FINAL STUDY REPORT

STUDY TITLE

AOAC Germicidal Spray Method

Test Organisms:

Escherichia coli O157:H7 (ATCC 43888)
Escherichia coli (ESBL) (ATCC BAA-196)

PRODUCT IDENTITY

Jymrsa
A1 Batch # ATD07 + B1 Batch # BTH07
and
A2 Batch # ATH07 + B2 Batch # ATH07

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

December 30, 2005

PERFORMING LABORATORY

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

SPONSOR

Jymrsa, Inc.
4738 42nd Avenue N.
Minneapolis, MN 55422

PROJECT NUMBER

A03441

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: Jymrsa, Inc.

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).

Submitter:_____ Date:_____

Sponsor:_____ Date:_____

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

QUALITY ASSURANCE UNIT SUMMARY

Study: AOAC Germicidal Spray Method

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical 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 date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director	Management
Critical Phase	December 13, 2005	December 13, 2005	December 30, 2005
Final Report	December 29, 2005	December 29, 2005	

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

Quality Assurance Auditor:_____ Date:_____

TABLE OF CONTENTS

STUDY PERSONNEL

STUDY DIRECTOR: Anne Stemper, B.S.

Professional personnel involved:

Scott R. Steinagel, B.S.	- Microbiology Laboratory Supervisor
Matthew Sathe, B.S.	- Research Assistant I
Peter Toll, B.S.	- Research Assistant I
Lisa Slusser, B.S.	- Research Assistant I
Katherine Sager, B.S.	- Research Assistant I

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Germicidal Spray Method

Project Number: A03441

Protocol Number: WBS01101905.GS

Sponsor: Jymrsa, Inc.
4738 42nd Avenue N.
Minneapolis, MN 55422

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

TEST SUBSTANCE IDENTITY

Test Substance Name: Jymrsa Spray

Lot/Batch(s): A1 Batch # ATD07 + B1 Batch # BTH07
and
A2 Batch # ATH07 + B2 Batch # ATH07

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 4, 2005
Study Initiation Date: November 8, 2005
Experimental Start Date: December 13, 2005
Experimental End Date: December 15, 2005
Study Completion Date: December 30, 2005

OBJECTIVE

The objective of this assay was to determine the effectiveness of spray products as disinfectants for contaminated surfaces in compliance with the U.S. Environmental Protection Agency requirements set forth in the Pesticide Assessment Guidelines.

SUMMARY OF RESULTS

Test Substance: Jymrsa (A1 Batch # ATD07 + B1 Batch # BTH07; A2 Batch # ATH07 + B2 Batch # ATH07)

Dilution: Equal parts of A1 were mixed with equal parts of B1
and
Equal parts of A2 were mixed with equal parts of B2

Test Organisms: *Escherichia coli* O157:H7 (ATCC 43888)
Escherichia coli (ESBL) (ATCC BAA-196)

Exposure Time: Ten minutes

Exposure Temperature: Room Temperature (20°C)

Organic Soil Load: 5% fetal bovine serum

Efficacy Result: Jymrsa demonstrated efficacy of two batches against *Escherichia coli* O157:H7, and therefore, meets the requirements set forth by the U.S. EPA for disinfectant label claims following a ten minute exposure period.

Jymrsa demonstrated efficacy of two batches against *Escherichia coli* (ESBL), 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 Organisms	ATCC #	Growth Medium
Escherichia coli O157:H7	43888	Synthetic Broth
Escherichia coli (ESBL)	BAA-196	Synthetic Broth

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

Recovery Media

Neutralizing Subculture Medium: Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 + 0.01% Catalase

Agar Plate Medium: Tryptic Soy Agar with 5% Sheep Blood

Reagents

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

Carriers

Glass slides (18 mm x 36 mm) were utilized as the carrier for this assay. The carriers were placed into a vessel and sterilized in an air oven for two hours at approximately 180°C. Individual sterile plastic petri dishes were matted with two pieces of filter paper. One sterile glass slide was transferred into each of the matted petri dishes.

TEST METHOD

Preparation of the Test Substance

Per Sponsor instruction, equal parts of A1 were mixed with equal parts of B1 and equal parts of A2 were mixed with equal parts of B2 prior to testing. The prepared test substances were homogenous as determined by visual observation and was used within three hours of preparation.

Preparation of the Test Organisms

The growth mediums were inoculated using a stock culture of each test organism. A minimum of four transfers were performed on consecutive days prior to use in testing procedures. For this assay, a 48-54 hour broth culture incubated at 35-37°C was utilized. The test cultures were thoroughly mixed and allowed to settle for a minimum of 10 minutes prior to use.

ATS Labs used the AB BIODISK Etest® Method to verify the antimicrobial susceptibility pattern of *Escherichia coli* Extended Spectrum Beta-Lactamase (ESBL). The same cultures used for the test were used to make a suspension equal to a 0.5 McFarland standard in 0.85% sterile saline. Each test organism suspension was streaked onto a Mueller Hinton agar plate using a sterile swab and rotating the plate 60º in between each inoculation. The Etest strip containg Cefotaxime (CT) and Cefotaxime + Clavulanic acid (CTL) and the Etest strip containing Ceftazidime (TZ) and Ceftazidime + Clavulanic acid (TZL) were both placed on each inoculated Mueller Hinton agar plate. The plates were incubated for 16-18 hours at 35-37ºC. Following incubation, the minimum inhibitory concentration (MIC) values for CT, CTL, TZ, and TZL were read where the respective inhibition ellipses intersect the strip. Two quality control strains were run concurrently with the test organisms to confirm the validity of the assay. *Escherichia coli* (ATCC 35218) served as the negative control and *Klebsiella pneumoniae* (ATCC 700603) served as the positive control for this test. The interpretation of the MIC values for the test organisms was determined using the Reading and Interpretation section included in the attached reference for AB BIODISK Etest® Method (see Table 6). The quality control results were determined using the specifications for the Etest ESBL CT/CTL and TZ/TZL strips (see Table 7) listed in the Quality Control section included in the attached reference for AB BIODISK Etest® Method. See Table 5 for test organism and quality control organism MIC results.

Addition of Organic Soil Load

A 0.25 mL aliquot of FBS was added to 4.75 mL of each broth culture to yield a 5% fetal bovine serum soil load.

Contamination of the Carriers

The soil load previously described was added to each culture. Individual glass slide carriers were each inoculated with 0.01 mL culture calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at a 40% relative humidity.

Exposure Conditions

For each prepared test substance, ten of the carriers were sprayed individually at staggered intervals with the test substance until saturated (3 pumps) at a distance of 6-8 inches. Each carrier remained in contact with the prepared test substance for ten minutes at room temperature (20°C) at a 10% relative humidity.

Test System Recovery
Following the exposure period, the remaining liquid was drained off. Each medicated carrier was then transferred using sterile forceps at identical staggered intervals to 20 mL aliquots of Letheen Broth + 0.07% Lecithin + 0.5% Twen 80 + 0.01% Catalase.

Incubation and Observation
The neutralized subcultures and controls were incubated for 48±4 hours at 35-37°C. The subcultures were stored at 2-8°C prior to examination. Following incubation (or incubation and storage), the subcultures were 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 lack of growth. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control
A representative uninoculated carrier was added to the neutralizing 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 subcultures containing 20 mL of neutralizing subculture medium. The subcultures containing the exposed carriers were 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 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 following 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 the 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 EPA efficacy performance requirements for label claims state that the disinfectant must kill the microorganisms 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:
No protocol deviations occurred during this study.

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. These original data include, but are not limited to, the following:

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

Test Substance Retention
The test substance will be returned 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), 2000. Germicidal Spray Products as Disinfectants, 961.02. *In* Official Methods of Analysis of the AOAC, Chapter 6, Seventeenth 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).

6. AB BIODISK Etest® ESBL for *in vitro* Confirmation of ESBL, Package Insert.

RESULTS

Control and Neutralization Results, see Tables 1-3
All data measurements including the culture purity, viability, organic soil load sterility, neutralizing subculture medium sterility, carrier sterility, neutralization confirmation and carrier population controls were within acceptance criteria.

Test Results, see Table 4

ANALYSIS

Jymrsa (A1 Batch # ATD07 + B1 Batch # BTH07 and A2 Batch # ATH07 + B2 Batch # ATH07), a trigger spray product, demonstrated no growth of *Escherichia coli* O157:H7 (ATCC 43888) in any of the 10 subcultures following a ten minute exposure period in the presence of a 5% fetal bovine serum soil load.

Jymrsa (A1 Batch # ATD07 + B1 Batch # BTH07 and A2 Batch # ATH07 + B2 Batch # ATH07), a trigger spray product, demonstrated no growth of *Escherichia coli* (ESBL) (ATCC BAA-196) in any of the 10 subcultures following a ten minute exposure period in the presence of a 5% fetal bovine serum soil load.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum soil load, Jymrsa (A1 Batch # ATD07 + B1 Batch # BTH07 and A2 Batch # ATH07 + B2 Batch # ATH07), a pump spray product, demonstrated efficacy against *Escherichia coli* O157:H7 as required by the U.S. EPA for disinfectant label claims following a ten minute exposure period.

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum soil load, Jymrsa (A1 Batch # ATD07 + B1 Batch # BTH07 and A2 Batch # ATH07 + B2 Batch # ATH07), a pump spray product, demonstrated efficacy against *Escherichia coli* (ESBL) 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	
	***Escherichia coli* O157:H7 (ATCC 43888)**	***Escherichia coli* (ESBL) (ATCC BAA-196)**
Purity Control	Pure	Pure
Viability Control	Growth	Growth
Carrier Sterility Control	No Growth	
Organic Soil Sterility Control	No Growth	
Neutralizing Subculture Medium Sterility Control	No Growth	

TABLE 2: CARRIER POPULATION CONTROL RESULTS

Test Organism	Date Performed	Result
Escherichia coli O157:H7 (ATCC 43888)	12/13/05	3.3×10^4 CFU/carrier
Escherichia coli (ESBL) (ATCC BAA-196)		3.3×10^5 CFU/carrier

CFU = Colony Forming Unit

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Date Performed	Inoculum (CFU)	Number Subcultures	
				Tested	Positive
Jymrsa, A1 Batch # ATD07 + B1 Batch # BTH07	*Escherichia coli* O157:H7 (ATCC 43888)	12/13/05	4	1	1
	Escherichia coli (ESBL) (ATCC BAA-196)		14	1	1
Jymrsa, A2 Batch # ATH07 + B2 Batch # ATH07	*Escherichia coli* O157:H7 (ATCC 43888)		4	1	1
	Escherichia coli (ESBL) (ATCC BAA-196)		14	1	1

CFU = Colony Forming Unit

TABLE 4: TEST RESULTS

Test Substance	Test Organism	Date Performed	Sample Dilution*	Number of Carriers	
				Exposed	Showing Growth**
Jymrsa, A1 Batch # ATD07 + B1 Batch # BTH07	*Escherichia coli* O157:H7 (ATCC 43888)	12/13/05	*See below*	10	0
	Escherichia coli (ESBL) (ATCC BAA-196)			10	0
Jymrsa, A2 Batch # ATH07 + B2 Batch # ATH07	*Escherichia coli* O157:H7 (ATCC 43888)			10	0
	Escherichia coli (ESBL) (ATCC BAA-196)			10	0

* Equal parts of A1 were mixed with equal parts of B1
Equal parts of A2 were mixed with equal parts of B2
** Number of carriers showing growth of the test organism.

TABLE 5: MINIMUM INHIBITORY CONCENTRATION (MIC) VERIFICATION OF ANTIBIOTIC RESISTANCE

Organism	MIC Value Cefotaxime (CT)	MIC Value Ceftaxime and Clavulanic acid (CTL)	MIC Value Ceftazidime (TZ)	MIC Value Ceftazidime and Clavulanic acid (TZL)	Interpretation Result
QC Organism: *Escherichia coli* (ATCC 35218)	≤0.25 μg/mL	0.032 μg/mL	≤0.5 μg/mL	0.064 μg/mL	Negative for ESBL
QC Organism: *Klebsiella pneumoniae* (ATCC 700603)	3 μg/mL	0.25 μg/mL	≥32 μg/mL	0.38 μg/mL	Positive for ESBL
Escherichia coli (ESBL) (ATCC BAA-196)	0.5 μg/mL	0.25 μg/mL	>32 μg/mL	1.0 μg/mL	Positive for ESBL
Escherichia coli O157:H7 (ATCC 43888)	≤0.25 μg/mL	0.064 μg/mL	≤0.50 μg/mL	0.25 μg/mL	Negative for ESBL

TABLE 6: REFERENCE TABLE FOR INTERPRETATION OF ESBL ETEST RESULTS

ESBL	MIC µg/mL Ratio*
Positive	CT ≥0.5 and CT/CTL ≥8 **OR** TZ ≥1 and TZ/TZL ≥8 **OR** "Phantom" zone or deformation of the CT or TZ ellipse
Negative	CT <0.5 or CT/CTL <8 **AND** TZ <1 or TZ/TZL <8

*from AB BIODISK Etest® Method Reading and Interpretation Section

TABLE 7: QUALITY CONTROL SPECIFICATIONS FOR ETEST ESBL CT/CTL AND TZ/TZL STRIPS

QC Organism*	MIC (µg/mL) Cefotaxime (CT)*	MIC (µg/mL) Cefotaxime + Clavulanic acid (CTL)*	ESBL Interpretation*
Escherichia coli (ATCC 35218)	≤0.25	0.016-0.064	Negative
Klebsiella pneumoniae (ATCC 700603)	1-4	0.125-1	Positive
QC Organism*	**MIC (µg/mL) Ceftazidime (TZ)***	**MIC (µg/mL) Ceftazidime + Clavulanic acid (TZL)***	**ESBL Interpretation***
Escherichia coli (ATCC 35218)	≤0.5	≤0.125	Negative
Klebsiella pneumoniae (ATCC 700603)	8-≥32	0.25-1	Positive

*from AB BIODISK Etest® Method Quality Control Section