FINAL STUDY REPORT

STUDY TITLE

Evaluation of Disinfectant Efficacy against a Biofilm – Single Tube Method

Test Organism:

Staphylococcus aureus (ATCC 6538)

PRODUCT IDENTITY

D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840;
D7 Part 1, Batch 17-391 + D7 Part 2, Batch 17-394 + D7 Part 3, Batch 18840;
D7 Part 1, Batch 17-392 + D7 Part 2, Batch 17-395 + D7 Part 3, Batch 18840

PROTOCOL NUMBER

DSS01082117.BFLM.2

AUTHOR

Carrie K. Bauer, B.S.
Study Director

STUDY COMPLETION DATE

March 16, 2018

PERFORMING LABORATORY

Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Decon7 Systems, LLC
8541 E. Anderson Dr, #106
Scottsdale, AZ 85255

PROJECT NUMBER

A24276

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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).

Company: Decon7 Systems, LLC

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 with the following exception:

Characterization of the compounds was performed by the Sponsor prior to use in the study, however, not per 40 CFR Part 160.

Submitter:_____ Date:_____

Sponsor:_____ Date:_____

Study Director:_____ Date: 3/16/18
Carrie K. Bauer, B.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: Evaluation of Disinfectant Efficacy against a Biofilm – Single Tube 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 in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol 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. The findings of these inspections have been reported to Management and the Study Director.

Phase Inspected	Date of Phase Inspection	Date Reported to Study Director	Date Reported to Management
Critical Phase Audit: Exposure Conditions	November 2, 2017	November 2, 2017	November 2, 2017
Draft Report	February 20, 2018 February 21, 2018 February 28, 2018	February 28, 2018	March 16, 2018
Final Report	March 13, 2018	March 13, 2018	

Quality Assurance Specialist: _____ Date: 3-16-18

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STUDY PERSONNEL

STUDY DIRECTOR: Carrie K. Bauer, B.S.

Professional personnel involved:

Shanen Conway, B.S.	- Manager, Study Director Operations
Jon Rossbach, M.S.	- Manager, Microbiology Laboratory Operations
Kristie Restrepo, B.S.	- Microbiologist
John Egan, B.S.	- Lead Microbiologist
Kyle Kuras, B.S.	- Microbiologist
Adam Meyer, B.S.	- Microbiologist
Benjamin Rafferty, B.S.	- Microbiologist
Matthew Kranz, B.S.	- Associate Microbiologist
Melanie Gorham, A.S.	- Associate Microbiologist
Alicia Doberstein, B.S.	- Associate Microbiologist
Miranda Quist, B.S.	- Associate Microbiologist

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Evaluation of Disinfectant Efficacy against a Biofilm – Single Tube Method

Project Number: A24276

Protocol Number: DSS01082117.BFLM.2

Sponsor: Decon7 Systems, LLC
8541 E. Anderson Dr, #106
Scottsdale, AZ 85255

Test Facility: Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name /Batches: D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840;
D7 Part 1, Batch 17-391 + D7 Part 2, Batch 17-394 + D7 Part 3, Batch 18840;
D7 Part 1, Batch 17-392 + D7 Part 2, Batch 17-395 + D7 Part 3, Batch 18840

Test Substance Characterization
Test substance characterization as to identity, strength, purity and uniformity, as applicable, however, not according to 40 CFR, Part 160, Subpart F (160.105), was documented prior to its use in the study. The Test Substance Certificate of Analysis Reports may be found in Attachments III-V. Test substance stability testing performed according to 40 CFR, Part 160, Subpart F (160.105), was documented prior to its use in the study.

STUDY DATES

Date Sample Received: September 14, 2017
Study Initiation Date: October 27, 2017
Experimental Start Date: November 2, 2017 (Start time: 2:01 pm)
Experimental End Date: February 13, 2018 (End time: 2:30 pm)
Study Completion Date: March 16, 2018

OBJECTIVE

The objective of this study was to evaluate disinfectant efficacy against a biofilm prepared using the CDC Biofilm Reactor using the Single Tube Method based on U.S. EPA MLB SOPs MB-19 and MB-20.

SUMMARY OF RESULTS

Test Substance:	D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840; D7 Part 1, Batch 17-391 + D7 Part 2, Batch 17-394 + D7 Part 3, Batch 18840; D7 Part 1, Batch 17-392 + D7 Part 2, Batch 17-395 + D7 Part 3, Batch 18840
Dilution:	Ready to use after mixing Parts 1, 2 and 3, defined as Part 1 : 49 parts + Part 2 : 49 parts + Part 3 : 2 parts
Test Organism:	*Staphylococcus aureus* (ATCC 6538)
Exposure Time:	10 minutes
Exposure Temperature:	21±2°C (20.5-21.0°C)
Efficacy Result:	D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840, demonstrated a 99.99999% (7.56 $\log_{10}$) reduction of *Staphylococcus aureus* biofilm following a 10 minute exposure time at 21±2°C (20.5°C). D7 Part 1, Batch 17-391 + D7 Part 2, Batch 17-394 + D7 Part 3, Batch 18840, demonstrated a 99.99999% (7.58 $\log_{10}$) reduction of *Staphylococcus aureus* biofilm following a 10 minute exposure time at 21±2°C (20.5°C). D7 Part 1, Batch 17-392 + D7 Part 2, Batch 17-395 + D7 Part 3, Batch 18840, demonstrated a 99.9999% (6.97 $\log_{10}$) reduction of *Staphylococcus aureus* biofilm following a 10 minute exposure time at 21±2°C (21.0°C).

TEST HISTORY

Testing of D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840 was initially performed on 11/2/17 and resulted in the carrier population and neutralization confirmation controls not meeting the acceptance criteria. All data from testing performed on 11/2/17 were deemed invalid and are presented in Attachment I, see protocol deviation 2.

On 1/12/18, Testing of D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840 was repeated and resulted in valid data presented in the body of the report except for the test carrier results. A different neutralizer and volume of neutralizer (96 mL) were used in testing. Due to the dilutions and volumes filtered and plated from the test carriers, a 6-log reduction could not be determined (see protocol deviation 1). The test carrier data from testing performed on 1/12/18 were deemed invalid and are presented in Attachment II.

On 1/19/18, Testing of D7 Part 1, Batch 17-391 + D7 Part 2, Batch 17-394 + D7 Part 3, Batch 18840 was performed and resulted in valid data presented in the body of the report. On 1/26/18, Testing of D7 Part 1, Batch 17-392 + D7 Part 2, Batch 17-395 + D7 Part 3, Batch 18840 was performed and resulted in valid data presented in the body of the report. On 2/9/18, Testing of D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840 was repeated and resulted in valid data presented in the body of the report.

STUDY MATERIALS

Test System

Test Organism	Designation #
Staphylococcus aureus	6538

The test organism used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Recovery Media

Neutralizer: D/E Neutralizing Broth + 2.3% Lecithin + 5% Tween + 1.0% catalase

Agar Plate Medium: Tryptic Soy Agar with 5% Sheep Blood (BAP)

Carriers

Previously used coupon carriers made of glass (borosilicate) were used. Coupons were checked for scratching, chipping, other damage or accumulated debris under 20X magnification using a magnifying glass. Carriers with visible damage were not used in testing. The carriers were submerged in laboratory soap diluted 1:100 in water and sonicated for approximately 5 minutes. The carriers were rinsed in deionized water. The carriers were submerged in deionized water and sonicated for approximately 1 minute. The rinsing and sonication procedure was repeated using deionized water until no soap was left on the carriers. The cleaned carriers were stored in a closed container prior to use in testing.

TEST METHOD

To satisfy the requirement to evaluate each test substance lot/batch on separate test dates. D7 Part 1, Batch 17-391 + D7 Part 2, Batch 17-394 + D7 Part 3, Batch 18840, was tested on 1/19/18. D7 Part 1, Batch 17-392 + D7 Part 2, Batch 17-395 + D7 Part 3, Batch 18840 was tested on 1/26/18. D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840 was performed on 2/9/18. Testing was performed the same on each test date, unless otherwise noted.

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor, after mixing Parts 1, 2 and 3, defined as Part 1 : 49 parts + Part 2 : 49 parts + Part 3 : 2 parts, see table below of volumes used. The prepared test substance was homogenous as determined by visual observation and was used within three hours of preparation. The test substance was equilibrated in a water bath at 21±2°C (20.5-21.0°C) for ≥10 minutes prior to use.

Test Date	D7 Part 1 (mL)	D7 Part 2 (mL)	D7 Part 3 (mL)
1/12/18	Batch 17-390 (49.0 mL)	Batch 17-393 (49.0 mL)	Batch 18840 (2.00 mL)
1/19/18	Batch 17-391 (49.0 mL)	Batch 17-394 (49.0 mL)	Batch 18840 (2.00 mL)
1/26/18	Batch 17-392 (49.0 mL)	Batch 17-395 (49.0 mL)	Batch 18840 (2.00 mL)
2/9/18	Batch 17-390 (49.0 mL)	Batch 17-393 (49.0 mL)	Batch 18840 (2.00 mL)

Preparation of Test Organism

A 10 µL aliquot of a thawed, vortex mixed cryovial of stock organism broth culture was added to an initial 10 mL of Tryptic Soy Broth (TSB). The tube was vortex mixed and incubated at 35-37°C under aerobic conditions for 24±2 hours. Following incubation, the initial suspension population of the culture was determined. In addition, a culture purity control was performed on the culture. (Refer to the Initial Suspension Population Control and Purity Control, respectively.)

The same procedures were followed for preparation of the test organism culture to be used for the neutralization confirmation control with the following exception: an initial suspension population and purity controls were not performed on this culture.

Preparation of CDC Biofilm Reactor

The CDC reactor was assembled with the selected carriers, autoclave-sterilized and was allowed to cool to room temperature prior to use.

Batch Phase Operation of CDC Biofilm Reactor
An operating volume of sterile Tryptic Soy Broth diluted 1:10 in sterile deionized water (which targeted a final concentration of 3 g/L) was aseptically added into the sterile, prepared reactor. The operating volume was the volume of broth required to fill the reactor to the drain spout after the rods and baffle were in place. This volume was determined to be 325-335 mL for the reactors used in testing. The reactor was placed onto a stir plate leaving any openings and tubing covered and/or clamped. The rod alignment pins were secured into the top notches on the reactor.

A 1.0 mL aliquot of prepared test organism was transferred into the reactor through one of the available top tubes or ports. The stir plate set to approximately 55-65 RPM was initiated. The reactor was incubated with rotation at 35-37°C (36.0-36.2°C) for 24±2 hours.

Continuous Flow Operation of CDC Biofilm Reactor
Into a sterile carboy containing approximately 20 L of sterile deionized water, a 667 mL aliquot of full strength Tryptic Soy Broth was aseptically added to generate a 20 L solution containing 1 g/L of Tryptic Soy Broth. This solution continuously flowed into the reactor following batch phase operation. Based on the working volume of the CDC Biofilm Reactor, the flow rate was determined by dividing the working volume by a 30 minutes residence time. A ±0.2 mL/minute rate was included for operational purposes. See table on the next page for working volumes and determined flow rates per test date. The actual flow rate was verified by measuring the volume of flow from the nutrient tubing line after 1 minute of operation. The pump was adjusted, as necessary, to ensure the proper flow rate. The connector was decontaminated with alcohol after flow verification. The flow break was ensured to be clamped in an upright position and the nutrient tubing line was aseptically connected from the carboy to the reactor. Once the flow had been verified, the clamp from the waste tubing line was removed and a continuous flow of nutrients was pumped into the reactor. Operation of the reactor was continued with a continuous flow of nutrients for 24±2 hours at 35-37°C (35.7-36.1°C) and rotation (set to 60 RPM).

Test Date	Working/Operating Volume of CDC Biofilm Reactor	Determined Flow Rate (mL/minute)	Actual Flow Rate (mL/minute)
1/19/18	335 mL	11.2	11.0
	325 mL	10.8	11.0
1/26/18	335 mL	11.2	11.0
	325 mL	10.8	11.0
2/9/18	335 mL	11.2	11.1

Preparation of Inoculated Carriers for Use in Testing
After continuous flow operation, randomly selected rods containing the carriers inoculated with biofilm were aseptically removed from the reactor.

The carriers were rinsed to remove planktonic cells by holding the rod in a vertical position over a sterile 50 mL conical tube containing 30 mL of sterile buffered water. The rod was immersed once into the buffered water, moved back and forth with minimal to no splashing and then immediately removed. A new sterile 50 mL conical tube containing 30 mL of buffered water was used for each rod.

Randomly selected, contaminated carriers were transferred to individual, sterile 250 mL conical tubes (see planned protocol deviation 3). To do this, the rod containing the carrier was held centered over an empty conical tube. The set-screw was loosened and the carrier was allowed to drop directly to the bottom of the tube. If the coupon did not freely drop, the carrier was pressed directly in the center using a flame-sterilized Allen wrench. Care was taken to avoid touching the top or inner sides of the conical tube with a contaminated carrier. If a tube was touched, a new tube and carrier was used as a replacement. To ensure that the maximum biofilm surface was in contact with the solution, the carrier was oriented (or adjusted to position) at an angle in the bottom of the tube. Five test carriers and three control carriers were evaluated per batch.

Preparation of the Ultrasonic Cleaner (Sonicator)
The ultrasonic cleaner was degassed at 100% power for ≥5 minutes prior to use. A conical tube rack was suspended in the bath so that the fluid levels were even with the water level. To sonicate the subcultures, the sonicator was set to 45 kHz, 10% power and the "sweep" function turned off.

Exposure Conditions
For each test carrier, a 4.0 mL aliquot of test substance was transferred into the tubes containing the carriers at staggered intervals. The carrier was ensured to be completely covered with test substance. Each tube was tapped to release any air bubbles; the tubes were not shaken. Each carrier was exposed for 10 minutes at 21±2°C (20.5-21.0°C). A water bath was utilized to achieve the exposure temperature.

Test System Recovery
Following the Sponsor specified exposure time, a 96.0 mL aliquot of neutralizer was transferred to each tube and each tube was vigorously shaken several times to mix the contents thoroughly.

Each tube was vortex-mixed on the highest setting for 30±5 seconds. The tubes were placed into a test tube rack suspended in an ultrasonic cleaner so that the liquid level in the tubes was even with the level of water in the bath. The tubes were sonicated for 30±5 seconds. Following sonication, the tubes were vortex-mixed for 30±5 seconds for a second time. The tubes were sonicated for 30±5 seconds for a second time and vortex-mixed for 30±5 seconds for a third time. The tubes represented the 10^0 dilution.

For treated carriers, ten-fold serial dilutions of the neutralized contents were prepared. An aliquot of 25 mL from the 10^0 dilution and the entire contents of the 10^{-1} dilution (10 mL) were individually processed through a 0.45 µm polyethersulfone filter unit pre-wetted with 20 mL of sterile phosphate buffered dilution water (PBDW). The 10^{-1} dilution tube was rinsed with approximately 10 mL of PBDW and filtered. The sides of each filter funnel were also rinsed with additional PBDW and filtered. The filters were aseptically plated onto the recovery agar medium.

Incubation and Observation
The plates were incubated at 36±1°C for 48±4 hours. The subcultures were placed at 2-8°C for 2-3 days prior to examination. Following incubation and storage, the subcultures were enumerated.

On 1/30/18 (from test date: 1/26/18) and 2/13/18 (from test date 2/9/18), representative test and positive control subcultures showing growth were visually examined, Gram stained and biochemically assayed to confirm or rule out the presence of the test organism.

STUDY CONTROLS

Purity Control
This control was performed on the day the biofilm reactor was inoculated. A "streak plate for isolation" was performed on the organism culture. The subculture plate was incubated for 48±4 hours at 36±1°C. Following incubation, the plate was 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.

Initial Suspension Population Control
The prepared test organism suspension used to inoculate the CDC Biofilm reactor was serially diluted and plated using 0.1 mL aliquots and standard microbiological techniques. The subculture plate was incubated for 48±4 hours at 36±1°C. Following incubation, the organism plates were observed and enumerated. This control is performed for informational purposes and therefore has no acceptance criterion.

Carrier Population Control
Three inoculated control carriers were treated as in the test, replacing the test substance with buffered water. Following neutralization, the tubes were vortex mixed and sonicated as in the test procedure. The neutralized tube represented the 10^0 dilution. Ten-fold serial dilutions were prepared and 0.1 mL aliquots of the 10^{-3} through 10^{-7} dilutions were spread plated in duplicate. The plates were incubated and enumerated. The acceptance criteria for this control is a mean log density of 7.5-9.0 CFU/coupon.

Neutralizer Sterility Control
A 1.0 mL aliquot of the neutralizer was plated as in the test and incubated. The acceptance criterion for this study control is a lack of growth.

Carrier Sterility Control
Prior to testing, a representative, uninoculated carrier was added to fluid thioglycollate medium (FTM) or other appropriate media. The vessel was incubated. The acceptance criterion is a lack of growth following incubation.

Neutralization Confirmation Control
The following neutralization confirmation control was performed concurrent with testing.

The prepared test organism was serially diluted to target 20-200 CFU (filter plates) per 0.1 mL. Multiple organism dilutions were prepared. The 10^{-5} and 10^{-6} test organism dilutions were used in the control.

Test Culture Titer (TCT)
Utilizing staggered timed intervals, 0.1 mL of diluted test organism was added to 100 mL of PBDW (in triplicate) and vortex mixed. This portion of the control is used for calculation purposes. The mixture was held for 10 minutes±30 seconds at 21±2°C. Individual 0.45 µm PES membrane filters were used and 25 mL aliquots from each tube were filtered. The acceptance criterion for this study control is growth.

Neutralizer Toxicity Treatment (NTT)
Utilizing staggered timed intervals, 0.1 mL of diluted organism was added to 100 mL of sterile neutralizer (in triplicate) and vortex mixed. The mixture was held for 10 minutes± 30 seconds at 21±2°C. Individual 0.45 µm PES membrane filters were used and 25 mL aliquots from each tube were filtered. The acceptance criterion for this study control is the average CFU/plate of a given test organism dilution for the NTT is within 50% of the average CFU/plate of the same test organism dilution for the TCT. A count less than 50% indicates that the neutralizer is harmful to the test organism. Note: counts higher than the average TCT (e.g., 120% of the average TCT) are also deemed valid, see protocol amendment 1.

Neutralization Confirmation Control Treatment (NCT)
Utilizing staggered timed intervals, 4 mL of test substance was added to 96.0 mL of neutralizer (in triplicate) and mixed. Within 10 seconds, 0.1 mL of diluted test organism was added and vortex mixed. Each mixture was held for 10 minutes±30 seconds at room temperature. Individual 0.45 µm PES membrane filters were used and 25 mL aliquots from each tube were filtered. The acceptance criterion for this study control is the average CFU/plate of a given test organism dilution for the NCT is within 50% of the average CFU/plate of the same test organism dilution for the TCT. Note: counts higher than the average TCT (e.g., 120% of the average TCT) are also deemed valid, see protocol amendment 1.

The filters from each treatment were aseptically plated onto the recovery agar medium.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The efficacy performance requirements for label claims require that the test substance demonstrate a minimum of a 6 Log_{10} reduction in numbers of the test organism as compared to the carrier population control.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number. If the population control exceeds an average log_{10} value of 9.0 and the test substance does not meet the performance criteria, the Sponsor may invalidate the study and repeat testing.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number. If the population control fails to meet the minimum requirement or if the neutralization control acceptance criteria is not met and the study fails to meet the efficacy requirements, repeat testing is not required.

PROTOCOL CHANGES

Protocol Amendments:

Amendment 1
For clarification and to align with the current EPA SOPs MB-20 and MB-19, the protocol is amended as follows:

STUDY CONTROLS

Neutralization Confirmation Control

Neutralizer Toxicity Treatment (NTT)
The acceptance criterion for this study control is the average CFU/plate of a given test organism dilution for the NTT is within 50% of the average CFU/plate of the same test organism dilution for the TCT. A count less than 50% indicates that the neutralizer is harmful to the test organism. Note: counts higher than the average TCT (e.g., 120% of the average TCT) are also deemed valid.

Neutralization Confirmation Control Treatment (NCT)
The acceptance criterion for this study control is the average CFU/plate of a given test organism dilution for the NCT is within 50% of the average CFU/plate of the same test organism dilution for the TCT. Note: counts higher than the average TCT (e.g., 120% of the average TCT) are also deemed valid.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{(\text{average number of colonies/plate@dilution}) \times (\text{dilution factor})}{(\text{volume plated in mL})}$$

Number of Organisms Surviving per Control Carrier

$$\text{CFU/carrier} = \left(\frac{\left[\frac{[(\text{Mean CFU for } 10^{-w}) + (\text{Mean CFU for } 10^{-x})]}{[(10^{-w}) + (10^{-x})]} \right]}{Y} \right) \times Z$$

where 10^{-w} and 10^{-x} are the dilutions plated
"*Y*" accounts for volume plated (0.1 mL)
"*Z*" is the volume of liquid (neutralizer + disinfectant) in the tube with the carrier (e.g. 40 mL)

Only applicable dilutions with countable numbers will be used.

Number of Organisms Surviving per Treated (test) Carrier

$$\text{CFU/carrier} = \left(\frac{\text{CFU per filter for } 10^0 + \text{CFU per filter for } 10^{-1}}{(a \times 10^0) + (b \times 10^{-1})} \right) \times Z$$

where *a* and *b* are the volumes filtered (typically: $10 \times 10^0 + 10 \times 10^{-1} = 11$)
"Z" is the volume of liquid (neutralizer + disinfectant) in the tube with the carrier (e.g. 40 mL)

For cases where there is no recovery for the treated coupons (10^0 and 10^{-1}) and only a sample of the 10^0 tube is filtered, substitute 0.5 CFU at the 10^0 dilution and scale up accordingly. If different dilutions were filtered, substitute 0.5 CFU at the lowest dilution and scale up accordingly.

For example:

$$\left(\frac{(0.5 \text{ CFU per filter for } 10^0) + (0 \text{ CFU per filter for } 10^{-1})}{[(10 \times 10^0) + (10 \times 10^{-1})]} \right) \times 40 \text{ mL} = 2 \text{ CFU/carrier}$$

For cases where there is no recovery for the treated coupons and the entire contents of the 10^0 tube is filtered, the LR is greater than or equal to the mean control counts.

Geometric Mean of Number of Organisms Surviving on Test or Control Carriers

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

Where: X equals CFU/carrier
N equals number of carriers

Log Reduction (LR)

$$\text{Log}_{10} \text{ Reduction} = \text{Log}_{10}(a) - \text{Log}_{10}(b)$$

Where:
a = geometric mean of the number of organisms surviving on the inoculated control carriers.
b = geometric mean of the number of organisms surviving on the test carriers.

Percent Reduction (PR)

$$PR = \frac{(a - b)}{a} \times 100$$

Where:
a = geometric mean of the number of organisms surviving on the inoculated control carriers.
b = geometric mean of the number of organisms surviving on the test carriers.

Recovery Difference

Average CFU in TCT – Average CFU in NCT or NTT
Used for the neutralization confirmation control.

Statistical Methods
None used.

The protocol is also amended to correct the EPA SOPs that are referenced from the draft versions to the current revisions as follows:

U.S. Environmental Protection Agency Office of Pesticide Programs, MLB SOP MB-19: Growing a Biofilm using the CDC Biofilm Reactor, May 2017.

U.S. Environmental Protection Agency Office of Pesticide Programs, MLB SOP MB-20: Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilm, May 2017.

Amendment 2
Amendment 1 is being amended to correct a typographical error. The Effective Date for amendment 1 should be February 14, 2018 per the raw data.

Protocol Deviations:

Deviation 1
On test date, 1/12/18, only a total of 1.1 mL of the total volume of the neutralized solution were filtered for test samples (9 mL from the 10^-1 dilution and 10 mL from the 10^-2 dilution). Per the protocol, when more than 36 mL of neutralizer is used, a minimum of 25% of the neutralized solution shall be filtered. There is no impact to the overall study since the test results were invalidated and testing of the lot of test substance involved was repeated and resulted in valid data.

Deviation 2
For carriers prepared on 10/30/17; during cleaning, the carriers were sonicated for 30 second intervals initially and between subsequent rinses. The carriers should have been sonicated for 5 minutes initially followed by 1 minute sonication intervals between rinsing per the protocol. There is no impact to the overall study, testing performed on 11/2/17 that used the carriers resulted in invalid data due to the neutralization confirmation control not meeting the acceptance criteria and the test was repeated.

Deviation 3
A deviation to the protocol was planned to use 250 mL conical tubes for carrier exposure to the test substance and for the carrier population control and neutralization controls to account for the larger volume of neutralizer used in the study. There is no impact to the overall intent of the study, the protocol allows for larger volumes of neutralizer. The conical shape of the larger tube still allowed the carrier to be positioned at an angle in the bottom of the tube ensuring maximum contact between the biofilm surface and the test substance (or appropriate solution).

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{\text{(average number of colonies/plate@dilution) x (dilution factor)}}{\text{(volume plated in mL)}}$$

Number of Organisms Surviving per Control Carrier

$$\text{CFU/carrier} = \left(\frac{\left[\frac{[(\text{Mean CFU for } 10^{-w}) + (\text{Mean CFU for } 10^{-x})]}{[(10^{-w}) + (10^{-x})]} \right]}{Y} \right) x\ Z$$

where 10^{-w} and 10^{-x} are the dilutions plated
"*Y*" accounts for volume plated (0.1 mL)
"*Z*" is the volume of liquid (neutralizer + disinfectant) in the tube with the carrier (e.g. 40 mL)

Only applicable dilutions with countable numbers will be used.

Number of Organisms Surviving per Treated (test) Carrier

$$\text{CFU/carrier} = \left(\frac{\text{CFU per filter for } 10^{0} + \text{CFU per filter for } 10^{-1}}{(a \times 10^{0}) + (b \times 10^{-1})} \right) x\ Z$$

where *a* and *b* are the volumes filtered (typically: $10 \times 10^{0} + 10 \times 10^{-1} = 11$)
"*Z*" is the volume of liquid (neutralizer + disinfectant) in the tube with the carrier (40 mL)

For cases where there is no recovery for the treated coupons (10^{0} and 10^{-1}) and only a sample of the 10^{0} tube is filtered, substitute 0.5 CFU at the 10^{0} dilution and scale up accordingly. If different dilutions were filtered, substitute 0.5 CFU at the lowest dilution and scale up accordingly.

For example:

$$\left(\frac{(0.5 \text{ CFU per filter for } 10^{0}) + (0 \text{ CFU per filter for } 10^{-1})}{[(10 \times 10^{0}) + (10 \times 10^{-1})]} \right) x\ 40\ mL = 2\ CFU/carrier$$

For cases where there is no recovery for the treated coupons and the entire contents of the 10^{0} tube is filtered, the LR is greater than or equal to the mean control counts.

Geometric Mean of Number of Organisms Surviving on Test or Control Carriers

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

Where: X equals CFU/carrier
N equals number of carriers

Log Reduction (LR)

Log_{10} Reduction = Log_{10} (a) – Log_{10} (b)

Where:
a = geometric mean of the number of organisms surviving on the inoculated control carriers.
b = geometric mean of the number of organisms surviving on the test carriers.

Percent Reduction (PR)

$$PR = \frac{(a - b)}{a} \times 100$$

Where:
a = geometric mean of the number of organisms surviving on the inoculated control carriers.
b = geometric mean of the number of organisms surviving on the test carriers.

Recovery Difference

Average CFU in TCT – Average CFU in NCT or NTT
Used for the neutralization confirmation control.

Statistical Methods
None used.

STUDY RETENTION

Record Retention
All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121 for a minimum of five years following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. The original data includes, but is not limited to, the following:

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

Test Substance Retention
The test substance will be discarded following study completion. It is the responsibility of the Sponsor to retain a sample of the test substance.

REFERENCES

1. U.S. Environmental Protection Agency. Draft Guidance to Assess the Efficacy of Antimicrobial Pesticide Products Intended to Control Public Health Biofilms on Hard, Non-Porous Surfaces. September 27, 2016.

2. U.S. Environmental Protection Agency Office of Pesticide Programs, MLB SOP MB-19: Growing a Biofilm using the CDC Biofilm Reactor, May 2017.

3. U.S. Environmental Protection Agency Office of Pesticide Programs, MLB SOP MB-20: Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilm, May 2017.

4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.

RESULTS

For Control and Neutralization Results, see Tables 1-4.
All data measurements/controls including the culture purity, carrier sterility, neutralizer sterility, carrier population and neutralization confirmation controls were within acceptance criteria

For Test Results, see Tables 5-7.

ANALYSIS

D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840, ready to use after mixing 49 mL of Part 1 + 49 mL of Part 2 + 2 mL of Part 3, demonstrated a 99.99999% (7.56 $\log_{10}$) reduction of a *Staphylococcus aureus* (ATCC 6538) biofilm following a 10 minute exposure time when tested at 21±2°C (20.5°C).

D7 Part 1, Batch 17-391 + D7 Part 2, Batch 17-394 + D7 Part 3, Batch 18840, ready to use after mixing 49 mL of Part 1 + 49 mL of Part 2 + 2 mL of Part 3, demonstrated a 99.99999% (7.58 $\log_{10}$) reduction of a *Staphylococcus aureus* (ATCC 6538) biofilm following a 10 minute exposure time when tested at 21±2°C (20.5°C).

D7 Part 1, Batch 17-392 + D7 Part 2, Batch 17-395 + D7 Part 3, Batch 18840, ready to use after mixing 49 mL of Part 1 + 49 mL of Part 2 + 2 mL of Part 3, demonstrated a 99.9999% (6.97 $\log_{10}$) reduction of a *Staphylococcus aureus* (ATCC 6538) biofilm following a 10 minute exposure time when tested at 21±2°C (21.0°C).

CONCLUSION

Under the conditions of this investigation, D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840; D7 Part 1, Batch 17-391 + D7 Part 2, Batch 17-394 + D7 Part 3, Batch 18840 and D7 Part 1, Batch 17-392 + D7 Part 2, Batch 17-395 + D7 Part 3, Batch 18840, ready to use after mixing 49 mL of Part 1 + 49 mL of Part 2 + 2 mL of Part 3, demonstrated efficacy against a *Staphylococcus aureus* (ATCC 6538) biofilm as required by the U.S. EPA following a 10 minute exposure time when tested at 21±2°C (20.5-21.0°C).

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			
	Staphylococcus aureus (ATCC 6538)			
	Test Date: 1/12/18	Test Date: 1/19/18	Test Date: 1/26/18	Test Date: 2/9/18
Purity Control	Pure	Pure	Pure	Pure
Carrier Sterility	No Growth	No Growth	No Growth	No Growth
Neutralizer Sterility	No Growth	No Growth	No Growth	No Growth

TABLE 2: INITIAL SUSPENSION POPULATION CONTROL RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)					
Test Date	Volume Plated	Dilution Factor			CFU/mL
		10^{-5}	10^{-6}	10^{-7}	
1/12/18	0.1 mL	T, T	106, 114	16, 12	1.10×10^{9}
1/19/18	0.1 mL	T, T	90, 84	15, 11	8.7×10^{8}
1/26/18	0.1 mL	T, T	126, 118	15, 7	1.22×10^{9}
2/9/18	0.1 mL	T, T	93, 94	10, 17	9.4×10^{8}

CFU = Colony Forming Units
T = Too Numerous To Count (>300 colonies)

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)						
Test Date	**Substance/Identity**	**Dilution**	**Neutralization Confirmation**		**50% of TCT**	**Pass/Fail (≥50% of TCT)**
			Results (CFU/filter)	**Average CFU**		
1/12/18 [a]	Test Culture Titer (TCT)	10^{-5}	T, T, T	26	13	Not applicable
		10^{-6}	25, 27, 27			
	Neutralizer Toxicity Control (NTT)	10^{-5}	T, T, T	25		Pass
		10^{-6}	29, 16, 31			
	D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840	10^{-5}	114, 100, 41	19		Pass
		10^{-6}	22, 19, 17			
1/19/18 [a]	Test Culture Titer (TCT)	10^{-5}	T, T, T	30	15	Not applicable
		10^{-6}	32, 27, 31			
	Neutralizer Toxicity Control (NTT)	10^{-5}	T, T, T	37		Pass
		10^{-6}	44, 30, 38			
	D7 Part 1, Batch 17-391 + D7 Part 2, Batch 17-394 + D7 Part 3, Batch 18840	10^{-5}	T, T, T	31		Pass
		10^{-6}	31, 33, 30			
1/26/18 [a]	Test Culture Titer (TCT)	10^{-5}	T, T, T	58	29	Not applicable
		10^{-6}	64, 60, 51			
	Neutralizer Toxicity Control (NTT)	10^{-5}	T, T, T	73		Pass
		10^{-6}	86, 78, 54			
	D7 Part 1, Batch 17-392 + D7 Part 2, Batch 17-395 + D7 Part 3, Batch 18840	10^{-5}	T, T, T	39		Pass
		10^{-6}	30, 36, 52			

CFU = Colony Forming Units
T = Too Numerous To Count (>200 colonies)
[a] Calculations performed using results from the 10^{-6} dilution

TABLE 4: CARRIER POPULATION CONTROL RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)
Volume Plated: 0.1 mL

Test Date: 1/12/18

Carrier #	Dilution Factor 10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	CFU/ carrier	Log_{10}	Geometric Mean (Average Log_{10})
1	60, 76	11, 8	3, 0	1, 0	0, 0	7.3×10^7	7.86	3.72×10^7 (7.57)
2	31, 31	5, 3	0, 0	0, 0	0, 0	3.2×10^7	7.51	
3	25, 19	1, 1	1, 1	0, 0	0, 0	2.2×10^7	7.34	

Test Date: 1/19/18

Carrier #	Dilution Factor 10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	CFU/ carrier	Log_{10}	Geometric Mean (Average Log_{10})
1	76, 92	7, 14	2, 2	0, 0	0, 0	8.7×10^7	7.94	7.59×10^7 (7.88)
2	58, 58	9, 12	3, 0	0, 1	0, 0	6.5×10^7	7.81	
3	76, 70	10, 9	1, 0	0, 1	0, 0	7.7×10^7	7.89	

Test Date: 1/26/18

Carrier #	Dilution Factor 10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	CFU/ carrier	Log_{10}	Geometric Mean (Average Log_{10})
1	39, 30	5, 2	1, 0	0, 1	2, 0	3.8×10^7	7.58	3.72×10^7 (7.57)
2	68, 65	6, 10	2, 0	0, 0	1, 0	6.9×10^7	7.84	
3	19, 17	3, 2	0, 0	0, 0	0, 0	1.9×10^7	7.28	

Test Date: 2/9/18

Carrier #	Dilution Factor 10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	CFU/ carrier	Log_{10}	Geometric Mean (Average Log_{10})
1	93, 86	19, 42	8, 7	30, 31	2, 0	1.4×10^8	8.15	9.55×10^7 (7.98)
2	54, 55	15, 21	55, 56	1, 4	0, 0	1.2×10^8	8.08	
3	47, 39	7, 7	3, 2	1, 3	0, 0	5.0×10^7	7.70	

CFU = Colony Forming Units

TABLE 5: TEST RESULTS FOR
D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840

Test Organism: *Staphylococcus aureus* (ATCC 6538)					
Test Date: 2/9/18					
Dilution (Volume filtered)	**Carrier #1**	**Carrier #2**	**Carrier #3**	**Carrier #4**	**Carrier #5**
10^0 (25 mL)	0	0	0	0	0
10^{-1} (10 mL)	2	0	0	0	0
CFU/Carrier	8×10^0	2×10^0	2×10^0	2×10^0	2×10^0
Log_{10} CFU/Carrier	0.90	0.30	0.30	0.30	0.30
Average Log_{10}	0.42				
Geometric Mean (CFU/Carrier)	2.63×10^0				
Reduction	99.99999%		$7.56\ log_{10}$		

CFU = Colony Forming Units

TABLE 6: TEST RESULTS FOR
D7 Part 1, Batch 17-391 + D7 Part 2, Batch 17-394 + D7 Part 3, Batch 18840

Test Organism: *Staphylococcus aureus* (ATCC 6538)					
Test Date: 1/19/18					
Dilution (Volume filtered)	**Carrier #1**	**Carrier #2**	**Carrier #3**	**Carrier #4**	**Carrier #5**
10^0 (25 mL)	0	0	0	0	0
10^{-1} (10 mL)	0	0	0	0	0
CFU/Carrier	2×10^0	2×10^0	2×10^0	2×10^0	2×10^0
Log_{10} CFU/Carrier	0.30	0.30	0.30	0.30	0.30
Average Log_{10}	0.30				
Geometric Mean (CFU/Carrier)	2.00×10^0				
Reduction	99.99999%		$7.58\ log_{10}$		

CFU = Colony Forming Units

TABLE 7: TEST RESULTS FOR D7 Part 1, Batch 17-392 + D7 Part 2, Batch 17-395 + D7 Part 3, Batch 18840

Test Organism: *Staphylococcus aureus* (ATCC 6538)					
Test Date: 1/26/18					
Dilution (Volume filtered)	**Carrier #1**	**Carrier #2**	**Carrier #3**	**Carrier #4**	**Carrier #5**
10^0 (25 mL)	1	7	0	0	0
10^{-1} (10 mL)	0	1	0	0	0
CFU/Carrier	4×10^0	3×10^1	2×10^0	2×10^0	2×10^0
Log_{10} CFU/Carrier	0.60	1.48	0.30	0.30	0.30
Average Log_{10}	0.60				
Geometric Mean (CFU/Carrier)	3.98×10^0				
Reduction	99.9999%		$6.97\ log_{10}$		

CFU = Colony Forming Units

ATTACHMENT I: INVALID DATA – 11/2/17

NOTE: Due to the carrier population and neutralization confirmation controls not meeting the acceptance criteria, this assay was repeated.

Date Performed: 11/2/17

Test Substance: D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840

Dilution: Ready to use after mixing Parts 1, 2 and 3, defined as Part 1 : 49 parts + Part 2 : 49 parts + Part 3 : 2 parts

Test Organism: *Staphylococcus aureus* (ATCC 6538)

Neutralizer: TAT Broth + 0.6% Lecithin + 4% Tween 80 + 0.2% Sodium Thiosulfate + 0.1% catalase

Exposure Time: 10 minutes

CONTROL RESULTS

Type of Control	Results
	***Staphylococcus aureus* (ATCC 6538)**
Purity Control	Pure
Carrier Sterility	No Growth
Neutralizer Sterility	No Growth

INITIAL SUSPENSION POPULATION CONTROL RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)				
Volume Plated	**Dilution Factor**			**CFU/mL**
	10^{-5}	**10^{-6}**	**10^{-7}**	
0.1 mL	87, 78	10, 8	3, 0	8.3×10^{7}

CFU = Colony Forming Units

NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)					
Substance/Identity	**Dilution**	**Neutralization Confirmation**		**50% of TCT**	**Pass/Fail (≥50% of TCT)**
		Results (CFU/filter)	**Average CFU**		
Test Culture Titer (TCT)	10^{-5}	38, 31, 30	33	17	Not applicable
	10^{-6}	1, 26, 8			
Neutralizer Toxicity Control (NTT)	10^{-5}	24, 20, 30	25		Pass
	10^{-6}	4, 3, 5			
D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840	10^{-5}	0, 0, 0	0		Fail
	10^{-6}	0, 0, 0			

CFU = Colony Forming Units

CARRIER POPULATION CONTROL RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)								
Volume Plated: 0.1 mL								
Carrier #	**Dilution Factor**					**CFU/ carrier**	**Log_{10}**	**Geometric Mean (Average Log_{10})**
	10^{-3}	**10^{-4}**	**10^{-5}**	**10^{-6}**	**10^{-7}**			
1	22, 24	3, 4	0, 0	0, 0	0, 0	9.7×10^{6}	6.99	1.23×10^{7} (7.09)
2	29, 37	3, 2	0, 0	0, 0	0, 0	1.3×10^{7}	7.11	
3	39, 31	1, 8	0, 1	0, 0	0, 0	1.5×10^{7}	7.18	

CFU = Colony Forming Units

TEST RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)					
Dilution (Volume filtered)	**Carrier #1**	**Carrier #2**	**Carrier #3**	**Carrier #4**	**Carrier #5**
10^0 (10 mL)	0	0	0	0	0
10^{-1} (9 mL)	0	0	0	0	0
CFU/Carrier	ND	ND	ND	ND	ND
Log_{10} CFU/Carrier	ND	ND	ND	ND	ND
Average Log_{10}	ND				
Geometric Mean (CFU/Carrier)	ND				
Reduction	ND		ND		

CFU = Colony Forming Units
ND = Not determined

ATTACHMENT II: INVALID DATA – 1/12/18

NOTE: Due to a deviation from the protocol for the total amount of neutralized solution from test carriers filtered and plated, this assay was repeated.

Date Performed: 1/12/18

Test Substance: D7 Part 1, Batch 17-390 + D7 Part 2, Batch 17-393 + D7 Part 3, Batch 18840

Dilution: Ready to use after mixing Parts 1, 2 and 3, defined as Part 1 : 49 parts + Part 2 : 49 parts + Part 3 : 2 parts

Test Organism: *Staphylococcus aureus* (ATCC 6538)

Neutralizer: D/E Neutralizing Broth + 2.3% Lecithin + 5% Tween + 1.0% catalase

Exposure Time: 10 minutes

TEST RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)					
Dilution (Volume filtered)	**Carrier #1**	**Carrier #2**	**Carrier #3**	**Carrier #4**	**Carrier #5**
10^0 (9 mL)	0	0	0	0	0
10^{-1} (10 mL)	0	0	0	0	0
CFU/Carrier	5×10^1	5×10^1	5×10^1	5×10^1	5×10^1
Log_{10} CFU/Carrier	1.70	1.70	1.70	1.70	1.70
Average Log_{10}	1.70				
Geometric Mean (CFU/Carrier)	5.01×10^1				
Reduction	>99.999%		5.87		

CFU = Colony Forming Units

ATTACHMENT III: TEST SUBSTANCE CERTIFICATE OF ANALYSIS – D7 Part 1

Baum's Castorine Co., Inc.
Manufacturing Chemists Since 1879

Certificate of Analysis D7 Part 1 8/7/2017

The active [Alkyl DimethylbenzeyQuat.] concentration is assayed using method BCQCSP-2.11.
Expiration date to all product is 08/01/2018.

batch number	%wt. Alkyl Dimethylbenzyl Ammonium Chloride (Active)	LCL	UCL
17-390	3.04	3.04	3.36
17-391	3.06	3.04	3.36
17-392	3.06	3.04	3.36

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ATTACHMENT IV: TEST SUBSTANCE CERTIFICATE OF ANALYSIS – D7 Part 2

Baum's Castorine Co., Inc.
Manufacturing Chemists Since 1879

8/7/2017

Certificate of Analysis D 7 Part 2

D 7 Part 2 is assayed for %wt.H_2O_2 using method BCQCSP – 6.44. Expiration date to all product is 07/28/2018.

batch number	%wt. H_2O_2	LCL	UCL
17-393	7.528	7.51	8.3
17-394	7.469	7.51	8.3
17-395	7.501	7.51	8.3

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ATTACHMENT V: TEST SUBSTANCE CERTIFICATE OF ANALYSIS – D7 Part 3

Allan Chemical Corporation
235 Margaret King Avenue
Ringwood, New Jersey 07456
Telephone: 1(973) 962-4014
Fax: 1(973) 962-6820
E-Mail: allanchem@allanchem.com

CERTIFICATE OF ANALYSIS

Product: DIACETIN
Manufacture Date: 10/13/15
Suggested Re-test Date: 10/12/17
Batch No: 18840

Property	Units	Specification	Results
Appearance		Clear liquid, free from suspended matter.	Satisfactory
Colour	Hazen	10 max	3
Acidity (as acetic acid)	% w/w	0.05 max	0.023
Saponification Value	mg KOH/g	542 – 605	574.2
Water Content	% w/w	0.2 max	0.03
Specific Gravity @ 20/20°C		1.180 – 1.195	1.186

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AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	2
Effective Date:	February 28, 2018
Sponsor:	Decon7 Systems, LLC 8541 E. Anderson Dr, #106 Scottsdale, AZ 85255
Test Facility:	Accuratus Lab Services 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Evaluation of Disinfectant Efficacy against a Biofilm – Single Tube Method
Protocol Number:	DSS01082117.BFLM.2
Project Number:	A24276

Modifications to Protocol:

Amendment 1 is being amended to correct a typographical error. The Effective Date for amendment 1 should be February 14, 2018 per the raw data.

Changes to the protocol are acceptable as noted.

Study Director

2/28/18
Date

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INITIALS DATE 3/16/18

AMENDMENT TO GLP TEST PROTOCOL

ACCURATUS —LAB SERVICES— THE ANTIMICROBIAL AUTHORITY

Amendment No.: 1

Effective Date: February 15, 2018

Sponsor: Decon7 Systems, LLC
8541 E. Anderson Dr, #106
Scottsdale, AZ 85255

Test Facility: Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

Protocol Title: Evaluation of Disinfectant Efficacy against a Biofilm – Single Tube Method

Protocol Number: DSS01082117.BFLM.2

Project Number: A24276

Modifications to Protocol:

For clarification and to align with the current EPA SOPs MB-20 and MB-19, the protocol is amended as follows:

STUDY CONTROLS

Neutralization Confirmation Control

Neutralizer Toxicity Treatment (NTT)
The acceptance criterion for this study control is the average CFU/plate of a given test organism dilution for the NTT is within 50% of the average CFU/plate of the same test organism dilution for the TCT. A count less than 50% indicates that the neutralizer is harmful to the test organism. Note: counts higher than the average TCT (e.g., 120% of the average TCT) are also deemed valid.

Neutralization Confirmation Control Treatment (NCT)
The acceptance criterion for this study control is the average CFU/plate of a given test organism dilution for the NCT is within 50% of the average CFU/plate of the same test organism dilution for the TCT. Note: counts higher than the average TCT (e.g., 120% of the average TCT) are also deemed valid.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{\text{(average number of colonies/plate@dilution) x (dilution factor)}}{\text{(volume plated in mL)}}$$

Number of Organisms Surviving per Control Carrier

$$\text{CFU/carrier} = \left(\frac{\left[\frac{[(\text{Mean CFU for } 10^{-w}) + (\text{Mean CFU for } 10^{-x})]}{[(10^{-w}) + (10^{-x})]} \right]}{Y} \right) \times Z$$

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where 10^{-w} and 10^{-x} are the dilutions plated
"Y" accounts for volume plated (0.1 mL)
"Z" is the volume of liquid (neutralizer + disinfectant) in the tube with the carrier (40 mL)

Only applicable dilutions with countable numbers will be used.

Number of Organisms Surviving per Treated (test) Carrier

$$\text{CFU/carrier} = \left[\frac{\text{CFU per filter for } 10^{0} + \text{CFU per filter for } 10^{-1}}{(a \times 10^{0}) + (b \times 10^{-1})} \right] \times Z$$

where *a* and *b* are the volumes filtered (typically: $10 \times 10^{0} + 10 \times 10^{-1} = 11$)
"Z" is the volume of liquid (neutralizer + disinfectant) in the tube with the carrier (e.g. 40 mL)

For cases where there is no recovery for the treated coupons (10^{0} and 10^{-1}) and only a sample of the 10^{0} tube is filtered, substitute 0.5 CFU at the 10^{0} dilution and scale up accordingly. If different dilutions were filtered, substitute 0.5 CFU at the lowest dilution and scale up accordingly.

For example:

$$\left[\frac{(0.5 \text{ CFU per filter for } 10^{0}) + (0 \text{ CFU per filter for } 10^{-1})}{[(10 \times 10^{0}) + (10 \times 10^{-1})]} \right] \times 40 \text{ mL} = 2 \text{ CFU/carrier}$$

For cases where there is no recovery for the treated coupons and the entire contents of the 10^{0} tube is filtered, the LR is greater than or equal to the mean control counts.

Geometric Mean of Number of Organisms Surviving on Test or Control Carriers

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

Where: X equals CFU/carrier
N equals number of carriers

Log Reduction (LR)

$$\text{Log}_{10} \text{ Reduction} = \text{Log}_{10}(a) - \text{Log}_{10}(b)$$

Where:
a = geometric mean of the number of organisms surviving on the inoculated control carriers.
b = geometric mean of the number of organisms surviving on the test carriers.

Percent Reduction (PR)

$$PR = \frac{(a - b)}{a} \times 100$$

Where:
a = geometric mean of the number of organisms surviving on the inoculated control carriers.
b = geometric mean of the number of organisms surviving on the test carriers.

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AMENDMENT TO GLP TEST PROTOCOL

ACCURATUS —LAB SERVICES— THE ANTIMICROBIAL AUTHORITY

Recovery Difference

Average CFU in TCT – Average CFU in NCT or NTT
Used for the neutralization confirmation control.

Statistical Methods
None used.

The protocol is also amended to correct the EPA SOPs that are referenced from the draft versions to the current revisions as follows:

U.S. Environmental Protection Agency Office of Pesticide Programs, MLB SOP MB-19: Growing a Biofilm using the CDC Biofilm Reactor, May 2017.

U.S. Environmental Protection Agency Office of Pesticide Programs, MLB SOP MB-20: Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilm, May 2017.

Changes to the protocol are acceptable as noted.

Study Director

2/20/18
Date

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(For Laboratory Use Only)
Accuratus Lab Services Project # A24276
Test Substance Tracking # [illegible] DSS01
9-15-17

10.30.17

ACCURATUS
—LAB SERVICES—

PROTOCOL

Evaluation of Disinfectant Efficacy against a Biofilm – Single Tube Method

Test Organism:

Staphylococcus aureus (ATCC 6538)

PROTOCOL NUMBER

DSS01082117.BFLM.2

PREPARED FOR

Decon7 Systems, LLC
8541 E. Anderson Dr, #106
Scottsdale, AZ 85255

PERFORMING LABORATORY

Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

DATE

August 21, 2017

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Evaluation of Disinfectant Efficacy against a Biofilm – Single Tube Method

PURPOSE
The purpose of this study is to evaluate disinfectant efficacy against a biofilm prepared using the CDC Biofilm Reactor using the Single Tube Method based on U.S. EPA Draft SOP MB-19 and MB-20.

TEST SUBSTANCE CHARACTERIZATION
According to (40 CFR, Part 160, Subpart F [160.105]) test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Accuratus Lab Services. Accuratus Lab Services will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once Accuratus Lab Services receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is September 8, 2017. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of October 13, 2017. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Accuratus Lab Services.

If a test must be repeated, or a portion of it, due to failure by Accuratus Lab Services to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of Accuratus Lab Services nor any of its employees are to be used in advertising or other promotion without written consent from Accuratus Lab Services.

The Sponsor is responsible for any rejection of the final report by the regulatory agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Accuratus Lab Services final report and notify Accuratus Lab Services of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Accuratus Lab Services will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM
In this method, the test system is represented by a biofilm grown with high shear and under continuous flow conditions using the CDC Biofilm reactor following the methods described in U.S. EPA Draft SOP MB-19. The efficacy of the disinfectant is evaluated using the single tube method described in U.S. EPA Draft SOP MB-20.

TEST PRINCIPLE
A biofilm grown on the surface of Sponsor selected carriers is exposed to the disinfectant in a closed system. After exposure, the carriers are neutralized and assayed for survivors. Appropriate culture purity, initial suspension population, carrier population, sterility, and neutralization confirmation controls are performed. The Standard Operating Procedure CGT-0040 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Growth Medium
Staphylococcus aureus	6538	Tryptic Soy Broth

The test organism to be used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Recovery Agar Medium: Tryptic Soy Agar (TSA)

Growth Medium
Tryptic Soy Broth may be prepared full strength (30 g/L) and diluted according to the protocol or prepared pre-diluted to the target concentrations listed in the protocol.

Carriers
Coupon carriers made of polycarbonate, stainless steel, glass (borosilicate) or other Sponsor selected material will be used. Coupons may be used once and discarded or reused repeatedly with proper cleaning and sterilization between use. Coupons will be checked for scratching, chipping, other damage or accumulated debris under at least 20X magnification using a magnifying glass or stereoscope. Carriers with visible damage will not be used in testing. Submerge the carriers in laboratory soap diluted 1:100 in water and sonicate the carriers for 5 minutes. Rinse the carriers in deionized water. Submerge the carriers in deionized water and sonicate for approximately 1 minute. Repeat the rinsing and sonication procedure in deionized water, as necessary, until no soap is left on the carriers. The cleaned carriers may be stored in a Petri dish prior to use in testing.

Preparation of Test Organism
Transfer 10 µL of a thawed, vortex mixed cryovial of stock organism broth culture to an initial 10 mL of full strength Tryptic Soy Broth. Vortex mix and incubate the initial culture at 35-37°C under aerobic conditions for 24±2 hours. Following incubation, determine the initial suspension population of the culture. In addition, perform a culture purity control on the culture.

Preparation of CDC Biofilm Reactor
The CDC reactor will be assembled with selected carriers, autoclave-sterilized and allowed to cool to room temperature prior to use, per SOP EQM-0049.

Batch Phase Operation of CDC Biofilm Reactor
An operating volume of sterile Tryptic Soy Broth diluted 1:10 in sterile deionized water (which targets a final concentration of 3 g/L) will be aseptically added into the sterile, prepared reactor. The operating volume is the volume of broth required to fill the reactor to the drain spout after the rods and baffle are in place. This volume should be approximately 330 mL and may be adjusted based on the reactor itself. Place the reactor onto a stir plate leaving any openings and tubing covered and/or clamped. Ensure the rod alignment pins are secured into the reactor top notches.

Vortex the 10 mL tube of the prepared test organism and add 1.0 mL into the reactor through one of the available top tubes or ports. Initiate the stir plate set to approximately 55-65 RPM. Incubate at 35-37°C with rotation for 24±2 hours. The actual operating temperature and rotation will be documented and reported.

Continuous Flow Operation of CDC Biofilm Reactor
The continuous flow operation should be run at 35-37°C. Preheat medium to maintain 35-37°C. Into a sterile carboy containing approximately 20 L of sterile deionized water, aseptically add 667 mL of concentrated Tryptic Soy Broth (30 g/L), then fill to 20 L to make a solution containing 1 g/L of Tryptic Soy Broth. Once the Tryptic Soy Broth is added, shake the carboy. This solution will continuously flow into the reactor following batch phase operation. Based on a working volume of 330 mL, the calculated flow rate is determined to be 11.0 ± 0.2 mL/minute. If an alternate working volume is added to the reactor, the flow rate is determined by dividing the working volume by a 30±2 minute residence time. (A ±0.2 mL/minute rate is included for operational purposes.) The actual flow rate will be verified by measuring the volume of flow from the nutrient tubing line after 1 minute of operation. The pump will be adjusted, as necessary, to ensure the proper flow rate. The connector may be decontaminated with alcohol after flow verification. Ensure the flow break is clamped in an upright position and the nutrient tubing line has been aseptically connected from the carboy to the reactor. Once the flow rate has been verified, remove the clamp from the waste tubing line and pump a continuous flow of nutrients into the reactor. Continue operating the reactor with a continuous flow of nutrients for 24±2 hours at 35-37°C and rotation (approximately 55-65 RPM). The actual operating temperature and rotation will be documented and reported.

Preparation of Inoculated Carriers for Use in Testing
After continuous flow operation, aseptically remove randomly selected rods containing the carriers with biofilm from the reactor.

Rinse the carriers to remove planktonic cells by holding the rod in a vertical position over a sterile 50 mL conical tube containing 30 mL of Phosphate Buffered Dilution Water (PBDW). Immerse the rod once into the PBDW, move back and forth with minimal to no splashing and then immediately remove. A new sterile 50 mL conical tube containing 30 mL of PBDW is used for each rod.

Transfer randomly selected contaminated carriers to individual, sterile 50 mL conical tubes containing a splashguard (for coupons exposed to test substance). To do this, hold the rod containing the carrier centered over an empty conical tube. Loosen the set-screw and allow the carrier to drop directly to the bottom of the tube. If the coupon does not freely drop, press directly in the center of the carrier. An Allen wrench may be used and should be flame-sterilized (or sterilized by another appropriate method), prior to use. Care should be taken to avoid touching the top or inner sides of the conical tube with a contaminated carrier. If a tube is touched, a new tube and carrier should be used as a replacement. Gently remove the splashguards from each tube using sterile forceps after each carrier has been deposited into the sterile tube. To ensure that the maximum biofilm surface is in contact with the solution, the carrier should be at an angle in the bottom of the tube. Adjust if necessary. Typically sets of five test and three control carriers are evaluated.

Preparation of Test Substance
The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the diluted test substance(s) shall be used within three hours of preparation. Equilibrate the test substance in a water bath at the Sponsor specified exposure temperature for ≥10 minutes prior to use.

Preparation of the Ultrasonic Cleaner (Sonicator)
The ultrasonic cleaner will be degassed at 100% power for ≥5 minutes prior to use. Suspend a conical tube rack in the bath so that the fluid levels will be even with the water level. To sonicate the subcultures, set the sonicator to 45 kHz, 10% power and the "sweep" function turned off.

Exposure Conditions
For each test carrier, transfer 4.0 mL of test substance down the side of the conical tubes containing the carriers at staggered intervals. Ensure the carrier is completely covered with test substance. Immediately after the addition of the test substance, fully expose the biofilm and release air bubbles by gently swirling the tube 1-2 times. Expose each carrier for the exposure time at room temperature. The actual exposure conditions will be clearly documented.

① ww, should be 30g/L ~~~ 10/27/17

Test System Recovery
Following the Sponsor specified exposure time, transfer 36 mL of neutralizer to each tube and vigorously shake the tube several times to mix the contents thoroughly. Additional neutralizer volumes may be needed for some test substances (e.g., highly acidic products).

Vortex mix each tube on the highest setting for 30±5 seconds. Place the tubes into a test tube rack suspended in an ultrasonic cleaner so that the liquid level in the tubes is even with the level of water in the bath. Sonicate the tubes for 30±5 seconds. Following sonication, vortex mix the tubes for 30±5 seconds for a second time. Sonicate the tubes for 30±5 seconds for a second time and vortex mix the tubes for 30±5 seconds for a third time. The tubes represent the 10^0 dilution.

Prepare ten-fold serial dilutions, in PBDW, of the neutralized contents. Filtration should be utilized to recover treated carriers and spread plating should be utilized for control carriers.

For treated carriers, filter 10 mL from the 10^0 dilution and the entire contents of the 10^{-1} dilution tube (10 mL) through a 0.45 µm PES filter membrane. Filter a minimum of 25% of the total volume of neutralizer if larger volumes are used; multiple filters may be used.

For filtration, pre-wet the membrane with approximately 20 mL of PBDW and filter the appropriate volume. If the entire contents are filtered, rinse the tube with approximately 10 mL of PBDW and filter the rinsate. Before placing the filter membrane on TSA, rinse the sides of the filter funnel with additional PBDW. To prevent air bubbles from forming under the filter, gently roll the filter onto the surface of the agar.

Incubation and Observation
Plates and subcultures will be incubated for 48±4 hours at 35-37°C. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination. Subcultures will be enumerated following incubation or storage. Use counts between 30-300 CFU/plate and 20-200 CFU/filter wherever possible.

Representative test subcultures showing growth may be subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism.

STUDY CONTROLS

Purity Control
This control will be performed on the day the biofilm reactor is inoculated. A "streak plate for isolation" will be performed on the organism culture. The subculture plate will be incubated for 48±4 hours at 35-37°C. Following incubation, each plate will be 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.

Initial Suspension Population Control
The prepared test organism suspension used to inoculate the CDC Biofilm reactor will be serially diluted and plated using standard microbiological techniques. The subculture plates will be incubated for 48±4 hours at 35-37°C. Following incubation, the organism plates will be observed and enumerated. This control is performed for informational purposes and therefore has no acceptance criterion.

Carrier Population Control
Inoculated control carriers will be treated as in the test, replacing the test substance with PBDW. If multiple time points are followed in the test, this control will be performed for the longest time point, minimally. Following neutralization, the tubes will be vortex mixed and sonicated as in the test procedure. The neutralized tube represents the 10^0 dilution. Ten-fold serial dilutions will be prepared and duplicate 0.1 mL aliquots of the 10^{-5} through 10^{-7} dilutions will be spread plated. The plates will be incubated and enumerated. This control should result in a mean log density of ~~8.0-9.0~~ CFU/coupon.
7.5-9.0
Revised per method 2013 10/27/17

Neutralizer Sterility Control
Prior to or concurrent with testing, a 1.0 mL aliquot of neutralizer will be plated onto appropriate agar and incubated. The acceptance criterion is a lack of growth following incubation.

Carrier Sterility Control
Prior to testing, or concurrent with testing, a representative, uninoculated carrier will be added to the neutralizer. The vessel will be mixed and 1.0 mL will be plated onto appropriate agar and incubated. The acceptance criterion is a lack of growth following incubation.

Neutralization Confirmation Control
The following neutralization confirmation control will be performed prior to testing or concurrent with testing. To represent worst-case conditions, only the most concentrated test substance dilution and/or shortest exposure time needs to be utilized in this control when multiple test substance concentrations or multiple exposure times are being evaluated in the study.

(3) Serially dilute the prepared test organism to target 30-300 CFU per 0.1 mL. Multiple organism dilutions may be prepared. *(Typically the 10^{-2} and 10^{-3} dilutions will provide a culture in range depending on expected titer. Alternate or partial dilutions may be used where appropriate.)* If all the organism dilution(s) used in this control fail to provide adequate numbers which coincides in a failure to meet the acceptance criterion for this study control, the control may be repeated in its entirety.

Test Titer Control (TCT)
Utilizing staggered timed intervals, add 0.1 mL of diluted test organism to 40 mL of PBDW (in triplicate) and vortex mix. This portion of the control is used for calculation purposes. Hold the mixture for 10 minutes±30 seconds at room temperature. After the contact time prepare a 1:10 dilution in PBDW. Vortex mix and plate duplicate 0.1 mL aliquots on TSA agar within 30 minutes of making the dilutions. The acceptance criterion for this study control is growth. If the test culture titer fails to yield countable numbers or if the culture titer is too low resulting in failing results, the entire neutralization confirmation control may be repeated in its entirety, as necessary, to properly validate neutralization.

Neutralizer Toxicity Treatment (NTT)
Utilizing staggered timed intervals, add 0.1 mL of diluted organism to 40 mL of sterile neutralizer (in triplicate) and vortex mix. Hold the mixture for 10 minutes±30 seconds at room temperature. After the contact time prepare a 1:10 dilution in PBDW. Vortex mix and plate duplicate 0.1 mL aliquots on TSA agar within 30 minutes of making dilutions. The acceptance criterion for this study control is growth within 0.5 $\log_{10}$ of the test culture titer (TCT) for at least one of the aliquots plated.

Neutralization Confirmation Control Treatment (NCT)
Utilizing staggered timed intervals, add 4 mL of test substance to 36 mL of neutralizer and mix. (1) Within 10 seconds, add 1.0 mL of diluted test organism and vortex mix. (2) Hold the mixture for 10 minutes at room temperature. After the contact time prepare a 1:10 dilution in PBDW. Vortex mix and plate duplicate 0.1 mL aliquots on TSA agar within 30 minutes of making dilutions. The acceptance criterion for this study control is growth within 0.5 $\log_{10}$ of the test culture titer (TCT) for at least one of the aliquots plated.

Alternatively, filtration may be used for the Neutralization Confirmation Controls. Filter 10 mL from each of the NCT, NTT and TCT treatment tubes through an individual 0.45 µm PES membrane. For larger neutralizer volumes, filter a minimum of 20% of the total volume. Multiple filters may be used.

(1) Revised per method; perform in triplicate ZMS 10/27/17
(2) Revised per method; should be 0.1 mL ZMS 10/27/17
(3) CC, target 20-200 CFU / 0.1mL for filter plates, use dilutions 10^{-5} and 10^{-6} ZMS 10/27/17

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

Accuratus Lab Services maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including test organism strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria

The efficacy performance requirements for label claims require that the test substance demonstrate a minimum of a 6 Log_{10} reduction in numbers of the test organism as compared to the carrier population control.

Control Acceptance Criteria

The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number. If the population control exceeds an average log_{10} value of 9.5 and the test substance does not meet the performance criteria, the Sponsor may invalidate the study and repeat testing.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number. If the population control fails to meet the minimum requirement or if the neutralization control acceptance criteria is not met and the study fails to meet the efficacy requirements, repeat testing is not required.

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

TEST SUBSTANCE RETENTION

It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

Down, should be 9.0 per method ZZZ 10/27/17

RECORD RETENTION

Study Specific Documents
All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services for a minimum of five years for GLP studies or a minimum of six months for all other studies following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. These original data include, but are not limited to, the following:

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

Facility Specific Documents
The following records shall also be archived at Accuratus Lab Services. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. U.S. Environmental Protection Agency. Draft Guidance to Assess the Efficacy of Antimicrobial Pesticide Products Intended to Control Public Health Biofilms on Hard, Non-Porous Surfaces. September 27, 2016.

2. U.S. Environmental Protection Agency Office of Pesticide Programs SOP Number: Draft MB-19, Standard Operating Procedure for Growing a Pseudomonas aeruginosa Biofilm using the CDC Biofilm Reactor, August 6, 2013.

3. U.S. Environmental Protection Agency Office of Pesticide Programs SOP Number: Draft MB-20, Standard Operating Procedure for Single Tube Method for Measuring Disinfectant Efficacy Against Biofilm Growth in the CDC Biofilm Reactor, August 6, 2013.

4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.

DATA ANALYSIS

Calculations

$$\text{CFU/mL} = \frac{\text{(average number of colonies/plate@dilution) x (dilution factor)}}{\text{(volume plated in mL)}}$$

Number of Organisms Surviving per Carrier

$$\text{CFU/carrier} = \frac{[(\text{CFU for } 10^{-x}) + (\text{CFU for } 10^{-y}) + (\text{CFU for } 10^{-z})]}{[(10^{-x}) + (10^{-y}) + (10^{-z})]} \times 10 \times 40$$

"10" accounts for volume plated (0.1 mL)
"40" is the volume of neutralizer in the tube with the carrier (40 mL)
where 10^{-x},10^{-y}, and 10^{-z} are the dilutions plated. Only applicable dilutions with countable numbers will be used.

Geometric Mean of Number of Organisms Surviving on Test or Control Carriers

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

Where: X equals CFU/carrier
N equals number of carriers

Log Reduction

$$\text{Log}_{10}\text{ Reduction} = \text{Log}_{10}(a) - \text{Log}_{10}(b)$$

Where:
a = geometric mean of the number of organisms surviving on the inoculated control carriers.
b = geometric mean of the number of organisms surviving on the test carriers.

Recovery Log_{10} Difference

Log_{10} (Average CFU in TCT) – Log_{10} (Average CFU in NCT or NTT)
Used for the neutralization confirmation control

Statistical Methods: None used.

STUDY INFORMATION

(All blank sections are verified by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)

Test Substance (Name & Batch Numbers) exactly as it should appear on final report:

(1) Test Date #1: D7 Part 1 Batch 17-390; D7 Part 2 Batch 17-393; D7 Part 3 Batch 18840
(1) Test Date #2: D7 Part 1 Batch 17-391; D7 Part 2 Batch 17-394; D7 Part 3 Batch 18840
(1) Test Date #3: D7 Part 1 Batch 17-392; D7 Part 2 Batch 17-395; D7 Part 3 Batch 18840

(1) **Expiration Date:** 7/28/2018

Product Description:

☑ Quaternary ammonia ☐ Peracetic acid ☐ Iodophor ☑ Peroxide
☐ Sodium hypochlorite ☐ Other _____

Approximate Test Substance Active Concentration (upon submission to Accuratus Lab Services):

(Part 3: 2.7% H2O2 7.78%)

(This value is used for neutralization planning only. This value is not intended to represent characterization values.)

Neutralization/Subculture Broth:

☐ _____
☑ Accuratus Lab Services' Discretion. By checking, the Sponsor authorizes Accuratus Lab Services, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions

☑ Room Temperature
☐ 2-8°C
☐ Other _____

Hazards

☐ None known: Use Standard Precautions
☑ Material Safety Data Sheet, Attached for each product
☐ As Follows: _____

Product Preparation

☑ No dilution required, Use as received (RTU) See below for mixing parts 1+2+3 ZMB 10/27/17
☐ *Dilution(s) to be tested:

_____ defined as _____ + _____
(example: 1 oz/gallon) (amount of test substance) (amount of diluent)

☐ Deionized Water (Filter or Autoclave Sterilized)
☐ Soft Tap Water (Filter or Autoclave Sterilized)
☐ AOAC Synthetic Hard Water: _____ PPM
☑ Other Mix Part 1 49% : Part 2 49% Part 3 2% 49 49 2

Part 1: 49 parts + Part 2: 49 parts + Part 3: 2 parts cc ZMB 10/27/17

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Towelette Products: ☑ Not Applicable (product is not towelette-based) OR
☐ A sufficient volume of liquid will be aseptically expressed by hand prior to use OR
☐ The liquid will be expressed by _____

Test Organism: ☑ *Staphylococcus aureus* (ATCC 6538)

Carrier Number: 5 test carriers per batch and 3 control carriers

Exposure Time: 10 Minutes

Exposure Temperature: 21±2°C
The recommended exposure temperature is 21±2°C. If an alternate condition is selected, the Sponsor may be responsible for population control failures that may result.

Carrier Material

☑ Glass (borosilicate) ☐ Polycarbonate
☐ Other: _____

(1) Revised per email ZMB 10/27/17

TEST SUBSTANCE SHIPMENT STATUS
(This section is for informational purposes only.)
❑ Test Substance is already present at Accuratus Lab Services.
❑ Test Substance has been or will be shipped to Accuratus Lab Services.
Date of expected receipt at Accuratus Lab Services:_____
❑ Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director)

COMPLIANCE
Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
❑ No (Non-GLP or Development Study)

PROTOCOL MODIFICATIONS
☑ Approved without modification
❑ Approved with modification

_____

_____

PROTOCOL ATTACHMENTS
Supplemental Information Form Attached - ❑ Yes ☑ No

SPONSOR DETERMINED TEST SUBSTANCE PERFORMANCE CRITERIA

❑ There is no required performance criterion.

☑ A minimum 6 $\log_{10}$ reduction in viable bacteria in biofilm should be observed

TESTING FACILITY MANAGEMENT VERIFICATION OF 40 CFR PART 160 SUBPART B (160.31(D))

Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing: ☑ **Yes** ❑ **No*** ❑ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ❑ **Yes** ☑ **No***

Optional Information to complete as applicable:
☑ A Certificate of Analysis (C of A) may be provided for each lot of test substance. If provided, the C of A will be appended to the report.
❑ Testing has been or will be conducted under protocol or study #:

_____

Stability testing has been or will be completed prior to or concurrent with efficacy testing:
☑ **Yes** ❑ **No*** ❑ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☑ **Yes** ❑ **No***

Optional Information to complete as applicable:
❑ Testing has been or will be conducted under protocol or study #:

_____

**If testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.*

Proprietary Information

APPROVAL SIGNATURES

SPONSOR:

NAME: Mr. Brian Narducci TITLE: Vice President of Operations

SIGNATURE: DATE: 9/11/17

PHONE: (480) 339 - 2858 EMAIL: bnarducci@decon7.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

Joseph Drake jdrake@decon7.com

Accuratus Lab Services:

NAME: Carrie K Bauer
Study Director

SIGNATURE: C K B
Study Director DATE: 10/27/17