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Volume _____

FINAL REPORT

AOAC Germicidal Spray Test Healthcare

Test Substance
PATH-AWAY Anit-Pathogenic Aerosol Solution

Lot Numbers
22020, 42020, 52020

Test Organism
Staphylococcus aureus, ATCC 6538
Pseudomonas aeruginosa, ATCC 15442
Salmonella enterica, ATCC 10708

Test Guidelines
EPA (2018) Guidelines 810.2000 and 810.2200 (D3)

Author
Travis R. Farley

Study Completion Date
08/04/20

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

Laboratory Project Identification Number
1029-101

Protocol Identification Number
GLOB.1.06.16.20

Sponsor
Global Infection Control Consultants LLC
23 Countryside Court
Bluffton, SC 29909

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: _____ Date: 04/08/2020

Printed Name of Signer: ARTHUR V. MARTIN

Printed Name of Company: GICC LLC

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: Travis R. Farley Date: 08/04/20
Typed Name: Travis R. Farley
Typed Name of Laboratory: Microbac Laboratories, Inc.

Sponsor Signature: Date: 08/04/2020
Printed Name: ARTHUR V. MARTIN
Printed Name of Company: GICC LLC

Submitter Signature: Date: 08/04/2020
Printed Name: ARTHUR V. MARTIN
Printed Name of Company: GICC LLC

AVM 8/4/2020

QUALITY ASSURANCE UNIT STATEMENT

The Quality Assurance Unit of Microbac has inspected Project Number 1029-101 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	07/06/20 07/08/20	07/08/20	07/08/20
In Process (Test)	07/08/20	07/08/20	07/08/20
Final Report	08/03/20	08/04/20	08/04/20

Jeanne M. Anderegg, RQAP-GLP
Quality Assurance Manager

08-04-2020
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 Healthcare
Project No.:	1029-101
Protocol No.:	GLOB.1.06.25.20
Test Method:	AOAC Official Method 961.02
Sponsor:	Global Infection Control Consultants LLC 23 Countryside Court Bluffton, SC 29909
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: 07/06/20 Experimental Start: 07/08/20 Experimental End: 07/11/20 Study Completion: See page 1

TEST SUMMARY (continued)

Test Substance: PATH-AWAY Anit-Pathogenic Aerosol Solution

- Lot No.: 22020, received at Microbac on 06/26/20 and assigned DS No. K889
- Lot No.: 42020, received at Microbac on 06/26/20 and assigned DS No. K890
- Lot No.: 52020, received at Microbac on 06/26/20 and assigned DS No. K891
- Physical Description: Liquid
- Storage Condition: Dark, Room Temperature
- Active Ingredient: Citrus Extract, Ascorbic Acid, and Glycerine USP
- Dilution: Ready to use
- Diluent: Not applicable

Test Conditions:

Organic Soil Load:	5% Heat-inactivated Fetal Bovine Serum
Contact Time:	5 minutes
Contact Temperature:	20±1°C (actual: 21°C)
Contact Relative Humidity:	66% RH

Challenge Organism: *Staphylococcus aureus*, ATCC 6538
Pseudomonas aeruginosa, ATCC 15442
Salmonella enterica, ATCC 10708

Incubation Time: 48±2 hours

Incubation Temperature: 36±1°C

Neutralizer: D/E Broth

TEST SUMMARY (continued)

Media and Reagents:	Synthetic Broth (SB) Nutrient Broth (NB) D/E Broth Phosphate Buffered Dilution Water (PBDW) Letheen Broth (LB) Heat-inactivated Fetal Bovine Serum Tryptic Soy Agar (TSA) Mannitol Salt Agar (MSA) MacConkey Agar (MCA) Xylose Lysine Deoxycholate Agar (XLDA) Gram Stain Reagents
Carriers:	Glass microscope slides (1" x 3" with a 1" x 1" area for inoculation)
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:

Angela L. Hollingsworth	Director of Microbiology
Kadesha Jordan	Associate Scientist I
Alexis Jackson	Associate Scientist I
Nicholas A. Horvat	Associate Scientist I
Bailey Xie	Associate Scientist I
Travis R. Farley	Scientist II/ Study Director
Hillary Kurland	Scientist I
Muhammad Bashir	Laboratory Manager Microbiology

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 for SA and SE (NB for PA); 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 (NB for PA) 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 pellicle formed in the PA culture was removed prior to carrier contamination by gently aspirating the pellicle away from the broth using vacuum removal. Care was taken to avoid harvesting the pellicle from the bottom of the tube. The culture was visible inspected for pellicle fragments. If pellicle fragments were present, the culture was not used for testing.

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. Heat-inactivated Fetal Bovine Serum was added to the culture to achieve an organic load of 5%.

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 40 minutes at 36°C and 46-48% Relative Humidity.

TEST PROCEDURES (continued)

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 47 minutes before testing. A 10 mL aliquot was taken from each lot and the temperature recorded in order to ensure that the Test Substances equilibrated to the room temperature. Prior to testing, the bottles were sprayed and the volume excreted was measured to ensure that all carriers were thoroughly wet.

Test:

Sixty 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.

Sterility controls:

One sterile carrier was added to a tube of 10 mL of neutralizer and incubated with the test in order to demonstrate the sterility of the media used in the study.

Carrier counts control:

For each challenge microorganism, per 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 for SA and SE (60±5 seconds for PA). 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.

TEST PROCEDURES (continued)

Neutralizer effectiveness control:

For each challenge microorganism, per lot of test substance, six 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.

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 the corresponding specialty media, 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 07/08/20 to 07/11/20. The study director signed the protocol on 07/06/20. The study completion date is the date the study director signed the final report. The individual test dates are as follows:

- Testing started at 9:47 am on 07/08/20 and ended at 12:00 pm on 07/11/20.

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

PROTOCOL CHANGES

Protocol Amendments:

No protocol deviations occurred during this study.

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 must be proven effective
- The sterility control must be negative for growth
- The viability control must be positive for growth
- The purity of the test culture must be confirmed based on the procedures employed for confirmation
    - The geometric mean of the Log_{10} density (LD) for the Carrier Counts must be between 1.0×10^5 and 3.2×10^6 CFU/carrier for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For *Salmonella enterica*, the range is between 1.0×10^4 and 3.2×10^5 CFU/carrier.
    - For average counts that are below the stipulated range, the test must be repeated if the performance standard is achieved.
    - For average counts that are below the stipulated range, the test does not need to be repeated if the performance standard is not achieved.
    - For average counts that are above the stipulated range, the test does not need to be repeated if the performance standard is achieved.
    - For average counts that are above the stipulated range, the test must be repeated if the performance standard is not 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 – 7. The challenge microorganism was confirmed by Gram Stain and colony morphology using the Viability Control streaks to be consistent with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella enterica*. 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
Staphylococcus aureus	22020	0/60
	42020	0/60
	52020	0/60
Pseudomonas aeruginosa	22020	0/60
	42020	0/60
	52020	0/60
Salmonella enterica	22020	0/60
	42020	0/60
	52020	0/60

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	Rep. 3	Rep. 4	Rep. 5	Rep. 6	
Staphylococcus aureus	22020	+	+	+	+	+	+	30
	42020	+	+	+	+	+	+	
	52020	+	+	+	+	+	+	
Pseudomonas aeruginosa	22020	+	+	+	+	+	+	34
	42020	+	+	+	+	+	+	
	52020	+	+	+	+	+	+	
Salmonella enterica	22020	+	+	+	+	+	+	18
	42020	+	+	+	+	+	+	
	52020	+	+	+	+	+	+	

Table 3
Viability Control

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

Microorganism	Replicate	Result
Staphylococcus aureus	1	+
	2	+
Pseudomonas aeruginosa	1	+
	2	+
Salmonella enterica	1	+
	2	+

RESULTS (continued)

Table 4
Sterility Controls

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

Source	Replicate	Result
Neutralizer + Carrier	1	0

Table 5
Carrier Counts
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
22020	Pre-Test	1	1.16×10^6	9.9×10^5	6.06	6.0
		2	8.10×10^5		5.91	
		3	1.38×10^6		6.14	
	Post-Test	1	9.00×10^5		5.95	
		2	8.70×10^5		5.94	
		3	8.30×10^5		5.92	
42020	Pre-Test	1	1.14×10^6	1.0×10^6	6.06	6.0
		2	1.44×10^6		6.16	
		3	9.50×10^5		5.98	
	Post-Test	1	8.20×10^5		5.91	
		2	9.90×10^5		6.00	
		3	8.90×10^5		5.95	
52020	Pre-Test	1	7.50×10^5	1.0×10^6	5.88	6.0
		2	1.10×10^6		6.04	
		3	1.41×10^6		6.15	
	Post-Test	1	7.40×10^5		5.87	
		2	1.17×10^6		6.07	
		3	8.00×10^5		5.90	

RESULTS (continued)

Table 6
Carrier Counts
Pseudomonas aeruginosa

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
22020	Pre-Test	1	3.16×10^5	3.2×10^5	5.50	5.5
		2	2.67×10^5		5.43	
		3	3.58×10^5		5.55	
	Post-Test	1	3.75×10^5		5.57	
		2	2.42×10^5		5.38	
		3	3.63×10^5		5.56	
42020	Pre-Test	1	2.53×10^5	2.9×10^5	5.40	5.5
		2	3.23×10^5		5.51	
		3	2.95×10^5		5.47	
	Post-Test	1	2.54×10^5		5.40	
		2	3.45×10^5		5.54	
		3	2.45×10^5		5.39	
52020	Pre-Test	1	7.40×10^5	4.5×10^5	5.87	5.6
		2	6.40×10^5		5.81	
		3	3.63×10^5		5.56	
	Post-Test	1	2.90×10^5		5.46	
		2	2.68×10^5		5.43	
		3	4.14×10^5		5.62	

RESULTS (continued)

Table 7
Carrier Counts
Salmonella enterica

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
22020	Pre-Test	1	5.15×10^5	3.4×10^5	5.71	5.5
		2	4.27×10^5		5.63	
		3	3.73×10^5		5.57	
	Post-Test	1	1.42×10^5		5.15	
		2	3.09×10^5		5.49	
		3	2.98×10^5		5.47	
42020	Pre-Test	1	1.98×10^5	1.9×10^5	5.30	5.3
		2	7.73×10^4		4.89	
		3	2.22×10^5		5.35	
	Post-Test	1	2.07×10^5		5.32	
		2	2.55×10^5		5.41	
		3	1.98×10^5		5.30	
52020	Pre-Test	1	3.14×10^4	1.6×10^5	4.50	5.0
		2	2.84×10^4		4.45	
		3	2.73×10^5		5.44	
	Post-Test	1	3.09×10^5		5.49	
		2	3.05×10^5		5.48	
		3	2.81×10^4		4.45	

PRODUCT EVALUATION CRITERIA

According to the US Environmental Protection Agency, the test substance passes the test if visible growth was observed in ≤ 1 replicate out of 60 for each lot and all controls must meet the test acceptance criteria.

CONCLUSIONS

When tested as described, PATH-AWAY Anit-Pathogenic Aerosol Solution, Lot Nos. 22020, 42020, and 52020 passed the AOAC Germicidal Spray Test Healthcare when *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella enterica* containing a 5% organic load, were exposed to the test substances for 2 minutes at 21°C and 55-60% 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.

APPENDIX I

MICROBAC®

Microbac Protocol

AOAC Germicidal Spray Test

Healthcare

Testing Facility
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
Global Infection Control Consultants LLC
23 Countryside Court
Bluffton, SC 29909

June 25, 2020

Microbac Protocol: GLOB.1.06.25.20

Microbac Project No.: 1029 - 101

AVM
6/30/2020

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 sprays and pressurized spray products as spot disinfectants for contaminated surfaces. The test is based on the Official Methods of Analysis (AOAC 961.02) (2012) and is required by EPA Product Performance Guidelines. This test meets the EPA OCSPP 810.2000 and 810.2200 Product Performance Test Guidelines (February 2018), the Series 810 2019 Frequently Asked Questions (FAQ) document (August 28, 2019), and Health Canada "Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs" as applicable.

TESTING CONDITIONS:

Sixty replicates, per lot, per microorganism will be evaluated using three lots of a single test substance. Glass carriers inoculated with *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella enterica* will be sprayed for the specified time and distance directed by the sponsor or label instructions and transferred into individual tubes containing neutralizing recovery broth.

MATERIALS:

A. Test, control and reference substances will be supplied by the sponsor of the study (see last page). As per CFR 40.160.105:

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance shall be determined for each lot and shall be documented by the sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented and retained by the sponsor.

- When relevant to the conduct of the study the solubility of each test, control, or reference substance shall be determined by the sponsor before the experimental start date. The stability of the test, control, or reference substance shall be determined by the sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis of each lot.

AUM

The test substance will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test substance such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures Microbac Laboratories, Inc. (Microbac) testing facility management that the test substance has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

Microbac will retain all unused test substances for a period of at least one year after completion of the test, and then discard them in a manner that meets the approval of the safety officer.

B. Materials supplied by Microbac, including, but not limited to:

1. Challenge microorganisms, required by the sponsor of the study:
   a. *Staphylococcus aureus*, ATCC 6538 (SA)
   b. *Pseudomonas aeruginosa*, ATCC 15442 (PA)
   c. *Salmonella enterica*, ATCC 10708 (SE)

2. Media and reagents:
   a. Synthetic Broth (SB)
   b. Nutrient Broth (NB)
   c. Neutralizer: Recovery broth with required neutralizer(s)
   d. Letheen Broth (LB)
   e. Heat-inactivated fetal bovine serum (if required)
   f. Phosphate Buffered Dilution Water (PBDW)
   g. Tryptic Soy Agar (TSA)
   h. Mannitol Salt Agar (MSA)
   i. MacConkey Agar (MCA)
   j. Xylose Lysine Deoxycholate Agar (XLDA)

3. Laboratory equipment and supplies, including glass microscope slides (1" x 3" with a 1" x 1" surface for contamination and treatment)

AVM

TEST SYSTEM IDENTIFICATION:

All test and control tube racks will be labeled with microorganism, test substance (if applicable) and project number prior to initiation of the study and during incubation. Petri dishes will be labeled with microorganism prior to initiation of the study and microorganism and project number during incubation. Test substance and usage will be traced according to SOPs extant in the laboratory.

EXPERIMENTAL DESIGN:

A. Inocula preparation:

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

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

The pellicle formed in the PA culture will be removed prior to carrier contamination by gently aspirating the pellicle away from the broth using a pipette or by vacuum removal. Care will be taken to avoid harvesting the pellicle from the bottom of the tube. The culture will be visibly inspected for pellicle fragments. If pellicle fragments are present, the culture will not be used for testing.

Each tube of inoculum will be agitated on a vortex mixer for 3-4 seconds, and then allowed to sit for 10 minutes. The upper portion of each culture will be removed, leaving any debris or clumps and transferred to a sterile flask and pooled.

If requested by the sponsor, heat-inactivated fetal bovine serum will be added to the culture to achieve an organic load of 5%.

B. Carrier preparation and inoculation:

The new carriers will be visually screened and discarded if visibly damaged (scratched, chipped or nicked). The carriers will be rinsed with 95% ethanol followed by a rinse with deionized water to remove oil and film on the slides. The carriers will be 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 will be 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 will be covered promptly, and the operation will be repeated for the rest of the carriers, for each microorganism. Carriers will be dried for 30-40 minutes at 36±1°C. The humidity level of the incubator during the drying phase required for the inoculated carriers will be monitored and reported.

C. Test substance preparation:

The test substance will be prepared and applied exactly as directed by the sponsor of the study. If mixing of components or dilution is required, the prepared test substance will be used within three hours for testing.

D. Test:

Note: The temperature and humidity level of the laboratory during the test phase will be monitored and reported.

Sixty carriers per organism will be sprayed in a horizontal position until thoroughly wet from 6"-8". Each carrier will be held in a horizontal position for the exposure time as specified by the sponsor. After the contact period, the excess liquid will be allowed to drain from the carrier and the carrier will be transferred to a tube containing 20 mL of Neutralizer and shaken thoroughly. For products with ≤ 1-minute contact time, the transfer will be made within ±3 seconds.

All subculture tubes containing the carriers will be incubated for 48±2 hours at 36±1°C. All observations will be recorded as growth or no growth.

E. Controls:

1. Sterility controls:

One sterile carrier will be added to a tube of Neutralizer and incubated with the test in order to demonstrate the sterility of the media used in the study.

2. Viability controls:

For each challenge microorganism, two inoculated, dried carriers will be independently transferred into tubes of Neutralizer and incubated with the test to serve as comparison for the test cultures.

3. Neutralizer effectiveness:

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

4. Carrier counts:

For each challenge microorganism, per lot, three inoculated, dried carriers will be randomly selected for carrier counts immediately (single set of three) before the testing and immediately (single set of three) after testing.

Inoculated, dried carriers will be placed individually into tubes containing 20 mL LB. The tubes will be immediately vortex mixed for 120±5 seconds for SA and SE (60±5 seconds for PA). After vortex mixing, serial ten-fold dilutions of each suspension will be performed in PBDW blanks. Duplicate one mL aliquots from selected dilutions will be plated in TSA pour plates. Diluting and plating will be completed within 2 hours after vortex mixing. All plates will be incubated with the test and the average CFU/carrier determined.

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5. Confirmation of challenge microorganism:

   All the viability controls and at least 20% of the test tubes showing growth will be streaked onto TSA plates (if three or less test tubes are positive within a group of 60 replicates, all positive tubes will be streaked onto TSA). The same tubes will also be streaked onto the corresponding specialty media: MSA (for SA), MCA (for PA), and XLDA (for SE). All plates will be incubated for 24±2 hours at 36±1°C. Gram stains will be performed from these streaks to confirm growth of the challenge microorganism.

PRODUCT EVALUATION CRITERIA:

According to the EPA, the test substance passes the test if visible growth is observed in ≤ 1 replicate out of 60 for each lot. All controls must meet the test acceptance criteria. There is no statistical method proposed for this protocol.

CALCULATIONS:

- The $\log_{10}$ density (LD) for each carrier will be determined based on the following:
  - Dilutions yielding counts to 300 CFU will be used.
  - Plate counts of 0 will be included in the calculations.
  - The CFU/mL (of broth) will be calculated:

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

Where 10^{-x}, 10^{-y}, 10^{-z} are the dilutions plated

- The CFU/carrier will be calculated by multiplying the CFU/mL by the volume of broth into which the challenge microorganism was harvested from the carrier by vortex mixing (20 mL).
- The LD for each carrier will be calculated by taking the Log_{10} of the density (per carrier).
- There is no statistical analysis for this test

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TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- Based on the AOAC method, the geometric mean of the carrier counts must be between 1.0 x 10^5 and 3.2 x 10^6 CFU/carrier for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For *Salmonella enterica*, the range is between 1.0 x 10^4 and 3.2 x 10^5 CFU/carrier. However, the geometric mean may fall outside this range and the test is still acceptable for evaluation if the geometric mean of the carrier counts are:
  - For average counts that are below the stipulated range, the test must be repeated if the performance standard is achieved.
  - For average counts that are below the stipulated range, the test does not need to be repeated if the performance standard is not achieved.
  - For average counts that are above the stipulated range, the test does not need to be repeated if the performance standard is achieved.
  - For average counts that are above the stipulated range, the test must be repeated if the performance standard is not achieved.
- The recovery broth with neutralizers must be proven effective
- The sterility controls must be negative for growth
- The viability control must be positive for growth.
- The purity of the challenge microorganism must be confirmed based on the procedures employed for confirmation.
- Regarding any presence of contamination in subculture media:
  - Contaminants are defined as microorganisms which are not the test organism that are present in the subculture media.
  - Methods such as Gram staining, colony morphology and biochemical assays may be used to identify the contaminant. The result will be reported to the EPA.
  - In the case where a contaminant and the test organism are both present in the subculture media, the outcome will be considered a positive carrier.
  - For a 60-carrier test: A test with one or more contaminated carrier(s) is invalid and may be repeated up to two times using another 60-carrier test.

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DATA PRESENTATION:

The final report will include the following information:

- The number of positive carriers.
- The average colony-forming units per carrier.
- The results of all controls.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned before initiation of the test. Resumes for technical personnel are maintained and are available on request. This study will be conducted at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164.

REGULATORY COMPLIANCE AND QUALITY ASSURANCE (GLP studies only):

This study will be performed in compliance with the US Environmental Protection Agency's Good Laboratory Practices (GLP) regulations, 40 CFR 160 (note: information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study unless otherwise stated).

The Quality Assurance Unit of Microbac will inspect the conduct of the study for GLP compliance. The dates of the inspections and the dates that findings are reported to the study management and study director will be included in the final report.

PROTOCOL AMENDMENTS AND DEVIATIONS:

Any protocol amendment(s) and protocol deviation(s) identified will be reported in project sheet(s) and included in the final report.

AUM

REPORT FORMAT:

The report will contain all items required by EPA 810.2200 and be compliant with EPA PR Notice 2011-3 (replaced PRN 86-5). Microbac employs a standard report format for each test design. Each final report will provide at least the following information:

- Sponsor identification
- Test substance identification
- Type of assay and project number
- Study start and end time (clock time)
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)

RECORDS TO BE MAINTAINED:

For all GLP studies, the original signed final report will be sent to the Sponsor.

A draft report will be provided to Sponsor for review prior to finalization of the report. All raw data, protocol, protocol modifications, test substance records, copy of final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test substance; challenge microorganism used, and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

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.

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MISCELLANEOUS INFORMATION: The following information is to be completed by sponsor before initiation of study:

A. Test substance information:

Test Substance Name	PATH-AWAY ANTI-PATHOGENIC AEROSOL SOLUTION		
Active ingredient(s)	SEE ATTACHED		
Lot No.	Lot No. 1	Lot No. 2	Lot No. 3
	22020	42020	52020
Dilution	☐ 1: _____ (1 part test substance + _____ parts diluent) ☑ Ready to Use ☐ Other: _____		
Diluent	☑ Not applicable (test substance is Ready to Use) ☐ _____ ppm ± 2.9% AOAC Hard Water ☐ Other: _____		
Concentration	☑ Lower Certified Limit (LCL) ☐ At or below nominal		

B. Test Conditions:

Contact time	5 MINUTES (must be ≤ 10 minutes
Contact temperature	Ambient Room Temperature (20±1C)
Test Substance Application	Spray until thoroughly wet from 6" – 8"

C. Organic load – serum (HI FBS) added to achieve 5% in the inoculum: ☑ yes ☐ no

D. Precautions/storage conditions: MSDS and/or C of A provided: ☑ yes ☐ no

STORE AT ROOM TEMP

Continued next page

REPORT HANDLING AND STUDY CONDUCT: ☑ EPA ☐ Health Canada, GLP

PROTOCOL APPROVAL:

Sponsor Signature: _____ Date: 6/30/2020

Sponsor Name: ARTHUR V. MARTIN Ph.D.

Study Director Signature: _____ Date: 7/6/20

Study Director Name (Print): Travis R. Farley

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Date Issued: 07/06/20 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No.1029-101

STUDY TITLE: AOAC Germicidal Spray Test Healthcare

STUDY DIRECTOR: Travis R. Farley
R. [illegible] 7/06/20
Signature Date

TEST SUBSTANCE(S):
PATH-AWAY Anti-Pathogenic Aerosol Solution

LOT NO.	DATE REC'D:	DS NO.
22020	06/26/20	K889
42020	06/26/20	K890
52020	06/26/20	K891

PERFORMING DEPARTMENT(S):
Applied Microbiology Laboratory

STORAGE CONDITIONS: Location: I5
■ Dark ■ Ambient Room Temperature
☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:

PROTECTIVE PRECAUTION REQUIRED: MSDS/SDS ■ Yes / ☐ No

PHYSICAL DESCRIPTION: ☐ Solid ■ Liquid ☐ Aerosol ☐ Other:

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 07/08/20 **TERMINATION DATE:** 07/11/20

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other:

SPONSOR: Global Infection Control Consultants LLC
23 Countryside Court Bluffton, SC 29909

CONTACT PERSON: Arthur V. Martin Ph. D.
Email: amartin@gicclic.com

TEST CONDITIONS:

Challenge organism(s):	*Staphylococcus aureus*, ATCC 6538 *Pseudomonas aeruginosa*, ATCC 15442 *Salmonella enterica*, ATCC 10708
Active ingredient(s):	See sponsor's attachment
Neutralizer(s):	D/E Broth
Contact Time(s):	5 minutes
Contact Temperature(s):	Ambient Room Temperature (20±1C)
Dilution(s):	Ready to Use
Organic Load:	■ Yes / ☐ No (Heat-inactivated Fetal Bovine Serum added to inoculum to achieve a 5% concentration)
Incubation Time(s):	48±2 hrs (test and controls), 24±2 hrs (streaks)
Incubation Temperature(s):	36±1C
Comments:	To apply test substance, spray until thoroughly wet using 4 pump sprays from a distance of 6"-8".

APPENDIX II

GLOBAL
Infection Control Consultants LLC

Certificate of Analysis

Product: Path-Away Anti-Pathogenic Aerosol Solution® 3% Solution Final Mix

Product Description: A proprietary non-metallic, organic antimicrobial and antifungal compound.
Complies with Federal and FDA Regulations 21 CFR 182.3013 and CFR 184.1540
USA EPA Registration Exempt as per FIFRA 25(b)

Chemical Description

Active Ingredients	Specifications	Result
Proprietary Citrus Extract CAS #92346-89-9	1.00 – 2.00%	1.15%
Ascorbic Acid CAS #50-81-7	1.25 - 1.75%	1.47%
Glycerine USP CAS #56-81-5	1.00 – 1.50%	1.11%
Inert Ingredients		
Citrus pulp CAS #68514-76-1	0.001 – 0.050%	0.010%
Dextrose CAS#492-62-6	0.05 – 0.25%	0.15%
Moisture CAS #7732-18-5	96.0 – 97.25%	96.11%

Physical Properties

Description	Specifications	Result
Appearance	Light to moderate golden viscous liquid	Light to moderate golden viscous liquid
Gardner Color –*Orbeco-Hellige Comparator*	3 – 9	N/A
Specific Gravity – *Optima OPD-E*	1.10 – 1.30	N/A
pH (d25°) – *Fisher Accumet AB150*	1.50 – 3.00	N/A
Flash Point (°F) - *Rapid Flash Closed-Cup Tester*	270 - 300	N/A
Infrared IR – *Spectrum Two Perkin/Elmer*	Reference Spectra	PASS

Batch Date: 2-10-2020 Batch # 22020

Shelf Life 5 – 7 years

Shipping Date: 6/25/2020
Shipped to: Microbac Laboratories

Name Arthur V. Martin Ph.D. President

Authorized Signature / Date 6/24/2020

Do NOT store in steel or metal containers. Store in plastic or glass containers only.

GLOBAL
Infection Control Consultants LLC

Certificate of Analysis

Product: Path-Away Anti-Pathogenic Aerosol Solution® 3% Solution Final Mix

Product Description: A proprietary non-metallic, organic antimicrobial and antifungal compound.
Complies with Federal and FDA Regulations 21 CFR 182.3013 and CFR 184.1540
USA EPA Registration Exempt as per FIFRA 25(b)

Chemical Description

Active Ingredients	Specifications	Result
Proprietary Citrus Extract CAS #92346-89-9	1.00 – 2.00%	1.15%
Ascorbic Acid CAS #50-81-7	1.25 - 1.75%	1.465%
Glycerine USP CAS #56-81-5	1.00 – 1.50%	1.02%
Inert Ingredients		
Citrus pulp CAS #68514-76-1	0.001 – 0.050%	0.015%
Dextrose CAS#492-62-6	0.05 – 0.25%	0.15%
Moisture CAS #7732-18-5	96.0 – 97.25%	96.20%

Physical Properties

Description	Specifications	Result
Appearance	Light to moderate golden viscous liquid	Light to moderate golden viscous liquid
Gardner Color –*Orbeco-Hellige Comparator*	3 – 9	N/A
Specific Gravity – *Optima OPD-E*	1.10 – 1.30	N/A
pH (d25°) – *Fisher Accumet AB150*	1.50 – 3.00	N/A
Flash Point (°F) - *Rapid Flash Closed-Cup Tester*	270 - 300	N/A
Infrared IR – *Spectrum Two Perkin/Elmer*	Reference Spectra	PASS

Batch Date: 4-15-2020 Batch # 42020

Shelf Life 5 – 7 years

Shipping Date: 6/25/2020
Shipped to: Microbac Laboratories

Name Arthur V. Martin Ph.D. President

Authorized Signature / Date 6/24/2020

Do NOT store in steel or metal containers. Store in plastic or glass containers only.

GLOBAL
Infection Control Consultants LLC

Certificate of Analysis

Product: Path-Away Anti-Pathogenic Aerosol Solution® 3% Solution Final Mix

Product Description: A proprietary non-metallic, organic antimicrobial and antifungal compound.
Complies with Federal and FDA Regulations 21 CFR 182.3013 and CFR 184.1540
USA EPA Registration Exempt as per FIFRA 25(b)

Chemical Description

Active Ingredients	Specifications	Result
Proprietary Citrus Extract CAS #92346-89-9	1.00 – 2.00%	1.30%
Ascorbic Acid CAS #50-81-7	1.25 - 1.75%	1.40%
Glycerine USP CAS #56-81-5	1.00 – 1.50%	1.20%
Inert Ingredients		
Citrus pulp CAS #68514-76-1	0.001 – 0.050%	0.005%
Dextrose CAS#492-62-6	0.05 – 0.25%	0.10%
Moisture CAS #7732-18-5	96.0 – 97.25%	95.995%

Physical Properties

Description	Specifications	Result
Appearance	Light to moderate golden viscous liquid	Light to moderate golden viscous liquid
Gardner Color –*Orbeco-Hellige Comparator*	3 – 9	N/A
Specific Gravity – *Optima OPD-E*	1.10 – 1.30	N/A
pH (d25°) – *Fisher Accumet AB150*	1.50 – 3.00	N/A
Flash Point (°F) - *Rapid Flash Closed-Cup Tester*	270 - 300	N/A
Infrared IR – *Spectrum Two Perkin/Elmer*	Reference Spectra	PASS

Batch Date: 5-19-2020 Batch # 52020

Shelf Life 5 – 7 years

Shipping Date: 6/25/2020
Shipped to: Microbac Laboratories

Name Arthur V. Martin Ph.D. President

Authorized Signature / Date 6/24/2020

Do NOT store in steel or metal containers. Store in plastic or glass containers only.