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FINAL REPORT

AOAC Germicidal Spray Test Additional Organism
Methicillin-Resistant *Staphylococcus aureus*

Test Substance
Total-Shield Plus

Lot Numbers
H21500823
H20300823

Test Organism
Methicillin-Resistant *Staphylococcus aureus*, ATCC 33591

Test Guidelines
EPA (2018) Guidelines 810.2000 and 810.2200 (E)

Author
Bailey Xie

Study Completion Date
09/28/20

Performing Laboratory
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Laboratory Project Identification Number
1056-102

Protocol Identification Number
MCG.2.07.26.20

Sponsor
McGowan Industries, Inc.
12285 World Trade Dr. STE O
San Diego, CA 92128

STATEMENT OF NO DATA CONFIDENTIALITY

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec.10(d)(1)(A), (B) or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Submitter signature: Thomas M McGowan Date: 10-7-20

Printed Name of Signer: _____

Printed Name of Company: _____

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The following is a detailed description of all differences between the practices used in the study and those required by 40 CFR part § 160:

- Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study.

Study Director Signature: _____ Date: 09/28/20
Typed Name: Bailey Xie
Typed Name of Laboratory: Microbac Laboratories, Inc.

Sponsor Signature: _____ Date: _____
Printed Name: _____
Printed Name of Company: _____

Submitter Signature: _____ Date: 10-2-20
Printed Name: _____
Printed Name of Company: _____

QUALITY ASSURANCE UNIT STATEMENT

The Quality Assurance Unit of Microbac has inspected Project Number 1056-102 to be in compliance with current Good Laboratory Practice regulations, (40 CFR § 160).

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below.

Phase Inspected	Date of Inspection	Date Reported to Study Director	Date Reported to Management
Protocol	09/02/20 09/14/20	09/14/20	09/14/20
In Process (Test)	09/14/20	09/14/20	09/14/20
Draft Final Report	09/24/20	09/24/20	09/24/20
Final Report	09/28/20	09/28/20	09/28/20

Jeanne M. Anderegg RQAP-GLP
Quality Assurance Manager

09-28-2020
Date

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TEST SUBSTANCE CHARACTERIZATION

Test Substance characterization as to the identity, strength, purity, solubility and composition, as applicable, according to 40 CFR, Part 160, Subpart F [160.105] was documented prior to its use in the study. The Characterization of Test Substance Summary of Results, provided by the sponsor, is found in Appendix II.

TEST SUMMARY

Study Title:	AOAC Germicidal Spray Test Additional Organism Methicillin-Resistant *Staphylococcus aureus*
Project No.:	1056-102
Protocol No.:	MCG.2.07.26.20
Test Method:	AOAC Official Method 961.02
Sponsor:	McGowan Industries, Inc. 12285 World Trade Dr. STE O San Diego, CA 92128
Testing Facility:	Microbac Laboratories, Inc. 105 Carpenter Drive Sterling, VA 20164
Study Objective:	This test is designed to prove germicidal effectiveness label claims for products registered with the Environmental Protection Agency and Canada (if applicable) as spray germicides. It evaluates the effectiveness of spray and pressurized spray products as spot disinfectants for contaminated surfaces.
Study Dates:	Study Initiation: 09/14/20 Experimental Start: 09/14/20 Experimental End: 09/17/20 Study Completion: See page 1

TEST SUMMARY (continued)

Test Substance: Total-Shield Plus

- Lot No.: H21500823, received at Microbac on 08/27/20 and assigned DS No. K1186
- Lot No.: H20300823, received at Microbac on 08/27/20 and assigned DS No. K1187
- Physical Description: Liquid
- Storage Condition: Dark, Ambient Room Temperature
- Active Ingredient: Phenol, Ethanol, and 2-Phenylphenol
- Dilution: Ready to use
- Diluent: Not applicable

Test Conditions:

Organic Soil Load:	Not applicable
Contact Time:	5 minutes
Contact Temperature:	20±1°C (actual: 21°C)
Contact Relative Humidity:	60% RH

Challenge Organism: Methicillin-Resistant *Staphylococcus aureus*, ATCC 33591

Incubation Time: 48±2 hours

Incubation Temperature: 36±1°C

Neutralizer: Letheen Broth containing 7% Polysorbate 80 and 1% Lecithin

Media and Reagents:
Synthetic Broth (SB)
Letheen Broth containing 7% Polysorbate 80 and 1% Lecithin
Phosphate Buffered Dilution Water (PBDW)
Letheen Broth (LB)
Tryptic Soy Agar (TSA)
Mueller Hinton Agar (MHA)
Oxacillin Antibiotic Disc
Gram Stain Reagents

Carriers: Glass microscope slides (1" x 3" with a 1" x 1" area for inoculation)

TEST SUMMARY (continued)

Study Design: This study was performed according to the signed protocol and project sheet(s) issued by the Study Director (see Appendix I).

Study Personnel:

Bailey Xie	Associate Scientist II/Study Director
Kadesha Jordan	Associate Scientist II
Nicholas A. Horvat	Associate Scientist II
Mary Manley	Associate Scientist I
Hilary Kurland	Scientist I

TEST PROCEDURES

Inoculum preparation:

A single frozen cryovial of stock culture was defrosted at room temperature and then briefly vortex mixed. A 10 µL aliquot of the thawed stock was added to a tube containing 10 mL of SB; the tubes were vortex mixed and incubated at 36±1°C for 24±2 hours. Daily transfers were made in for at least one but no more than five consecutive days.

For the final subculture transfer, tubes containing 10 mL of SB were inoculated with 10 µL of culture per tube and incubated at 36±1°C. After 48-54 hours, cultures were used for contaminating the carriers.

The cultures were agitated on a Vortex-type mixer for 3-4 seconds and then allowed to sit for 10 minutes. The upper portion of each culture was removed, leaving behind any debris or clumps and transferred to a sterile tube.

TEST PROCEDURES (continued)

Carrier preparation:

New carriers were visually screened and discarded if visibly damaged (scratched, chipped or nicked). The carriers were rinsed with 95% ethanol followed by a rinse with deionized water to remove oil and film on the slides. The carriers were sterilized by placing in evaporating dishes matted with two pieces of filter paper, heating them in a hot air oven for two hours at 180°C, cooling and storing them at room temperature until use.

Using a positive displacement pipet, a 0.01 mL (10 µL) aliquot of each culture was transferred onto a one-square inch area on the sterile carriers (in Petri dishes) and immediately spread uniformly over the entire area with a sterile glass rod. Each dish was promptly covered and the operation was repeated for the rest of the carriers. Carriers were dried for 30 minutes at 36°C and 41-44% Relative Humidity.

Test Substance:

The test substance was received ready to use from the sponsor. The test substance was allowed to equilibrate to room temperature for at least 10 minutes before testing. Prior to testing, the bottles were sprayed and the volume excreted was measured to ensure that all carriers were thoroughly wet.

Test:

Ten carriers per lot were sprayed in a horizontal position until thoroughly wet from a distance of 6-8". Each carrier was held in a horizontal position for the contact time. After the contact time, the excess liquid was allowed to drain from the carrier, the carrier was transferred to a tube containing 20 mL of neutralizer and shaken thoroughly. All subculture tubes containing the carriers were incubated for 48±2 hours at 36±1C.

Sterility controls:

One sterile carrier was added to a tube of 20 mL of neutralizer and incubated with the test in order to demonstrate the sterility of the media used in the study.

TEST PROCEDURES (continued)

Carrier counts control:

For each lot, triplicate replicates were processed before and immediately after processing the test.

Dried inoculated carriers were placed individually into tubes containing 20 mL of LB. The tubes were immediately vortex mixed for 120±5 seconds. After vortex mixing, serial ten-fold dilutions of each suspension were performed in 9 mL PBDW blanks. Duplicate one-mL aliquots from selected dilutions (10^{-2}, 10^{-3}, 10^{-4}) were plated in TSA pour plates. Diluting and plating were completed within 2 hours after vortex mixing. All plates were incubated with the test and the average CFU/carrier was determined.

Verification of antibiotic resistance:

An individual MHA plate was streaked with the prepared challenge organism in a crosshatch pattern and the appropriate antibiotic disc was added on the agar plate. The plate was incubated for 24±2 hours at 36±1C. Upon completion of incubation, the plate was observed and the zone of inhibition (the area immediately surrounding the antibiotic disc) was measured and documented. Using the Zone Diameter Interpretive Standards provided by the manufacturer of the antibiotic discs, the zone of inhibition will determine the organism to be Resistant, Intermediate, or Susceptible.

Neutralizer effectiveness control:

For each challenge microorganism, per lot of test substance, two sterile carriers were exposed to the test substance for the contact time evaluated, and then transferred into individual tubes of 20 mL of neutralizer. To each tube, fewer than 100 colony forming units (CFU) of the challenge microorganism were added and the count of the bacteria inoculated into these tubes was confirmed in duplicate TSA pour plates. The tubes and plates were incubated with the test.

TEST PROCEDURES (continued)

Viability control:

Two inoculated carriers were independently transferred into tubes of 20 mL of neutralizer and incubated with the test to serve as a comparison of the test cultures.

Challenge microorganism confirmation:

All of the Viability Control tubes and at least 20% of the test tubes showing growth were streaked on TSA and incubated for 24±2 hours at 36±1°C. Plates were examined for colony morphology characteristic of the test organism. Gram stains were performed from these streaks to confirm growth of the challenge microorganism.

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, from 09/14/20 to 09/17/20. The study director signed the protocol on 09/14/20. The study completion date is the date the study director signed the final report. The individual test dates are as follows:

- Testing started at 9:07 am on 09/14/20 and ended at 12:00 pm on 09/17/20.

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

PROTOCOL CHANGES

Protocol Amendments:

1. The study protocol inadvertently did not include the Verification of Antibiotic Resistance control using the challenge microorganism. The following will be performed:

   An individual MHA plate will be streaked with the prepared challenge organism in a crosshatch pattern and the appropriate antibiotic disc(s) will be added on the agar plate. The plate will be incubated for 24±2 hours at 36±1°C. Upon completion of incubation, the plate will be observed and the zone of inhibition (the area immediately surrounding the antibiotic disc) will be measured and documented. Using the Zone Diameter Interpretive Standards provided by the manufacturer of the antibiotic discs, the zone of inhibition will determine the organism to be Resistant, Intermediate, or Susceptible. The final determination, along with the specific disc(s) used, will be reported in the final report.
   Appropriate antibiotic disc(s): Oxacillin.

   Furthermore, in the Media and Reagents section, Mannitol Salt Agar (MSA) will be replaced with Mueller Hinton Agar (MHA). The appropriate antibiotic disc should be listed as Oxacillin

   This amendment serves to add this required control and will not have any negative impact on the study.

2. On Page 7 of the protocol, the Test Acceptance Criteria section contains the statement, "(for all three organisms)". This is a typographical error, and this statement should not be included, as there is only one microorganism being tested.

3. On Page 6 of the protocol, the Confirmation of challenge microorganism section should include the sentence: "Plates were examined for colony morphology characteristic of the test organism".

Protocol Deviations:

No protocol deviations occurred during this study.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test substance records, the final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

TEST ACCEPTANCE CRITERIA

The test was considered acceptable for evaluation of the test results if the criteria listed below are satisfied.

- The recovery broth with neutralizers was proven effective
- The sterility control was negative for growth
- The viability control was positive for growth
- The purity of the test culture was confirmed based on the procedures employed for confirmation
- The geometric mean of the Log_{10} density (LD) for the Carrier Counts should be between 1.0×10^4 and 1.0×10^5 CFU/carrier. Average results that do not meet this criterion will be interpreted based on the following:
    - Testing must be repeated if the counts are below the range and the performance standard is achieved; or, the counts are above the range and the performance standard is not achieved.
    - Testing does not need to be repeated if the counts are below the range and the performance standard is not achieved; or, the counts are above the range and the performance standard is achieved

CALCULATIONS

The $\log_{10}$ density (LD) for each carrier was determined based on the following:

- Dilutions yielding counts up to 300 CFU were used
- Plate counts of 0 were included in the calculations
- The CFU/mL was calculated as:

$$\text{CFU/mL} = \frac{(\text{avg. CFU for } 10^{-x}) + (\text{avg. CFU for } 10^{-y}) + (\text{avg. CFU for } 10^{-z})}{(10^{-x} + 10^{-y} + 10^{-z})}$$

where: 10^{-x}, 10^{-y} and 10^{-z} are the dilutions plated
CFU = Colony Forming Units

- CFU/carrier = CFU/mL x volume of neutralizer (20)
- The Average $\log_{10}$ Carrier Count Control =

$$\frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_3}{N}$$

where: X equals CFU/carrier set
N equals number of control carrier sets

RESULTS

Results are presented in Tables 1 – 6. The challenge microorganism was confirmed by Gram Stain and colony morphology using the Viability Control streaks to be consistent with Methicillin-Resistant *Staphylococcus aureus.* The Sterility Controls exhibited no growth. The Neutralizer Effectiveness Control exhibited growth.

Table 1
Test Results
Results Expressed as Number of Tubes Exhibiting Growth / Total Number of Tubes

Microorganism	Lot No.	Results
Methicillin-Resistant *Staphylococcus aureus*	H21500823	0/10
	H20300823	0/10

RESULTS (continued)

Table 2
Neutralizer Effectiveness

Results Expressed as Growth (+) or No Growth (0) and Colony-Forming Units (CFU)/mL

Microorganism	Lot No.	Tube Result		Average CFU/Tube
		Rep. 1	Rep. 2	
Methicillin-Resistant *Staphylococcus aureus*	H21500823	+	+	29
	H20300823	+	+	

Table 3
Viability Control

Results Expressed as Growth (+) or No Growth (0)

Microorganism	Replicate	Result
Methicillin-Resistant *Staphylococcus aureus*	1	+
	2	+

Table 4
Sterility Controls

Results Expressed as Growth (+) or No Growth (0)

Source	Replicate	Result
Neutralizer + Carrier	1	0

Table 5
Antibiotic Resistance

Microorganism	Antibiotic Disc	Zone of Inhibition Size (mm)	Resistance Determination
Methicillin-Resistant *Staphylococcus aureus*	Oxacillin	0mm	Resistant

RESULTS (continued)

Table 6
Carrier Counts
Methicillin-Resistant *Staphylococcus aureus*

Results Expressed as Average Colony Forming Units (CFU) / Carrier and Mean Log_{10} Density

Lot No.	Phase	Rep.	CFU/ Carrier	Mean Test CFU/Carrier	Log Density (Log_{10} CFU/Carrier)	Mean Test Log_{10} Density
H21500823	Pre-Test	1	1.10×10^6	1.1×10^6	6.04	6.0
		2	1.38×10^6		6.14	
		3	1.66×10^6		6.22	
	Post-Test	1	2.56×10^5		5.41	
		2	6.70×10^5		5.83	
		3	1.28×10^6		6.11	
H20300823	Pre-Test	1	1.42×10^6	1.4×10^6	6.15	6.1
		2	1.66×10^6		6.22	
		3	1.74×10^6		6.24	
	Post-Test	1	8.20×10^5		5.91	
		2	1.24×10^6		6.09	
		3	1.64×10^6		6.21	

PRODUCT EVALUATION CRITERIA

According to the US Environmental Protection Agency, the test substance passes the test if no visible growth is observed in any of the 10 replicates for each lot and all controls must meet the test acceptance criteria.

CONCLUSIONS

When tested as described, Total-Shield Plus, Lot Nos. H21500823 and H20300823 passed the AOAC Germicidal Spray Test Additional Organism when Methicillin-Resistant *Staphylococcus aureus* was exposed to the test substances for 5 minutes at 21°C and 60% RH.

All of the controls met the criteria established for a valid test. These conclusions are based on observed data.

REFERENCES

1. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides, Guidance for Efficacy Testing, February 2018.

2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces - Guidance for Efficacy Testing, 2019.

3. *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 961.02, Germicidal Spray Products as Disinfectants, Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417

4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces - Guidance for Efficacy Testing, 2019.

5. U.S. Environmental Protection Agency, Office of Pesticide Programs, Microbiology Laboratory, Environmental Science Center, Ft. Meade, MD, Standard Operating Procedure for Germicidal Spray Products as Disinfectants (GSPT): Testing of *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella enterica*. SOP Number: MB-06-09. Date Revised: 09-29-17.