FINAL STUDY REPORT

STUDY TITLE

AOAC Use-Dilution Method

Test Organism:

Klebsiella pneumoniae - NDM-1 positive
(CDC 1000527)

PRODUCT IDENTITY

EST-PAGS0000021
Lot # 019142345 (≥60 days old) and Lot # 014925082

DATA REQUIREMENTS

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

AUTHOR

Anne Stemper, B.S.
Study Director

STUDY COMPLETION DATE

February 9, 2011

PERFORMING LABORATORY

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

SPONSOR

Esther Material Technology Co., Ltd.
No. 8 XinZhan Rd., Qianzhen Distr.
Koahsiung, Taiwan 80672

PROJECT NUMBER

A10782

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: Esther Material Technology Co., Ltd.

Company Agent: _____

_____
Title

_____ Date: _____
Signature

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) and antibiotic sensitivity testing performed at the University of Minnesota Physicians Outreach Laboratory.

Submitter:_____ Date:_____

Sponsor:_____ Date:_____

Study Director:_____ Date: 2.9.11
Anne Stemper, B.S.

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 of Phase Inspection	Date Reported to Study Director	Date Reported to Management
Critical Phase Audit	January 13, 2011	January 13, 2011	February 1, 2011
Final Report	February 8, 2011	February 8, 2011	February 9, 2011

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

Quality Assurance Auditor: Judy Heidemann Date: 2-9-11

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STUDY PERSONNEL

STUDY DIRECTOR: Anne Stemper, B.S.

Professional personnel involved:

Amy S. Jeske, B.S.	- Manager, Microbiology Operations
Scott R. Steinagel, B.S.	- Manager, Microbiology Laboratory Operations
Matthew Sathe, B.S.	- Research Scientist I
Adam W. Pitt, B.S.	- Research Assistant II
Peter Toll, B.S.	- Research Assistant II
Kathryn Thomas, B.S.	- Research Assistant I
Lindsey Peach, B.A.	- Research Assistant I

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Use-Dilution Method

Project Number: A10782

Protocol Number: EMA01100710.UD.2

Sponsor: Esther Material Technology Co., Ltd.
No. 8 XinZhan Rd., Qianzhen Distr.
Koahsiung, Taiwan 80672

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

TEST SUBSTANCE IDENTITY

Test Substance Name: EST-PAGS0000021

Lot/Batch(s): Lot # 019142345 (≥60 days old) and Lot # 014925082

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: November 19, 2010
Study Initiation Date: January 6, 2011
Experimental Start Date: January 13, 2011
Experimental End Date: January 27, 2011
Study Completion Date: February 9, 2011

OBJECTIVE

The objective of this study was to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method was in compliance with the requirements of the U.S. Environmental Protection Agency (EPA).

SUMMARY OF RESULTS

Test Substance: EST-PAGS0000021, Lot # 019142345 (≥60 days old) and Lot # 014925082

Dilution: Ready to use (RTU)

Test Organism: *Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527)

Exposure Time: 10 minutes

Exposure Temperature: 20±1°C (20.0°C)

Organic Soil Load: No organic soil load required

Number of Carriers: 10 per batch

Efficacy Result: EST-PAGS0000021 demonstrated efficacy of two lots against *Klebsiella pneumoniae* - NDM-1 positive, and therefore, meets the requirements set forth by the U.S. EPA for disinfectant label claims following a 10 minute exposure time at 20±1°C (20.0°C).

TEST MATERIALS

Test System/Growth Media

Test Organism	Designation #	Growth Medium	Incubation Parameters
Klebsiella pneumoniae - NDM-1 positive	1000527	Nutrient Broth	35-37°C, aerobic

The microorganism used in this study was obtained from the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia.

Recovery Media

Neutralizing Subculture Medium: Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 (primary and secondary)

Agar Plate Medium: Tryptic Soy Agar with 5% Sheep Blood (BAP)

Carriers

Carriers were screened according to the AOAC Official Method of Analysis and all carriers positive for growth were discarded. Only penicylinders which demonstrated no growth during screening were used in this test. Stainless steel penicylinders were pre-soaked overnight in 1.0 N NaOH, washed in water until neutral and autoclaved in deionized water.

TEST METHOD

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor.

Ten (10.0) mL aliquots of the test substance were transferred to sterile Morton closure tubes 25 x 150 mm tubes, placed in a 20±1°C (20.0°C) waterbath and allowed to equilibrate for ≥10 minutes.

Preparation of Test Organism

From a stock slant, an initial tube of culture broth was inoculated. This culture was termed the "initial broth" suspension. From the initial broth suspension a minimum of three daily transfers were performed on consecutive days prior to use in the testing procedure. The appropriate growth medium was subcultured using a daily transfer (more than 3, but less than 30 transfers) of the test organism.

A 48-54 hour broth culture incubated at 35-37°C was prepared. The test cultures were thoroughly mixed and allowed to stand for ≥10 minutes prior to use.

Antibiotic sensitivity testing was performed using a representative culture from the day of testing to verify the stated antibiotic resistance pattern. This testing was performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. This testing was not performed under EPA Good Laboratory Practices (40 CFR Part 160). See Attachment I.

Contamination of Carriers

The penicylinders were transferred to the culture and immersed for 15 minutes in a prepared suspension, at a ratio of 1 carrier per 1.0 mL of culture. The inoculated carriers were then dried on filter paper in a sterile Petri dish at 35-37°C for 40 minutes at 55% relative humidity.

Exposure Conditions

For each lot of test substance, contaminated and dried carriers were individually transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the requested dilution. The carriers were exposed for 10 minutes at 20±1°C (20.0°C).

Test System Recovery

Following the Sponsor specified exposure time, each medicated carrier was then transferred by hook needle at identical staggered intervals to 10 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 ≥30 minutes after subculture of the first carrier.

Incubation and Observation

All subculture tubes and plates were incubated for 48±4 hours at 35-37°C. Subcultures were stored at 2-8°C for two days prior to examination. Following incubation and storage, 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.

Carrier Sterility Control
A representative uninoculated carrier was added to the neutralizing subculture medium. The subculture medium containing the carrier was incubated and visually 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 for growth. 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 colony forming units (CFU) of the test organism, 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 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 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.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The U.S. EPA efficacy performance requirements for label claims state that the test substance 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 Amendment:
Per Sponsor's request, this protocol is amended to clarify that no organic soil load is required for this study.

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculation

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.

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), 2006. Use-Dilution Methods 964.02, 955.14, and 955.15.
2. Association of Official Analytical Chemists (AOAC), 2005. Germicidal and Detergent Sanitizing Action of Disinfectants Method 960.09 [Preparation of Synthetic Hard Water].
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 1-3 and
All data measurements/controls including the culture purity, viability, neutralizing subculture medium sterility, carrier sterility, neutralization confirmation, and carrier population were within acceptance criteria.

For Test Results, see Table 4.

ANALYSIS

EST-PAGS0000021, Lot #019142345 (≥60 days old) and Lot #014925082, ready to use, demonstrated no growth of *Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527) in any of the 10 primary subculture tubes and no growth in any of the 10 secondary subculture tubes following a 10 minute exposure time at 20±1°C (20.0°C).

STUDY CONCLUSION

Under the conditions of this investigation, ready to use, demonstrated efficacy against *Klebsiella pneumoniae* - NDM-1 positive as required by the U.S. EPA for disinfectant label claims following a 10 minute exposure time at 20±1°C (20.0°C).

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
	***Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527)**
Purity Control	Pure
Viability Control	Growth
Neutralizing Subculture Medium Sterility Control	No Growth
Carrier Sterility Control	No Growth

TABLE 2: CARRIER POPULATION CONTROL RESULTS

Test Organism	Date Performed	Result
Klebsiella pneumoniae - NDM-1 positive (CDC 1000527)	1-13-11	3.8×10^5 CFU/carrier

CFU = Colony Forming Unit

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Date Performed	Average CFU Added	Number of Subculture Tubes Tested	Number of Subculture Tubes Positive
EST-PAGS0000021 Lot # 014925082	*Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527)	1-13-11	26	1	1
EST-PAGS0000021 Lot # 019142345 (≥60 days old)				1	1

CFU = Colony Forming Unit

TABLE 4: TEST RESULTS

Test Substance	Test Organism	Date Performed	Sample Dilution*	Number of Carriers	
				Exposed	Showing Growth**
EST-PAGS0000021 Lot # 014925082	*Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527)	1-13-11	RTU	1°=10 2°=10	1°=0 2°=0
EST-PAGS0000021 Lot # 019142345 (≥60 days old)				1°=10 2°=10	1°=0 2°=0

* RTU = Ready to use.
** Number of carriers showing growth of the test organism.
1° Primary Subculture
2° Secondary Subculture

ATTACHMENT I: ANTIBIOTIC SENSITIVITY TESTING RESULTS

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University of Minnesota Physicians
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ATS Labs
Attn: Chris Slitts
1285 Corporate Ctr Dr, Suite 110
Eagan, MN 55121

PATIENT NAME	PATIENT ID	DOB	SEX	STATUS	DESTINATION
ATSLABS, A10781A10782	Z1590-69	01/01/1900	U	Final	DZ1590

PHYSICIAN	COLLECT DATE & TIME	DATE OF SERVICE	EXTRACT DATE/TIME	PAGE
UNKNOWN, PHYSICIAN	01/14/2011 00:00 (a)	01/17/2011 14:04	01/27/2011 03:31	1

REQUISITION NO.	EXTERNAL ID
10079.Z1590	

COMMENTS:

Diagnostic Procedure	Result In Range	Result Out of Range	Units	Reference Range
Referral sensitivity Collected on: 01/14/2011 00:00				
TRANSPORT TIME	86.1			
Specimen Description	Culture plate			
Culture	Klebsiella pneumoniae			
Report status	FINAL 01/26/2011			
Susceptibility Collected on: 01/14/2011 00:00				
Organism:	Klebsiella pneumoniae			
Method	E Test			
Ertapenem	>32.0 Resistant			
Susceptibility Collected on: 01/14/2011 00:00				
Organism:	Klebsiella pneumoniae			
Method	MIC			
Amikacin	>=64 Resistant			
Ampicillin	>=32 Resistant			
Ampicillin/Sulbactam	>=32 Resistant			
Cefazolin	>=64 Resistant			
Cefepime	>=64 Resistant			
Ceftazidime	>=64 Resistant			
Ceftriaxone	>=64 Resistant			
Ciprofloxacin	>=4 Resistant			
Gentamicin	>=16 Resistant			
Imipenem	>32.0 Resistant			
Levofloxacin	>=8 Resistant			
Tobramycin	>=16 Resistant			
Trimethoprim/Sulfa	>=16/304 Resistant			
Meropenem	32.0 Resistant			
Piperacillin/Tazo	>256.0 Resistant			
End of Report				

EXACT COPY
INITIALS AS DATE 29-11

ATSLABS, A10781A10782 01/27/2011 03:31 DZ1590

ATS LABS

ATS LABS

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	1-11-11
Sponsor:	Esther Material Technology Co., Ltd. No. 8 XinZhan Rd., Qianzhen Distr. Koahsiung, Taiwan 80672
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	AOAC Use-Dilution Method
ATS Labs Protocol Number:	EMA01100710.UD.2
ATS Labs Project Number:	A10782

Modifications to Protocol:

Per Sponsor's request, this protocol is amended to clarify that no organic soil load is required for this study.

Changes to the protocol are acceptable as noted.

EXACT COPY
INITIALS M DATE 2-9-11

Study Director

1-11-11
Date

(For Laboratory Use Only)
ATS Labs Project # A10782
LC 7/10/11

PROTOCOL

AOAC Use-Dilution Method

Test Organism:

Klebsiella pneumoniae - NDM-1 positive (CDC 1000527)

PROTOCOL NUMBER

EMA01100710.UD.2

PREPARED FOR

Esther Material Technology Co., Ltd.
No. 8 XinZhan Rd., Qianzhen Distr.
Koahsiung, Taiwan 80672

EXACT COPY
INITIALS M DATE 2-9-11

PERFORMING LABORATORY

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

PREPARED BY

Lynsey Wieland, B.S.
Research Scientist I

DATE

October 7, 2010

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

AOAC Use-Dilution Method

SPONSOR: Esther Material Technology Co., Ltd.
No. 8 XinZhan Rd., Qianzhen Distr.
Koahsiung, Taiwan 80672

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

PURPOSE

The purpose of this study is to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the following: The U. S. Environmental Protection Agency (EPA).

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. 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 October 25, 2010. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of November 22, 2010. 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 regulatory agencies 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

Regulatory agencies require that a specific organism 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 organism. This is accomplished in the laboratory by treating the target organism 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 organism cells dried on a surface of stainless steel carriers is exposed to the test substance for a specified exposure time. After exposure, the carriers are transferred to vessels containing neutralizing subculture media and assayed for survivors. Appropriate viability, culture purity, sterility, carrier population and neutralization confirmation controls are performed. The current version of Standard Operating Procedure CGT-4400 reflects the methods which shall be used in this study.

Protocol Number: EMA01100710.UD.2 Esther Material Technology Co., Ltd.
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TEST METHOD

Test Organism	Designation #	Growth Medium	Incubation Parameters
Klebsiella pneumoniae - NDM-1 positive	CDC 1000527	Nutrient Broth	35-37°C, aerobic

The test organism to be used in this study was obtained from the Centers for Disease Control and Prevention (CDC) Atlanta, Georgia.

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 deionized water.

Preparation of Test Organism
From a stock slant, an initial tube of culture broth will be inoculated. This culture is termed the "initial broth suspension." From this initial broth suspension, a minimum of three daily transfers will be performed on consecutive days prior to use in testing procedure. The appropriate growth medium will be subcultured using a daily transfer (more than 3, but less than 30 transfers) of the test organism.

A 48-54 hour broth culture incubated at the parameters listed above will be prepared.

The test cultures will be thoroughly mixed and allowed to stand for ≥10 minutes prior to use.

An organic soil load may be added to the test culture per Sponsor's request. Antibiotic sensitivity testing will be performed using a representative organism from the day of testing to verify the stated antibiotic resistance pattern. This testing may be performed at the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. If not performed by ATS Labs, testing will not be performed under EPA Good Laboratory Practices (40 CFR Part 160) and will be exempt from the GLP compliance statement.

Contamination of Carriers
The penicylinders will be transferred to the culture and immersed for 15 minutes in a 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. 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.

Test System Recovery
Following the Sponsor specified exposure time 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.

Incubation and Observation
All subcultures and controls are incubated for 48±4 hours at 35-37°C (or other appropriate time/temperatures).

Following incubation, the subcultures 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 neutralized subcultures 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 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 visually 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 for growth. 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 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. 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.

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.

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 the aliquots spread 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.

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 organism 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 U.S. EPA efficacy performance requirements for label claims state that the test substance 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. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number.

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism 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.

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.

TEST SUBSTANCE RETENTION

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.

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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), 2006. Use-Dilution Methods 964.02, 955.14, and 955.15.
2. Association of Official Analytical Chemists (AOAC), 2005. Germicidal and Detergent Sanitizing Action of Disinfectants Method 960.09 [Preparation of Synthetic Hard Water].
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

Calculation

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.

JAN-04-2011 TUE 06:57 PM P. 007/009

STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Sponsor (Date/Initial): Esther Material Technology Co., Ltd. Per Sponsor clarifications, this protocol was reviewed on 1-4-11.

Test Substance (Name & Batch Numbers, *including ≥60 day old batch* - exactly as it should appear on final report): EST-PAGS000021: Lot# 014925082, Lot# 01914 2345 AM 1-6-11

Specify ≥60 day old batch: Lot# 01914 2345

Expiration Date: Lot# 01914 2345 EXP. Aug 30, 2012 / Lot# 014925082 Oct. 14, 2012

Product Description:

- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☑ Other: Nano-Silver

Test Substance Active Concentration (upon submission to ATS Labs): 1000 ppm

Neutralization/Subculture Broth:

☐ _____

☑ ATS Labs' Discretion. By checking, the Sponsor authorizes ATS Labs, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Box Checked per Sponsor clarification AM 1-6-11

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)
- ☐* Dilution(s) to be tested:

_____ defined as _____ + _____
(example: 1 oz/gallon) (amount of test substance) (amount of diluent)

- ☐ Deionized Water (Filter or Autoclave Sterilized)
- ☐ Tap Water (Filter or Autoclave Sterilized)
- ☐ AOAC Synthetic Hard Water: _____ PPM
- ☐ Other _____

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

Shaken the test substance well prior to use in testing added per Sponsor clarification AM 1-6-11

Test Organism: *Klebsiella pneumoniae* - NDM-1 positive (CDC 1000527)

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: Nov. 19, 2010 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: Ms. No-Ya Nora Hung TITLE: Product Design & Q.C. Senior Manager

SIGNATURE: _____ DATE: Jan. 4, 2011

PHONE: +886-7-8232265 FAX: +886-7-8230065 EMAIL: noyahung@gmail.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

ATS LABS:

NAME: Anne Stempien
Study Director

SIGNATURE: _____ DATE: 1-6-11
Study Director