_____
☐ Sender (if other than Sponsor):_____

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

☑ Approved without modification
☐ Approved with modification - Supplemental Information Form Attached - ☐ Yes ☐ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Brian Kalamanka TITLE: CEO

SIGNATURE: [signature] DATE: 21 MARCH 2007

PHONE: 303-373-2698 FAX: 303-373-2699 EMAIL: bjk@deepsolutions.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

COPY TO: DR. MATTHEW BROOKS

ATS LABS:

NAME: Amy S. Jenke
Study Director

SIGNATURE: Amy S. Jenke
Study Director DATE: 3/23/07