FINAL STUDY REPORT

STUDY TITLE

AOAC Germicidal Spray Method

Test Organisms:

Staphylococcus aureus - MRSA (ATCC 33592)
Staphylococcus aureus - VISA (HIP 5836)
Staphylococcus epidermidis (ATCC 12228)
Enterococcus faecalis Vancomycin Resistant (ATCC 51299)

PRODUCT IDENTITY

Jymrsa MDF-200
Lot AA18-BG04 and Lot 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

WorldWide BioSolutions, Inc.
13570 Grove Drive #281
Maple Grove, MN 55311

PROJECT NUMBER

A02757

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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

Company: WorldWide BioSolutions, Inc.

Company Agent: Todd Leonard M.D.

President
Title

Signature

Date: 3/25/05

ATS LABS

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: 3/25/06

Sponsor: _____ Date: 3/25/06

Study Director: _____ Date: 3/24/05
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 16, 2005	March 15, 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: Brenda Sim Date: 3/24/05

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STUDY PERSONNEL

STUDY DIRECTOR: Amy Jeske, B.S.

Professional personnel involved:

Scott R. Steinagel, B.S.	- Microbiology Laboratory Supervisor
Adam W. Pitt, B.S.	- Research Assistant II
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: A02757

Protocol Number: WBS01012105.GS.1

Sponsor: WorldWide BioSolutions, Inc.
13570 Grove Drive #281
Maple Grove, MN 55311

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

TEST SUBSTANCE IDENTITY

Test Substance Name: Jymrsa MDF-200

Lot/Batch(s): Lot AA18-BG04 and Lot 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 MDF-200 (Lot AA18-BG04 and Lot FSCP05-AA11-24)
Dilution:	Equal parts A & B mixed together
Test Organisms:	*Staphylococcus aureus* - MRSA (ATCC 33592) *Staphylococcus aureus* - VISA (HIP 5836) *Staphylococcus epidermidis* (ATCC 12228) *Enterococcus faecalis* Vancomycin Resistant (ATCC 51299)
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 *Staphylococcus aureus* – MRSA, *Staphylococcus aureus* – VISA, *Staphylococcus epidermidis, and Enterococcus faecalis* Vancomycin Resistant as required by the U.S. EPA for disinfectant label claims.

STUDY MATERIALS

Test System/Growth Media

Test Organism	ID #	Growth Medium
Staphylococcus aureus - MRSA	ATCC 33592	Synthetic Broth
Staphylococcus aureus - VISA	HIP 5836	Trypticase Soy Agar + Vancomycin
Staphylococcus epidermidis	ATCC 12228	Synthetic Broth
Enterococcus faecalis Vancomycin Resistant	ATCC 51299	Fluid Thioglycollate

Staphylococcus aureus – MRSA, *Staphylococcus epidermidis*, and *Enterococcus faecalis* Vancomycin Resistant used in this study were obtained from the American Type Culture Collection (ATCC), Manassas, VA. The Vancomycin Intermediate Resistant *Staphylococcus aureus* (VISA) used in this study was obtained from the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia.

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)

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 *Staphylococcus aureus* – MRSA, *Staphylococcus epidermidis*, and *Enterococcus faecalis* Vancomycin Resistant, the appropriate growth medium was inoculated using a stock culture of the test organism. A minimum of four transfers was performed on consecutive days prior to use in testing procedures. A 48-54 hour broth culture incubated at 35-37°C was prepared.

For Vancomycin Intermediate Resistant *Staphylococcus aureus* (VISA), a stock culture was used to inoculate multiple agar plates. The plates were incubated for 2-5 days at 35-37°C. Following incubation, the agar plates were swabbed to suspend the organisms. The suspensions from all plates were collected using Butterfield's Buffer and matched a 0.5 McFarland turbidity standard.

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

An organic soil load was added to the test cultures per Sponsor's request. Antimicrobial susceptibility testing was performed for *Staphylococcus aureus* – MRSA, Vancomycin Intermediate Resistant *Staphylococcus aureus* (VISA), and *Enterococcus faecalis* Vancomycin Resistant utilizing a representative culture from the day of testing to verify the antimicrobial resistance pattern stated.

Staphylococcus aureus - MRSA (ATCC 33592) and *Enterococcus faecalis* Vancomycin Resistant (ATCC 51299) were purchased from the American Type Culture Collection (ATCC) by ATS Labs. ATS Labs verified that the organism was resistant by performing a Kirby Bauer Susceptibility assay under GLP conditions. The organisms were subcultured onto a BAP plate and were incubated for approximately 24 hours at 35-37°C. Following incubation, a suspension of each test organism was prepared equal to a 0.5 McFarland Standard in 0.85% sterile saline. The suspensions were streaked onto individual Mueller Hinton agar plates. An oxacillin disc was placed in the center of the *Staphylococcus aureus* - MRSA inoculated Mueller Hinton plate. A vancomycin disc was placed in the center of the *Enterococcus faecalis* Vancomycin Resistant inoculated Mueller Hinton plate. The plates were inverted and incubated for ≥ 24 hours at 35-37°C. Following incubation, the zone of inhibition was measured using a calibrated caliper. A control organism, *Staphylococcus aureus* (ATCC 25923), was run concurrently with each test organism to confirm the validity of the assay. The interpretation of the zone of inhibition is based on established NCCLS performance standards. See Tables 5 and 6 for results.

Vancomycin Intermediate Resistant *Staphylococcus aureus* (HIP 5836), was purchased from the CDC by ATS Labs. ATS Labs verified that the organism was intermediate resistant by performing an E test assay under GLP conditions. The same culture used for the test was used to make a suspension equal to a 0.5 McFarland Standard in 0.85% sterile saline. The suspension was streaked onto Mueller Hinton agar rotating the plate 60° in between each inoculation. A vancomycin strip was placed in the center of the inoculated Mueller Hinton plate. The plate was incubated for 24 ± 2 hours at 35-37°C. Following incubation, the minimum inhibitory concentration (MIC) was read where the edge of the inhibition ellipse intersects the side of the strip. A control organism, *Staphylococcus aureus* (ATCC 29213), was run concurrently with the test organism to confirm the validity of the assay. The interpretation of the MIC value is based on ATS Labs SOP CGT-4055, current revision. See Table 7 for results.

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 culture using a calibrated pipettor. The cultures used for *Staphylococcus aureus* – MRSA, *Staphylococcus epidermidis*, and *Enterococcus faecalis* Vancomycin Resistant were 48-54 hours old. The culture used for Vancomycin Intermediate Resistant *Staphylococcus aureus* was harvested from 5 day old agar plates. 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 25 minutes at 35-37°C with a 40% humidity and for the final 5 minutes of drying the humidity was adjusted to give a final humidity of 67%. (See Deviation)

Exposure Conditions
For the 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 8.8% 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 20.0 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 were incubated for 48±4 hours at 35-37°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 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:

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.

   It is also being amended to clarify the test substance name listed on page 8 of the protocol. The test substance should read Jymrsa MDF-200 Lot AA18-BG04 and Jymrsa MDF-200 Lot FSCP05-AA11-24.

2. This protocol is being amended to correct a typographical error on page 3 of the protocol. In the Contamination of Carriers section it states that a 48-54 hour culture will be used to inoculate the test carriers. This is incorrect. The glass slide carriers will each be inoculated with 0.01 mL of a 48-54 hour culture of *Staphylococcus aureus* – MRSA, *Staphylococcus epidermidis*, and *Enterococcus faecalis* Vancomycin Resistant, a suspension made from 2-5 day old agar plate growth will be used for Vancomycin Intermediate Resistant *Staphylococcus aureus*.

Protocol Deviations:

The protocol states that the slides will be dried for 30-40 minutes at 35-37°C with 60-70% humidity. This was inadvertently not followed for the entire drying period. The carriers were dried at 35-37°C with 40% humidity for the first 25 minutes of the drying period. The error was corrected for the last 5 minutes of drying and the final humidity was 67%. Because the carrier counts were not impacted by the deviation, and because the carrier counts were higher than the minimum requirements this deviation had no impact on the validity of the study.

DATA ANALYSIS

Calculations

Carrier Population Control Calculation:

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

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. 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.

For Confirmation of Antibiotic Resistance, See Tables 5 - 7.

ANALYSIS

Jymrsa MDF-200 (Lot AA18-BG04 and Lot FSCP05-AA11-24), a trigger spray product, mixed together with equal parts A & B, demonstrated no growth of *Staphylococcus aureus* - MRSA (ATCC 33592), *Staphylococcus aureus* - VISA (HIP 5836), *Staphylococcus epidermidis* (ATCC 12228), or *Enterococcus faecalis* Vancomycin Resistant (ATCC 51299) in any of the 10 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 MDF-200 (Lot AA18-BG04 and Lot FSCP05-AA11-24), a trigger spray product, demonstrated efficacy against *Staphylococcus aureus* - MRSA, *Staphylococcus aureus* - VISA, *Staphylococcus epidermidis*, and *Enterococcus faecalis* Vancomycin Resistant 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.

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TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control	Results			
	***Staphylococcus aureus* - MRSA (ATCC 33592)**	***Staphylococcus aureus* - VISA (HIP 5836)**	***Staphylococcus epidermidis* (ATCC 12228)**	***Enterococcus faecalis* Vancomycin Resistant (ATCC 51299)**
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
Staphylococcus aureus – MRSA (ATCC 33592)	03/15/05	1.20×10^6 CFU/carrier
Staphylococcus aureus – VISA (HIP 5836)		2.43×10^5 CFU/carrier
Staphylococcus epidermidis (ATCC 12228)		4.4×10^6 CFU/carrier
Enterococcus faecalis Vancomycin Resistant (ATCC 51299)		2.2×10^5 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, MDF-200 Lot AA18-BG04	*Staphylococcus aureus* - MRSA (ATCC 33592)	03/15/05	16	1	1
	Staphylococcus aureus - VISA (HIP 5836)		4	1	1
	Staphylococcus epidermidis (ATCC 12228)		40	1	1
	Enterococcus faecalis Vancomycin Resistant (ATCC 51299)		2	1	1
Jymrsa, MDF-200 Lot FSCP05-AA11-24	*Staphylococcus aureus* - MRSA (ATCC 33592)		16	1	1
	Staphylococcus aureus - VISA (HIP 5836)		4	1	1
	Staphylococcus epidermidis (ATCC 12228)		40	1	1
	Enterococcus faecalis Vancomycin Resistant (ATCC 51299)		2	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, MDF-200 Lot AA18-BG04	*Staphylococcus aureus* - MRSA (ATCC 33592)	03/15/05	Equal parts A & B mixed together	10	0
	Staphylococcus aureus - VISA (HIP 5836)			10	0
	Staphylococcus epidermidis (ATCC 12228)			10	0
	Enterococcus faecalis Vancomycin Resistant (ATCC 51299)			10	0
Jymrsa, MDF-200 Lot FSCP05-AA11-24	*Staphylococcus aureus* - MRSA (ATCC 33592)			10	0
	Staphylococcus aureus - VISA (HIP 5836)			10	0
	Proteu epidermidis (ATCC 12228)			10	0
	Enterococcus faecalis Vancomycin Resistant (ATCC 51299)			10	0

* Number of carriers showing growth of the test organism.

TABLE 5: ANTIBIOTIC RESISTANCE CONFIRMATION FOR VANCOMYCIN RESISTANT *ENTEROCOCCUS FAECALIS*

Organism (ATCC)	**Zone of Inhibition (mm)**	**NCCLS* Resistant Range (mm)**
Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51299)	6.14	≤ 14
Quality Control Organism	**Zone of Inhibition (mm)**	**NCCLS* Acceptable Range (mm)**
Staphylococcus aureus (ATCC 25923)	18.04	17-21

*NCCLS = National Committee for Clinical Laboratory Standards

TABLE 6: ANTIBIOTIC RESISTANCE CONFIRMATION FOR METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

Organism (ATCC)	**Zone of Inhibition (mm)**	**NCCLS* Resistant Range (mm)**
Methicillin Resistant *Staphylococcus aureus* (ATCC 33592)	6.25	≤ 10
Quality Control Organism	**Zone of Inhibition (mm)**	**NCCLS* Acceptable Range (mm)**
Staphylococcus aureus (ATCC 25923)	18.62	18 - 24

*NCCLS = National Committee for Clinical Laboratory Standards

TABLE 7: ANTIBIOTIC RESISTANCE CONFIRMATION FOR VANCOMYCIN INTERMEDIATE *STAPHYLOCOCCUS AUREUS*

Organism	**MIC Value (µg/mL)**	**Interpretation**
Vancomycin Resistant *Staphylococcus aureus* (HIP 5836)	8	Intermediate
Quality Control Organism	**MIC Value (µg/mL)**	**Interpretation**
Staphylococcus aureus (ATCC 29213)	1.5	Pass

MIC Interpretive Standards

Antimicrobial Agent	**Test Organism**	**Susceptible (µg/mL)**	**Intermediate (µg/mL)**	**Resistant (µg/mL)**	***S. aureus* 29213(QC) (µg/mL)**
Vancomycin	Aerobic bacteria	≤ 4	8-16	≥ 32	0.5-2