FINAL STUDY REPORT

STUDY TITLE

AOAC Germicidal Spray Method

Test Organisms:

Pseudomonas aeruginosa (ATCC 15442)
Enterobacter aerogenes (ATCC 15038)
Proteus mirabilis (ATCC 9240)
Klebsiella pneumoniae (ATCC 4352)

PRODUCT IDENTITY

Jymrsa
AA18-BG04 and FSCP05-AA11-24

DATA REQUIREMENTS

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

AUTHOR

Amy Jeske, B.S.
Study Director

STUDY COMPLETION DATE

March 24, 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

A02758

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 procedures 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:_____
Amy Jeske, 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 non-clinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and a standard protocol. 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	March 17, 2005	March 17, 2005	March 24, 2005
Final Report	March 23, 2005	March 23, 2005	

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

Quality Assurance Auditor:_____ Date:_____

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STUDY PERSONNEL

STUDY DIRECTOR: Amy Jeske, 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

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Germicidal Spray Method

Project Number: A02758

Protocol Number: WBS01012105.GS.2

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): AA18-BG04 and FSCP05-AA11-24

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 23, 2005
Study Initiation Date: February 24, 2005
Experimental Start Date: March 15, 2005
Experimental End Date: March 17, 2005
Study Completion Date: March 24, 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 (AA18-BG04 and FSCP05-AA11-24)

Dilution: Equal parts A & B mixed together

Test Organisms: *Pseudomonas aeruginosa* (ATCC 15442)
Enterobacter aerogenes (ATCC 15038)
Proteus mirabilis (ATCC 9240)
Klebsiella pneumoniae (ATCC 4352)

Exposure Time: Ten minutes

Exposure Temperature: Room temperature

Organic Soil Load: 5% fetal bovine serum

Efficacy Result: Jymrsa demonstrated efficacy of two (2) lots against *Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus mirabilis* and *Klebsiella pneumoniae* as required by the U.S. EPA for disinfectant label claims.

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium
Pseudomonas aeruginosa	15442	Nutrient Broth
Enterobacter aerogenes	15038	Tryptic Soy Broth
Proteus mirabilis	9240	Nutrient Broth
Klebsiella pneumoniae	4352	Nutrient Broth

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

Recovery Media

Neutralizing Subculture Medium: Letheen Broth with 0.07% Lecithin, 0.5% Tween 80 and 0.05% Catalse

Agar Plate Medium: Tryptic Soy Agar with 5% Sheep Blood (BAP) for *Pseudomonas aeruginosa, Enterobacter aerogenes* and *Klebsiella pneumoniae.*
MacConkey Agar for *Proteus mirabilis*

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 Test Substance
The test substances were prepared by mixing 400 mL of Part A with 400 mL of Part B in a clean, ATS provided trigger spray bottle. The prepared test substance was homogenous as determined by visual observation and was used within three hours of preparation.

Preparation of Test Organism
For each test organism, the appropriate growth medium was inoculated using a stock culture of the test organism. A minimum of four transfers were performed on consecutive days prior to use in testing procedures.

A 48-54 hour broth culture incubated at 35-37°C for *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumoniae* and at 25-30°C for *Enterobacter aerogenes* was prepared.

On the day of use, the pellicle was aspirated from the *Pseudomonas aeruginosa* culture. The test cultures were thoroughly mixed and allowed to stand for ≥10 minutes prior to use.

Addition of Organic Soil Load
A 0.1 mL aliquot of FBS was added to 1.9 mL of each broth culture to yield a 5% fetal bovine serum soil load.

Contamination of Carriers
The glass slide carriers were each inoculated with 0.01 mL of a 48-54 hour culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide. The dishes were covered immediately and the procedure repeated until all slides were individually inoculated. The slides were allowed to dry for 30-40 minutes at 35-37°C at a 40% relative humidity.

Exposure Conditions
For each prepared test substance, 10 of the carriers were sprayed individually at staggered intervals with the test substance for 3 pumps until thoroughly wet at a distance of 6-8 inches. Each carrier remained in contact with the prepared test substance for ten minutes at room temperature (20.0°C) and at a 9.0% 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 with 0.07% Lecithin, 0.5% Tween 80 and 0.05% Catalase.

Incubation and Observation
The neutralized subcultures and controls for *Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumoniae* were incubated for 48±4 hours at 35-37°C. The neutralized subcultures and controls for *Enterobacter aerogenes* were incubated for 48±4 hours at 25-30°C. Following incubation 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:
This protocol is being amended for two reasons.

1. It is being amended to correct an error on page 8 of the protocol. The Dilution/Concentrations to be tested box under Product Preparation should be checked. The test substance should be prepared by mixing equal parts of A and B together.

2. It is also being amended to correct an error on page 3 of the protocol in the Contamination of Carriers section. The slides inoculated with *Enterobacter aerogenes* will be allowed to dry for 30-40 minutes at 35-37°C.

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

Carrier Population Control Calculation:

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

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. Certified copy of final study report.
2. Original signed protocol.
3. Any protocol amendments.
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 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), 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).

RESULTS

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

For Test Results, see Table 4.

ANALYSIS

Jymrsa (AA18-BG04 and FSCP05-AA11-24), a trigger spray product, mixed together with equal parts A & B, demonstrated no growth of *Pseudomonas aeruginosa* (ATCC 15442), *Enterobacter aerogenes* (ATCC 15038), *Proteus mirabilis* (ATCC 9240) or *Klebsiella pneumoniae* (ATCC 4352) in any of the 10 primary subcultures 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, Jymrsa (AA18-BG04 and FSCP05-AA11-24), a trigger spray product, demonstrated efficacy against *Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus mirabilis* and *Klebsiella pneumoniae* as required by the U.S. EPA for disinfectant label claims.

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			
	***Pseudomonas aeruginosa* (ATCC 15442)**	***Enterobacter aerogenes* (ATCC 15038)**	***Proteus mirabilis* (ATCC 9240)**	***Klebsiella pneumoniae* (ATCC 4352)**
Purity Control	Pure	Pure	Pure	Pure
Viability Control	Growth	Growth	Growth	Growth
Organic Soil Sterility Control	No Growth			
Neutralizing Subculture Medium Sterility Control	No Growth			
Carrier Sterility Control	No Growth			

TABLE 2: CARRIER POPULATION CONTROL RESULTS

Test Organism	Date Performed	Result
Pseudomonas aeruginosa (ATCC 15442)	03/15/05	1.7×10^6 CFU/carrier
Enterobacter aerogenes (ATCC 15038)		7.4×10^6 CFU/carrier
Proteus mirabilis (ATCC 9240)		5.4×10^4 CFU/carrier
Klebsiella pneumoniae (ATCC 4352)		1.53×10^6 CFU/carrier

CFU = Colony Forming Unit

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Date Performed	Inoculum (CFU/mL)	Number of Subcultures Tested	Number of Subcultures Positive
Jymrsa AA18-BG04	*Pseudomonas aeruginosa* (ATCC 15442)	03/15/05	4	1	1
	Enterobacter aerogenes (ATCC 15038)		14	1	1
	Proteus mirabilis (ATCC 9240)		4	1	1
	Klebsiella pneumoniae (ATCC 4352)		5	1	1
Jymrsa FSCP05-AA11-24	*Pseudomonas aeruginosa* (ATCC 15442)		4	1	1
	Enterobacter aerogenes (ATCC 15038)		14	1	1
	Proteus mirabilis (ATCC 9240)		4	1	1
	Klebsiella pneumoniae (ATCC 4352)		5	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 AA18-BG04	*Pseudomonas aeruginosa* (ATCC 15442)	03/15/05	Equal parts A & B mixed together	10	0
	Enterobacter aerogenes (ATCC 15038)			10	0
	Proteus mirabilis (ATCC 9240)			10	0
	Klebsiella pneumoniae (ATCC 4352)			10	0
Jymrsa FSCP05-AA11-24	*Pseudomonas aeruginosa* (ATCC 15442)			10	0
	Enterobacter aerogenes (ATCC 15038)			10	0
	Proteus mirabilis (ATCC 9240)			10	0
	Klebsiella pneumoniae (ATCC 4352)			10	0

* Number of carriers showing growth of the test organism.