STUDY TITLE

Germicidal and Detergent Sanitizing Action of Disinfectants

Study Identification Number

GLP1833

Protocol Number

P2014

Product Identity

Test Substance: D7 Part 1 (A)
Lots: 17-390, 17-391, 17-392

Test Substance: D7 Part 2 (B)
Lots: 17-393, 17-394, 17-395

Test Substance: D7 Part 3
Lot: 20335

Test Microorganism

Staphylococcus aureus ATCC 6538

Data Requirements

U.S. EPA 40 CFR § 158
EPA OCSPP 810.2300

Author

Nicholas Garcia, B.S.
Study Director

Study Completion Date

29NOV2017

Testing Facility

Microchem Laboratory
1304 West Industrial Blvd.
Round Rock, Texas 78681

Study Sponsor

Brian Narducci
Decon 7
8541 East Anderson Drive, Suite 106
Scottsdale, AZ 85255

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: _____

Agent/Submitter: _____

Title: _____

Date: _____

Signature: _____

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets U.S. Environmental Protection Agency's Good Laboratory Practice Standards and requirements for 40 CFR § 160 with the following exception:

1. Records concerning test substance characteristics (i.e. composition, purity, stability, strength, solubility) are maintained by the Study Sponsor. The test substance certificate(s) of analysis may be found attached to this report for reference.

Study Director

Company: Microchem Laboratory

Name: Nicholas Garcia, B.S.

Title: Study Director

Signature: _____ Date: 29Nov2017

Study Sponsor

Company: Decon 7

Name: Brian Narducci

Title: Study Sponsor

Signature: _____ Date: 11/29/17

Submitter

Company:

Name:

Title:

Signature: _____ Date: _____

QUALITY ASSURANCE STATEMENT

Study Title: Germicidal and Detergent Sanitizing Action of Disinfectants

Study ID#: GLP1833

The following quality assurance audits were conducted in accordance with Good Laboratory Practice Standards outlined in 40 CFR §160 and reported to management and the Study Director:

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
In Phase (Treatment and Neutralization of Test Suspension)	16NOV2017	16NOV2017	17NOV2017
Draft Report	21NOV2017	21NOV2017	22NOV2017
Final Report	28NOV2017	28NOV2017	28NOV2017

Quality Assurance Unit:

Signature: Travis Chesser Date: 29NOV2017

Name: Travis Chesser, B.S.
Title: Quality Assurance Specialist

PERSONNEL INVOLVED IN THE STUDY

Study Director
Name: Nicholas Garcia, B.S.
Company: Microchem Laboratory
Title: Study Director

Scientific Director
Name: Benjamin Tanner, Ph.D.
Company: Microchem Laboratory
Title: Scientific Director

Assisting Laboratory Personnel
Name: Hamza Boukhriss, B.S.
Company: Microchem Laboratory
Title: Technician

Name: Beth Richard, B.S.
Company: Microchem Laboratory
Title: Technician

Name: Megan Rutland, B.S.
Company: Microchem Laboratory
Title: Technician

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FINAL STUDY REPORT SUMMARY

Study Title
Germicidal and Detergent Sanitizing Action of Disinfectants

Study Identification Number
GLP1833

Protocol Number
P2014

Test Microorganism
Staphylococcus aureus ATCC 6538

Study Sponsor
Brian Narducci
Decon 7
8541 East Anderson Drive, Suite 106
Scottsdale, AZ 85255

Testing Facility
Microchem Laboratory
1304 West Industrial Blvd.
Round Rock, Texas 78681

Study Director
Nicholas Garcia, B.S.

Study Completion Date
29NOV2017

Study Objective
To determine, using the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants method (AOAC Official Method 960.09), the antimicrobial efficacy of preparations of D7 Part 1 (A) (Lots: 17-390, 17-391, 17-392), D7 Part 2 (B) (Lots: 17-393, 17-394, 17-395), and D7 Part 3 (Lot: 20335) against *S. aureus* ATCC 6538 at a 30 ± 2 seconds contact time at ambient room temperature.

Study Conclusion
Preparations of D7 Part 1 (A) (Lots: 17-390, 17-391, 17-392), D7 Part 2 (B) (Lots: 17-393, 17-394, 17-395), and D7 Part 3 (Lot: 20335) demonstrated ≥99.999% reduction against *S. aureus* ATCC 6538 when compared to the initial numbers control after a 30 ± 2 seconds contact time at ambient room temperature.

FINAL STUDY REPORT

Important Dates

Study Initiation Date: 16NOV2017
Experimental Start Date/Time: 16NOV2017 / 1243
Experimental End Date/Time: 17NOV2017 / 1617

Test Substance Information

Name: D7 Part 1 (A)
Date of Manufacture: 01AUG2017
Date Received: 05SEP2017
Expiration Date: 01AUG2018
Lots: 17-390
Active Ingredient (Concentration): Alkyl Dimethylbenzyl Ammonium Chloride (3.04%)
17-391
Active Ingredient (Concentration): Alkyl Dimethylbenzyl Ammonium Chloride (3.06%)
17-392
Active Ingredient (Concentration): Alkyl Dimethylbenzyl Ammonium Chloride (3.06%)

Name: D7 Part 2 (B)
Date of Manufacture: 28JUL2017
Date Received: 05SEP2017
Expiration Date: 28JUL2018
Lots: 17-393
Active Ingredient (Concentration): Hydrogen Peroxide (7.528%)
17-394
Active Ingredient (Concentration): Hydrogen Peroxide (7.469%)
17-395
Active Ingredient (Concentration): Hydrogen Peroxide (7.501%)

Name: D7 Part 3
Date of Manufacture: 14SEP2016
Date Received: 05SEP2017
Expiration Date: 14SEP2018
Lot: 20335
Active Ingredient: Diacetin

See below for Lot combinations for a final of 49% D7 Part 1 (A), 49% D7 Part 2 (B), and 2% D7 Part 3:

Test Substance Preparation Ratio	Part 1 Lot# (Volume Used)	Part 2 Lot# (Volume Used)	Part 3 Lot# (Volume Used)	Total Volume Prepared
49:49:2	17-390 49.0 ml	17-393 49.0 ml	20335 2 ml	100 ml
	17-391 49.0 ml	17-394 49.0 ml	20335 2 ml	100 ml
	17-392 49.0 ml	17-395 49.0 ml	20335 2 ml	100 ml

FINAL STUDY REPORT (cont.)

Test Parameters

Microorganism:	*Staphylococcus aureus* ATCC 6538
Daily Subculture Medium:	Nutrient Agar A
Daily Subculture Inc. Time:	24 ± 2 hours
Subculture Number(s):	2 (for Neutralization Verification and Efficacy Test)
Test Culture Medium:	Nutrient Agar B
Test Culture Inc. Time:	24 ± 2 hours
Test Culture Dilution:	Test culture was diluted 4.0 ml into 9.0 ml Phosphate buffered dilution water prior to use in the study.
Culture Incubation Temp.:	36 ± 1°C
Number of Test Replicates:	1 per lot
Number of Control Replicates:	1 per test microorganism
Test Substance Diluent:	400 ppm AOAC Synthetic Hard Water
Test Substance Dilution:	Test substance was diluted at a ratio of 49:49:2 (49 parts D7 part 1 (A) + 49 parts D7 part 2 (B) + 2 parts D7 part 3). After combined, a further 1:5 dilution (1 part combination : 4 parts AOAC Hard Water) was performed. Test substance was thoroughly mixed for 20 seconds and used within 3 hours of preparation.
Number Neutralization Rep.:	1 per lot
Inoculation Volume:	1.0 ml
Sanitization Contact Time:	30 ± 2 seconds
Contact Temperature:	Room Temperature (23 ± 2°C)
Neutralization Broth:	2X Dey-Engley Neutralizing Broth supplemented to contain 1.0% Tween 80 and 1.0% catalase
Plate Incubation Temp.:	36°C ± 1°C
Plate Incubation Time:	25 Hours and 49 Minutes

Test Method

The test was conducted according to the attached protocol unless noted on page 10 of this report.

Test Description

A sterile 250 ml Erlenmeyer flask containing 99.0 ml of test substance was swirled in a circular motion to create enough residual motion to prevent pooling of the test microorganism during the inoculation process. 1.0 ml of the test culture was added halfway between the center and side of the flask immediately after swirling and during the residual swirling period. The flask was swirled thoroughly immediately after addition of the test microorganism. 1.0 ml of the test substance exposed to test culture was removed aseptically and neutralized at 30 seconds ± 2 seconds by addition to 19.0 ml sterile neutralization broth and was mixed well. A total of four, 1.0 ml and four, 0.100 ml volumes of harvested, neutralized test substance were pour plated with sterile tryptic soy agar to determine viable CFU/ml after the contact time. All plates were incubated at 36 ± 1°C for 24-30 hours.

PROTOCOL CHANGES

Protocol Amendments

No protocol amendments were issued for this study.

Protocol Deviations

No protocol deviations were noted for this study.

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CONTROLS

Initial Numbers Control

One flask containing 99.0 ml sterile Phosphate Buffered Dilution Water (PBDW) was inoculated with 1.0 ml of prepared test culture as performed in the test and harvested within 30 seconds. After neutralization, serial dilutions were made in PBDW out to 10^{-6}. Four, 1.0 ml and four, 0.100 ml volumes of the 10^{-6} tube were pour plated with sterile tryptic soy agar to determine the starting concentrations of test microorganism in CFU/ml.

Neutralization Control

A 1.0 ml volume of test substance of each lot was added separately to 19.0 ml volumes of neutralization broth to represent the neutralization test. A 0.100 ml volume of diluted test culture was added directly to each neutralization test tube within 30 seconds of adding the test substance, to 20.0 ml PBDW (neutralization control), and to 20.0 ml neutralizer (toxicity control). All tubes were vortex mixed and plated in duplicate to determine CFU/ml. The neutralization test result was directly compared to the neutralization control result for each lot of test substance.

Media Sterility Control

A 1.0 ml aliquot of PBDW, PBS supplemented with Tween 80 (PBST), test substance diluent, and neutralization broth was plated to determine media sterility. One plate containing only the growth medium used in this study was incubated to determine media sterility.

Media Growth and Culture Purity Control

A loopful of the test microorganism culture was struck to sterile tryptic soy agar to achieve isolated colonies in order to confirm culture purity as well as serve as the media growth control for this study.

Test Substance Sterility Control

A 1.0 ml aliquot of each lot of test substance was plated to determine test substance sterility.

STUDY ACCEPTANCE CRITERIA

The experimental success (controls) criteria follow:

- The test microorganism must demonstrate a concentration of 1 x 10^7 CFU/ml corresponding to a mean log density of 7.0 and not above 1 x 10^8 CFU/ml corresponding to a mean log density of 8.0
- The plates for the neutralization viability control, neutralization toxicity control, and all test substance lots evaluated demonstrate 10-100 CFU.
- The media sterility controls are negative for growth.
- To demonstrate neutralization, the differences between the three neutralizer treatments should not exceed 1 log.
- The test microorganism demonstrates a pure culture based on morphological characteristics typical of selected test microorganisms.

The U.S. EPA performance criterion for sanitization follows:

- The test substance must demonstrate a 99.999% reduction when compared to the averaged Initial Numbers Control within 30 seconds.

Retesting guidance for disinfection follows:

- When a test fails and the $\log_{10}$ density of the test microorganism is below 7.0, no retesting is necessary.
- When a test passes and the $\log_{10}$ density of the test microorganism is above 8.0, no retesting is necessary.
- When a test fails and the $\log_{10}$ density of the test microorganism is above 8.0, retesting may be conducted.

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CALCULATIONS AND STATISTICAL ANALYSIS

Test and Initial Numbers Control Calculation

- CFU/ml = $\frac{\text{(avg. CFU for } 10^{-x}) + \text{(avg. CFU for } 10^{-y})}{10^{-x} + 10^{-y}} \times 2$
- Plates were counted between 0 – 300 CFU, when possible, and recorded.
  A multiplication factor of 2 was used in calculations to account for the increased neutralization dilution ratio from 1:10 to 1:20.

Neutralization Control Calculation

- CFU/ml = [(Plate Count 1 + Plate Count 2)/ 2]
- Plates were counted between 0 – 300 CFU, when possible, and recorded.

Percent Reduction was calculated as follows:

- Percent Reduction = 100 [(B-A)/B]

  A= The average of the number of viable CFU/ml recovered from each treated test substance replicate at each contact time.

  B= The average of the number of viable CFU/ml recovered from the initial numbers control.

Log Reduction was calculated as follows:

- Log Reduction = Mean $\log_{10}$ numbers control – mean $\log_{10}$ treated sample

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STUDY RECORD AND TEST SUBSTANCE RETENTION

Study Record Retention

The study report and corresponding raw data will be held in the archives of Microchem Laboratory for at least two years after the study completion date. Afterward, Microchem reserves the right to transfer the documents to the Sponsor at Sponsor's expense. After record retention periods as described by section 195 of EPA and FDA GLP regulations have elapsed, unnecessary documentation may be destroyed.

Test Substance Retention

The test substance may be returned to the Study Sponsor at Study Sponsor's request and expense within 30 days of study completion. If the Study Sponsor does not request return of the sample, it will be destroyed >90 days after study completion.

RESULTS

Table 1

The following were initial numbers enumeration results for efficacy testing conducted on 16NOV2017.

Test Microorganism	CFU/mL
S. aureus ATCC 6538	1.82E+07

Table 2

The following were the test results for D7 Part 1 (A) (Lots: 17-390, 17-391, 17-392), D7 Part 2 (B) (Lots: 17-393, 17-394, 17-395), D7 Part 3 (Lot: 20335) when tested against *S. aureus* ATCC 6538 at a 30 seconds ± 2 seconds contact time. Efficacy testing was conducted on 16NOV2017.

Test Microorganism	Contact Time	Test Substance	CFU/mL	Percent Reduction Compared to Initial Numbers Control
S. aureus ATCC 6538	30 ± 2 seconds	D7 Part 1 (A) Lot: 17-390 D7 Part 2 (B) Lot: 17-393 D7 Part 3 Lot: 20335	<2.00E+01	>99.99989%
		D7 Part 1 (A) Lot: 17-391 D7 Part 2 (B) Lot: 17-394 D7 Part 3 Lot: 20335	<2.00E+01	>99.99989%
		D7 Part 1 (A) Lot: 17-392 D7 Part 2 (B) Lot: 17-395 D7 Part 3 Lot: 20335	<2.00E+01	>99.99989%
Note: The limit of detection for this assay was 20 CFU/ml. Results observed to be below the limit of detection for this assay were noted as <2.00E+01 in the data table.				

RESULTS (cont.)

Table 3

The following were the neutralization results for D7 Part 1 (A) (Lots: 17-390, 17-391, 17-392), D7 Part 2 (B) (Lots: 17-393, 17-394, 17-395), D7 Part 3 (Lot: 20335) against *S. aureus* ATCC 6538. Neutralization confirmation tubes were inoculated within 30 seconds of neutralization and held for at least 2 minutes prior to enumeration. Neutralization Validation was performed on 16NOV2017.

Test Microorganism	Test Substance	CFU/ml	Log_{10} CFU/ml	Neutralization Valid?
S. aureus ATCC 6538	Neutralization Control	53.5	1.73	Yes
	Toxicity Control	49.0	1.69	
	D7 Part 1 (A) Lot: 17-390 D7 Part 2 (B) Lot: 17-393 D7 Part 3 Lot: 20335	53.5	1.73	
	D7 Part 1 (A) Lot: 17-391 D7 Part 2 (B) Lot: 17-394 D7 Part 3 Lot: 20335	42.5	1.63	
	D7 Part 1 (A) Lot: 17-392 D7 Part 2 (B) Lot: 17-395 D7 Part 3 Lot: 20335	52.5	1.72	

Table 4

The following were the incubation times and temperature ranges for the test materials incubated in this study for D7 Part 1 (A) (Lots: 17-390, 17-391, 17-392), D7 Part 2 (B) (Lots: 17-393, 17-394, 17-395), D7 Part 3 (Lot: 20335).

Incubation Temperature Ranges	Test Culture	Transfer Date and Time	Culture Incubation Time
36 ± 1 °C	Initial Culture From Microbial Library Stock (Transfer 1)	14NOV2017 / 1321	23 Hours 00 Minutes
	Daily Transfer (Transfer 2)	15NOV2017 / 1242	23 Hours 22 Minutes

RESULTS (cont.)

Table 5

The following were the incubation times and temperature ranges for the test materials incubated in this study for D7 Part 1 (A) (Lots: 17-390, 17-391, 17-392), D7 Part 2 (B) (Lots: 17-393, 17-394, 17-395), D7 Part 3 (Lot: 20335).

Incubation Temperature Range	Test Materials	Incubation Duration
36 ± 1 °C	Test Enumeration Plates, Control Enumeration Plates, Neutralization Verification Plates, Media Sterility Controls, Test Substance Sterility, and Purity Streak Plate	25 Hours 49 Minutes

Table 6

The following were the results from the purity, growth and sterility controls.

Test Microorganism	Control	Result
S. aureus ATCC 6538	Growth Control	Growth
	Purity Control	Pure
	PBDW Sterility	Sterile
	Hard Water Sterility	Sterile
	PBST Sterility	Sterile
	Neutralization Broth Sterility	Sterile
	D7 Part 1 (A) Lot: 17-390 D7 Part 2 (B) Lot: 17-393 D7 Part 3 Lot: 20335	Sterile
	D7 Part 1 (A) Lot: 17-391 D7 Part 2 (B) Lot: 17-394 D7 Part 3 Lot: 20335	Sterile
	D7 Part 1 (A) Lot: 17-392 D7 Part 2 (B) Lot: 17-395 D7 Part 3 Lot: 20335	Sterile
	TSA Sterility	Sterile

STUDY CONCLUSION

For Study Identification Number GLP1833, test substance D7 Part 1 (A) (Lots: 17-390, 17-391, 17-392), D7 Part 2 (B) (Lots: 17-393, 17-394, 17-395), D7 Part 3 (Lot: 20335) were diluted per the approved protocol and were tested against *S. aureus* ATCC 6538. A total of 1 flask per preparation was inoculated and the test culture was exposed to each combination of test substance at ambient room temperature for a contact time of 30 ± 2 seconds and then chemically neutralized.

Test substance preparation for D7 Part 1 (A) (Lot:17-390), D7 Part 2 (B) (Lot: 17-393), and D7 Part 3 (Lot: 20335) demonstrated a >99.999% reduction when the test substance enumeration results were compared to the initial numbers control after a contact time of 30 ± 2 seconds against *S. aureus* ATCC 6538.

Test substance preparation for D7 Part 1 (A) (Lot:17-391), D7 Part 2 (B) (Lot: 17-394), and D7 Part 3 (Lot: 20335) demonstrated a >99.999% reduction when the test substance enumeration results were compared to the initial numbers control after a contact time of 30 ± 2 seconds against *S. aureus* ATCC 6538.

Test substance preparation for D7 Part 1 (A) (Lot:17-392), D7 Part 2 (B) (Lot: 17-395), and D7 Part 3 (Lot: 20335) demonstrated a >99.999% reduction when the test substance enumeration results were compared to the initial numbers control after a contact time of 30 ± 2 seconds against *S. aureus* ATCC 6538.

The prepared test substances met the U.S. EPA Product Performance criteria for sanitizing previously cleaned food-contact surfaces against *S. aureus* ATCC 6538.

The study was carried out in compliance with the approved protocol (P2014) and all experimental controls met the established acceptance criteria unless otherwise stated on page 10 of this report.

REFERENCES

1. "Association of Official Analytical Chemists" *AOAC Official Method 960.09*. Germicidal and Detergent Sanitizing Action of Disinfectants. Revised First Action 2013

2. U.S. EPA Product Performance Guidelines, *OCSPP 810.2300: Sanitizers for Use on Hard Surfaces-Efficacy Data Recommendations.* (Sept. 4, 2012).

3. U.S. EPA Product Performance Guidelines*, OCSPP 810.2000: Public Health Uses of Antimicrobial Agents* (Sept. 4, 2012).

PROTOCOL

Protocol for GLP Germicidal and Detergent Sanitizing Action of Disinfectants
P2014

Test Microorganism(s)
Staphylococcus aureus ATCC 6538

Product Identity
Test Substance: D7 Part 1 (A)
Lot Numbers: 17-390, 17-391, 17-392

Test Substance: D7 Part 2 (B)
Lot Numbers: 17-393, 17-394, 17-395

Test Substance: D7 Part 3
Lot Numbers: 20335

Data Requirement
U.S. EPA 40 CFR Part 158
U.S. EPA OCSPP 810.2300

Study Sponsor
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Microchem Laboratory
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Protocol Number
P2014

Study Director
Nicholas Garcia, B.S.

Date
10NOV2017

PROTOCOL (cont.)

I. Introduction

This document details the materials and procedure for evaluating the antimicrobial efficacy of a sanitizer using the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Food Contact Surfaces under GLP testing conditions. This document also explains the terms and conditions of testing.

II. Purpose

The purpose of this study is to document the efficacy of the test substance against the test system (microorganism) under the parameters specified in this protocol.

III. Justification for the Selection of Test System (Microorganism)

The test microorganism listed on page 1 of this protocol is designated as a required microorganism for the test method AOAC Germicidal and Detergent Sanitizing Action of Disinfectants as well as per EPA Product Performance Test Guidelines, OCSPP 810.2300, Sanitizers for Use on Hard Surfaces–Efficacy Data Recommendations and other related EPA guidance.

IV. Terms and Conditions

Studies by Microchem Laboratory are conducted in accordance with general terms and conditions posted on www.MicrochemLab.com/terms

Prior to study initiation, Microchem Laboratory must receive the approved and signed protocol, test substance and payment. Changes to the signed, approved protocol will require amendment and may incur additional fees. Cancellation of the study any time after the protocol has been signed will result in a cancellation fee of up to 100% of the total study cost, to be determined by laboratory management at its sole discretion.

Microchem Laboratory may repeat studies, free of charge, in the event of unintended protocol non-conformance, if the non-conformance is determined by the Study Director to have affected the study outcome. If the neutralization system specified for a study is not adequate, the study will be deemed "inconclusive" and the Study Sponsor will be responsible for the cost of the study. In addition, the Study Sponsor is responsible for the cost of all studies performed to confirm the outcome of a previous study and for ensuring that the study will meet their regulatory objectives.

The Study Sponsor must obtain written consent from Microchem Laboratory to use or publish its protocols, study reports (or parts thereof), logo or employee names for marketing purposes.

Test substance characterization as to content, stability, etc., is the responsibility of the Study Sponsor. The test substance shall be characterized by the sponsor prior to the completion of this study.

PROTOCOL (cont.)

V. Test Substance Identification, Characterization, and Handling

All test substances used to substantiate antimicrobial efficacy claims will be manufactured or otherwise tested at the lower certified limit (LCL).

Test Substance Name: – D7 Part 1 (A)
Test Substance Lot Number(s) – 17-390
Ingredient(s) & Concentration(s) – Alkyl Dimethylbenzyl Ammonium Chloride 3.04%
Test Substance Manufacture Date – 01AUG2017
Test Substance Expiration Date –01AUG2018

Test Substance Name: – D7 Part 1 (A)
Test Substance Lot Number(s) – 17-391
Ingredient(s) & Concentration(s) – Alkyl Dimethylbenzyl Ammonium Chloride 3.06%
Test Substance Manufacture Date – 01AUG2017
Test Substance Expiration Date –01AUG2018

Test Substance Name: – D7 Part 1 (A)
Test Substance Lot Number(s) – 17-392
Ingredient(s) & Concentration(s) – Alkyl Dimethylbenzyl Ammonium Chloride 3.06%
Test Substance Manufacture Date – 01AUG2017
Test Substance Expiration Date –01AUG2018

Test Substance Name: – D7 Part 2 (B)
Test Substance Lot Number(s) – 17-393
Ingredient(s) & Concentration(s) – Hydrogen Peroxide 7.528%
Test Substance Manufacture Date – 28JUL2017
Test Substance Expiration Date – 28JUL2018

Test Substance Name: – D7 Part 2 (B)
Test Substance Lot Number(s) – 17-394
Ingredient(s) & Concentration(s) – Hydrogen Peroxide 7.469%
Test Substance Manufacture Date – 28JUL2017
Test Substance Expiration Date – 28JUL2018

Test Substance Name: – D7 Part 2 (B)
Test Substance Lot Number(s) – 17-395
Ingredient(s) & Concentration(s) – Hydrogen Peroxide 7.501%
Test Substance Manufacture Date – 28JUL2017
Test Substance Expiration Date – 28JUL2018

Test Substance Name: – D7 Part 3
Test Substance Lot Number(s) – 20335
Ingredient(s) & Concentration(s) – Diacetain
Test Substance Manufacture Date – 14SEP2016
Test Substance Expiration Date – 14SEP2018

PROTOCOL (cont.)

Special Handling Requirements — None

Test substances and devices are handled as follows:

- The test substance is stored at ambient (room) temperature under fluorescent lighting or in a cabinet.
- The test substance is shaken or otherwise mixed well immediately prior to use (if applicable).
- The test substance is handled safely in accordance with the chemical risks it may pose, stated in the MSDS or by the Study Sponsor during the course of pre-study communication.

VI. Study Parameters, Incorporated by Reference

Number of Tests Comprising the Study — 3 (1 Test per Test Substance Lot per Test Microorganism)
Control Replicates – 1 per Test Microorganism
Test Replicates — 1 per Lot, per Test Microorganism
Test Substance Form — Dilution Required
(49:49:2). 49 parts D7 Part 1 (A) + 49 parts D7 Part 2 (B) + 2 parts D7 Part 3
After 3 parts are combined, a further 1:5 dilution is performed (1 part combination + 4 parts diluent)
Test Substance Diluent — 400 ± 10 ppm AOAC Synthetic Hard Water
Test Temperature — Room Temperature (Approximately 23°C ± 2 °C)
Contact Time — 30 seconds ± 2 seconds
Neutralization Broth — To be noted in final report

Proposed Experimental Start Date: 15NOV2017
Proposed Experimental Termination Date: 22NOV2017

VII. Test System (Microorganism)

Staphylococcus aureus ATCC 6538

VIII. Materials

- Test substance(s).
- Pure culture of test system (microorganism).
- Sterile filter flask, Buchner funnel, sufficient quantity of sterile Whatman (or equivalent) #2 90cm filter paper.
- Sufficient number of clean, sterile 250 ml wide-mouth Erlenmeyer flasks, sufficient quantity of sterile 25 x 150 mm test tubes.
- Sufficient quantity of sterile Petri dishes.
- Sufficient quantity of sterile neutralizer.
- Sufficient quantity of sterile Phosphate buffer dilution water (PBDW).
- Sufficient quantity of sterile 1x PBS with 0.1% Tween 80.

PROTOCOL (cont.)

- Sufficient quantity of sterile tap water.
- Sufficient volume of sterile Tryptic Soy Agar for bacterial enumerations.
- Sufficient quantity of sterile Nutrient agar "A" (3 g of beef extract, 5 g peptone, and 15 g agar in 1L H_2O) prepared by dispensing 10 ml volumes in 25 x 150mm test tubes. Slant to cool.
- Sufficient quantity of sterile Nutrient agar "B" (3 g beef extract, 5 g peptone, and 30 g agar in 1L H_2O) prepared by dispensing 25 ml into sterile Petri dishes.
- Sufficient quantities of glacial acetic acid (17.4M), 0.1N sodium thiosulfate, 0.28 N iodine solution, sterile DI water, potassium iodide, and 0.5% starch solution for titration.
- Sufficient quantity of calibrated micropipettes and appropriately sized sterile micropipette tips.
- Appropriate volume of 95% ethanol.
- Inoculating loop.
- Bunsen burner, microbiological incinerator, or micro-torch as appropriate to ensure rapid and complete flame-sterilization of transfer loops.
- Automatic pipettor (PipetAid or similar) and various sizes of sterile serological pipettes.
- Sufficient quantity of sterile 50ml centrifuge tubes.
- Certified satellite clock.
- Calibrated digital timer.
- Vacuum pump.
- Incubator capable of sustaining temperature of 36 ± 1° C.

IX. Procedure

Preparation of Test Culture

- A daily culture is initiated on a Nutrient Agar "A" slant from a library stock culture and is incubated at 36 ± 1° C for 24 ± 2 hours. Only one daily transfer is required prior to initiating the test culture.
- Surface growth from the last daily transfer is washed with 5 ml PBDW, dislodged from the slant using a sterile rod (or equivalent), and added to 99 ml PBDW.
- A 0.200 ml volume of the dilute suspension is added to each of a minimum of 5 Nutrient Agar "B" plates in order to create a bacterial lawn.
- Plates are incubated at 36 ± 1°C for 24 ± 2 hours. These cultures represent the test culture for use in this study.
- After incubation, 5 ml of 1x PBS + 0.1% Tween 80 is added to each plate and the culture is dislodged from the plate using a sterile rod (or equivalent).
- Washed surface growth is pooled in a sterile 50 ml conical centrifuge tube (or equivalent).
- The pooled microorganism suspension is passed through a pre-wet Whatman No.2 (or equivalent) sterile filter paper and collected in a sterile vessel as needed.
- Using PBDW, each test culture is standardized to give an approximate average of $1 \times 10^9 - 1 \times 10^{10}$ CFU/ml.

PROTOCOL (cont.)

Preparation of Neutralization/Elution media

- Before the test begins, a sufficient volume of the appropriate neutralizer is prepared and steam sterilized.

Preparation of AOAC synthetic hard water solution

- From each 1000 mL of sterile deionized (DI) water (as measured by 1L volumetric flask), a volume equal to the total volume of AOAC hard water reagents added in the steps below is removed by serological pipette. For example, if 4 mL of solution "1" and 4 mL of solution "2" are to be added, then 8 mL of sterile water is removed.
- The concentration in PPM of hard water to be made is divided by 100. That is the volume, in mL, of AOAC hard water solution "1" will be needed to make 1000 mL of hard water.
- Based on the calculation above, an appropriate volume of AOAC solution "1" is added to the sterile water, and mixed.
- The appropriate volume of solution "2" is then added and mixed.
- An appropriate volume of the synthetic hard water is removed and titrated. If necessary, the solution may be diluted with sterile water or augmented with parts of solution "1" and "2" to achieve the study sponsor requested hard water level. In any case, the hard water concentration of the final solution is to be determined by titration and recorded.

Preparation of Test Substance

- Test substance is prepared by dilution.
  - The following ratio is used to prepare the test substance 49:49:2. This is equivalent to 49 parts D7 Part 1 (A) + 49 parts D7 Part 2 (B) + 2 parts D7 Part 3.
  - The solution is stirred or mixed well for 15-20 seconds.
  - After the solution has mixed, a further 1:5 dilution is prepared by diluting 1 part of prepared solution into 4 parts of test substance diluent.
- Test substance is used within 3 hours of preparation.
- All flasks that will receive test substance are thoroughly cleaned and steam sterilized prior to use. A 99.0 ml volume of test substance is added to a 250 ml wide mouth Erlenmeyer flask.
- One flask is prepared for each lot of test substance for efficacy testing.
- The test vessels are allowed to equilibrate to test temperature for ≥ 10 minutes.
- The test substance is to be used within 3 hours of preparation.

Preparation of Initial Numbers Control

- All flasks that will receive control substance are thoroughly cleaned and steam sterilized prior to use.
- A 99.0 ml volume of sterile PBDW is added to a 250 ml wide-mouth Erlenmeyer flask.
- The control vessel is then allowed to equilibrate to test temperature for ≥10 minutes.
- One flask is prepared per test microorganism.

PROTOCOL (cont.)

Inoculation of Test Substance with Test Culture

- Flasks containing 99.0 ml Test Substance are swirled in a circular motion to create enough residual motion to prevent pooling of the test microorganism during the inoculation process.
- A 1.0 ml volume of test culture is added halfway between the center and side of the flask immediately after swirling and during the residual swirling period.
- The flask is swirled thoroughly immediately after addition of the test microorganism.
- A 1.0 ml volume of test substance exposed test culture is removed and neutralized at the contact time by addition to 19.0 ml neutralization broth and is mixed well.
- A total of four (4) 1.0 ml and four (4) 0.100 ml volumes of harvested, neutralized test substance are plated using standard pour or spread plating techniques to determine viable CFU/ml after the contact time.
- Additional dilutions may be performed and plated at the discretion of the Study Director.

Initial Numbers Control

- Flasks containing 99.0 ml sterile PBDW are swirled in a circular motion to create enough residual motion to prevent pooling of the test microorganism during the inoculation process.
- A 1.0 ml volume of test culture is added halfway between the center and side of the flask immediately after swirling and during the residual swirling period.
- The flask is swirled thoroughly immediately after addition of the test microorganism.
- A 1.0 ml volume of PBDW exposed to test culture (Initial Numbers Control) is removed within 30 seconds and added to 19.0 ml neutralization broth and is mixed well. This tube corresponds to the 10^{-1} dilution tube.
- Serial dilutions are made in 9.0 ml PBDW out to 10^{-6}.
- A total of four (4) 1 ml volumes and four (4) 0.1 ml volumes of the 10^{-6} dilution tube, representing dilutions of 10^{-6} and 10^{-7}, respectively, are plated to determine initial viable CFU/ml at the time of treatment (Initial Numbers Control).

Neutralization Confirmation Test

- A 1:10 serial dilution of each test culture is performed in sterile PBDW to yield a microorganism concentration of approximately 1 x 10^{4} CFU/ml.
- A 20.0 ml volume of sterile PBDW is prepared to represent the neutralization viability control.
- A 20.0 ml volume of sterile neutralizer is prepared to represent the neutralization toxicity control.
- A 1.0 ml volume of test substance is added directly to 19.0 ml neutralization broth to represent the neutralization confirmation treatment. The neutralization confirmation treatment tubes are inoculated within 30 seconds of adding the test substance to the neutralization tube.
- All neutralization tubes are inoculated with 0.1 ml of the dilute test culture, held for at least 2 minutes, and vortex mixed.
- Two 1.0 ml aliquots are plated using appropriate growth media and incubated.

PROTOCOL (cont.)

Media Sterility Control(s)

- A 1 ml aliquot of PBS with 0.1% Tween 80 is plated to sterile growth medium and incubated alongside enumeration plates to verify sterility at the time of test.
- A 1 ml aliquot of test substance diluent (if used) is plated to sterile growth medium and incubated alongside enumeration plates to verify sterility at the time of test.
- A plate containing only growth medium used in this study is incubated alongside enumeration plates to verify sterility at the time of test.
- A 1 ml aliquot of PBDW is plated to sterile growth medium and incubated alongside enumeration plates to verify sterility at the time of test.
- A 1 ml aliquot of neutralizer is plated to sterile growth medium and is incubated alongside enumeration plates to verify sterility at the time of test.

Test Substance Sterility Control

- A 1 ml aliquot of prepared Test Substance per lot is plated to determine sterility at the time of test.

Media Growth and Culture Purity Control

- A loopful of the test microorganism culture is struck to the appropriate growth agar to achieve isolated colonies in order to confirm culture purity based on morphology, as well as serve as the media growth control for this study.

Incubation of Plates and Controls

- All enumeration and control plates are incubated for 24-30 hours at 36 ± 1°C.

X. Calculations

Test and Initial Numbers Control Calculation

- $\text{CFU/ml} = \frac{(\text{avg. CFU for } 10^{-x}) + (\text{avg. CFU for } 10^{-y})}{10^{-x} + 10^{-y}}$
- Plates are counted between 0 – 300 CFU, when possible, and recorded.

Neutralization Control Calculation

- CFU/ml = [(Plate Count 1 + Plate Count 2)/ 2]
- Plates are counted between 00 – 300 CFU, when possible, and recorded.

Percent Reduction is calculated as follows:

PROTOCOL (cont.)

- Percent Reduction = 100 [(B-A)/ B]

  A= The average of the number of viable CFU/ml recovered from each treated test substance replicate at each contact time.
  B= The average of the number of viable CFU/ml recovered from the initial numbers control.

Log Reduction is calculated as follows:

Log Reduction = Mean $\log_{10}$ numbers control – mean $\log_{10}$ treated sample

XI. Success Criteria

- The experimental success (controls) criteria follow:
  - The test microorganism must demonstrate a concentration of 1×10^7 CFU/ml corresponding to a mean log density of 7.0 and not above 1×10^8 CFU/ml corresponding to a mean log density of 8.0
  - The plates for the neutralization viability control, neutralization toxicity control, and all test substance lots evaluated demonstrate 10-100 CFU.
  - The media sterility controls are negative for growth.
  - To demonstrate neutralization, the differences between the three neutralizer treatments should not exceed 1 log.
  - The test microorganism demonstrates a pure culture based on morphological characteristics typical of selected test microorganism.

- The U.S. EPA performance criterion for sanitization follows:
  - The test Substance must demonstrate a 99.999% reduction when compared to the averaged Initial Numbers Control within 30 seconds.
-
- Retesting guidance for disinfection follows:
  - When a test fails and the $\log_{10}$ density of the test microorganism is below 7.0, no retesting is necessary.
  - When a test passes and the $\log_{10}$ density of the test microorganism is above 8.0, no retesting is necessary.
  - When a test fails and the $\log_{10}$ density of the test microorganism is above 8.0, retesting may be conducted.

XII. Reporting

- Results are reported accurately and fully, in accordance with EPA GLP (40 CFR Part 160). A draft report will be provided for review by the Study Sponsor prior to study completion.

XIII. Data and Sample Retention

- The study report and corresponding raw data will be held in the archives of Microchem Laboratory for at least two years after the study completion date. Afterward, Microchem reserves the right to transfer the documents to the Sponsor at Sponsor's expense. After record retention periods as described by section 195 of EPA and FDA GLP regulations have elapsed, unnecessary documentation may be destroyed.

PROTOCOL (cont.)

- The test substance may be returned to the Study Sponsor at Study Sponsor's request and expense within 30 days of study completion. If the Study Sponsor does not request return of the sample, it will be destroyed >90 days after study completion.

XIV. Quality Control

- The study is conducted in accordance with Microchem Laboratory's Quality Management System and will undergo a full quality assurance review. All protocol amendments will be fully recorded and reported, as well as any deviations from the protocol.

XV. References

- "AOAC International." *AOAC Official Method 960.09.* Germicidal and Detergent Sanitizing Action of Disinfectants. Revised First Action 2013.
- U.S. EPA Product Performance Test Guidelines OCSPP 810.2300: Sanitizers for use on Hard Surfaces – Efficacy Data Recommendations. September 4, 2012.

XVI. Protocol Approval

"I, the Study Sponsor, have read and understand the study protocol. By signing this protocol I am certifying that the information and parameters accurately describe the test(s) to be completed in accordance with Good Laboratory Practice Standards (GLPS) stipulated by 40 CFR 160. I have also read, understand and agree to the terms and conditions listed in the protocol."

Study Sponsor/Representative Signature Approving Protocol

Brian Narducci, Study Sponsor, Decon 7 — Date: 11/13/2017

Nicholas Garcia, Study Director, Microchem Laboratory, LLC — Date: 16 Nov 2017

CERTIFICATE OF ANALYSIS

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

CERTIFICATE OF ANALYSIS (cont.)

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